Dosage Levels: 0 (vehicle control), 10, 50 and 300 mg/kg/day (22/sex/dose group).

Observations/Measurements: Animals were observed for survival and clinical signs of toxicity twice daily during drug treatment. Body weights of males were obtained once a week throughout the study period; females were weighed before mating and then on days 0, 6, 13, 18 and 20 of gestation. Food consumption was determined for all animals weekly before mating and in pregnant females on days 0, 6, 8, 13, 17 and 19 of gestation. Males and females receiving the same dose were mated for 3 weeks on a one-to-one basis and mating was confirmed by presence of a copulation plug. The copulatory index (# of animals copulated x 100/# of animals cohabitated) and fertility index (# of pregnant animals x 100/# animals that copulated) were calculated for each group. Males were killed and necropsied after 15 weeks of dosing. Their reproductive and main organs were examined grossly. The testes, epididymides, seminal vesicles and ventral prostate were weighed and relative organ weights were calculated. Females were necropsied on day 20 of gestation. The uteri, ovaries and other main organs were examined grossly. The numbers of corpora lutea, implants, dead embryos/fetuses and live fetuses were counted. The sex ratios (# males x 100/# males and females) were determined. The live fetuses were examined for external abnormalities and were weighed. One-third of the live fetuses were examined for visceral abnormalities and the skeletons from the remaining two-thirds were stained with dye for examination of skeletal malformations.

Results: No treatment-related deaths occurred and no general signs of toxicity were noted; one control male and one control female died due to intubation errors. In drug-treated males, terminal body weights were decreased dose-dependently (-4.6%, -5%, -7% for LD, MD and HD, respectively) from control; body weights of females were unaffected by treatment. No treatment-related effects on food consumption were noted. No differences were noted in copulatory index (100% for control and each treated group) or fertility index (100% for control and each treated group). There were no treatment-related differences from control in the numbers of corpora lutea, implants, percent implantation losses, number of live fetuses, fetal weights and sex ratios (Table 38). At necropsy, no treatment related abnormalities were observed in any group of dams. External, visceral or skeletal examination of fetuses revealed no treatment-related abnormalities. Although body weights in the 50 and 300 mg/kg/day males were slightly lower than those in the control group at study termination, relative organ (testes, epididymides, ventral prostate or seminal vescicles) weights were not significantly different from control.

Table 38. C-Section Data (Gestation Day 20)

Parameter	Dose Group (mg candesartan cilexetil/kg/day)			
	0 (control)	10	50	300
Corpora Lutea (mean #)	16.1	16.7	16.4	16.5
Implants (mean #)	14.3	15.1	15.3	15.2
Pre-implantation loss (%)	10.8	9.3	6.4	7.5
Post-implantation loss (%)	7.3	6.0	4.9	5.5
Live Fetuses (mean #)	13.3	14.2	14.6	14.4
Fetal Weight (mean gm)				
Male	3.01	3.11	3.05	3.01
Female	2.79	2.94	2.86	2.82
Sex Ratio (% males)	53.6	49.5	47.7	48.6

Developmental Toxicity Study in Rats

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1322/TE
Study Dates: Beginning date 2/25/92. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Presumed pregnant female Jcl:Wistar rats (12-15 wks old, 198-252 gm, at initiation of dosing).

Drug Administration: Candesartan cilexetil (Lot #M464-012) was suspended in 5% gum arabic aqueous solution and administered orally by gavage to pregnant females from day 6 to day 17 of gestation.

Dosage Levels: 0 (vehicle control), 10, 30 and 100 mg/kg/day (33-36F/dose group).

Observations/Measurements: All dams were observed for survival and clinical signs of toxicity twice daily during the treatment period. Maternal body weights were measured on days 0, 6, 8, 10, 13, 15, 18 and 20 of gestation and on days 0, 4, 7, 14 and 22 of lactation. Food consumption was recorded on days 0, 6, 8, 10, 13, 15 and 18 of gestation. On day 20 of gestation, 20-23 dams in each group were killed and their uteri, ovaries and main organs

were examined. The number of corpora lutea and implants were counted; the numbers of dead embryos/fetuses and live fetuses were determined. The sex ratio (# of males x 100/# of males and females) was calculated. Live fetuses were weighed and examined for external abnormalities. Approximately one-third of the fetuses were examined for visceral abnormalities and the other two-thirds were for examined for skeletal malformations. Eleven to 13 dams in each group were allowed to deliver naturally. The duration of gestation was calculated and the number of live and dead newborn pups were counted, weighed, sexed and examined for external abnormalities. On day 4 of lactation litters were reduced to 8 pups each (4M & 4F, if possible). The F_1 pups were were observed for physical development (body weight, pinna detachment, incisor eruption, eye opening) during lactation. At weaning, 2 males and 2 females were necropsied and examined for skeletal abnormalities. The remaining 2 males and 2 females from each litter were observed during further physical (body weight, testes descent, vaginal opening), behavioral (determined from locomotor activity, open field and water T-maze performance) and sexual (reproductive performance) development. After the reproductive ability assessment, the F_1 animals were necropsied and the epididymis was examined for the presence of sperm and the ovary for the development of follicles and corpora lutea.

Results: No deaths occurred in F_0 dams during the study. Body weight gain was reduced (-13%) from control from day 18 to day 20 of gestation in the 100 mg/kg/day group. In the 30 and 100 mg/kg/day F_0 dams, food consumption was decreased (-9%, -13%, respectively) from control on day 18 of gestation. No treatment-related abnormalities were observed at necropsy of F_0 dams on day 20 of gestation. The numbers of corpora lutea and implantations, percent pre- and post-implantation losses, numbers of live fetuses, fetal weights and sex ratios in the treated groups were not significantly different from those in the control group (Table 40). No treatment-related external, visceral or skeletal abnormalities of fetuses were observed.

Table 40. Maternal/Fetal Necropsy Results (Gestation Day 20)

Parameter	Dose Group (mg candesartan cilexetil/kg/day)			
	0 (22)*	10 (23)*	30 (20)*	100 (23)*
Corpora Lutea (mean #)	15.6	15.8	15.5	16.2
Implants (mean #)	14.9	14.8	14.7	15.7
Pre-implantation loss (%)	4.8	5.8	5.0	3.2
Post-implantation loss (%)	6.4	4.1	7.5	6.7
Live Fetuses (mean #)	13.9	14.2	13.6	14.7
Fetal Weight (mean gm)				
Male	2.97	3.06	3.05	2.92
Female	2.80	2.85	2.86	2.75
Sex Ratio (% males)	46.9	51.7	54.1	49.2

* Numbers in parentheses represent the number of dams examined

In F_0 dams allowed to deliver, no treatment-related effects were observed in gestation duration, numbers of pups/litter, numbers of live pups/litter, birth index (# newborns X 100/# implants) and viability index (# live pups on day 4 X 100/ # newborns)(Table 41).

Table 41. F_0 Dams/ F_1 Pups Parturition Results

Parameter	Dose Group (mg candesartan cilexetil/kg/day)			
	0	10	30	100
# F_0 Dams	13	11	13	13
Gestation Duration (days)	21.5	21.8	21.8	21.8
Newborns/Litter (mean #)	13.8	13.5	13.3	11.8
Birth Index (%)	95.2	92.0	91.6	92.3
Live Pups/Litter (mean #)				
Day 0	13.3	12.3	11.2	10.8
Day 4	12.8	11.4	10.8	10.3
Viability Index (%)				
Day 4	96.9	92.6	96.2	96.0
Day 22*	99.0	100.0	100.0	99.0

* Based on survival of Day 4 culled groups (8/litter)

Treatment had no significant effects on the physical and behavioral development or on the sexual maturation (reproductive ability) of F_1 animals. Necropsy of matured F_1 animals revealed no treatment-related effects in either sex (Tables 42-44)

Table 42. Physical Development of F_1 Pups

Parameter	Dose Group (mg candesartan cilexetil/kg/day)			
	0	10	30	100
Pinna Detachment, Day 4 (% animals)[a]	100	100	98.8	100
Incisor Eruption, Day 13 (% animals)[a]	95.2	97.7	93.3	100
Eye Opening, Day 17 (% animals)[a]	100	100	92.3	100
Testes Descent, Day 26 (% animals)[b]	100	86	92	85
Vaginal Opening, Day 40 (% animals)[b]	100	100	96	100

[a] Based on 125-171 animals/dose group
[b] Based on 22-26 males or females/dose group

Table 43. Behavioral Development of F_1 Pups

Parameter		Dose Group (mg candesartan cilexetil/kg/day)			
		0	10	30	100
Locomotor Activity (activity counts)	M	1436	1343	1363	1409
	F	1410	1282	1360	1409
Water-T-Maze (completion time, sec) Trial #1	M	54.5	42.3	46.5	40.8
	F	57.4	46.9	38.7	33.6
Trial #3	M	22.8	32.3	25.5	25.4
	F	24.5	33.9	25.5	27.3

