Table 78. Organ Weights

Organ	Dose Group (mg/kg/day)							
	0 (Control)		300		1000		3000	
	M	F	M	F	M	F	M	F
Heart Weight								
Absolute Wt., gm	0.80	0.52	0.67*	0.45*	0.61*	0.43*	0.60*	0.43*
Relative Wt.[a]	0.29	0.36	0.25*	0.26*	0.26*	0.31*	0.26*	0.31*
Kidney Weight								
Absolute Wt., gm	1.80	1.11	2.01*	1.18	1.86*	1.18	1.77	1.20
Relative Wt.[a]	0.66	0.76	0.76*	0.83*	0.79*	0.84*	0.77*	0.88*

[a] Organ weight/body weight ratio
* Statistically significant difference from concurrent control value ($p<0.05$)

Histopathology: Higher than control incidences of hypertrophy of the juxtaglomerular (JG) cells and intimal proliferation of the interlobular artery in the kidney were observed in candesartan cilexetil-treated groups (Table 79). The severity of JG cell hypertrophy seen with 300 mg candesartan cilexetil/kg/day in males (mild hypertrophy in 7/10 and moderate hypertrophy in 3/30) increased with higher doses (mild in 1/10 and moderate in 9/10 after 1000 mg/kg/day and mild in 2/10 and moderate in 8/10 after 3000 mg/kg/day).

Table 79. Renal Histopathology Incidence[a]

Renal Pathology	Sex	Dose Group (mg/kg/day)			
		0 (Control)	300	1000	3000
Basophilic Renal Tubule	M	8/10	4/10	3/10	2/10
	F	2/10	4/10	3/10	2/10
JG Cell Hypertrophy	M	0/10	10/10	10/10	10/10
	F	0/10	10/10	10/10	9/10
Proliferation of Interlobular Artery	M	0/10	2/10	10/10	9/10
	F	0/10	4/10	2/10	3/10

[a] # Affected/ # Examined

Plasma Drug Levels: Plasma concentrations of candesartan associated with candesartan cilexetil treatment increased dose-dependently; candesartan levels at 8PM were higher than those at 8AM and at 2PM in each treated group at the end of week 1 (Table 80). No apparent plasma drug level differences between sexes were detected and no evidence of candesartan accumulation with repeated administration was observed. Though unchanged candesartan cilexetil was also measured at the end of week 13, it was not detected (detection limit= 0.1 ug/ml) in plasma in any treated group.

Table 80. Plasma Concentrations (ug/ml) of Candesartan in Rats Treated with Oral Candesartan Cilexetil[a].

Dose (mg/kg)	Sex	Week 1			Week 6	Week 13
		8 AM	2 PM	8PM	8 PM	8 PM
300	M	7.4	8.1	9.2	8.7	8.5
	F	7.7	7.3	8.0	8.5	8.3
	M & F	7.6	7.7	8.6	8.6	8.4
1000	M	19.7	17.0	25.6	29.0	30.6
	F	22.6	18.0	24.0	23.5	23.9
	M & F	21.2	17.8	24.8	26.3	27.3
3000	M	45.7	46.9	51.9	66.7	65.2
	F	47.0	32.3	53.0	57.0	64.5
	M & F	46.4	39.6	52.4	61.9	64.9

[a] Mean values for M or F, n= 3; for M & F, n=6.

24-Month Carcinogenicity Study of Dietary Candesartan Cilexetil in Rats

Study Facility: Takeda Chemical Industries, Inc., Yamaguchi, Japan
Study No.: T3055
Study Dates: Initiation of dosing: 3/12/92 End of Necropsy: 3/25/94

GLP Compliance: GLP compliance statement included.

Animals: Male and female F344/Jcl rats (6 weeks old; M=89-111 gm, F=73-94 gm at initiation of dosing).

Drug Administration: Candesartan cilexetil (Lot #M464-013,-104, -015, -016, -017, -018, -019, -021, -022 and -023) was incorporated in the diet; the concentrations were adjusted weekly based on the most recent body weight and food consumption values.

Dose Levels: 0 (control 1), 0 (control 2), 100, 300 and 1000 mg candesartan cilexetil/kg/day (50/sex/group); additional 10/sex/candesartan cilexetil dose group used for determination of plasma drug levels.

Observations/Measurements: Animals were observed twice daily for survival and clinical signs of toxicity. Palpations for masses were done weekly and mass location, consistency and size were noted for each animal. Body weight measurements were obtained at initiation of dosing, weekly from week 1 to 14, every 4 weeks from week 15 to 70 and then weekly, thereafter, to study termination. Food consumption was determined every week from week 1 to 14, every 4 weeks thereafter and for weeks 52 and 104. Individual drug intake values (mg/kg body weight/day) were

calculated weekly on the basis of food consumed, drug concentration in the diet and body weight. At necropsy, blood samples were obtained from the abdominal aorta of all surviving animals and all animals killed *in extremis* for hematology analysis. Gross pathological examinations were performed on all animals that died spontaneously, were killed in a moribund state or were sacrificed at the end of the study. Sections of major organs and tissues (listed in Appendix A, pg. 8) from all animals in the control and 1000 mg/kg/day groups and from rats which died spontaneously or were killed *in extremis* in the course of the study were examined microscopically. Sections of liver, kidneys, adrenal glands, stomach, spleen, testes and uterus and any tissue that showed gross neoplastic lesions from the 100 and 300 mg/kg group were also examined microscopically.

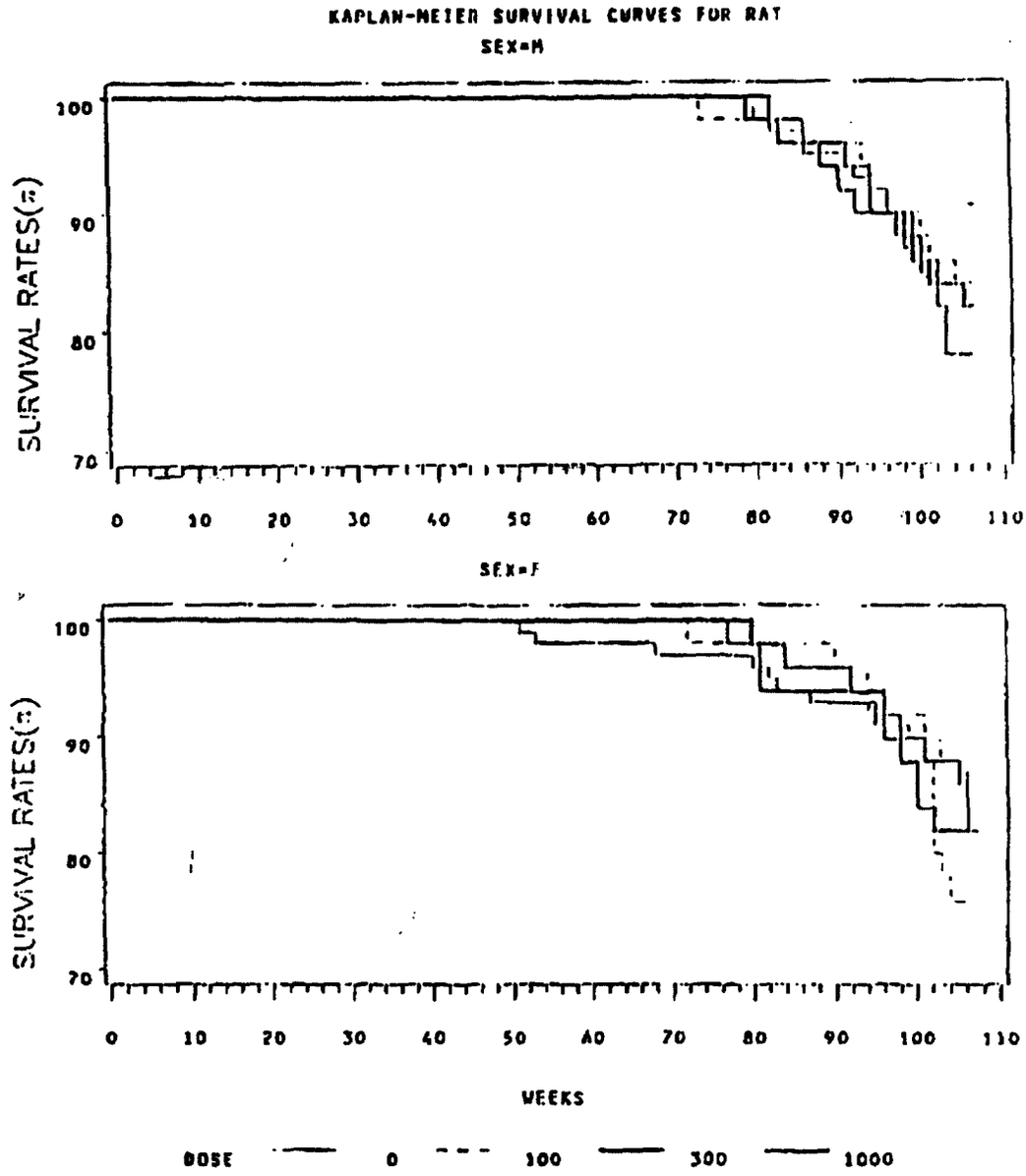
Results

Mortality and Survival: Forty-six male rats and 44 female rats died or were killed *in extremis* during the course of the study. There were no significant differences in survival rates among the control and treated groups of either sex (Table 81, Fig 13).

Table 81. Animal Survival

Sex	Male					Female				
Dose (mg/kg/day)	0	0	100	300	1000	0	0	100	300	1000
No rats/group	50	50	50	50	50	50	50	50	50	50
# Found dead	5	7	6	6	6	5	7	10	5	8
# Killed *in extremis*	3	3	2	5	3	1	1	2	4	1
Total	8	10	8	11	9	6	8	12	9	9
Survival Rate %	84	80	84	78	82	88	84	76	82	82

Figure 13.. Animal Survival Curves



Body Weight: Significantly lower than control body weights and body weight gains were noted for males in all treatment groups beginning week 1 of the dosing period (Table 82; Fig. 14). The body weight effects were dose-dependent and maximal around weeks 70-78 of the study; by study termination, body weights among treated male groups were comparable to control. For female rats, there was a lower than control body weight gain at the high dose early in the dosing period , but the mean weights of high dose and control groups differed by less than 5%, with no statistically significant difference, at the end of the treatment period.

