Drug Administration: Candesartan cilexetil (Lot # M464-043) and hydrochlorothiazide (Lot # M373-001) were suspended in 5% gum arabic solution and administered orally by gavage. Control animals received 5% gum arabic solution.

Dose Levels:

Treatment Group	#/sex/group	Oral Dose (mg/kg)
Vehicle (5% gum arabic soln.)	3	3ml/kg
Candesartan cilexetil	3	4
HCTZ	3	30
Candesartan cilexetil/HCTZ	3	4/30

Observations/Measurements: Animals were observed for mortality and clinical signs of toxicity 3 times daily during the dosing period. Body weights were measured prior to dosing, on the 1st day of dosing and then weekly thereafter. Food consumption was measured daily. Water intake was measured for a 24-hour period prior to dosing and then once a week during the dosing period. Urinalysis was performed on urine collected for 22 hours pretest and in months 1, 2 and 3 of the dosing period. Ophthalmoscopic examinations were performed on all dogs pretest and in month 3 of the dosing period. Electrocardiograms were recorded on all animals 2 weeks prior to dosing and in months 1 and 3 of the dosing period. Blood was obtained from the cephalic vein of each animal pretest and in months 1, 2 and 3 of the dosing period for hematology and blood chemistry analyses. Venous blood samples were also obtained at 1, 2, 4, 8 and 24 hours after the 1st, 28th and 91st dose (all groups except the control group) for measurement of candesartan and for HCTZ. Dogs were sacrificed the day after the last dose and examined for external and visceral pathology. Major organs were weighed. Sections of major organs and tissues (Appendix A) were fixed on slides and examined for microscopic pathology.

Results

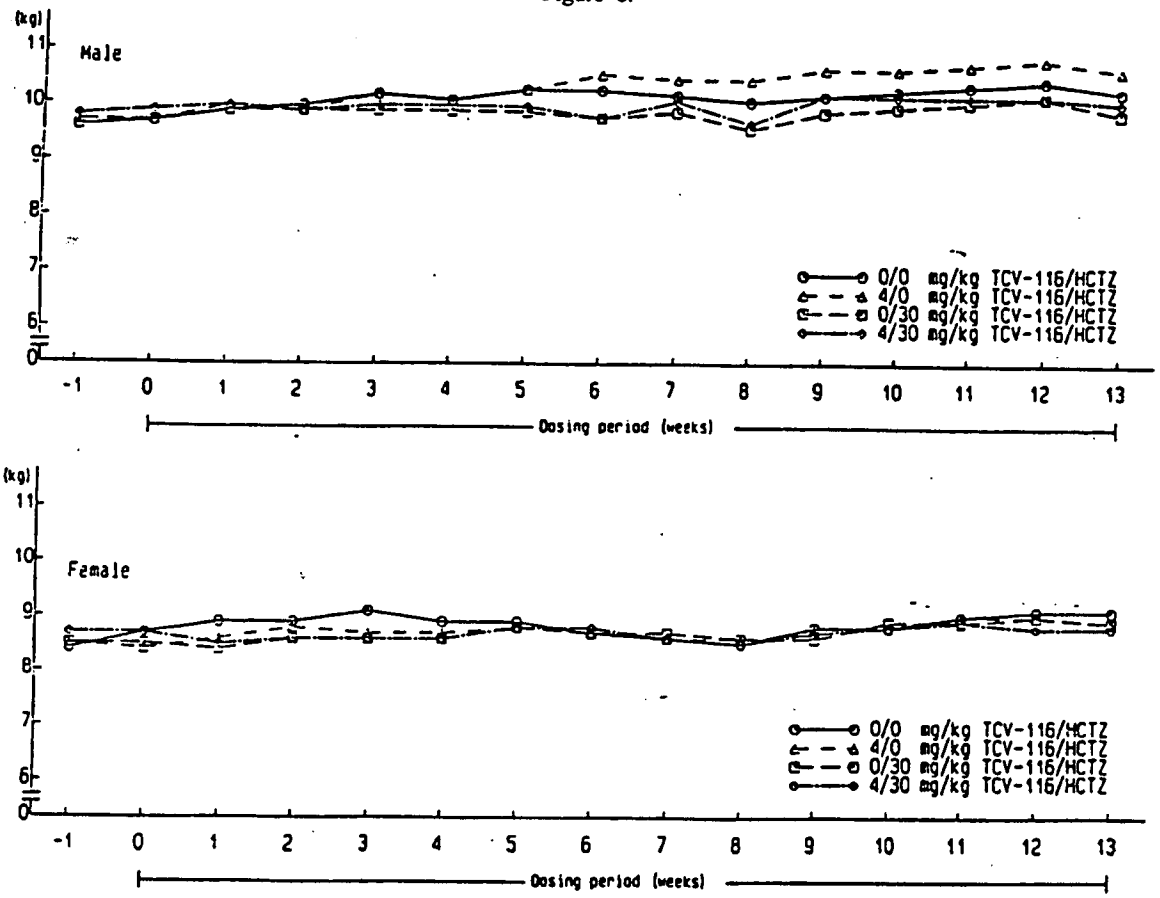
Mortality and Clinical Signs

No animals died during the study. No clinical signs of toxicity were observed in any of the groups throughout the study.