APPEARS THIS WAY
ON ORIGINAL

Table 44. Reproductive Performance of F_1 Animals

Parameter	Dose Group (mg candesartan cilexetil/kg/day)			
	0	10	30	100
# Pairs Mated	13	10	12	10
Fertility Index (%)[a]	100	100	92	100
Gestation Duration (days)	21.9	21.7	21.7	21.6
# Implants/Dam	13.5	14.2	14.8	13.6
Birth Index (%)[b]	94.5	94.6	93.2	95.9
Live Pups (mean #)	10.8	12.1	12.2	11.7
Pup Weight, Day 0 (gm) M F	5.3 5.3	5.6 5.2	5.6 5.3	5.7 5.3
Viability Index, Day 4 (%)[c]	95.8	98.0	97.9	97.7

[a] # females pregnant/# females mated
[b] # born (live + dead)/# implants
[c] # live pups on Day 4/# live pups on Day 0

Note: A supplemental oral developmental toxicity study (1457/TE) in rats was conducted in compliance with GLP regulations by Takeda Chemical Industries, Inc, Japan (initiated on 8/07/92). This study, conducted similarly to study 1322/TE, investigated the developmental toxicity of a higher oral dose (300 mg/kg/day only) of candesartan cilexetil administered to Jcl:Wistar rats from day 6 to day 17 of gestation. No deaths or other adverse effects were observed in the treated dams compared to vehicle control (35F/group); however decreases in food consumption (-13%) and body weight gain (-20%) were detected at gestation day 20. In dams subjected to C-section (22/drug or vehicle) on gestation day 20, no differences from control numbers of corpora lutea, implants, implantation losses or adverse effects on embryo-fetal mortality, sex ratios, fetal weights, morphological observation of placentae, or external, visceral or skeletal features of the fetuses were observed in the treated group. In dams allowed to deliver normally (12/drug or vehicle), no adverse effects were noted on gestation duration, delivery, nursing behavior or necropsy findings. Body weights of F_1 pups were lower (-11.8% for females and -15.2% for males) than control at time of weaning. No adverse effects on neonatal viability, physical, behavioral or sexual development were seen in the treated group compared to control. Based on these results and those of the previous study, it is concluded that the no-observed-adverse-effect-level of candesartan cilexetil is 30 mg/kg/day for dams, 300 mg/kg/day for fetuses and 100 mg/kg/day for pups.

Peri-and Post-natal Toxicity Study in Rats

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1403/PE
Study Dates: Beginning date 6/25/92. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals:Presumed pregnant female Jcl:Wistar rats (12-15 wks old, 190-256 gm at initiation of dosing).

Drug Administration: Candesartan cilexetil (Lot #M464-016) was suspended in 5% gum arabic aqueous solution and administered orally by gavage to pregnant females from day 15 of gestation through day 21 of lactation.

Dosage Levels: 0 (vehicle control), 10, 50 and 300 mg/kg/day (22-23F/dose group).

Observations/Measurements: All dams were observed for survival and clinical signs of toxicity twice daily during the treatment period. Maternal body weights were measured on days 0, 6, 13, 15, 17 and 20 of gestation and on days 0, 4, 7, 14 and 22 of lactation. Food consumption was measured on gestation days 0, 6, 15, 17 and 20 and on lactation days 0, 4, 7, 14 and 20. All dams were allowed to deliver naturally and the duration of gestation was noted. Following delivery, the numbers of live and dead newborns were counted and pups examined for external abnormalities. When the litter size was nine or more, the litter was reduced to 8 pups (4/sex whenever possible) by culling on day 4 of lactation. The dams were necropsied at weaning (day 22 of lactation) and the numbers of implantation sites counted and main internal organs examined macroscopically. At weaning, 2 males and 2 females from each litter were raised for evaluation of physical, behavioral and reproductive development. The remaining pups were necropsied and examined for external, visceral and skeletal malformations. After evaluation of reproductive performance, the F_1 animals were necropsied, the epididymis examained for the presence of sperm and the ovaries examined for the development of follicles and corpora lutea.

Results: No treatment-related deaths occurred and no signs of toxicity were observed; one dam in the 300 mg/kg/day group died due to an intubation error. Body weights of treated groups at the end of gestation and lactation were comparable to control; body weights of high dose dams were slightly lower (-5%) than control on day 14 of lactation. Food consumption was decreased (15-25%) in high dose dams on lactation days 7, 14 and 20. The duration of gestation and and the number of newborns in the treated groups were comparable to control. By day 4 of lactation, all pups died in one litter in the 50 mg/kg/day group and in one litter in the 300 mg/kg/day group. By day 14 of lactation,

all pups died in 3 litters in the 50 mg/kg/day group and in two litters in the 300 mg/kg/day group. The mean numbers of live pups on day 4 of lactation and the viability index on day 22 of lactation for high dose treated animals were significantly lower than control (Table 45). Drug treatment had no effect on pup birth weights but pup weights in the high dose group were significantly lower than control on day 14 of lactation and pup weights in the mid and high dose groups were significantly lower than control on day 22 of lactation.

Table 45. F_0 Dams/F_1 Pups Parturition Results

Parameter		Dose Group (mg/kg/day)			
		0	10	50	300
# F_0 Dams		23	23	22	22
Gestation Duration (days)		21.6	21.6	21.6	21.7
Newborns/dam (mean #)		14.0	13.7	13.3	12.6
Birth Index[a] (%)		94.3	88.5	91.0	89.0
Live Pups/Litter (mean #)					
Day 0		12.7	12.4	11.6	11.2
Day 4		12.4	11.3	10.1	9.4*
Viability Index (%)					
Day 4[b]		90.4	91.0	87.7	89.2
Day 22[c]		96.4	90.3	81.3	80.3*
Pup Weight (mean gm)					
Day 0	M	5.4	5.5	5.5	5.6
	F	5.0	5.1	5.1	5.2
Day 4	M	8.2	8.4	8.5	8.4
	F	7.8	7.8	8.0	8.0
Day 14	M	26.2	24.3	23.6	21.7*
	F	25.2	23.1	23.7	20.9*
Day 22	M	46.9	41.6	38.2*	35.5*
	F	44.1	39.8	38.5*	34.2*

* Significantly lower than control ($p<0.05$)
[a] # born (live + dead)/# implants
[b] # live pups/# live pups on Day 0
[c] Based on survival of Day 4 culled groups (8/litter)

No treatment-related abnormalities were observed in dams at necropsy; the numbers of implants in the treated groups were comparable to the control value. No treatment-related external and skeletal abnormalities were observed on examination of weanlings. In visceral examinations of weanlings, the incidences of hydronephrosis, 9.4%, 26% and 31.4%, in the 10, 50 and 300 mg/kg/day groups, respectively, were increased compared to control (1.1%).

No significant treatment-related adverse effects were noted on the physical, behavioral or reproductive development of the F_1 offspring (Tables 46-48).

Table 46. Physical Development of F_1 Pups

Parameter	Dose Group (mg/kg/day)			
	0	10	50	300
Pinna Detachment, Day 4 (% animals)[a]	100	100	99.2	98.4
Incisor Eruption, Day 13 (% animals)[a]	97.8	96.6	90.8	95.0
Eye Opening, Day 17 (% animals)[a]	100	99.3	100	98.6
Testes Descent, Day 26 (% animals)[b]	97.7	88.6	89.2	88.9
Vaginal Opening, Day 40 (% animals)[b]	100	97.7	97.2	94.1

[a] Based on 210-286 animals/dose group
[b] Based on 32-44 males or females/dose group

Table 47. Behavioral Development of F_1 Pups

Parameter		Dose Group (mg/kg/day)			
		0	10	50	300
Pupillary Reflex, Day 4 (% animals)	M	100	100	100	100
	F	100	100	100	94.4
Pain Response (% animals)	M	100	100	100	100
	F	100	100	100	100
Preyer's Reflex (% animals)	M	100	100	100	100
	F	100	100	100	100
Locomotor Activity (activity counts)	M	1474	1379	1195*	1376
	F	1466	1270	1236	1278
Water-T-Maze (completion time,sec) Trial #1	M	45.6	60.4	61.4	59.9
	F	42.7	57.0	47.1	52.1
Trial #3	M	28.5	30.4	29.8	35.1
	F	34.6	33.3	34.6	29.5

* Significantly lower than control value ($p<0.05$)

Table 48. Reproductive Performance of F_1 Animals

Parameter	Dose Group (mg/kg/day)			
	0	10	50	300
# Pairs Mated	21	21	18	18
Fertility Index (%)[a]	100	81	76.5	83.3
Gestation Duration (days)	21.8	21.7	22.0	22.1*
# Implants/Dam	13.6	15.3	13.7	12.8
Birth Index (%)[b]	94.1	92.3	88.8	88.1
Live Pups/Dam (mean #)	11.9	12.3	10.2	10.7
Pup Weight, Day 0 (gm) M	5.8	5.5	5.7	5.9
F	5.3	5.0	5.3	5.5
Viability Index, Day 4 (%)[c]	95.5	94.0	83.9	85.1

[a] # females pregnant/# females mated
[b] # born (live + dead)/# implants
[c] # live pups on Day 4/# live pups on Day 0

At necropsy of matured F_1 animals and F_1 animals that died after weaning, the incidence of hydronephrosis was increased in all drug-treated groups (2/89, 28/89, 70/72 and 70/70 in C, LD, MD and HD, respectively).