Table 82. Body Weight, gm (% difference from control value)

Dosing Period	Male Dose Group (mg/kg/day)[a]				Female Dose Group (mg/kg/day)[a]			
	0	100	300	1000	0	100	300	1000
Week 0	100	100	100	100	84	84	84	83
Week 1	125	121*(-3.2)	121*(-3.2)	121*(-3.2)	98	97	97	97
Week 26	336	311*(-7.4)	307*(-8.6)	301*(-10)	174	172	170	168*(-3.4)
Week 52	389	353*(-9.3)	348*(-11)	338*(-13)	202	202	200	195*(-3.5)
Week 78	400	365*(-8.8)	356*(-11)	345*(-14)	243	240	239	232*(-4.5)
Week 104	346	344	344	329	262	270	264	251

* Significantly different from 0 (control) value ($p < 0.05$)

[a] Initial group size: n=100 for 0 (control); n=50 for all other groups

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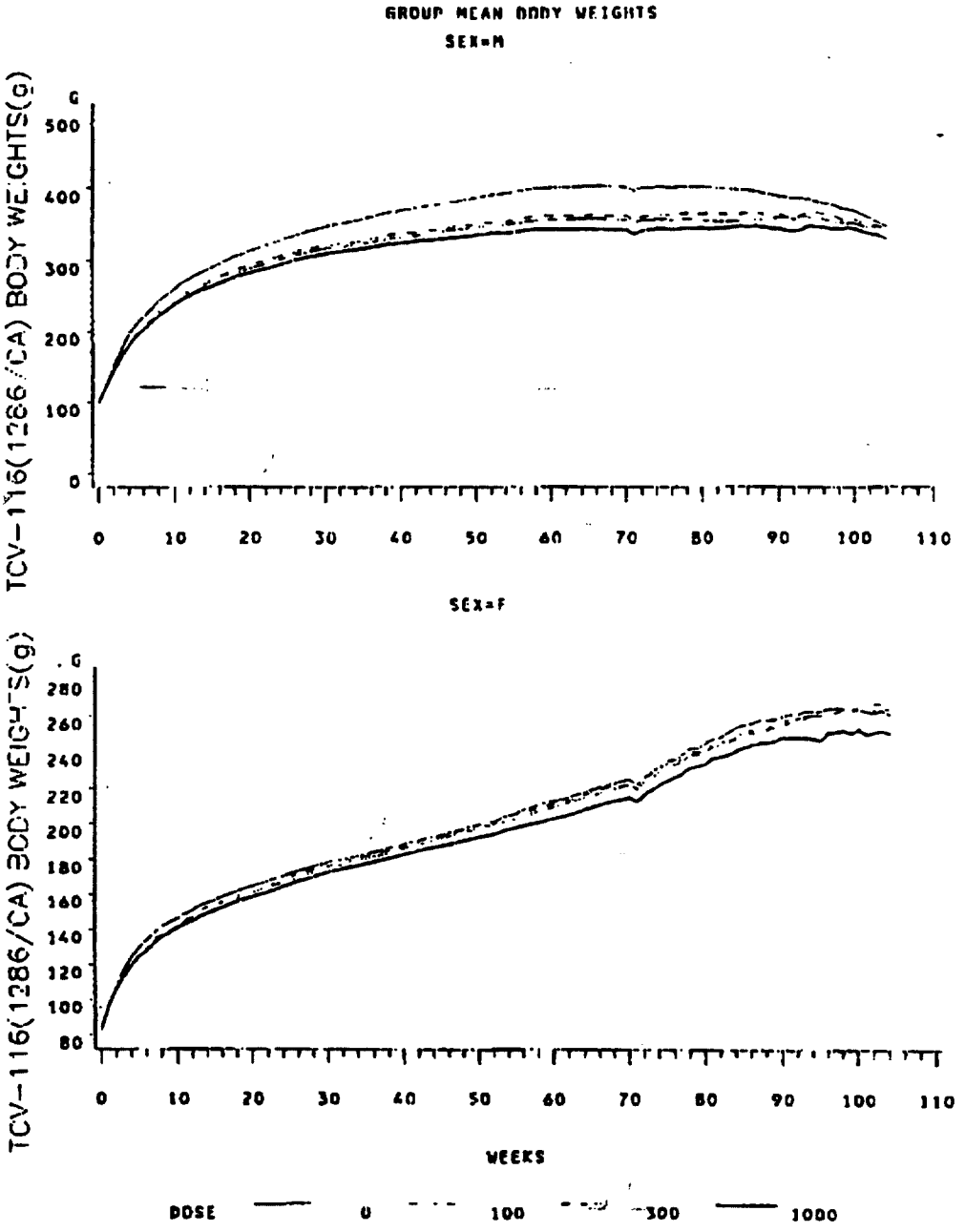
Figure 14. Body Weight Curves



Food Consumption: Significantly lower than control food consumption was noted for males in all treatment groups throughout the first 74 weeks of the study; by week 78, only the mid dose and high dose males showed significantly lower than control food consumption. Food consumption by candesartan cilexetil-treated females was comparable to control throughout most of the study (Table 83).

Table 83. Food Consumption, gm/rat/wk (% difference from control value)

Dosing Period	Male Dose Group (mg/kg/day)[a]				Female Dose Group (mg/kg/day)[a]			
	0	100	300	1000	0	100	300	1000
Week 1	88	80*(-9.1)	82*(-6.8)	81*(-8.0)	75	72*(-4.0)	73*(-2.7)	74
Week 26	110	105*(-4.5)	104*(-5.4)	104*(-5.4)	75	74	74	74
Week 52	115	111*(-3.5)	109*(-5.2)	109*(-5.2)	79	79	79	79
Week 78	109	108	104*(-4.6)	106*(-2.8)	87	87	88	87
Week 104	99	96	101	101	88	93	91	90

* Significantly different from 0 (control) value ($p < 0.05$)
[a] Initial group size: n=100 for 0 (control); n=50 for all other groups

Drug Intake: The achieved doses were within 10% of the intended dosages (Table 84).

Table 84. Achieved Doses of Candesartan Cilexetil, mean mg/kg/day

Dosing Period	Intended Dose (mg/kg/day)-Males			Intended Dose (mg/kg/day)-Females		
	100	300	1000	100	300	1000
Week 1	91.1	280.0	926.5	91.4	280.5	926.2
Week 26	100.4	300.3	1008.1	103.1	305.3	1015.9
Week 52	100.2	296.5	986.4	98.5	295.7	1081.6
Week 78	102.1	294.0	1001.0	102.9	312.2	1023.2
Week 104	96.3	294.2	991.2	102.8	303.0	1002.3
Week 1-104	100.7	302.7	1012.5	101.5	306.0	1021.0

Hematology: Slight and non-dose-related, but significantly lower than control numbers of platelets were noted in males in all the treatment groups. Slight and non-significantly lower than control erythroid values were noted in females treated with 1000 mg candesartan cilexetil/kg/day (Table 85).

Table 85. Hematology Values

Hematology Parameter	Sex	Dose Group (mg/kg/day)*			
		0 (Control)	100	300	1000
RBC Count, X 10^6/µl	M F	8.21 7.31	8.68 7.31	8.81 7.14	8.60 6.86
Hematocrit, %	M F	47.3 42.8	49.1 43.0	49.4 42.1	48.1 40.0
Hemoglobin, gm%	M F	14.5 13.2	15.1 13.3	15.1 13.0	14.9 12.5
Platelet Count, 10^4/µl	M F	65.9 54.5	49.2* 50.6	50.6* 51.6	51.6* 50.5

* Significantly differerent from control value ($p<0.05$).

Gross Pathology: Higher than control incidences of an enlarged spleen and a rough kidney surface were noted in high dose males (Table 86). The incidences of all other macroscopic lesions were comparable among control and treated groups.

Table 86. Macroscopic Lesion Incidence, # rats affected/# rats examined

Macroscopic Lesion	Sex	Dose Group (mg/kg/day)				
		0 (C-1)	0 (C-2)	100	300	1000
Enlarged Spleen	M F	8/50 4/50	9/50 7/50	7/50 3/50	9/50 4/50	12/50 2/50
Rough Kidney Surface	M F	39/50 11/50	34/50 12/50	42/50 12/50	42/50 9/12	48/50 11/50

Histopathology: Neoplastic Lesions

The sponsor's analysis revealed that the numbers of tumor-bearing animals and total primary neoplasms including benign, malignant and hematopoietic neoplasms were not significantly affected by candesartan cilexetil treatment (Table 87).

Table 87. Tumor Data for Rats[a]

Measurement	Sex	Dose Group (mg candesartan cilexetil/kg/day)				
		0 (C-1)	0 (C-2)	100	300	1000
No. of Animals	M	50	50	50	50	50
	F	50	50	50	50	50
No. of Tumor-Bearing Animals	M	50	50	50	50	50
	F	45	44	45	43	45
Primary Neoplasms						
Total	M	117	112	92	91	96
	F	89	88	85	68	86
Benign	M	104	102	75	78	79
	F	70	67	68	50	68
Malignant[b]	M	5	2	8	5	5
	F	14	14	13	12	16
Hematopoietic	M	8	8	9	8	12
	F	5	7	4	6	2

[a] No significant differences between treated and control groups; sponsor's analysis. [b] Excluding hematopoietic neoplasms

In female rats treated with candesartan cilexetil, the incidence of pheochromocytoma (benign), showed a positive trend (p= 0.044) and there were statistically significant pairwise differences between control and the 100 (p=0.036) and 1000 mg/kg/day (p=0.036) groups by the sponsor's analysis. There were no significant differences (p>0.05) between female combined control and treated groups in the incidences of malignant pheochromocytoma. When pheochromocytomas (benign) were combined with malignant pheochromocytomas, a statistically significant difference between control and the 1000 mg/kg/day (p=0.043) group was also detected. No evidence of candesartan cilexetil-related hyperplasia of the adrenal medulla was found (Table 88). There was no significant trend and no significant pairwise differences (p>0.05) in pheochromocytoma incidence between combined control and treated males.

FDA statistical analysis (Appendix B) showed no statistically significant trend (p>0.025) and no significant pairwise difference between combined control and the high dose group in the incidence of benign, malignant or the combination of benign and malignant pheochromocytoma for female or male rats.

Table 88. Hyperplasia and Tumors of the Adrenal Medulla

Parameter	Dose Group (mg candesartan cilexetil/kg/day)				
	0 (C-1)	0 (C-2)	100	300	1000
No. of Males	50	50	50	50	50
Males with Focal Hyperplasia	37	37	38	30	33
Males with Pheochromacytoma					
Benign	10	5	5	5	4
Malignant	0	1	0	0	0
Benign or Malignant	10	6	5	5	4
No. of Females	50	50	50	50	50
Females with Focal Hyperplasia	16	14	14	17	15
Females with Pheochromacytoma					
Benign	0	0	3*	1	3*
Malignant	1	0	0	0	1
Benign or Malignant	1	0	3*	1	4*

*Statistically different from combined control groups ($p<0.05$); sponsor's analysis

For male rats treated with candesartan cilexetil, the incidence of bilateral interstitial cell tumors of the testes showed, in the sponsor's analysis, a positive trend ($p=0.004$) and a statistically significant pairwise difference between combined control and the 1000 mg/kg/day dose group ($p=0.007$). However, there were no statistically significant differences ($p>0.05$) when the incidence of bilateral interstitial cell tumors were combined with unilateral tumors of the interstitial cells (Table 89).

FDA statistical analysis revealed no statistically significant trend ($p>0.005$) or significant pairwise difference between combined control and the 1000 mg/kg/day group in the incidence of combined bilateral and unilateral testicular interstitial cell tumors.

Table 89. Testicular Interstitial Cell Tumors

Parameter	Dose Group (mg candesartan cilexetil/kg/day)				
	0 (C-1)	0 (C-2)	100	300	1000
Interstitial Cell Tumors, Benign					
Bilateral[a]	40/50	44/50	46/50	46/50	49/50*
Unilateral[a]	5/50	4/50	2/50	3/50	0/50
Total[a]	45/50	48/50	48/50	49/50	49/50

[a] Values represent # rats affected/# rats examined (%)

* Significantly different from control value ($p< 0.05$); sponsor's analysis

An increase (non-significant) above control incidence (3/50 *vs* 1/50) of renal tubular tumors was seen in males receiving 300 mg candesartan cilexetil/kg/day; no renal tumors were seen in the 100 or 1000 mg/kg/day groups and none were observed in females of any group. The incidences of renal tubule hyperplasia in candesartan cilexetil-treated groups were comparable to control. Thus, the sponsor concludes the renal tumors appear to be unrelated to drug treatment (Table 90). FDA

statistical analysis revealed no significant increase above control incidence of renal tubular tumors among male or female treated groups.

Table 90. Renal Tubule Hyperplasia and Tumors in Male Rats[a]

Parameter	Dose Group (mg candesartan cilexetil/kg/day)				
	0 (C-1)	0 (C-2)	100	300	1000
No. of Animals	50	50	50	50	50
Hyperplasia, Simple[a]	6	4	2	3	2
Hyperplasia, Complex[a]	0	3	2	1	3
Tubular Adenoma, Clear Cell[a]	0	0	0	1	0
Tubular Adenoma, Basophilic[a]	0	1	0	0	0
Tubular Carcinoma, Basophilic[a]	0	0	0	2	0

[a] Values represent # animals affected

The incidences of all other tumors observed in candesartan cilexetil-treated groups were comparable to control.