Note: A supplemental oral peri- and post-natal toxicity study (1537/PE) in rats was conducted in compliance with GLP regulations by Takeda Chemical Industries, Inc., Japan (initiated on 1/11/93). This study, conducted similarly to study 1403/PE investigated peri-and post-natal toxicity of oral doses of 0.4, 2 and 10 mg/kg/day of candesartan cilexetil (Lot #M464-016) administered to Jcl:Wistar rats from day 15 of gestation to day 21 of lactation to determine a no-effect dose for hydronephrosis. In dams, no deaths occurred and no adverse effects on clinical signs, gestation duration, delivery, nursing behavior or necropsy findings were observed in treated groups compared to vehicle control (22F/dose group). Lower than control birth index (88% vs. 95%) and neonatal viability index (75% vs 92% at day 4 of lactation) were noted in the 10 mg/kg/day group. In F_1 pups, body weight in the 10 mg/kg/day group was lower (-10.5% for females and -19% for males) than control at time of weaning. By the end of the lactation period, all the pups of 5 out of 22 dams in the 10 mg/kg/day group died. The incidence of hydronephrosis was higher than control (26.8% vs. 3.6%) in combined results from weanlings and mature F_1 animals from the 10 mg/kg/day dose group; however the incidences in the 0.4 and 2 mg/kg/day groups were comparable to contol. No adverse effects on external features or physical development of F_1 animals were noted in any group.

Developmental Toxicity Study in Rabbits

Study Facility: Takeda Chemical Industries, Inc.; Japan
Study No: 1448/TE
Study Dates: Beginning date 8/07/92. Ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Presumed pregnant female Kbl:JW rabbits (14 wks old, 2.86-3.73 kg, at initiation of dosing).

Drug Administration: Candesartan cilexetil (Lot #M464-016) was suspended in 5% gum arabic aqueous solution and administered orally by gavage to pregnant females from day 6 to day 18 of gestation.

Dosage Levels: 0 (vehicle control), 0.3, 1.0 and 3.0 mg/kg/day (13-15F/dose group).

Observations/Measurements: All dams were observed for survival and clinical signs of toxicity twice daily during the treatment period and then once daily until study termination. Maternal body weights were measured on days 0, 6, 9, 12, 15, 19, 22, 25 and 28 of gestation. Food consumption was recorded on days 0, 6, 9, 12, 15, 18, 22, 25 and 27 of gestation. Blood samples were taken from the ear vein of each dam on days 6 and 19 of gestation for measurement of plasma urea nitrogen and creatinine. Blood samples were also taken one hour after dosing on days 6, 13 and 18 of gestation for measurement of plasma levels of the active metabolite, candesartan. All dams were killed on day 28 of gestation and their uteri, ovaries and main organs were examined. The number of corpora lutea, implants, dead embryos/fetuses and live fetuses were counted. The sex ratio (# of males x 100/# of males and females) was calculated. Live fetuses were weighed and examined for external, visceral and skeletal abnormalities.

Results: One high dose (3 mg/kg/day) dam aborted her litter on day 20 of gestation and died 2 days later; another dam in this dose group died on day 23 of gestation. Two other dams (one each in the 1 and 3 mg/kg/day dose groups) died due to intubation errors. Body weights decreased about 0.5 kg after day 15 of gestation in the two non-surviving high-dose dams; no adverse effect on body weight was observed in surviving dams. Increases from control levels of plasma urea nitrogen and creatinine were detected in high dose dams; however, the increases were primarily ascribed to the 2 dams that subsequently died. No significant increases in these blood chemistry parameters were noted among surviving high dose dams. Petechia in the stomach, discoloration of the kidneys and liver and red foci in the gallbladder were observed in the two dead dams during macroscopic examination. In surviving dams, there were no significant differences from control in the

numbers of corpora lutea or implantations (Table 49). No adverse effects were observed on fetal mortality, sex ratio, fetal weight or at external, visceral and skeletal examination of live fetuses. The mean number of live fetuses/dam in the 1 mg/kg/day group (6.3) was lower than the number in the control group (9.2); however, the difference was considered to be unrelated to treatment due to the lack of dose-dependency (essentially no difference in the 3 mg/kg/day group).

Table 49. Rabbit Maternal/Fetal Necropsy Results*

Parameter	Dose Group (mg/kg/day)			
	0	0.3	1.0	3.0
Number of Dams	15	15	13	11[a]
Corpora lutea (mean #)	10.9	9.6	9.9	11.1
Implants (mean #)	9.7	7.3	7.4	9.3
Pre-implantation loss (%)	9.6	22.5	25.2	15.7
Post-implantation loss (%)	5.4	2.6	11.9	6.0
Live fetuses/dam (mean #)	9.2	7.1	6.3	8.7
Sex ratio (% males)	51.5	61.0	58.2	57.3
Fetal weight (mean gm)	37.5	40.3	41.3	39.8

* No significant differences between drug teated and corresponding control values.
[a] Excludes 2 dams that died prior to scheduled necropsy

Plasma concentrations of the active metabolite, candesartan, in dams increased dose-dependently; the plasma metabolite concentrations increased in all groups from gestation day 13 to gestation day 18 (Table 50).

Table 50. Plasma Levels of Candesartan* in Rabbits Given Oral Candesartan Cilexetil

Dose Group (mg/kg/day)	Plasma Concentration (ng/ml)		
	GD 6	GD 13	GD18
0.3	17	10	41
1.0	84	73	143
3.0	212	318	636

* Plasma levels determined one hour after dosing

Developmental Toxicity Study in Mice

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: 1947/TE
Study Dates: Initiation Date: 12/14/94; ending date not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Presumed pregnant female Jcl:ICR mice (28-41 gm at initiation of dosing)

Drug Administration: Candesartan cilexetil (Lot #M464-032) was suspended in 5% gum arabic aqueous solution and administered orally by gavage to pregnant females from day 6 to day 15 of gestation.

Dosage Levels: 0 (vehicle control), 10, 100 and 1000 mg/kg/day (16-20F/dose group; additional 39 F/candesartan cilexetil-treated group for measurement of plasma candesartan levels).

Observations/Measurements: All dams were observed for survival and clinical signs of toxicity twice daily during the treatment period. Maternal body weights and food consumption were measured on days 0, 6, 8, 10, 12, 14 and 16 of gestation. On day 18 of gestation, the dams were killed by cervical dislocation and the uterus, ovaries and main organs were examined. The numbers off corpora lutea, implants, resorptions, placental remnants, dead embryos/fetuses, live fetuses and sex ratio (# males X 100/# males and females) were recorded. The live fetuses were weighed and examined for external abnormalities. Approximately one-half of the live fetuses were examined for visceral abnormalities and the other one-half were examined for skeletal abnormalities.

Results:

Mortality and Clinical Signs
One animal in the high-dose toxicokinetic satellite group died from a dosing error. One control group mouse delivered prematurely on day 18 of gestation. No other deaths or signs of toxicity were observed in any group.

Body Weight and Food Consumption
Body weight and food consumption among candesartan-treated groups were not significantly different from control (Tables 51 & 52).

Table 51. Mean Body Weights, gm

Gestation Day	Dose group, mg candesartan cilexetil/kg/day			
	0 (Control)	10	100	1000
0	35.0	35.0	34.8	35.7
6	36.0	36.3	36.0	36.8
12	43.0	43.4	43.1	43.1
16	63.1	64.8	63.9	64.3

Table 52. Mean Food Consumption, gm

Gestation Day	Dose group, mg candesartan cilexetil/kg/day			
	0 (Control)	10	100	1000
0	3.8	4.5	4.2	4.1
6	6.4	6.4	6.5	6.4
12	8.2	7.7	7.8	7.9
16	10.1	10.0	9.7	9.9

Necropsy

There were no significant differences in the numbers of corpora lutea, implants, embryo/fetal mortality, numbers of live fetuses, fetal body weight or sex ratio between the control and any candesartan cilexetil-treated group (Table 53).