Non-Neoplastic Lesions: Increase above control incidences of juxtaglomerular cell hypertrophy and intimal proliferation in the interlobular arteries of the kidney were observed in candesartan cilexetil-treated groups. Also, increases above control incidence and severity of atrophy of zona glomerulosa cells in the adrenal glands were observed in candesartan-treated rats of both sexes from all treated groups (91). The incidences of all other non-neoplastic lesions observed in candesartan cilexetil-treated groups were comparable to control.

Table 91. Non-Neoplastic Lesions

Lesion	Sex	Dose Group (mg candesartan cilexetil/kg/day)				
		0 (C-1)	0 (C-2)	100	300	1000
Kidney *	M					
J-G Cell Hypertrophy		0	0	50	50	50
Interlobular Artery Intimal Proliferation		0	0	50	50	50
Adrenal Gland *						
Zona Glomerulosa Cell Atrophy						
Mild		17	17	9	4	5
Moderate		1	4	17	27	14
Marked		2	0	23	19	29
Liver *						
Hepatic Granuloma		14	16	19	22	25
Kidney *	F					
J-G Cell Hypertrophy		0	0	50	50	50
Interlobular Artery Intimal Proliferation		0	0	43	43	50
Adrenal Gland *						
Zona Glomerulosa Cell Atrophy						
Mild		8	12	23	27	23
Moderate		0	0	11	15	18
Marked		0	0	0	1	3
Liver *						
Hepatic Granuloma		32	31	28	34	38

* Values represent # rats affected (N=50/dose group)

Plasma Drug Levels: Plasma levels of candesartan increased with increasing doses of candesartan cilexetil measured at treatment weeks 19, 52 and 78 (Table 92). Plasma concentrations of candesartan during weeks 52 and 78 were higher at 8AM than at 2PM with no apparent gender differences in plasma levels at any given dose level. No evidence of drug accumulation was noted in males or females through the 78 week treatment period.

Table 92. Plasma Candesartan Concentrations (ug/ml) in Rats Treated with Candesartan Cilexetil*

Dose (mg/kg/day)	Sex	Wk 19	Wk 52		Wk 78	
		8 AM	8 AM	2 PM	8 AM	2 PM
100	M	5.83	6.31	3.62	6.91	4.09
	F	6.32	7.84	4.28	6.08	4.12
	M & F	6.08	7.07	3.95	6.50	4.11
300	M	13.7	16.5	9.77	18.0	12.9
	F	20.0	19.3	10.3	21.4	15.4
	M & F	15.3	17.8	10.0	19.7	14.2
1000	M	37.0	43.3	28.1	33.6	24.3
	F	42.1	41.2	27.0	49.8	28.5
	M & F	39.5	42.3	27.6	41.7	26.4

* n=3 rats/group/sampling time

The Mouse Studies

13-Week Oral Dose Rangefinding Toxicity Study in Mice

Study Facility: Takeda Chemical Industries, Ltd., Osaka, Japan
Study No.: T3068
Study Dates: Initiation of dosing: 8/17/92 End of dosing: 11/17/92

GLP Compliance: GLP compliance statement included.

Animals: Male and female B6C3F1/CrlBR mice (5 weeks old, M=17.9-22.0 gm; F=16.0-19.2 gm)

Drug Administration: Candesartan cilexetil (Lot # M464-016) was suspended in aqueous 5% gum arabic solution and administered to mice orally by gavage.

Dose Levels: 0, 10, 30, 100 and 300 mg candesartan cilexetil/kg/day (10/sex/group; additional 21/sex/candesartan cilexetil groups were used for plasma drug level measurements).

Note: High dose based on results from a 13-week oral (dietary) toxicity study (#T3176) which showed significantly lower than control body weight gain (-32% and -17% in M & F, respectively) and higher than control incidence of renal tubule basophilia (no incidence in control versus 90% and 60% incidence in M & F, respectively) at a dose of 1000 mg candesartan cilexetil/kg/day.

Observations/Measurements: Animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were measured prior to dosing, twice weekly for 4 weeks, then weekly thereafter. Food consumption was measured weekly. Venous blood was obtained from all animals under ether anesthesia at the end of the dosing period for hematology and blood chemistry analyses. Blood samples were obtained at the end of the dosing period from the inferior vena cava of mice in the satellite groups under ether anesthesia at 0.25, 0.5, 1, 2, 8 and 24 hours after dosing (n=3 at each sampling point). At the end of the dosing period the animals were weighed, exsanguinated, examined for gross pathology and major organs weighed. Sections of major organs and tissues (listed in Appendix A, pg. 9) were obtained for all animals and were examined microscopically.

Results

Mortality and Clinical Signs: No animals died during the study. No treatment-related clinical signs of toxicity were observed.

Body Weight: Body weights among candesartan cilexetil-treated animals were comparable to control. (Fig. 15, Table 93).

Figure 15. Body Weight Curves

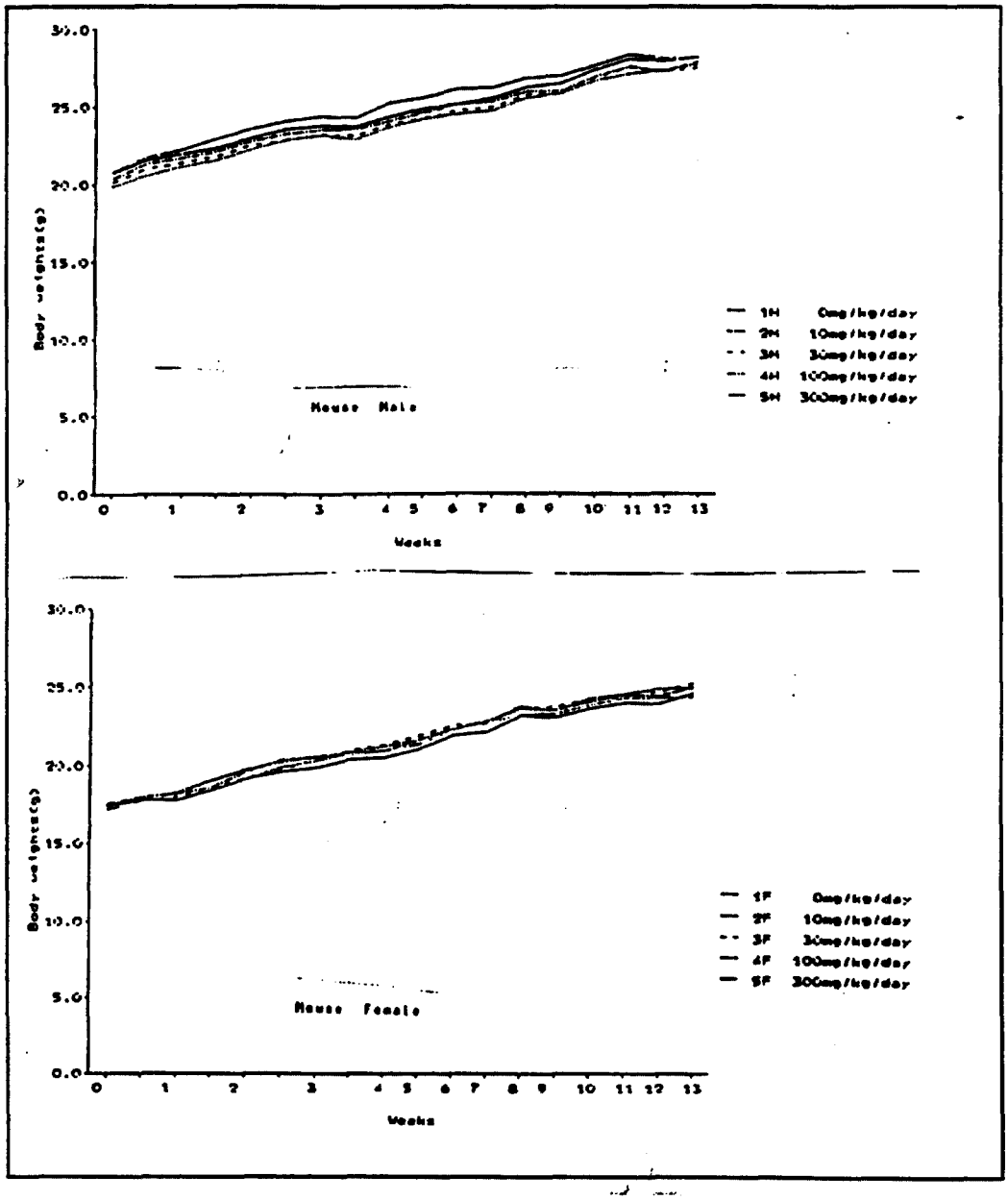


Table 93. Body Weight, gm[a]

Dose Group (mg/kg/day)	Sex	Day 0	Week 2	Week 7	Week 13
0	M	20.7	22.9	25.5	28.1
10		19.8	22.2	24.7	27.7
30		20.1	22.4	24.9	27.5
100		20.3	22.7	25.3	27.8
300		20.8	23.5	26.2	28.1
0	F	17.4	19.1	22.2	24.7
10		17.5	19.1	2.9	25.1
30		17.4	19.2	22.7	25.4
100		17.6	19.6	22.9	25.1
300		17.2	19.7	22.8	24.5

[a] Values are the means from 10 animals.

Food Consumption: Food consumption among candesartan cilexetil-treated groups was comparable to control (Table 94).

Table 94. Food Consumption, gm/mouse/wk[a]

Dose Group (mg/kg/day)	Sex	Treatment Period			
		Week 1	Week 4	Week 7	Week 13
0	M	30.6	29.6	29.3	27.5
10		29.0	28.7	28.2	26.9
30		29.9	29.2	28.8	27.3
100		29.3	28.9	28.4	27.5
300		29.8	30.1	28.7	27.0
0	F	25.5	28.6	28.2	28.7
10		25.9	29.2	29.0	28.5
30		25.4	28.9	28.4	29.3
100		25.8	29.1	28.6	28.4
300		25.9	28.4	28.5	28.1

[a] Values are the means from 10 animals.

Hematology and Clinical Chemistry: Lower than control erythrocyte counts, hematocrit and hemoglobin concentrations were observed among all candesartan cilexetil-treated male groups. In female treated groups, these hematologic parameters tended to be lower than control but the differences were not dose-dependent and not statistically significant (Table 95).

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Table 95. Hematology Values[a]

Hematology Parameter	Sex	Dose Group (mg/kg/day)				
		0 (Control)	10	30	100	300
RBC Count, X $10^6/\mu l$	M F	9.64 9.48	9.21* 8.99	9.19* 9.21	8.97* 8.83	8.99* 9.06
Hematocrit, %	M F	48.8 48.0	46.7* 45.9	46.7* 47.2	45.8* 45.4	45.9* 46.5
Hemoglobin, gm%	M F	14.0 13.9	13.4* 13.4	13.3* 13.6	13.1* 13.3	13.1* 13.6

[a] Values are the means from 10 mice
* Significantly different from control value ($p<0.05$).

Plasma urea nitrogen values for females in the 100 and 300 mg candesartan cilexetil/kg/day groups were elevated above control. Higher than control total protein was noted in females treated with 300 mg candesartan cilexetil/kg/day and higher than control levels of ALP were observed in the 300 mg/kg/day male group and in the 100 and 300 mg/kg/day female groups (Table 96).

Table 96. Blood Chemistry Values[a]

Hematology Parameter	Sex	Dose Group (mg/kg/day)				
		0 (Control)	10	30	100	300
Urea Nitrogen, mg %	M F	25.1 15.4	25.2 17.1	24.6 18.1	24.8 20.9*	25.6 22.5*
Total Protein, g%	M F	4.78 4.43	4.58 4.35	4.68 4.45	4.56 4.68	5.01 4.90*
ALP, u/L	M F	145 197	138 193	149 210	150 225*	173* 249*

[a] Values are the means from 10 mice
* Significantly different from control value ($p<0.05$).

Organ Weights: Lower than control absolute and/or relative heart weights were observed in all treated male groups and in females in the 30, 100 and 300 mg candesartan cilexetil/kg/day groups (Table 97). Other organ weights among treated groups were comparable to control.

Table 97. Heart Weights[a]

Parameter	Sex	Dose Group (mg/kg/day)				
		0 (Control)	10	30	100	300
Absolute Heart Wt., gm	M F	0.125 0.105	0.108* 0.101	0.108* 0.098	0.105* 0.091*	0.104* 0.093*
Relative Heart Wt. (Body wt. ratio)	M F	0.45 0.43	0.39* 0.40	0.39* 0.40*	0.38* 0.37*	0.37* 0.38*

[a] Values are the means from 10 mice
* Significantly different from control value ($p<0.05$).

Histopathology: Higher than control incidence of hyperplasia of the juxtaglomerular (JG) cells of the kidney was seen in males and females in each candesartan cilexetil treated group, and renal tubular basophilia was seen in males and females receiving 30 mg candesartan cilexetil/kg/day or more (Table 98).

Table 98. Kidney Histopathology[a]

Kidney Lesion	Sex	Dose Group (mg/kg/day)				
		0 (Control)	10	30	100	300
Tubular Epithelium Basophilia	M	0/10	0/10	3/10	4/10	7/10
	F	0/10	1/10	5/10	8/10	5/10
J-G Cell Hyperplasia	M	0/10	3/10	10/10	10/10	10/10
	F	0/10	8/10	10/10	10/10	10/10

[a] Values represent # mice affected/# mice examined.

Plasma Drug Levels: The concentration of unchanged candesartan cilexetil detected in plasma was very low relative to that of the two major metabolites, candesartan and M-II (Table 99). The plasma concentrations of M-I and M-II increased with increasing dose; the concentrations of each metabolite were higher in females than in males at each dosage level.

Table 99. Toxicokinetics in Mice Treated With Candesartan Cilexetil

Measured Compound	Group (mg/kg/d)	Sex	Tmax (hr)	Cmax (ug/ml)	AUC_{0-24} (ug.hr/ml)
Unchanged Drug	300	M	0.25	0.006	0.005
		F	0.25	0.009	0.003
Metabolite M-I (Candesartan)	10	M	0.25	1.69	2.9
		F	0.50	3.09	6.2
	30	M	0.25	5.35	6.6
		F	0.25	9.75	17.3
	100	M	0.25	20.45	26.4
		F	0.25	31.44	54.8
	300	M	0.25	52.14	68.5
		F	0.25	84.33	120.3
Metabolite M-II	10	M	0.5	0.055	0.053
		F	0.25	0.091	0.127
	30	M	0.5	0.222	0.289
		F	0.5	0.399	0.665
	100	M	0.5	1.012	1.139
		F	0.5	1.108	1.865
	300	M	0.25	1.471	1.372
		F	0.25	2.390	2.648

Values are the means from 3 animals

104-Week Oral (Gavage) Carcinogenicity Study in Mice

Study Facility:
Study No: T3322
Study Dates: Initiation of dosing: 2/11/93; End of necropsy: 2/24/95

GLP Compliance: GLP compliance statement included.

Animals: Male and female B6C3F1/CrlBR mice (M=20-25 gm; F=16-23 gm)

Drug Administration: Candesartan cilexetil (Lot #M464-018) was suspended in vehicle (5% gum arabic in deionized water) and administered orally by gavage. Drug solutions were prepared weekly and stored under refrigeration. Analyses of drug preparations were conducted weekly for the first 4 weeks then monthly thereafter.

Dose Levels: 0 (control 1), 0, (control 2), 3, 10, 30 and 100 mg candesartan cilexetil/kg/day (60/sex/group); additional animals included for toxicokinetic analyses= 84/sex/group for the 3, 30 and 100 mg/kg/day dose groups and 40/sex/group for the 10 mg/kg/day dose group.
Note: Sponsor's selection of high dose (100 mg/kg/day) was based upon findings from the 13-week dose rangefinding study which showed higher than control incidence of renal tubule basophilia in mice receiving doses ≥30 mg candesartan cilexetil/kg/day.

Observations/Measurements: Animals were observed 2 to 3 times daily for mortality and clinical signs of toxicity. Body weights were recorded prior to dosing, weekly for the first 14 weeks of dosing and then once every 4 weeks thereafter. Food consumption was measured weekly for the first 14 weeks then once every 4 weeks thereafter. Blood samples were collected via cardiac puncture from all surviving mice at study termination for hematology analysis. Blood samples were collected from satellite mice at 26, 52, 78 (at varying intervals up to 24 hours postdosing) and 103 weeks (at 1 hour postdosing) for toxicokinetic analysis. All main study mice sacrificed *in extremis* and all surviving mice at study termination were euthanized by carbon dioxide inhalation and examined macroscopically for external and internal abnormalities. Sections of major organs and tissues (listed in Appendix A, pg. 10) from control groups 1 and 2, the 100 mg/kg/day treated group and all main study mice that died or were sacrificed *in extremis* were examined microscopically. In addition, sections of kidney, tissue masses and all gross lesions from all main study mice were examined microscopically.

Results

Analysis of Drug Suspensions: The (overall) mean drug concentrations found in the formulations prepared for study weeks 1-4 and 8 and every 4 weeks thereafter were 101%, 104% 104% and 103% of the nominal concentrations for the 3, 10 30 and 100 mg candesartan cilexetil/kg/day dosage groups, respectively.

Survival and Clinical Signs: Survival of candesartan cilexetil-treated groups was comparable to control (Table 100). Sponsor's statistical analysis of the survival data did not reveal any drug-related effects.

Table 100. Animal Survival

Dose Group, mg/kg/day	# Surviving mice/Total # mice (%)	
	Male	Female
0 (control 1)	53/60 (88%)	41/60 (68%)
0 (control 2)	50/60 (83%)	45/60 (75%)
3	49/60 (82%)	48/60 (80%)
10	49/60 (82%)	44/60 (73%)
30	47/60 (78%)	44/60 (73%)
100	49/60 (82%)	45/60 (75%)

No treatment-related clinical signs of toxicity were observed.

Body Weights: In both sexes, statistically significant lower than control mean body weight values were noted for the 30 and 100 mg candesartan cilexetil/kg/day groups (Fig. 16; Table 101). The lower than control body weights of the males were apparent beginning approximately in week 22; for females, lower than control body weights became evident at about week 18.

Figure 16. Body Weight Curves

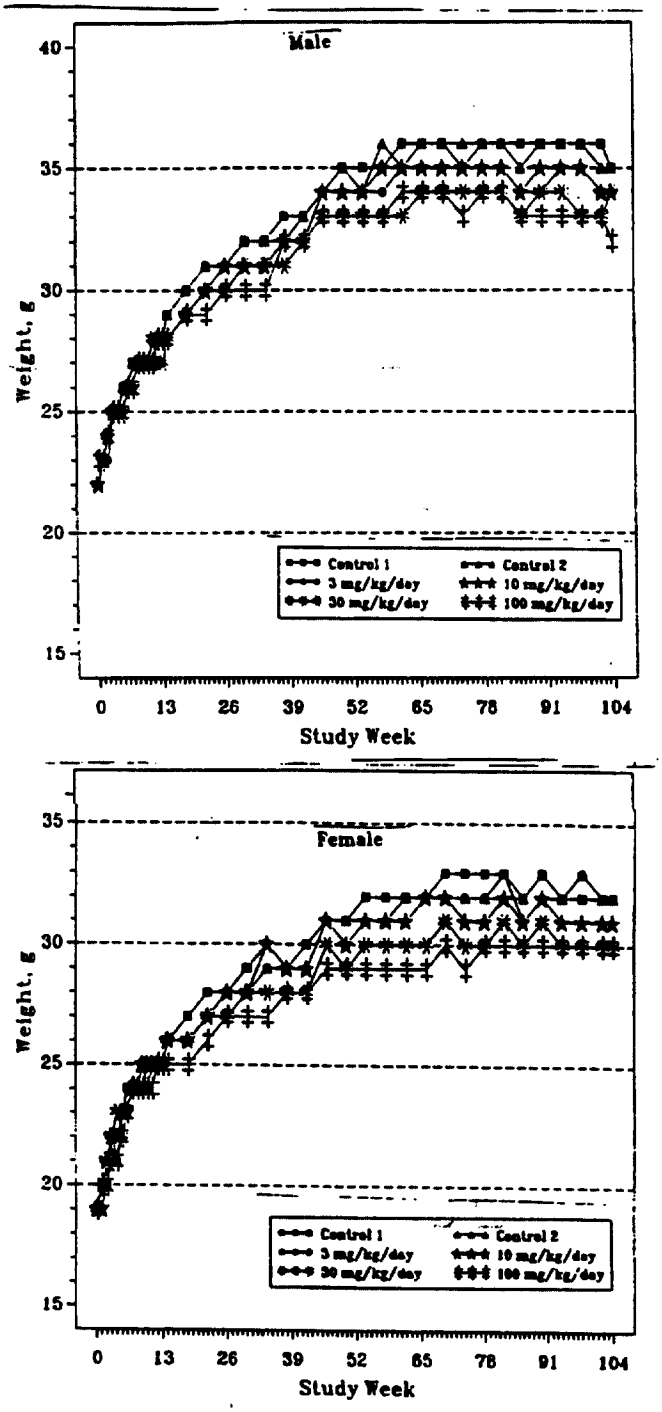

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Table 101. Body Weights, gm

Treatment Week	Sex	Dose Group, mg/kg/day					
		0 (C-1)	0 (C-2)	3	10	30	100
Week 0	M	22	22	22	22	22	23
	F	19	19	19	19	19	19
Week 26	M	31	31	31	31	30	30
	F	28	28	28	28	27	27[a,b] (-3.6)
Week 54	M	35	34	34	34	33[a] (-5.7)	33[a] (-5.7)
	F	32	31	31	31	30	29[a] (-9.4)
Week 78	M	36	36	35	35	34	34[a,b] (-5.6)
	F	33	32	32	31	30[a,b] (-9.1,-6.3)	30[a,b] (-9.1,-6.3)
Week 104	M	35	35	34	34	34	32[a,b] (-8.6)
	F	32	32	32	31	30[a,b] (-6.3)	30[a,b] (-6.7)

[a] Significantly different from 0 (C-1), p<0.05.
[b] Significantly different from 0 (C-2), p<0.05
Values in parentheses are the % differences from C-1 and/or C-2 where applicable.

Food Consumption: Food consumption among candesartan cilexetil-treated groups was comparable to that seen in the control groups.

Hematology: Lower than control levels of hemoglobin and hematocrit were noted in males treated with 100 mg candesartan cilexetil/kg/day. Females in the 30 and 100 mg/kg/day groups had lower than control concentrations of hemoglobin (Table 102).

Table 102. Hematology Values

Parameter	Sex	Dose Group, mg/kg/day					
		0 (C-1)	0 (C-2)	3	10	30	100
RBC Counts, $X10^6/mm^3$	M	9.21	9.04	9.27	9.12	8.94	8.69
	F	8.53	8.40	8.58	8.16	8.00	8.17
Hemoglobin, g/dl	M	14.2	14.1	14.0	14.1	13.7	13.2[a,b]
	F	13.6	13.5	13.5	13.0	12.8[a,b]	12.9
Hematocrit, %	M	41.8	41.3	41.7	41.6	40.0	38.7[a]
	F	39.2	38.8	39.4	37.3	36.7	37.2

Values are the means derived at terminal sacrifice from 47-53 males/group and 41-48 females/group.
[a] Significantly different from 0 (C-1), p<0.05
[b] Significantly different from 0 (C-2), p<0.05

Macroscopic Pathology: Increase above control incidences of renal cysts or granular surface of the kidneys were noted in candesartan cilexetil-treated mice (Table 103). All other incidences of

macroscopic findings among treated mice were comparable to those in control groups.