Table 53. Maternal/Fetal Necropsy Results

Parameter	Dose Group, mg candesartan cilexetil/kg/day			
	0 (Control)	10	100	1000
Number of dams	18	20	16	18
Corpora lutea, mean #	15.4	15.4	15.1	15.5
Implants, mean #	14.7	14.6	14.6	14.4
Pre-implantation loss, mean %	4.7	5.1	3.3	7.4
Post-implantation loss, mean %	9.8	4.4	7.2	4.4
Live fetuses, mean #	13.3	13.9	13.5	13.8
Sex ratio, (M/M + F) %	50.5	49.0	49.5	53.7
Fetal weight, mean gm				
Male	1.47	1.52	1.51	1.50
Female	1.42	1.46	1.44	1.42

No significant differences were noted in the frequency of external abnormalities between control and any candesartan cilexetil-treated group. Examination for visceral and skeletal abnormalities were conducted for the control and high dose groups; visceral and skeletal examinations were not performed for the low- and mid-dose groups because no treatment-related abnormalities were seen

in fetuses in the high dose group (Table 54).

Table 54. External, Visceral and Skeletal Examination Results

Parameter	Dose Group, mg candesartan cilexetil/kg/day			
	0 (Control)	10	100	1000
External Abnormalities, %	1.6 (239)[a]	1.7 (278)	1.3 (216)	0.8 (248)
Visceral				
Abnormalities, %	0.0 (129)	NE[b]	NE	0.0 (135)
Variations, %	16.3 (129)	NE	NE	10.7 (135)
Skeletal				
Abnormalities, %	0.9 (110)	NE	NE	0.0 (113)
Variations, %	47.8 (110)	NE	NE	44.7 (113)

[a] Numbers in parentheses are the total # fetuses examined.
[b] NE= not examined.

Toxicokinetics

Cmax and AUCs of the active metabolite, candesartan, increased, non-dose-proportionally, with increasing dose when measured on days 6 and 15 of gestation. No accumulation of drug with repeated dosing was observed (Table 55).

Table 55. Toxicokinetics of Candesartan after Oral Administration of Candesartan Cilexetil to Pregnant Mice.

Dose Group, mg/kg/day	Dosing Day	Candesartan Pharmacokinetic Parameter		
		Tmax, hr	Cmax, µg/ml	AUC_{0-24}, µg.hr/ml
10	GD6	1	2.20	5.8
	GD15	1	2.35	8.0
100	GD6	0.5	10.5	35.4
	GD15	1	10.1	39.9
1000	GD6	0.25	26.8	124.3
	GD15	1	26.7	123.8

GENOTOXICITY

Ames Bacterial Mutagen Test of Candesartan Cilexetil

Study Facility: Takeda Analytical Research Labs., Osaka, Japan
Study No: 91/MH
Study Dates: 8/8/91-10/25/91

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Bacterial Strains: Salmonella typhymurium strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2uvrA.

The mean number of revertant colonies was determined for each concentration (2-4 plates/conc.) of candesartan cilexetil and for the positive controls in the presence and absence of metabolic activation and compared to vehicle controls. The test agent was judged to be positive for mutagenic activity when the mean number of revertant colonies on the treated plates was dose-dependent and, for at least one concentration, two or more times the mean number of revertant colonies on the concurrent control plate.

Results: Candesartan cilexetil did not cause a significant increase (equal to or greater than twice concurrent vehicle control value) in the number of revertant colonies of any of the bacterial tester strains in the presence or absence of metabolic activation. The positive controls were mutagenic under the conditions of this assay (Table 56 & 57).

Table 56. Bacterial Mutagen Test (No Metabolic Activation)

Treatment	Conc/ Plate	Mean# Revertant Colonies/Plate (n=2-4)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		32	161	11	33	12
DMSO		31	136	13	28	11
Candesartan cilexetil[a]	4.9 ug		172	13	30	9
	9.8		156	13	22	14
	19.5		137	10	28	10
	39		141	10	37	13
	78		134	14	26	10
	156	29	106[c]	10[c]	23[c]	11[c]
	313	29				
	625	24				
	1250	29				
	2500	19[d]				
	5000	26[d]				
DAPA[b]	25 ug	558	1062		1629	499
AF-2[a]	10 ng	149	574		455	
	40					
ENNG[b]	5 ug			137		
AZI[b]	0.5 ug			462		
9AA[a]	80 ug					464

[a] DMSO vehicle [b] Water vehicle
[c] Cytotoxic effect on colony growth
[d] Precipitate formed

APPEARS THIS WAY
ON ORIGINAL

Table 57. Bacterial Mutagen Test (With Metabolic Activation)

Treatment	Conc/Plate	Mean# Revertant Colonies/Plate (n=2-4)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		34	143	16	47	27
DMSO		37	134	14	49	20
Candesartan cilexetil[a]	78 ug	-	140	-	-	-
	156	39	136	10	50	25
	313	31	141	14	44	28
	625	35	121	11	28	26
	1250	28	126[c]	16	31	21
	2500	29[c]	93[c,d]	11[c]	41[c]	10[c]
	5000	38[c,d]		10[c,d]	31[c,d]	5[c,d]
2AA[a]	1 ug		662			
	2			198		
	4	139				
BP[a]	5 ug		870		331	170
DEN[b]	5 uL	247				
CPP[b]	200 ug			410		

[a] DMSO vehicle [b] Water vehicle
[c] Cytotoxic effect on colony growth
[d] Precipitate formed

Note: A replicate assay using the same concentrations of candesartan cilexetil, positive and vehicle controls was conducted with and without metabolic activation. The results of the replicate assay were comparable to the above results and showed no positive mutagenic effects of candesartan cilexetil.

APPEARS THIS WAY
ON ORIGINAL

Ames Bacterial Mutagen Test of Active Metabolite (Candesartan; CV-11974)

Study Facility: Takeda Analytical Research Labs., Osaka, Japan
Study No: 63/MH
Study Dates: 12/12/90-3/11/91

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Bacterial Strains: Salmonella typhymurium strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2uvrA.

The mean number of revertant colonies was determined for each concentration (2 plates/conc.) of candesartan and for the positive controls in the presence and absence of metabolic activation and compared to vehicle control. The test agent was judged to be positive for mutagenic activity when the mean number of revertant colonies on the treated plates was dose-dependent and, for at least one concentration, two or more times the mean number of revertant colonies on the concurrent control plate.

Results: Candesartan did not cause a significant increase (equal to or greater than twice concurrent vehicle control value) in the number of revertant colonies of any of the bacterial tester strains in the presence or absence of metabolic activation. The posiitve controls were mutagenic under the conditions of this assay (Tables 58 & 59a).

Table 58. Bacterial Mutagen Test (No Metabolic Activation)

Treatment	Conc/ Plate	Mean# Revertant Colonies/Plate (n=2-4)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		25	172	18	61	16
DMSO		20	144	16	53	19
Candesartan [a]	156	18	184	19	41	17
	313	12	172	16	51	17
	625	27	163	12	51	10
	1250	26	152	17	50	10
	2500	18	171	16	51	18
	5000	23	154	14	52	11
DAPA[b]	25 ug	289	546		783	387
AF-2[a]	20 ng	416	1108			
	40				326	
ENNG[b]	5 ug			89		
AZI[b]	0.5 ug			335		
9AA[a]	40 ug					249

[a] DMSO vehicle [b] Water vehicle

APPEARS THIS WAY
ON ORIGINAL

Table 59a. Bacterial Mutagen Test (With Metabolic Activation)

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate (n=2)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		30	152	14	60	21
DMSO		27	138	10	60	30
Candesartan[a]	156 ug	28	140	11	67	26
	313	30	142	15	68	31
	625	27	126	15	73	33
	1250	30	148	19	69	20
	2500	33	142	13	65	32
	5000	26	147	13	75	23
2AA[a]	1 ug		630			
	2			169	950	148
	4	361				
BP[a]	5 ug		1309		545	148
DEN[b]	5 uL	294				
CPP[b]	200 ug			540		

[a] DMSO vehicle [b] Water vehicle

Note: A replicate assay using the same concentrations of candesartan, positive and vehicle controls was conducted with and without metabolic activation. The results of the replicate assay were comparable to the above results and showed no mutagenic effects of the active metabolite, candesartan.

Bacterial Mutagen Test with Candesartan Metabolite, M-II

Study Facility: Takeda Analytical Research Labs., Osaka, Japan
Study No: 160/MH
Study Dates: 10/15/92-11/30/92

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Bacterial Strains: Salmonella typhymurium strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2uvrA.

The mean number of revertant colonies was determined for each concentration (2 plates/conc.) of the M-II metabolite and for the positive controls in the presence and absence of metabolic activation and compared to vehicle control. The test agent was judged to be positive for mutagenic activity when the mean number of revertant colonies on the treated plates was dose-dependent and, for at least one concentration, two or more times the mean number of revertant colonies on the concurrent control plate.