Table 103. Macroscopic Renal Pathology Incidence

Parameter	Sacrifice Group	Sex	Dose Group (mg/kg/day)					
			0 (C-1)	0 (C-2)	3	10	30	100
# of Mice Examined	DOS[a] SS[a]	Male	7 53	10 50	11 49	11 49	13 47	11 49
# with Renal Cysts	DOS SS	Male	0 0	0 2	0 1	0 5	0 10	3 20
# with Granular Surface on Kidney	DOS SS		0 6	0 2	0 5	0 8	1 9	1 15
# of Mice Examined	DOS SS	Female	19 41	15 45	13 47	17 43	17 43	15 45
# with Renal Cysts	DOS SS	Female	2 0	0 0	0 2	0 0	1 7	1 3
# with Granular Surface on Kidney	DOS SS		0 0	0 0	2 2	0 7	2 10	2 4

[a] DOS= Mice that died or were sacrificed in extremis.
SS= Mice that survived to scheduled sacrifice.

Microscopic Pathology:

Neoplastic Lesions

The number of tumor-bearing animals and total numbers of primary neoplasms, including benign and malignant neoplasms, were not significantly affected by candesartan cilexetil treatment (Table 104).

Table 104. Mouse Tumor Data

Measurement	Sex	Dose Group (mg candesartan cilexetil/kg/day)					
		0 (C-1)	0 (C-2)	3	10	30	100
No. of Animals Examined	M	60	60	60	60	60	60
	F	60	60	60	60	60	60
No. of Tumor-Bearing Animals	M	31	35	31	21	27	32
	F	31	31	21	32	23	28
Primary Neoplasms							
Total	M	46	61	58	48	60	63
	F	125	128	98	139	90	59
Benign	M	23	33	20	11	18	30
	F	23	17	5	17	6	17
Malignant	M	20	15	31	26	15	10
	F	44	27	54	34	12	15
Hematopoietic Neoplasms	M	3	13	7	11	27	23
	F	58	84	39	88	72	27

The type, onset and incidence of benign and/or malignant tumors in candesartan cilexetil-treated animals were, according to the sponsor, not significantly different from control.

Review of the tumor data by the FDA Statisticians (Appendix B; Statistical Review) also showed no significant positive trend or increase above control incidence of tumors among candesartan treated groups.

Non-Neoplastic Lesions

Higher than control incidence and severity of microscopic renal effects were noted among candesartan cilexetil-treated mice (Table 105).

Table 105. Non-neoplastic Renal Histopathology Incidence[†]

Observation	Dose Group (mg/kg/day)											
	0 (C-1)		0 (C-2)		3		10		30		100	
	M	F	M	F	M	F	M	F	M	F	M	F
Cystic Dilatation												
Mild	0	1	0	0	0	0	0	0	0	13[b,d]	0	20[b,d]
Moderate	0	0	0	0	0	0	0	0	0	23[b,d]	0	6[a,c]
Severe	0	0	0	0	0	0	0	0	0	0	0	4
Cystic Hyperplasia	3	1	6	0	17[b,d]	0	15[b,c]	1	17[b,c]	1	28[b,d]	1
JG Cell Hyperplasia/Hypertrophy												
Trace	0	0	0	0	32[b,d]	33[b,d]	18[b,d]	0	0	0	0	0
Mild	0	0	0	0	6[a,c]	14[b,d]	37[b,d]	6[a,c]	18[b,d]	1	2	0
Moderate	0	0	0	0	0	2	0	47[b,d]	39[b,d]	20[b,d]	28[b,d]	9[b,d]
Severe	0	0	0	0	0	0	0	0	0	36[b,d]	26[b,d]	51[b,d]
Arterial Hypertrophy	0	0	0	0	0	0	0	0	0	2	0	11[a,c]
Chronic Nephritis												
Trace	44	15	36	8	43	36[b,d]	27	1	6	3	2	4
Mild	3	2	1	0	6	2	26[b]	33[b,d]	26[b,d]	27[b,d]	17[b,d]	41[b,d]
Moderate	0	0	0	0	0	0	0	16[b,d]	23[b,d]	21[b,d]	38[d]	7[a,d]
Severe	0	0	0	0	0	0	0	0	0	0	1	1

[†] Values represent # mice affected (# mice examined=60/sex/group)
[a] Significantly different from control-1; ($p<0.05$); [b] ($p<0.01$)
[c] Significantly different from control-2; ($p<0.05$); [d] ($p<0.01$)

Toxicokinetics: Plasma concentrations of candesartan (metabolite M-1) increased with increasing dose at each sampling week (Weeks 26, 52, 78 and 103; Table 106). The plasma concentrations of candesartan in females tended to be higher than those achieved with the same doses in males. No intra-dose-level differences were seen in Cmax and AUC for candesartan following repeated dosing with candesartan cilexetil, except for slight duration-related increases in AUC in the 100 mg/kg/day male group.

Table 106. Toxicokinetic Parameters for Candesartan after Oral Doses of Candesartan Cilexetil*

Candesartan Cilexetil Dose Group (mg/kg/day)	Dosing Week	Male		Female	
		Cmax, µg/ml	AUC_{0-24}, µg.hr.ml	Cmax, µg/ml	AUC_{0-24}, µg.hr.ml
3	Wk 26	0.27	0.5	0.63	0.9
	Wk 52	0.27	0.4	0.36	0.7
	Wk 78	0.27	0.6	0.40	1.3
	Wk 103[a]	0.19	-	0.43	-
10	Wk 26	0.96	1.9	1.4	3
	Wk 52[b]	0.66	-	1.7	-
	Wk 78[b]	0.72	-	1.0	-
	Wk 103[a]	0.63	-	2.2	-
30	Wk 26	2.7	5	4.0	7
	Wk 52	2.1	5	4.1	8
	Wk 78	2.7	9	4.9	15
	Wk 103[a]	3.0	-	5.6	-
100	Wk 26	4.4	9	9.3	16
	Wk 52	1.5	17	8.8	24
	Wk 78	7.4	22	9.9	26
	Wk 103[a]	6.6	-	11.0	-

[a] Single measurement only (1 hour postdose)
[b] Determination made from 2 points (0.5 and 1 hour post dose).
- Insufficient data points for determination.
* Unless otherwise indicated, values are derived from 3 males and 3 females/sampling time.

OVERALL SUMMARY AND EVALUATION

Candesartan cilexetil is a prodrug that is rapidly converted *in vivo* to its pharmacologically active metabolite, candesartan, during its absorption from the intestinal tract. Candesartan exhibits antihypertensive properties through antagonism of vascular angiotensin II (AT_1 subtype) receptors.

Oral candesartan cilexetil elicited dose-dependent antihypertensive effects in SHR (0.01-10 mg/kg), in renal hypertensive rats (0.01-10 mg/kg) and in renal hypertensive dogs (0.3-1 mg/kg); cardiac rate was unaffected with these antihypertensive doses of candesartan cilexetil. In DOCA/salt hypertensive rats in which plasma renin activity is typically suppressed, candesartan cilexetil caused no significant antihypertensive effect. In renal hypertensive dogs, candesartan cilexetil produced antihypertensive effects independent of plasma renin levels.

Candesartan shows selectivity (>500X) for the angiotensin AT_1 receptor subtype versus the AT_2 receptor subtype as determined by radioligand receptor binding. The prodrug ester is generally devoid of angiotensin II receptor binding activity. Candesartan was shown to block, non-competitively, angiotensin II induced vascular contractions *in vitro* and angiotensin II induced pressor responses *in vivo*. In rats, marked increases in plasma renin activity are a consequence of blockade of angiotensin II receptors following a single oral dose of 0.1 mg candesartan cilexetil/kg.

Candesartan cilexetil and/or its active metabolite were evaluated for ancillary pharmacologic properties and found to be devoid of actions on the central, somatic and autonomic nervous systems, the gastrointestinal system and the pulmonary system.

Bioavailability of candesartan in fasted rats and dogs was modest (~20%) following oral administration of 1 mg candesartan cilexetil/kg. Decreased bioavailability was observed in fed dogs (~5%) but not fed rats. Candesartan, at concentrations of 0.01 - 10 ug/ml, is found highly bound to plasma protein in rats (99.6%-99.8%), dogs (96.7%-97.5%) and humans (99.4%-99.6%); this may be the reason for the low penetration of the drug into the brain and spinal cord of rats. In mice, rats, dogs and monkeys, the major metabolite of candesartan cilexetil is formed by hydrolysis of the ester moiety to yield candesartan., the active carboxylic acid metabolite. In rats and dogs, candesartan is further conjugated to its carboxylic acid and tetrazol-N glucuronides, major excretory products. A fourth metabolite, formed by O-de-ethylation of candesartan, was identified in rat and dog feces. In humans, candesartan is the major metabolic product detected, constituting 70%-80% of candesartan cilexetil metabolic products identified in urine and feces of human subjects. The O-de-ethylated metabolite of candesartan was the only other human metabolic product detected. The predominant route of elimination of candesartan in rats, dogs and humans is via the feces (>90%for rats and dogs and ~67% for humans); approximately one-third of

the fecal elimination of candesartan in rats is the result of biliary excretion of drug. The occurrence and extent of biliary excretion of candesartan in mice, rabbits, dogs and man was not determined. Only a small fraction (1%-4%) of the absorbed radioactive dose is eliminated via the urine in rats whereas approximately 33% is eliminated via the urine in humans. The half-life of candesartan after an oral dose of 1 mg candesartan cilexetil/kg is 3.8 hours and 4.3 hours in rats and dogs, respectively; the half-life of candesartan after oral administration of 8 mg candesartan cilexetil to humans was 9.3 hours. Half-life values for candesartan in mice, rabbits and monkeys were not provided (often an insufficient number of data points hindered this determination).

Acute toxicity studies in mice, rats and dogs revealed that high oral doses (2000 mg/kg) of candesartan cilexetil are tolerated without overt adverse effects. Single dose toxicity studies have also been conducted in mice and rats with parenteral administration of candesartan, candesartan metabolites and cyclohexanediol, the cilexetil hydrolyzed product.. High IV or IP doses of these agents (≥910 mg/kg of candesartan, ≥1000 mg/kg of metabolite M-II and ≥1240 mg/kg of cyclohexanediol) in mice and rats caused ataxia, respiratory depression, decreased locomotor activity and death.

Repeat oral dose toxicity studies were conducted in rats, up to 26 weeks with administration by gavage and up to 2 years with dietary administration, both studies at doses as high as 1000 mg/kg/day. The main drug-related findings in the 26-week rat study were dose-related erosions of the glandular mucosa of the stomach in males (30% and 70% incidence rates with 100 and 1000 mg/kg/day, respectively) and to a lesser extent in females (40% incidence rate with 1000 mg/kg/day). Renal tubular basophilia, renal tubular hypertrophy and renal cellular infiltration occurred in males at doses ≥ 10 mg/kg/day. Kidney and stomach pathologies were not observed in a 4-wk study in rats with doses of up to 300 mg/kg/day. Decreases in erythroid parameters were noted in males and females at doses ≥100 mg/kg/day and blood urea nitrogen increased dose-dependently from concurrent control values at doses ≥10 mg/kg/day (males and females) in the 26-wk study. Decreases in erythroid parameters and increases in urea nitrogen occurred with doses as low as 30 mg/kg/day in the 4-wk study. Despite these effects, no severe toxicity or treatment-related deaths were seen in either the 4-wk or the 26-wk study. The no-observed-effect-level for gastric mucosal erosion, the most critical toxicity observed in rats given candesartan cilexetil by gavage, was 10 mg/kg/day and 100 mg/kg/day for males and females, respectively; the steady-state $AUC_{0\text{-}24hr}$ values associated with these doses were 81 and 727 ug.hr/ml, respectively. Dietary administration of 100, 300 and 1000 mg candesartan cilexetil/kg/day for up to 2 years was associated with marginal reductions of erythroid parameters in the mid- and high dose females and slightly higher than control incidence of enlarged spleen and rough kidney surface in high dose males. The gastric mucosal erosion observed when candesartan cilexetil was administered by gavage for 26 weeks was not observed when the drug was mixed in the feed for up to 2 years. Histopathologic findings in the 2-year rat study

included J-G cell hypertrophy, renal interlobular artery proliferation and atrophy of adrenal gland zona glomerulosa cells in males and females treated with ≥100 mg candesartan cilexetil/kg/day. When candesartan cilexetil was given orally to rats at doses up to 300 mg/kg/day in the 4-wk study and up to 1000 mg/kg/day in the 26-wk study, the AUC for candesartan increased with increasing dose. However, in the 26 week study, at doses ≥100 mg candesartan cilexetil/kg/day, the increase in AUC for candesartan was not dose-proportional. Also, the AUC for candesartan increased slightly with repeated administration of 1000 mg candesartan cilexetil/kg/day.

Repeated oral dose toxicity studies were conducted in dogs. The longest duration and highest dose evaluated (both in the same study) was 52 weeks at 300 mg candesartan cilexetil/kg/day. There was no mortality or overt toxicity. Treatment-related effects were primarily regeneration of renal tubular epithelium in dogs receiving 300 mg/kg/day (for 4 or more weeks), renal JG cell hyperplasia in all dogs receiving doses ≥20 mg/kg/day (for 26 or more weeks) and increases in blood urea nitrogen with doses ≥100 mg/kg/day (for 4 or more weeks). Postmortem examination of dogs after 52 weeks of treatment with candesartan cilexetil revealed no histopathology other than effects described for the kidney. The incidences and degrees of effects on the kidney and on blood urea nitrogen were not increased as treatment duration increased from 4 weeks to 52 weeks; the juxtaglomerular renal effects were considered adaptive and reflective of the pharmacological effects associated with angiotensin receptor blockade. Except for the JG effects and small increases in BUN, the 100 mg/kg/day dose is considered to be the no-observed-toxic-effect-level; the AUC_{0-24} value associated with this dose at week 52 is 5875 ng.hr/ml for males and 10466 ng.hr/ml for females. The regeneration of renal tubular epithelium seen in males and females with 300 mg candesartan cilexetil/kg/day appears to reflect drug-induced renal damage and this dose can be considered as a maximally tolerated dose in dogs for the 52 week treatment period. When candesartan cilexetil was given orally to dogs over 4, 26 or 52 weeks at doses of 2.4 to 300 mg/kg/day, the AUC for candesartan increased with increasing dose. With repeated administration of 300 mg candesartan cilexetil/kg/day, the AUC for candesartan also increased, suggesting that drug accumulation occurs with this dose level in dogs.

In a 4-wk toxicity study in monkeys (the longest treatment duration evaluated in monkeys), increased urea nitrogen levels were noted at oral doses as low as 2.4 mg/kg/day. Hypertrophy of the JG cells was noted at the 12 mg/kg/day dose. There were no apparent effects on erythroid parameters except in one of four males in the 300 mg/kg/day dose group. The poor or moribund condition of 3/4 monkeys dosed with 300 mg/kg/day appeared to be associated with emesis and/or reduced food consumption. There were no apparent gross or microscopic pathologies that could be attributed to the poor health monkeys in the high dose group.

Some of the effects observed in the repeat dose toxicity studies were considered to be

secondary to the pharmacologic activity of candesartan cilexetil and are typical for agents of this pharmacologic class. The drug-related increases in JG cell hypertrophy, renal tubular dilatation and kidney weight are considered adaptive responses to blockade of the renin-angiotensin system and have also been seen with ACE inhibitors. The sponsor notes from a published report (Hricik *et al*, N Engl J Med, 308:373-76, 1983) that the increased plasma urea nitrogen and creatinine levels are likely related to effects of candesartan on the autoregulation of glomerular filtration in the presence of marked reductions in renal artery perfusion pressure. The decreases in erythroid parameters in the mouse, rat and dog are known effects associated with inhibition of the renin-angiotensin system and are believed to be related to reduced erythropoietin levels. The decreased heart weight is considered to be due to decreased cardiac workload as a result of decreased peripheral resistance. The primary signs of toxicity of orally administered candesartan cilexetil were gastric erosion (seen after 4 weeks in dogs with 300 mg/kg/day and after 26 weeks in rats with 1000 mg/kg/day) and basophilic epithelium of the renal tubules (seen in rats after 26 weeks with 10 mg/kg/day and in dogs at 52 weeks with 100 mg/kg/day). Basophilic staining of the renal tubular epithelium is indicative of tubular regeneration and tissue repair. Collectively, the elevations in urea nitrogen and creatinine, the appearance of urinary casts and the presence of renal tubular regeneration indicate that the kidney is a target organ for toxicity during treatment with candesartan cilexetil.

Dose selection for the 2-year rat dietary administration carcinogenicity study was based on results from the 13-week dose-rangefinding study which evaluated dietary doses of 300, 1000 and 3000 mg candesartan cilexetil/kg/day. The high dose of 3000 mg/kg/day corresponded to 5% in the diet. Mean body weights of males treated with 1000 and 3000 mg/kg/day were significantly lower than control (12% and 14% lower, respectively, at the end of week 13). Mean body weights of treated females did not differ significantly from control. Body weight gains from initial weight to weight at 13 weeks were 3%, 18% and 21% lower than control for males and 4%, 8% and 10% lower than control for females in the 300, 1000 and 3000 mg/kg/day groups, respectively. Plasma urea nitrogen was significantly higher than control in males and females treated with 1000 and 3000 mg candesartan cilexetil/kg/day. Erythroid parameters were significantly lower than control in males and females treated with 1000 and 3000 mg candesartan cilexetil/kg/day. Pronounced pharmacologic effects on the kidney (JG cell hypertrophy, renal artery proliferation and increased kidney relative weight), consequences of blockade of the renin-angiotensin system, were observed in males and females in all treated groups. Based on the reductions in body weight and body weight gain and the exaggerated pharmacologic effects on the kidney, the 3000 mg/kg/day dose was regarded by the sponsor to likely exceed a maximally tolerated dose for the 24-month study. Therefore, the sponsor selected 1000 mg candesartan cilexetil/kg/day as an appropriate high dose for the 24-month rat carcinogenicity study. (No concurrence from the division was sought by either the drug's developer, Takeda Chemical Co., or the sponsor/licensee, Astra Merck) Toxicokinetic information obtained during this study was limited to AM and PM plasma drug

concentrations at 3 treatment intervals (wks 1, 6 and 13) and no AUC values were derived. However, the 1000 mg candesartan cilexetil/kg/day dose is approximately 330X the maximum recommended human dose on a mg/m^2 basis.

In the 24-month rat carcinogenicity study, dietary administration of doses as high as 1000 mg candesartan cilexetil/kg/day did not adversely affect survival. The average achieved candesartan cilexetil daily doses were 100%-102% of the intended daily doses. Plasma concentrations of the primary active metabolite, candesartan, increased with increasing doses (dose-proportional with the 100 and 300 mg/kg/day doses, less than dose-proportional with the 1000 mg/kg/day dose). Mean body weights in all male treated groups were lower (dose-dependently) than control beginning week 1 and reaching maximal differences around weeks 70-78 of the study; the body weight decrement between high dose males and control was 14% at study week 78. Although significantly lower than control body weights were detected in treated females, the maximum body weight decrement from control was less than 5%. Food consumption was significantly lower (non-dose-dependent) in all male dose groups throughout the first 70-78 weeks; treated females showed only slight and transient reductions from control food consumption. The sponsor's analysis showed no significant differences between candesartan cilexetil and control groups in the number of tumors/rat or in the number of tumor-bearing rats. For individual tumor types, the sponsor's analysis showed a positive trend and statistically significant pairwise increased incidence of benign pheochromocytoma between female control and the low- and high-dose groups; the incidence at the mid-dose (300 mg/kg/day) was not statistically significant. No significant differences were observed in the incidences of malignant pheochromocytoma in treated female groups and no significant differences were observed in the incidences of benign or malignant pheochromocytoma in control and treated male groups. FDA statistical analysis showed no significant positive trend or significant pairwise difference between combined control and the high dose group incidences of benign, malignant or the combination of benign and malignant pheochromocytoma for female or male rats. Although the sponsor's analysis revealed that the incidence of bilateral interstitial cell tumors of the testes showed a positive trend, no statistically significant differences were detected when the incidences of bilateral and unilateral interstitial cell tumors were combined. Given that the incidence rate for interstitial cell tumors is quite high in this strain of rats (93%), it appears unlikely that this statistically significant difference represents a drug-related effect. FDA statistical analysis also showed no significant positive trend or significant pairwise difference between combined control and the high dose group incidence of combined bilateral and unilateral testicular interstitial cell tumors. *[The Executive Carcinogenicity Committee, at its 2/10/98 meeting, determined that the rat study is acceptable, with dose selection based on a large multiple of human exposure in males and females and attainment of an MTD in males; the committee concluded that there was no evidence of carcinogenic potential in rats.]* The non-neoplastic lesions (JG cell hypertrophy, renal artery intimal proliferation and adrenal zona glomerulosa cell atrophy) observed in candesartan-treated animals are tissue responses

secondary to the pharmacologic blockade of the renin angiotensin system and are not regarded as overt toxicities.

Dose selection for the 24-month mouse gavage administration carcinogenicity study was based on results of the 13-week dose-rangefinding study which evaluated oral (gavage) doses of 10, 30, 100 and 300 mg candesartan cilexetil/kg/day *(selection of 300 mg/kg/day as the highest dose in this rangefinding study was influenced by the results of a 13-week dietary study in which a dose of 1000 mg candesartan cilexetil/kg/day resulted in significantly lower [32% and 17% lower in M & F, respectively] than control body weight gain and significantly higher than control incidence of renal tubular basophilia [90% in males and 60% in females vs 0% in control males and females]).* Body weights and food consumption among candesartan cilexetil treated groups in the gavaging experiment were comparable to control. Lower than control erythroid parameters were observed in all candesartan treated male groups; erythroid parameters in treated females were only slightly lower than control. Plasma urea nitrogen and ALP in females treated with 100 and 300 mg candesartan cilexetil/kg/day were significantly elevated above control (dose-related). Significantly higher than control ALP levels were seen in the 300 mg/kg/day male group. Higher than control incidences of hyperplasia of the JG cells of the kidney were seen in males and females in all candesartan cilexetil treated groups and higher than control incidences of renal tubular basophilia were observed in males and females receiving doses ≥30 mg candesartan cilexetil/kg/day. Toxicokinetic assessment showed that, with any given dose level, the AUC for the active metabolite, candesartan, in treated females was approximately 2X that seen in males. Based on the higher than control incidences of renal tubular basophilia seen with doses ≥30 mg/kg/day, the sponsor selected 100 mg candesartan cilexetil/kg/day as an appropriate high dose for the 2-year mouse carcinogenicity study. This dose represents 16X the maximum recommended human dose of 32 mg/day on a mg/m^2 basis.

In the 104-week mouse carcinogenicity study, oral doses of 3, 10, 30 and 100 mg candesartan cilexetil/kg/day had no adverse effect on animal survival. Mean body weights for the 30 and 100 mg/kg/day male and female dose groups were statistically significantly lower than control. The lower than control mean body weights of males were apparent beginning in week 22 and for females at about week 18. The 100 mg/kg/day dose of candesartan cilexetil was associated with a maximum body weight decrement of 8.6% for males (study week 104) and 9.4% for females (study week 54). Erythroid parameters were slightly lower than control in males given 100 mg candesartan cilexetil/kg/day and in females treated with 30 and 100 mg/kg/day. Macroscopic examination revealed higher than control incidences of renal cysts and granular kidney surface in males and females at doses ≥10 mg/kg/day. The sponsor's analysis showed no statistically significant differences between treated and control groups in the numbers of tumor-bearing animals or in the total number of tumors. Both the sponsor's and FDA statistical analyses showed no significant positive trend or significant increase above control incidence of tumors among candesartan cilexetil treated male and female groups.

Toxicokinetic analysis showed Cmax and AUC of candesartan increased with increasing doses of candesartan cilexetil. Except for slight duration-related increases in AUC in the 100 mg/kg/day male group, no intra-dose-level increases in candesartan Cmax and AUC were seen following repeated dosing with candesartan cilexetil.