Results: Concentrations of 156 to 5000 ug/plate of the candesartan metabolite, M-II, caused no significant increases in the number of revertant colonies of any of the bacterial tester strains in the presence or absence of metabolic activation. The positive controls were mutagenic under the conditions of this assay (Tables 59b & 59c)).

Table 59b. Bacterial Mutagen Test (No Metabolic Activation)

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate (n=2-4)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		34	170	18	32	17
DMSO		30	151	16	29	16
Candesartan metabolite, M-II [a]	156ug	27	139	15	30	27
	313	32	163	15	33	19
	625	27	131	12	39	13
	1250	36	170	16	28	15
	2500	28	170	15	27	21
	5000	24	160	16	36	15
DAPA[b]	25 ug	597	1125		1647	455
AF-2[a]	10 ng	179	635			
	40				478	
ENNG[b]	5 ug			498		
AZI[b]	0.5 ug			497		
9AA[a]	40 ug					458

[a] DMSO vehicle [b] Water vehicle

Table 59c. Bacterial Mutagen Test (With Metabolic Activation)

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate (n=2)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		40	191	18	48	27
DMSO		45	175	18	52	23
Candesartan metabolite, M-II[a]	156 ug	45	188	13	44	30
	313	40	181	15	42	31
	625	46	174	16	51	24
	1250	47	180	15	42	25
	2500	35	179	21	42	31
	5000	35	159	15	51	30
2AA[a]	1 ug		1196		848	
	2			248		319
	4	435				
BP[a]	5 ug		1029		251	153
DEN[b]	5 uL	186				
CPP[b]	200 ug			395		

[a] DMSO vehicle [b] Water vehicle

Bacterial Mutagenicity Assay of Candesartan Cilexetil and Impurities

Study Facility: Takeda Chemical Industries, Inc., Japan
Study No: T3325
Study Dates: Assay initiation: 12/27/95; Assay termination: Not stated.

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Bacterial Strains: *Salmonella typhymurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2uvrA.

Procedure: A mixture of candesartan cilexetil and chemically related products (impurities) contained in the bulk drug used for product manufacture was evaluated for mutagenic activity in the bacterial reverse mutation assay.

The mean number of revertant colonies was determined for each concentration (2-4 plates/conc..) of candesartan cilexetil and for the positive controls in the presence and absence of metabolic activation and compared to vehicle controls. The test agent was judged to be positive for mutagenic activity when (1) the number of revertant colonies in the solvent control was in the range of the historical control value, (2) the mean number of revertant colonies on the treated plates was dose-dependent and, (3) for at least one concentration, two or more times the mean number of revertant colonies on the concurrent control plate.

THIS PAGE
WAS
DETERMINED
NOT
TO BE
RELEASABLE

Results: Candesartan cilexetil did not cause a significant increase (equal to or greater than twice concurrent vehicle control value) in the number of revertant colonies of any of the bacterial tester strains in the presence or absence of metabolic activation (Tables 60 & 61). The positive controls were mutagenic under the conditions of this assay.

Table 60. Bacterial Mutagen Test (No Metabolic Activation)

Treatment	Conc.*/ Plate	Mean # Revertant Colonies/Plate (n=2-4)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		38	160	16	52	8
DMSO		35	139	10	48	9
Candesartan cilexetil + impurities[a]	1.2ug					9
	2.4		130	12		9
	4.9		137	12		6
	9.8		132	16		7
	19.5		125	12		6
	39		114	8		7
	78		121	7		
	156	33			40	
	313	30			39	
	625	27			37	
	1250	44			42	
	2500	28			46	
	5000	27			31	
DAPA[b]	25 ug	494	744		1221	365
AF-2[a]	10 ng	134	419			
	40				287	
ENNG[b]	5 ug			2164		
AZI[b]	0.5 ug			452		
9AA[a]	80 ug					490

* Concentration for candesartan cilexetil + impurities is for the mixture.
[a] DMSO vehicle [b] Water vehicle

APPEARS THIS WAY
ON ORIGINAL

Table 61 Bacterial Mutagen Test (With Metabolic Activation)

Treatment	Concn/ # Plate	Mean # Revertant Colonies/Plate (n=2-4)				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water		41	145	14	56	14
DMSO		36	140	11	58	18
Candesartan cilexetil + impurities[a]	78 ug					22
	156	41	138	9	55	21
	313	37	144	18	50	19
	625	36	111	11	48	18
	1250	31	121	10	58	9
	2500	35	135	13	51	11
	5000	35	76	8	49	-
2AA[a]	1 ug		701		432	
	2			305		195
	10	1141				
BP[a]	5 ug		1015		320	149
DEN[b]	5 uL	404				
CPP[b]	200 ug			437		

* Concentration of candesartan cilexetil + impurities is for the mixture.

[a] DMSO vehicle [b] Water vehicle

Note: A replicate assay using the same concentrations of candesartan cilexetil mixture, positive and vehicle controls was conducted with and without metabolic activation. The results of the replicate assay were comparable to the above results and showed no positive mutagenic effects of the mixture of candesartan cilexetil and related impurities.

Mouse Lymphoma Cell Assay of Candesartan Cilexetil

Study Facility:
Study No: T3075
Study Dates: Assay initiation: 2/13/95; Assay termination: 5/04/95

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: L5178Y $TK^{+/-}$ mouse lymphoma cells

The test substance was considered to be mutagenic if (1) the mutant frequency in the vehicle control fell within the normal range but not more than 3 times the historical control mean value, (2) the mutant frequency at one or more doses was significantly greater than that of the negative control, (3) there was a significant dose-relationship as indicated by linear trend analysis and (4) the positive findings were reproducible in a repeat assay.

Results: In the cytotoxicity rangefinding study, relative cell survival at candesartan cilexetil concentrations ≥125 ug/ml was zero with or without S-9; at a concentration of 62.5 ug/ml, relative cell survival was zero without S-9 (~50% cell survival at 40 ug/ml) and 54% with S-9. In the mutation assay, candesartan cilexetil produced no significant increases in mutant frequencies at concentrations up to 50 ug/ml in the absence of S-9 and 75 ug/ml in the presence of S-9 (Table 62). Significant increases in mutant frequencies were observed with the positive controls. Thus, candesartan cilexetil was not mutagenic in this assay.

Table 62 . Mouse Lymphoma Assay of Candesartan Cilexetil

Treatment, ug/ml	S-9	% Relative Cell Survival		Mutant Frequency, #/10^6 cells	
		Exp.#1	Exp.#2	Exp #1	Exp. #2
Vehicle	-	100	100	157	207
Candesartan cilexetil					
1.56	-	84		141	
3.125	-	101		120	
6.25	-	99		145	
10.0	-	-	99	-	127
12.5	-	84.5	-	182	-
20	-	-	103	-	163
25	-	-	-	-	152
30	-	-	93	-	195
40	-	-	60	-	168
50	-	2	8	X[a]	167
4-Nitroquinoline-1-oxide					
0.05	-	88	104		
0.10	-	56	67		
Vehicle	+	100	100	149	153
Candesartan cilexetil					
6.25	+	86		128	
12.5	+	100		147	
25	+	107		127	
30	+	-	87	-	119
40	+	-	87	-	121
50	+	48	77	148	179
60	+	-	32	-	188
70	+	-	20	-	162
75	+	2	7	X	X
100	+	0.8	-	X	
Benzo(a)pyrene					
2.0	+	61	71	1018	1201
3.0	+	45	59	1869	1790

[a] X= Excluded from testing due to excessive cytotoxicity.

Mouse Lymphoma Assay of Active Metabolite, Candesartan

Study Facility:
Study No: TCJ1/TK
Study Dates: 9/03/92-10/07/92

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: L5178Y $TK^{+/-}$ mouse lymphoma cells

. The test substance was considered to be mutagenic if 1) the mutant frequency in the solvent control fell within the normal range but not more than 3 times the historical mean value, 2) the mutant frequency at one or more concentrations was significantly greater than the solvent control, 3) there was a significant dose-relationship as indicated by the linear trend analysis and 4) the positive findings were reproducible in a repeat assay.

Results: In the rangefinding test, cell survival at a candesartan concentration of 2500 ug/ml was 60% in the absence of metabolic activation and 70% in the presence of metabolic activation; higher doses produced a variable range (31%-85%) of cytotoxicity. No statistically significant increases in mutant frequency were observed following treatment with candesartan at concentration levels up to 5000 ug/ml in the absence or presence of metabolic activation (Table 63).

Table 63. Mouse Lymphoma Cell Assay of Candesartan

Treatment (ug/ml)	S-9	% Cell Survival		Mutant Frequency (#/10^6 cells)	
		Exp #1	Exp #2	Exp #1	Exp #2
Vehicle	-	100	100	247	210
Candesartan					
500	-	96	ND[b]	ND	ND
1000	-	90	95	248	197
2000	-	90	90	258	184
3000	-	79	105	228	198
4000	-	68	93	275	250
5000	-	19	72	374	299
NQO[a]					
0.05	-	90	81	633	1079
0.1	-	91	74	777	750
Vehicle	+	100	100	239	229
Candesartan					
500	+	70	ND	ND	ND
1000	+	62	94	244	182
2000	+	73	93	274	211
3000	+	57	104	210	240
4000	+	31	93	332	219
5000	+	15	56	ND/LS[c]	294
Benzo(a)pyrene					
2.0	+	64	70	1119	1295
3.0	+	44	59	1286	1260

[a] 4-nitroquinoline-1-oxide
[b] Not determined
[c] Not determined due to low cell survival

APPEARS THIS WAY
ON ORIGINAL

Mouse Lymphoma Assay of Candesartan Metabolite, M-II

Study Facility:
Study No: TCJ2/TK
Study Dates: 2/19/93-6/10/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: L5178Y $TK^{+/-}$ mouse lymphoma cells in culture

The test substance was considered to be mutagenic if 1) the mutant frequency in the solvent control fell within the normal range but not more than 3 times the historical mean value, 2) the mutant frequency at one or more concentrations was significantly greater than the solvent control, 3) there was a significant dose-relationship as indicated by the linear trend analysis and 4) the positive findings were reproducible in a repeat assay.

Results: In the rangefinding test, cell survival at M-II concentration of 2500 ug/ml was approximately 70% in the absence of metabolic activation and 50% in the presence of metabolic activation; at a concentration of 5000 ug/ml, relative cell survival was 11% and 18% with and without metabolic activation, respectively. No statistically significant increases in mutant frequency were observed following treatment with candesartan metabolite M-II at doses up to 5000 ug/ml in the absence and presence of metabolic activation (Table 64).

Table 64 Mouse Lymphoma Cell Assay of Metabolite M-II

Treatment (ug/ml)	S-9	% Cell Survival		Mutant Frequency (#/10^6 cells)	
		Exp.#1	Exp.#2	Exp.#1	Exp. #2
Vehicle	-	100	100	163	229
M-II Metabolite					
312	-	97	ND[b]	155	
500	-	ND	109		165
625	-	94	ND	153	
1000	-	ND	109		258
1250	-	76	ND	158	
2000	-	ND	71		161
2500	-	45	ND	216	
3000	-	ND	62		217
4000	-	ND	72		303
5000	-	2	ND	ND/LS[c]	
NQO[a]					
0.05	-	63	85	623	665
0.1	-	64	47	761	794
Vehicle	+	100	100	168	96
M-II Metabolite					
312	+	95	ND	156	
500	+	ND	88		ND
625	+	89	ND	123	
1000	+	ND	35		86
1250	+	79	ND	169	
2000	+	ND	58		95
2500	+	37	ND	165	
3000	+	ND	60		110
4000	+	ND	51		144
5000	+	12	33	ND/LS[c]	99
Benzo(a)pyrene					
2.0	+	107	60	517	312
3.0	+	79	61	800	488

[a] 4-nitroquinoline-1-oxide
[b] Not determined
[c] Not determined due to low cell survival

APPEARS THIS WAY
ON ORIGINAL

Chinese Hamster Lung In Vitro Cytogenetic Assay of Candesartan

Study Facility: Takeda Drug Safety Research Labs., Osaka, Japan
Study No.: 1344/GE
Study Dates: 3/31/92-11/15/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: Chinese hamster lung (CHL) cells from a newborn female.

A cell having one or more chromosomal aberrations was recorded as an aberrant cell. The frequency of aberrant cells in each dosage group was compared for statistically significant difference from vehicle control by the Fisher's exact probability test.

Results: Incubation of candesartan with CHL cells for 6 hours with or without metabolic activation caused no adverse effect on cell viability as indicated by the mitotic indexes (Table 65). Exposure of the cell cultures for 24 and 48 hours reduced mitotic activity relative to saline controls. With 48 hours of exposure to candesartan concentrations equal to or greater than 1.25 mM and with 24 hours of exposure to candesartan concentrations equal to or greater than 2.5 mM, concentration-dependent reductions in mitotic indexes (relative to control) were observed. Six hours of exposure to candesartan, with or without metabolic activation, resulted in no significant increase in aberration frequency at any of the tested concentrations (up to 10 mM). Significant increases in the frequency of cells with chromosomal aberrations were detected with 24 hours of exposure to candesartan concentrations of 2.5 mM and 5.0 mM and with 48 hours of exposure to candesartan concentrations of 1.25 mM and 2.5 mM (Table 66). These candesartan concentrations that produced increases in chromosomal aberration frequencies correspond to concentrations that produced decreases in mitotic activity. The sponsor considers these positive clastogenic effects as cytotoxicity mediated and not related to a direct clastogenic effect of the drug.

Table 65. Candesartan Effects on Mitotic Index

Treatment (mM)	S-9	Mitotic Index (% of Control)		
		6-Hr	24-Hr	48-Hr
Saline	-	100	100	100
	+	100		
Candesartan				
0.625	-	99	111	103
	+	108		
1.25	-	87	120	79
	+	105		
2.5	-	97	89	48
	+	108		
5.0	-	97	43	23
	+	104		
10	-	117	17	12
	+	106		

APPEARS THIS WAY
ON ORIGINAL

Table 66. CHL In Vitro Cytogenetic Assay of Candesartan

Treatment (mM)	Exposure Period (hr)	S-9	Chromosomal Aberration Frequency (% Cells)[a]
Saline	6	-	0.5
	6	+	1.0
Candesartan			
2.5	6	-	0.5
5.0	6	-	0.5
10.0	6	-	1.5
2.5	6	+	0.0
5.0	6	+	1.5
10.0	6	+	0.5
Cyclophosphamide			
3.6×10^{-2}	6	-	0.0
	6	+	22.0*
Saline	24	-	0.0
	48	-	0.5
Candesartan[b]			
1.25	24	-	2.0
2.5	24	-	2.5*
5.0	24	-	7.5*
0.625	48	-	3.0
1.25	48	-	13.5*
2.5	48	-	25.5*
Mitomycin C			
7.5×10^{-3}	24	-	43.0*
3.75×10^{-3}	48	-	22.5*

[a] Based on 200 cells/dose group
[b] Due to to severe reduction in mitotic activity, concentrations >5.0 mM with 24-Hr exposure and >2.5 mM with 48-exposure were not tested.
* Significantly greater than concurrent saline control ($p<0.05$)

Chinese Hamster Lung In Vitro Cytogenetic Assay of Candesartan Metabolite, M-II

Study Facility: Takeda Drug Safety Research Labs., Osaka, Japan
Study No.: 1582/GE
Study Dates: 5/10/93-2/23/94

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: Chinese hamster lung (CHL) cells from newborn female.

A cell having one or more chromosomal aberration was recorded as an aberrant cell. The frequency of aberrant cells in each dosage group was compared for statistically significant difference from vehicle control by the Fisher's exact probability test.

Results: The effects of candesartan metabolite, M-II, on mitotic activity are shown in Table 67.

Table 67. Candesartan Metabolite, M-II, Effects on Mitotic Index

Treatment (mM)	S-9	Mitotic Index (% of Control)		
		6-Hr	24-Hr	48-Hr
Saline	-	100	100	100
	+	100		
M-II Metabolite				
0.375	-			98
	+			
0.625	-		108	
	+			
0.75	-			65
	+			
1.25	-	93	88	
	+	95		
1.5	-			69
	+			
2.5	-	82	66	
	+	94		
3.0	-			25
	+			
5.0	-	92	32	
	+	101		
10	-	115		
	+	78		

Incubation of M-II metabolite with CHL cells for 6 hours at concentration up to 5 mM with or without metabolic activation caused no adverse effect on cell viability as indicated by the mitotic indexes. With 6 hours of exposure, and only in the presence of metabolic activation, mitotic activity was reduced at an M-II concentration of 10 mM. With 48 hours exposure to M-II concentrations equal to or greater than 0.75 mM, and with 24 hours exposure to concentrations equal to or greater than 1.25 mM, concentration-dependent reductions in mitotic indexes (relative to control) were observed.

Table 68. CHL In Vitro Cytogenetic Assay of Candesartan Metabolite, M-II

Treatment (mM)	Exposure Period (hr)	S-9	Chromosomal Aberration Frequency (% Cells)[a]
Saline	6	-	0.5
	6	+	1.0
M-II Metabolite			
2.5	6	-	0.5
5.0	6	-	1.0
10.0	6	-	1.5
2.5	6	+	0.5
5.0	6	+	0.5
10.0	6	+	1.0
Cyclophosphamide			
3.6×10^{-2}	6	-	0.5
	6	+	24.0*
Saline	24	-	0.5
	48	-	0.5
M-II Metabolite[b]			
1.25	24	-	1.5
2.5	24	-	2.5
5.0	24	-	10.0*
0.375	48	-	1.0
0.75	48	-	5.0*
1.5	48	-	4.5*
3.0	48	-	28.5*
Mitomycin C			
7.5×10^{-3}	24	-	35.0*
3.75×10^{-3}	48	-	29.0*

[a] Based on 200 cells/dose group

[b] Due to to severe reduction in mitotic activity, concentrations >5.0 mM with 24-Hr exposure and >3.0 mM with 48-exposure were not tested.