The evidence from the 13-week dose-rangefinding study used by the sponsor to conclude that 300 candesartan cilexetil/kg/day may have exceeded a maximally tolerated dose for the mouse does not appear very convincing. In the absence of overt toxicity or any adverse effects on body weight or body weight gain, the sponsor regarded renal histopathology (dose-related renal tubular basophilia) and its potential to accelerate renal disease as the basis for limiting the high dose in their 2-year mouse study to 100 mg/kg/day. Based on this division's experience with numerous ACE inhibitors and A-II antagonists and their associated renal histopathology, we would not have shared this level of concern in making the high dose selection. Nevertheless, results from the 2-year study may allow one to consider the study as adequate based on candesartan cilexetil-induced significant body weight decrements in both males (up to 8.6%) and females (up to 9.4%) that occurred after week 13. In addition, evidence of drug-induced acceleration of renal disease (chronic nephritis and cystic dilatation) appears to validate the sponsor's basis for limiting the high dose to 100 mg candesartan cilexetil/kg/day. *[The Executive Carcinogenicity Assessment Committee, at its 2/10/98 meeting, determined that the mouse study is acceptable, with dose selection based on attainment on a MTD. The committee concluded that there was no evidence of carcinogenic potential in mice.]*

Effects of orally administered candesartan cilexetil on fertility, reproduction, embryo, fetal and neonatal development and maturation were evaluated in a comprehensive battery of reproductive toxicity studies. In a fertility study in rats, 10-300 mg candesartan cilexetil/kg/day (beginning 9 weeks before mating in males and 2 weeks before mating in females) had no effect on male or female mating or fertility. In a developmental toxicity study in rats, oral doses of 10-100 mg/kg/day (GD 6 - GD 17) had no effect on embryo/fetal mortality, fetal development, or growth, maturation and reproductive ability of the F_1 offspring. Other than body weight suppression in dams and offspring at 300 mg/kg/day, there were no effects. In a developmental toxicity study in rabbits, maternal toxicity (decreased body weight and death) resulted after oral doses of 3 mg/kg/day of candesartan cilexetil. Organ toxicities detected at necropsy of non-surviving dams included petechia in the stomach, discoloration of the liver and kidneys and red foci in the gallbladder. Despite the maternal toxicity, no adverse effects on fetal survival, fetal weight or external, visceral and skeletal development were observed. Developmental toxicity was also assessed in the mouse at doses as high as 1000 mg/kg/day (GD6-GD15). No maternal toxicity was observed and no adverse effects on fetal survival, fetal weight or external, visceral and skeletal development were observed. In a peri- and post-natal toxicity study in rats, oral doses of 0.4 to 300 mg candesartan cilexetil/kg/day (GD 15 - LD 21) caused no overt maternal toxicity except for slight reductions in food consumption and a slight

decrease (5%) relative to concurrent control body weight at the 300 mg/kg/day dosage level. Neonatal survival was reduced by maternal treatment with doses ≥10 mg/kg/day. These same doses elicited dose-related increased incidences of hydronephrosis in weanling and matured F_1 animals. This effect was not evident when dams were dosed with candesartan cilexetil during early or mid-pregnancy.

A battery of *in vitro* and *in vivo* genotoxicity tests were conducted to evaluate the genotoxic potential of candesartan cilexetil, candesartan and the O-de-ethylated metabolite (M-II) of candesartan. No mutagenic or clastogenic potential was detected. Results of these studies are summarized below.

Summary of Genotoxicity Studies

Genotoxicity Assay	Activity/Concentration or Dose		
	Candesartan cilexetil	Candesartan	Metabolite M-II
Ames Test (*in vitro*)	Negative 4.9-5000 ug/plate 1.2-5000 ug/plate[d]	Negative 156-5000 ug/plate	Negative 156-5000 ug/plate
Mouse Lymphoma L5178Y Cells (*in vitro*)	Negative 1.56-75 ug/ml	Negative 500-5000 ug/ml	Negative 312-5000 ug/ml
CHL Cell Cytogenicity (*in vitro*)		Negative 2.5-10 mM[a] 1.25mM[b] 0.625 mM[c]	Negative 2.5-10 mM[a] 1.25 mM[b] 0.375 mM[c]
Mouse Micronucleus (*in vivo*)	Negative 500-2000 mg/kg	Negative 187.5-750 mg/kg	
Rat Hepatocyte UDS (*in vitro*)	Negative 300-3000 mg/kg		
Chinese Hamster Ovary Gene Mutation (*in vitro*)		Negative 0.31-5 mg/ml	

[a] Based on 6-hr drug exposure period
[b] Based on 24-hr drug exposure period; cytotoxic at higher concentrations
[c] Based on 48-hr drug exposure period; cytotoxic at higher concentrations
[d] Test conducted with candesartan cilexetil with related compounds (impurities).

For purposes of comparing animal and human exposures of candesartan, the sponsor's pharmacokinetic data showed a mean candesartan $AUC_{0\text{-}24hr}$ (at steady state) of 2880 ng.hr/ml achieved with oral administration of a maximum recommended daily human dose (32 mg) of candesartan cilexetil to hypertensive patients (described in the Human Pharmacokinetics section of the NDA, Vol 06-001-057, Clinical Study # SH-AHC-0003).

LABELING

Those sections in the proposed labeling that refer to non-clinical studies that were covered by this review were reviewed and considered acceptable with the following exceptions:

The following statements under CLINICAL PHARMACOLOGY, *Mechanism of Action,* 4th paragraph, are considered inappropriate (potency comparisons with other agents) or provide little information relevant to the drug's clinical benefit and should be deleted:

"Candesartan binds to the AT_1 receptor with an affinity 80-fold greater than losartan, 10-fold greater than losartan's active metabolite, and 7-fold greater than angiotensin II."

"The antagonism of candesartan at the AT_1 receptor is insurmountable, and is not diminished by increasing concentrations of angiotensin II." [*The same information can be provided by incorporating the term "non-competitive" in the first sentence of the same paragraph " Candesartan produces specific cardiovascular effects through direct, non-competitive, antagonism of angiotensin II at the AT_1 receptor"]*

"Candesartan exhibits a slow rate of dissociation from the AT_1 receptor in vitro, contributing to an inherently long duration of action. Candesartan's distinctive receptor-binding characteristics, including high affinity for the receptor and slow receptor-dissociation rate, contribute to its insurmountable effect and long duration of action as an AT_1 receptor antagonist in animal models of cardiovascular function and in humans."

Under WARNINGS, *Fetal/Neonatal Morbidity and Mortality,* the sponsor provides a brief description of the results of developmental and neonatal toxicity studies. The sponsor's proposed text reads as follows:

"Although there is no clinical experience with the use of ATACAND in pregnant women, studies have demonstrated late fetal and neonatal injury in the kidneys of animals exposed to either candesartan, other angiotensin II receptor antagonists or ACE inhibitors. The mechanism is believed to be pharmacologically mediated through effects on the renin-angiotensin-aldosterone system during late gestation and during lactation. It is not known whether candesartan is excreted in human milk. However, candesartan is present in the milk of lactating rats. In rats, it has been demonstrated that candesartan passes across the placental barrier and is distributed in the fetus".

In order to provide a more detailed description of the developmental and neonatal toxicity studies, and in order to place doses associated with adverse effects in animals in the context of human exposure, the text should be revised as follows:

"There is no clinical experience with the use of ATACAND in pregnant women. Oral doses ≥ 10 mg candesartan cilexetil/kg/day administered to pregnant rats during late gestation and continued through lactation were associated with reduced survival and an increased incidence of hydronephrosis in the offspring. The 10 mg/kg/day dose in rats is approximately 2.8 times the maximum recommended daily human dose (MRHD) of 32 mg on a mg/m^2 basis (comparison assumes human body weight of 50 kg). Candesartan cilexetil given to pregnant rabbits at an oral dose of 3 mg/kg/day (approximately 1.7 times the MRHD on a mg/m^2 basis) caused maternal toxicity (decreased body weight and death) but, in surviving dams, had no adverse effects on fetal survival, fetal weight or on external, visceral or skeletal development. No maternal toxicity or adverse effects on fetal development were observed when oral doses up to 1000 mg candesartan cilexetil/kg/day (approximately 138 times the MRHD on a mg/m^2 basis) were administered to pregnant mice."

[*The sentences " It is not known whether candesartan is excreted in human milk. However, candesartan is present in the milk of lactating rats." should be deleleted from this section because it also appears in the labeling section* ***Nursing Mothers*** "]

Under PRECAUTIONS, *Carcinogenesis, Mutagenesis, Impairment of Fertility,* the sponsor's proposed text includes the following:

"Candesartan cilexetil was not carcinogenic when administered at maximally tolerated doses to rats and mice for 104 weeks. The maximally tolerated doses (1000 mg/kg/day in rats, 100 mg/kg/day in mice) provided plasma levels (Cmax) of candesartan that were approximately 123-times and 16 to 29-times in rats and mice, respectively, the exposure of a 50 kg human given a dose of 32 mg per day.

Candesartan cilexetil was not genotoxic in the microbial mutagenesis and mammalian cell mutagenesis assays and in the *in vivo* chromosomal aberration and rat unscheduled DNA synthesis assays. In addition, candesartan was not genotoxic in the microbial mutagenesis, mammalian cell mutagenesis and *in vitro* and *in vivo* chromosome aberration assays.

Fertility and reproductive performance were not affected in studies with male and female rats given oral doses of up to 300 mg/kg/day (83-times the maximum daily human dose of 32 mg on a body surface area basis)."

The sponsor's statement on carcinogenic potential compares rodent and human exposures on the basis of Cmax. AUC values, when available, are preferable to Cmax values in determining multiples of human exposure. The sponsor has derived AUCs for candesartan in the mouse carcinogenicity study [candesartan mean $AUCs_{0\text{-}24hr}$ at the high dose (averages of values obtained in treatment weeks 26, 52 and 78) were 16 and 22 ug.hr/ml for male and female mice, respectively] but has not derived AUCs for candesartan in the rat study. In the rat carcinogenicity study, plasma candesartan levels, determined at 8AM and at 2PM in treatment weeks 52 and 78, showed no apparent gender or treatment week differences. Based on the two mean plasma candesartan concentrations ($Conc_{8AM}$ of 42 ug/ml and $Conc_{2PM}$ of 27 ug/ml), an AUC for the period covering this 6-hour interval is determinable ($AUC_{8AM\text{-}2PM}$= 207,000 ng.hr/ml). As this 6-hr AUC is >70 times the 24-hr human AUC (2880 ng.hr/ml) following the MRHD of 32 mg, the high dose candesartan exposure in the rat for an equivalent 24-hour period must also be greater than 70 times the human exposure from the MRHD. Accordingly, the paragraph describing the carcinogenicity results should be revised to read as follows:

"There was no evidence of carcinogenicity when candesartan cilexetil was orally administered to mice and rats for up to 104 weeks at doses up to 300 and 1000 mg/kg/day, respectively. Rats received the drug by gavage whereas mice received the drug by dietary administration. These (maximally tolerated) doses of candesartan cilexetil provided systemic exposures to candesartan (AUCs) that were, in mice, approximately 7 times and, in rats, more than 70 times the exposure in man at the maximum recommended daily human dose (32 mg).

The sponsor's labeling statements on genotoxic potential and effects on fertility are acceptable as written.

Under OVERDOSAGE, the sponsor's proposed text includes the following:

"No lethality was observed in acute toxicity studies in mice, rats and dogs given single oral doses of up to 2000 mg/kg of candesartan cilexetil. In mice given single oral doses of candesartan, the minimum lethal dose was 1000-2000 mg/kg."

The above statement should be revised to read as follows:

"No lethality was observed in acute toxicity studies in mice, rats and dogs given single oral doses of up to 2000 mg/kg of candesartan cilexetil. In mice given single oral doses of the primary metabolite, candesartan, the minimum lethal dose was greater than 1000 mg/kg but less than 2000 mg/kg."

RECOMMENDATION

From a preclinical safety perspective, this new drug application for candesartan cilexetil is approvable with the recommended changes in labeling.