* Significantly greater than concurrent saline control ($p<0.05$)

Six hours exposure to the M-II metabolite caused no significant increase in chromosomal aberration frequency at any concentration tested, with or without metabolic activation (Table 68). Significant increases in the frequency of cells with chromosomal aberrations were detected with 24 hours exposure to a concentration of 5.0 mM and with 48 hours exposure to concentrations of 0.75, 1.5 and 3.0 mM. These M-II metabolite concentrations that produced increases in chromosomal aberration frequencies correspond to concentrations that produced decreases in mitotic activity. The sponsor considers these positive clastogenic effects as cytotoxicity mediated and not related to a direct clastogenic effect of the drug.

Mouse Micronucleus Assay of Candesartan Cilexetil

Study Facility: Takeda Drug Safety Research Labs., Osaka, Japan
Study No: 1087/GE
Study Dates: 1/31/91-3/17/91

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male C3HxSWV F_1 mice (7 wks old, 32-36 gm at initiation of dosing)

Drug Administration: Candesartan cilexetil (Lot # M464-006) was suspended in 5% gum arabic aqueous solution and administered to the mice orally by gavage.

Dose Levels: 0 (vehicle), 500, 1000 and 2000 mg/kg/day (5 mice/group) for 2 consecutive days.

Observations/Measurements: Mice were killed 24 hours after the last treatment, one femur was removed from each animal and bone marrow cells extracted. Suspensions of bone marrow cells were smeared on slides, fixed and examined for the presence of micronuclei in polychromatic erythrocytes. The frequency of micronucleated cells in each candesartan cilexetil treated group was compared with that in the vehicle control group for statistically significant differences. Mitomycin C, given as a single dose of 2 mg/kg IP, was used as the positive control.

Results: Candesartan cilexetil caused no significant increase in the frequency of micronucleated polychromatic erythrocytes at any dose level when compared to vehicle control (Table 69).

Table 69. Micronucleus Assay of Candesartan Cilexetil

Treatment	Dose (mg/kg)	% Micronucleated Polychromatic Erythrocytes
Vehicle Control	-	0.19
Candesartan Cilexetil	500 1000 2000	0.16 0.20 0.17
Mitomycin-C	2.0	3.98*

* Significantly higher than vehicle control

Mouse Micronucleus Assay of Candesartan

Study Facility: Takeda Drug Safety Research Labs., Osaka, Japan
Study No: 1476/GE
Study Dates: 9/16/92-11/15/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male and female ICR mice (9 weeks old; M=32-40 gm, F=28-36 gm at initiation of dosing).

Drug Administration: Candesartan (Lot # A07807-03522) was prepared as a solution in physiologic saline (1N NaOH used for solubilization) and administered to the mice intravenously.

Dose Levels: 0 (vehicle), 187.5, 375 and 750 mg/kg/day (5/sex/group) for 2 consecutive days.

Note: Highest dose used is more than half the LD_{50} as determined from preliminary experiment (6/10 deaths with single dose of 1000 mg/kg).

Observations/Measurements: Mice were killed 24 or 48 hours after the last treatment, one femur was removed from each animal and bone marrow cells extracted. Suspensions of bone marrow cells were smeared on slides, fixed and examined for the presence of micronuclei in polychromatic erythrocytes. The frequency of micronucleated cells in each candesartan-treated group was compared with that in the vehicle control group for statistically significant differences. Mitomycin C, given as a single dose of 2 mg/kg IP, was used a the positive control.

Results: Candesartan caused no significant increase in the frequency of micronucleated polychromatic erythrocytes when compared to vehicle control at any dose except at the lowest dose in females at 48 hours post-treatment (Table 70). This singular positive effect was not seen at higher doses and is not likely to be related to candesartan treatment.

Table 70. Micronucleus Assay of Candesartan

Treatment	IV Dose (mg/kg)	Post-Dose Time (hr)	% Micronucleated Polychromatic Erythrocytes[a]	
			Males	Females
Saline	-	24	0.12	0.20
		48	0.21	0.16
Candesartan	187.5	24	0.21	0.18
	375	24	0.13	0.21
	750	24	0.14	0.14
	187.5	48	0.25	0.64*
	375	48	0.13	0.14
	750	48	0.15	0.11
Mitomycin-C	2.0 IP	24	6.42*	7.42*

[a] Based on 2000 polychromatic erythrocytes/mouse
* Significantly higher than saline control

In Vivo/In Vitro Unscheduled DNA Synthesis Assay of Candesartan Cilexetil

Study Facility:
Study No.: 15206-0-494
Study Dates: 8/11/92-10/02/92

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Animals: Male Fisher 344 rats (190-229 gm at initiation of dosing).

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

Dose Levels: 0 (vehicle), 300, 600, 1000 and 3000 mg/kg (3 rats/dose/exposure period)

Observations/Measurements: This assay is designed to measure unscheduled DNA synthesis (UDS) in rat liver cells using an autoradiographic technique.

The test compound was considered to be active in the UDS assay if a dose of the drug produced 1) an increase in the mean net nuclear count of at least 5 grains/nucleus above concurrent vehicle control and 2) an increase in the percent of nuclei with 5 or more grains by at least 10% above concurrent vehicle control.

Results: Candesartan cilexetil, administered by oral gavage, was unassociated with significant effects on the nuclear labeling of rat primary hepatocytes 2-hr or 12-hr after dosing (Table 71). Thus, candesartan cilexetil was inactive in this assay.

Table 71. Rat Hepatocyte UDS Assay of Candesartan Cilexetil

Treatment (mg/kg)	Mean Net Nuclear Grains (#)		% Cells with ≥5 NNG[a]	
	2-Hr	12-Hr	2-Hr	12-Hr
Vehicle	0.16	0.26	1.78	5.33
Candesartan cilexetil				
300	- 0.58	-0.41	1.33	2.22
600	-0.47	-0.13	2.43	6.89
1000	-0.45	0.04	0.89	4.44
3000	-0.19	0.44	1.56	3.11
Dimethylnitrosamine				
10	21.5	16.1	96.4	89.8

[a] NNG= Net nuclear grains

Chinese Hamster Ovary Gene Mutation Assay of Candesartan

Study Facility: Takeda Drug Safety Research Labs., Osaka, Japan
Study No: 1393/GE
Study Dates: 5/25/92-3/30/93

GLP Compliance: Statement indicates that this study was conducted in compliance with GLP regulations.

Cell Culture: Chinese hamster ovary K_1 (CHO) cells in monolayer culture.

The mutation frequency in each candesartan-treated group was compared for statistically significant differences from vehicle control.

Results: Candesartan produced no significant increases in the mean number of mutant colonies up to the highest tested concentration of 5 mg/ml, either in the absence or presence of metabolic activation (Table 72).

Table 72. CHO Gene Mutation Assay of Candesartan

Treatment (mg/ml)	S-9	Mean # Mutant Colonies		Relative Cell Survival (%)	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Saline Vehicle	-	9.0	5.0	77	85
	+	7.5	2.0	80	88
Candesartan					
0.3125	-	9.5	3.0	88	82
0.625	-	4.0	2.0	77	79
1.25	-	2.0	2.0	77	73
2.5	-	4.5	2.5	81	78
5.0	-	4.5	5.0	64	49
0.3125	+	13	2.5	76	84
0.625	+	8.5	3.5	81	71
1.25	+	2.5	5.5	74	66
2.5	+	4.0	0.5	76	75
5.0	+	5.5	2.5	74	75
EMS[a] 0.4	-	458	543	64	64
3-MC[b] 0.002	+	848	951	85	64

[a] Ethyl methanesulfonate [b] 3-Methylcholanthrene

CARCINOGENICITY

The Rat Studies

13-Week Dietary Rangefinding Study in Rats

Study Facility: Takeda Chemical Industries, Ltd., Osaka, Japan
Study No.: T2959
Study Dates: Initiation of dosing: 5/29/91; End of dosing 8/29/91

GLP Compliance: GLP compliance statement included.

Animals: Male and female F344/Jcl rats (5 weeks old;M=83-97gm, F=76-89gm at initiation of dosing)

Drug Administration: Candesartan cilexetil (Lot #M464-010) was administered to the animals in the diet; the amount of the test compound that was added to the diet was determined from the previous weekly body weight and food consumption data for each group and sex.

Dose Levels: 0, 300, 1000 and 3000 mg/kg/day (10/sex/dose group; additional 4/sex/dose group used for toxicokinetic evaluation).