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NDA 20,838
HFD-110
HFD-110/CSO
HFD-110/AProakis
HFD-110/CResnick
HFD-345/EButler
Accepted by LM on 2-17-98

APPENDIX A

Tissues/Organs Examined Microscopically

Tissues/Organs Examined from 4-Week Oral Toxicity Study in Rats

The following tissues from all animals in groups 1-4 were fixed in 10% buffered neutral formalin:

adrenal glands	salivary glands (submaxillary and sublingual)
brain	seminal vesicles
duodenum	skin
esophagus	small intestine
eye ball	spinal cord
femurs (with bone marrow)	spleen
Harder's glands	sternum
heart	stomach
kidneys	testes
large intestine (cecum)	thymus
liver	thyroid glands and parathyroid glands
lungs (with bronchi)	tongue
lymph node (mesenteric)	trachea
mammary glands	urinary bladder
ovaries	uterus
pancreas	vagina
pituitary gland	other organs and tissues with gross changes
prostate (ventral)	

In the case of the animals from the control and 300 mg/kg groups, all the above tissues and organs were embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined by microscopy. In the case of the animals from 30 and 100 mg/kg groups, the liver and kidney were examined by microscopy. In the case of paired organs, only the left organ was processed for microscopy unless lesions were found in the right at necropsy.

Tissues/Organs Examined from 26-Week Oral Toxicity Study in Rats

The following tissues from all animals in Groups 1-5 were fixed in 10% buffered neutral formalin:

- adrenal glands
- brain
- duodenum
- esophagus
- eye balls
- femurs (with bone marrow)
- Harderian glands
- heart
- kidneys
- large intestine (cecum and colon)
- liver
- lungs (with bronchi)
- lymph node (mesenteric)
- mammary gland regions
- ovaries
- pancreas
- pituitary gland
- prostate (ventral)
- seminal vesicles
- skin
- small intestine
- spinal cord
- spleen
- sternum
- stomach
- sublingual glands
- submandibular glands
- testes
- thymus
- thyroid glands and parathyroid glands
- tongue
- trachea
- urinary bladder
- uterus
- vagina

All the above tissues and organs of all animals in the control and 1000 mg/kg groups, the liver, kidney and stomach of all animals in the 1, 10 and 100 mg/kg groups were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H·E) and examined light microscopically.

Tissues/Organs Examined from 4-Week Oral Toxicity Study in Dogs

Samples of the following tissues from all animals were fixed in 10% neutral buffered formalin.

—brain	—spinal cord
—heart	—lungs
—bronchus	—liver
—gallbladder	—kidneys
—spleen	—testes
—ovaries	—prostate
—uterus	—vagina
—urinary bladder	—pituitary
—adrenals	—thyroid glands
—parathyroid glands	—submaxillary glands
—sublingual glands	—lacrimal glands
—stomach	—duodenum
—small intestine	—large intestine
—pancreas	—tongue
—esophagus	—thymus
—trachea	—lymph node, mesenteric
—sternum	—femur (with bone marrow)
—skin	—mammary gland region

The eyeballs were pre-fixed in 4% glutaraldehyde in a 0.1M phosphate buffered solution (pH 7.1) and post-fixed in 10% neutral buffered formalin. All the above tissues were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H.E.) and examined by microscopy. The kidney sections from of one male and one female in each group (B733, B734, B728, B722, B748, B753, B747 and B743) were stained with Sudan

Tissues/Organs Examined from 26-Week Oral Toxicity Study in Dogs

After gross observations and organ weight measurement, samples of the following organs/tissues from all animals were fixed in 10% neutral buffered formalin.

- brain
- heart
- gallbladder
- spleen
- ovaries
- uterus
- urinary bladder
- adrenals
- parathyroids
- sublingual glands
- stomach
- small intestine
- pancreas
- esophagus
- trachea
- sternum with bone marrow
- skin
- eyeballs
- any other organs and tissues with gross changes
- spinal cord
- lungs with bronchus
- liver
- kidneys
- testes
- prostate
- vagina
- pituitary
- thyroids
- submaxillary glands
- lacrimal glands
- duodenum
- large intestine
- tongue
- thymus
- lymph node, mesenteric
- femur with bone marrow
- mammary gland region

The eyeballs were fixed for 15 min in 4% glutaraldehyde in a 0.1 M phosphate buffered solution (pH 7) and post-fixed in 10% neutral buffered formalin.

All the above-mentioned organs/tissues were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H.E.), and examined by microscopy. Additional sections of the kidney from all animals were examined after periodic acid Schiff's reaction (PAS), Berlin blue staining or Schmori's reaction.

Tissues/Organs Examined from 52-Week Oral Toxicity Study in Dogs

After gross observations and organ weight measurement, samples of the following organs/tissues from all animals were fixed in 10% neutral buffered formalin.

- brain
- heart
- gallbladder
- spleen
- ovaries
- uterus
- urinary bladder
- adrenals
- parathyroids
- sublingual glands
- stomach
- small intestine
- pancreas
- esophagus
- trachea
- sternum with bone marrow
- skin
- eyeballs
- any other organs and tissues with gross changes
- spinal cord
- lungs with bronchus
- liver
- kidneys
- testes
- prostate
- vagina
- pituitary
- thyroids
- submaxillary glands
- lacrimal glands
- duodenum
- large intestine
- tongue
- thymus
- mesenteric lymph node
- femur with bone marrow
- mammary gland region

The eyeballs were fixed for 15 min. in 2.5% glutaraldehyde in 10% neutral buffered formalin and post-fixed in 10% neutral buffered formalin.

12) Histopathology

All the above mentioned organs/tissues were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H.E.) and examined by microscopy. Additional sections of the kidney from all animals were examined after Azan staining.

Tissues/Organs Examined from 4-Week Oral Toxicity Study in Monkeys

Sections from the following tissues and organs from all monkeys were examined microscopically:

-adrenal glands
-bone marrow
-duodenum
-eye
-esophagus
-femurs
-gallbladder
-heart
-kidney
-lymph nodes (mesenteric)
-large intestine
-lacrimal glands
-liver
-lung
-mammary glands
-ovary
-parathyroid gland
-pancreas
-pituitary gland
-prostate gland
-skin
-seminal vescicle
-spleen
-sternum
-stomach
-submandibular gland
-small intestine
-spinal cord
-sublingual gland
-tongue
-trachea
-thymus
-testis
-uterus
-urinary bladder
-vagina

Tissues/Organs Examined from 13-Week Oral Toxicity (Dose Rangefinding) Study in Rats

The following tissues from all animals were fixed in 10% buffered neutral formalin:

adrenal glands	seminal vesicle
brain	skin
duodenum	small intestine
esophagus	spinal cord
eye balls	spleen
femurs (with bone marrow)	sternum
Harderian glands	stomach
heart	sublingual glands
kidneys	submandibular glands
large intestine (cecum and colon)	testes
liver	thymus
lungs (with bronchi)	thyroid glands and parathyroid glands
lymph node (mesenteric)	tongue
mammary gland regions	trachea
ovaries	urinary bladder
pancreas	uterus
pituitary gland	vagina
prostate	

All the above tissues and organs of all animals in the control and 3000 mg/kg groups, the liver and kidney of all animals in the 1000 and 300 mg/kg groups and the lung of one male (No. 22) in the 1000 mg/kg group, in which white focus was observed visually, were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H·E) and examined light microscopically.

Tissues/Organs Examined from 104-Week Carcinogenicity Study in Rats

Liver	Ovaries
Kidneys	Prostate
Heart	Uterus
Lung with bronchi	Seminal vesicles
Spleen	Vagina
Trachea	Urinary bladder
Tongue	Brain
Esophagus	Spinal cord
Stomach and duodenum	Eyeballs
Small intestine	Harderian glands
Large intestine (colon)	Skin
Salivary glands (submandibular and sublingual)	Mammary glands
	Pituitary gland
Pancreas	Thyroid and parathyroid glands
Thymus (thymic region)	Adrenal glands
Lymph nodes (mesenteric)	Sternum with bone marrow
Testes	Femurs with bone marrow

Other organs and tissues with gross neoplastic changes

After proper fixing, all the above tissues and organs from all animals in the control and 1000 mg/kg groups and from rats which died spontaneously or were killed *in extremis* in the course of the study were routinely processed, embedded in paraffin, sectioned, stained with hematoxylin and eosin and then examined by light microscopy. The liver, kidneys, adrenal glands, stomach, spleen, testes and uterus and all gross neoplastic lesions from the 300 and 100 mg/kg groups were also examined.

Selected paraffin-embedded sections of the kidney from 3 control males (1M-15, 2M-62 and 2M-69) and 4 males receiving 1000 mg/kg/day (5M-213, 5M-240, 5M-246 and 5M-247) were stained with PAS, elastica van Gieson, Masson's trichrome and acid fuchsin orange G (AFOG) [7] stains for detailed examinations.

Tissues/Organs Examined from 13-Week Oral Toxicity (Dose Rangefinding) Study in Mice

The following tissues from all animals in Group 1-5 were fixed in 10% buffered neutral formalin:

adrenal glands
brain
duodenum
esophagus
eye balls
femurs (with bone marrow)
Harderian glands
heart
kidneys
large intestine (cecum and colon)
liver
lungs (with bronchi)
lymph node (mesenteric)
mammary gland regions
ovaries
pancreas
pituitary gland
prostate (ventral)
seminal vesicles
skin
small intestine
spinal cord
spleen
sternum
stomach
sublingual glands
submandibular glands
testes
thymus
thyroid glands and parathyroid glands
tongue
trachea
urinary bladder
uterus
vagina

The liver, kidney, spleen, bone marrow and testis of all animals in Groups 1-5 were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H.E) and examined light microscopically.

Tissues/Organs Examined from 104-Week Carcinogenicity Study in Mice

4.4.2. Microscopic

Representative samples of protocol-designated organs and tissues were processed for the preparation and microscopic examination of hematoxylin-eosin stained paraffin sections. A full complement of organs and tissues was prepared and microscopically examined for all main study mice in control groups 1 and 2 and in the 100 mg/kg/day treated group that were euthanized at study termination, and on all those mice from all main study groups that died or were sacrificed *in extremis* during the course of the study. In addition, sections were prepared for microscopic examination of kidney, tissue masses and all gross lesions from all main study mice.

The following list constitutes the full complement of organs and tissues collected from all mice:

- Adrenal (2)
- Bone with bone marrow (femur)
- Bone with bone marrow (sternum)
- Brain (fore, mid and hind)
- Eye including optic nerve (2)[a]
- Gallbladder
- Gastrointestinal tract:
  - esophagus
  - stomach (glandular and nonglandular)
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)
  - testis with epididymis (2)
- Gross lesions
- Harderian gland (2)[a]
- Heart
- Kidney (2)
- Lacrimal gland, exorbital (2)
- Liver (2 sections examined; all lobes collected)
- Lung with mainstem bronchi (collected whole, 1 section examined containing all lobes)[b]
- Lymph nodes: mandibular, mesenteric and regional when applicable[c]
- Mammary gland[d]
- Pancreas
- Pituitary
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular with sublingual and parotid (2)
- Sciatic nerve
- Skeletal muscle (thigh)
- Skin
- Spinal cord (cervical, thoracic and lumbar)
- Spleen
- Thymus[d]
- Thyroid/parathyroid (2)[e]
- Tissue masses
- Tongue
- Trachea
- Urinary bladder[f]
- Uterus (both horns)
- Uterus, cervix
- Vagina

[a] Tissue fixed in Davidson's fixative.
[b] Lungs were inflated with formalin via the trachea.
[c] When a tissue mass was present, the lymph node draining the region of the mass was examined.
[d] At times these tissues could not be identified with the unaided eye because of physiologic variation in size. However, tissue from the region was fixed for microscopic evaluation.
[e] Parathyroids could not always be identified macroscopically. They were examined microscopically if in the plane of section and in all cases where they were noted as grossly enlarged.
[f] Urinary bladder was inflated with formalin and opened for examination after fixation.

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