Note: The high dose was set at 3000 mg/kg/day, corresponding to 5% of the diet.

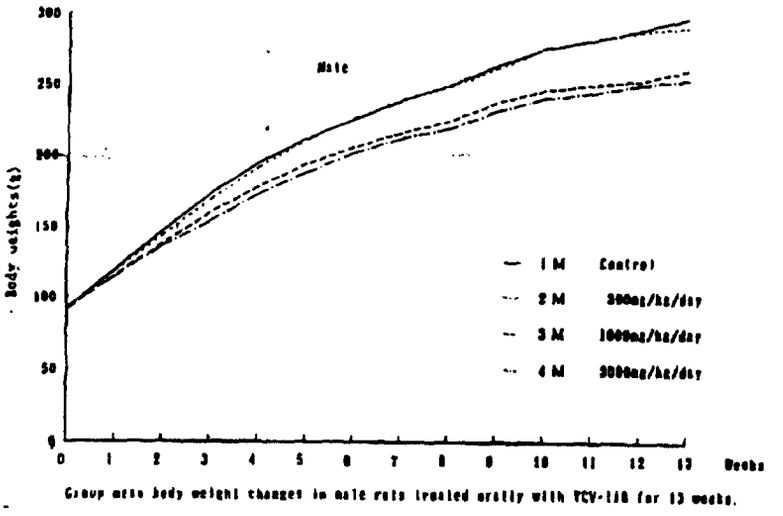
Observations/Measurements: Animals were observed daily for mortality and clinical signs of toxicity. Body weights were measured prior to dosing, twice weekly for 4 weeks, then weekly thereafter. Food consumption was measured weekly; drug intake was calculated from the food consumption data. Ophthalmoscopic examination was performed for 5 rats/sex in each group prior to dosing and on day 86 of the dosing period. Four-hour (9a.m.-1p.m.) urine samples were collected on day 89 of the dosing period for urinalysis. Arterial blood was obtained from all animals under ether anesthesia at the end of the dosing period for hematology and blood chemistry. At the end of the dosing period the animals were exsanguinated and examined for gross pathology. Sections of major organs and tissues (listed in Appendix A, pg. 7) were obtained for all animals; only those sections from control and high dose groups and the sections of liver and kidneys for all dose groups were examined microscopically. Blood samples were obtained from animals in the toxicokinetic satellite groups via the external jugular vein under ether anesthesia on day 7 (8 a.m., 2 p.m. and at 8 p.m.), and weeks 6 and 13 (8 p.m.) for measurement of plasma level of candesartan (M-I metabolite).

Results

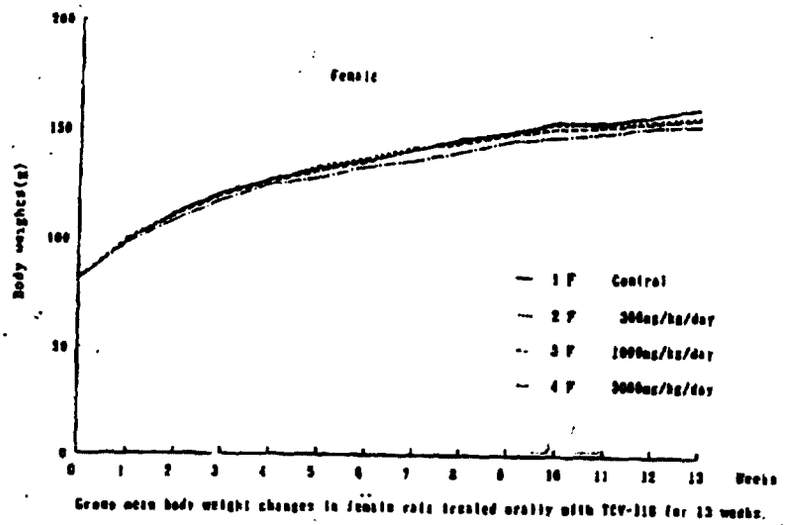
Mortality and Clinical Signs: No animals died during the study. No treatment-related clinical signs of toxicity were observed.

Body Weight: Significantly lower than control body weights were observed for males treated with 1000 and 3000 mg candesartan cilexetil/kg/day from week 2 to week 13 of dosing (Fig. 12, Table 73). Slightly lower than control body weights were observed for females in the 3000 mg/kg dose group; however, the latter difference was not statistically significant. The gains from initial body weights to those at week 13 were 3%, 18% and 21% lower than the control value for males and 4%, 8% and 10% lower for females in the 300, 1000 and 3000 mg/kg/day dose groups (statistical significance for differences in body weight gain not determined).

Figure 12. Body Weight Curves



Group mean body weight changes in male rats treated orally with TCV-116 for 13 weeks.



Group mean body weight changes in female rats treated orally with TCV-116 for 13 weeks.

Table 73. Body Weight, gm

Dose Group (mg/kg/day)	Sex	Day 0	Week 2	Week 7	Week 13
0	M	91	147	230	297
300		91	144	239	291
1000		90	138*	216*	261*
3000		92	136*	212*	254*
0	F	81	112	141	161
300		82	112	142	157
1000		82	111	141	156
3000		81	109	136	153

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

Food Consumption and Drug Intake: A statistically significant reduction from control food consumption was observed for males in each treated group from week 1 through week 6; thereafter, statistically significant reductions were noted sporadically in the 1000 mg/kg group. No treatment-related effects on food consumption were noted for female rats (Table 74).

Table 74. Food Consumption, gm/rat/wk

Dose Group (mg/kg/day)	Sex	Treatment Period			
		Week 1	Week 4	Week 7	Week 13
0	M	90	108	112	113
300		84*	108	113	115
1000		84*	98*	105	102*
3000		82*	97*	107	110
0	F	76	79	80	81
300		76	79	79	79
1000		75	77	77	78
3000		76	79	79	83

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

Drug intake during week 1 was below the target value because of unexpected decreases in food consumption; however, drug intake was generally within acceptable levels (±10%) from week 2 to the end of the dosing period (Table 75).

Table 75. Drug Intake, mg/kg/day

Dose Group (mg/kg/day)	Sex	Treatment Period			
		Week 1	Week 4	Week 7	Week 13
300	M	246	351	296	300
1000		834	1115	1004	1005
3000		2470	3366	3198	3052
300	F	262	311	282	296
1000		855	1029	941	1029
3000		2600	3230	2841	3034

Ophthalmology: No treatment-related ophthalmologic effects were observed.

Urinalysis: Urine values (pH, protein, ketones, urobilinogen and incidence of occult blood, erythrocytes, leukocytes and epithelial cells) in treated animals were comparable to those of control.

Hematology and Clinical Chemistry: Lower than control values in erythrocyte counts, hemoglobin concentration and hematocrit were observed in females receiving 300 mg candesartan cilexetil/kg/day and in both sexes receiving 1000 and 3000 mg candesartan cilexetil/kg/day (Table 76).

Table 76. Hematology Values

Hematology Parameter	Dose Group (mg/kg/day)							
	0 (Control)		300		1000		3000	
	M	F	M	F	M	F	M	F
RBC counts, x 10^6	9.85	9.66	9.71	9.02*	9.15*	8.97*	8.91*	8.75*
Hemoglobin, gm/dl	15.1	15.7	14.0	14.9*	14.2*	14.9*	14.0*	14.5*
Hematocrit, %	42.5	44.6	42.2	42.0*	40.4*	41.8*	39.6*	40.8*

* Statistically significant difference from concurrent control value ($p<0.05$)

Plasma urea nitrogen was increased in each treated group except for females in the 300 mg/kg/day group. Plasma LDH, AST and ALT values in females given 1000 and 3000 mg candesartan cilexetil/kg/day were higher than control (Table 77). In males, higher than control levels of inorganic phosphorus were noted in all candesartan cilexetil- treated groups, and higher than control levels of cholesterol were noted the 3000 mg/kg/day group.

Table XX. Blood Chemistry Values

Blood Chemistry Parameter	Dose Group (mg/kg/day)							
	0 (Control)		300		1000		3000	
	M	F	M	F	M	F	M	F
Urea Nitrogen, mg%	16.9	17.8	27.9*	21.9	34.7*	24.4*	37.5*	25.3*
LDH, U/l	103	65	100	100	78	166*	97	130*
AST, U/l	105	87	105	119	90	179*	104	154*
ALT, U/l	51	44	49	61	40	90*	45	75*
Inorganic Phosphorus, mg%	6.2	5.0	6.7*	5.2	6.8*	5.3	6.8*	5.2
Total Cholesterol, mg%	49	64	53	67	53	66	57*	59

* Statistically significant difference from concurrent control value ($p<0.05$).

Gross Pathology and Organ Weights: Macroscopic examination showed no candesartan cilexetil-related pathology. Lower than control heart weight and higher than control relative kidney weights were noted in each treated group for both sexes (Table 78).