Body Weight

No treatment-related effects on mean body weights of males or females were observed (Fig. 6).

Figure 6.



Body weight changes of beagle dogs treated orally with TCV-116/Hydrochlorothiazide for 13 weeks

Food Consumption

Food consumption among treated males and females was comparable to control.

Water Intake and Urinalysis

Higher than control water intake during the latter half of the study was noted in candesartan cilexetil, HCTZ and candesartan cilexetil/HCTZ treated male groups; water intake by treated females was comparable to control

Although urinalysis parameters among treated groups did not differ significantly from control, slightly higher than control urine volume was noted in males given candesartan cilexetil, HCTZ or candesartan cilexetil/HCTZ and in females given candesartan cilexetil or candesartan cilexetil/HCTZ. Slight increases in sodium, potassium and chloride excretion among treated groups were also seen (Table 46).

Table 46. Urinalysis Results (Treatment month 3).

Parameter	Sex	Treatment Group, mg/kg			
		Control, 0	C. cilexetil, 4	Hctz, 30	C. cilexetil/Hctz, 4/30
Urine Volume, ml/22hr	M	133	210	243	210
	F	193	270	193	257
Osmolarity, mOs/kg	M	1607	1272	988	968
	F	1345	1438	1146	1050
Sodium, mEq/22hr	M	19.0	28.2	23.6	15.9
	F	26.4	26.1	22.2	23.6
Potassium, mEq/22hr	M	32.7	32.3	28.7	24.8
	F	23.2	38.9	28.0	30.0
Chloride, mEq/22hr	M	26.0	29.8	33.5	26.4
	F	28.7	32.7	27.3	33.4

Hematology

Lower than control hemoglobin concentration was noted among females treated with candesartan cilexetil or candesartan cilexetil/HCTZ and slightly lower than control hematocrit was seen in females given candesartan cilexetil/HCTZ (Table 47).

Table 47. Hematology Results (Treatment month 3).

Parameter	Sex	Treatment Group, mg/kg			
		Control, 0	C. cilexetil, 4	Hctz, 30	C. cilexetil/Hctz, 4/30
RBC counts, $x10^6/mm^3$	M	687	687	699	693
	F	733	683	705	661
Hemoglobin, g/dl	M	16.2	16.2	16.9	15.9
	F	18.0	16.3*	17.1	15.8*
Hematocrit, %	M	46	46	47	45
	F	50	46	48	44*

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

Blood Chemistry

Significantly lower than control levels of serum potassium were noted in month 3 of treatment in males and females treated with HCTZ or candesartan cilexetil/HCTZ Lower than control levels of serum calcium and chloride were seen sporadically during months 1 and 2 of treatment in males and/or females receiving HCTZ or candesartan/HCTZ and these effects were considered to be related to the diuretic effect of HCTZ and toxicologically insignificant.

Ophthalmoscopy

No ocular abnormalities were observed in any of the animals throughout the study.

Electrocardiography

No electrocardiographic changes from predose levels in treated or control animals were observed.

Organ Weights

Mean absolute and relative organ weights among treated groups were comparable to control.

Macroscopic Pathology

No treatment-related gross lesions were detected.

Microscopic Pathology

Histopathologic examination revealed mild hypertrophy of the J-G cell of the kidneys in all of the animals treated with candesartan cilexetil or candesartan cilexetil/HCTZ. No other treatment related histopathology was observed.

Toxicokinetics

Cmax and AUC values of HCTZ were not affected by combination treatment, sex or duration of dosing. Cmax and AUC values of candesartan in the candesartan cilexetil/HCTZ group were slightly higher than those in the candesartan cilexetil group; however, this difference was resulted from large values contributed by one male dog (Table 48-49).

Table 48. Toxicokinetics of Candesartan After Oral Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ to Dogs.

Treatment Group mg/kg	Dosing Day	Males (n=3)			Females (n=3)		
		Tmax (hr)	Cmax (ng/ml)	AUC_{0-24} (ng.hr/ml)	Tmax (hr)	Cmax (ng/ml)	AUC_{0-24} (ng.hr/ml)
C. Cilexetil 4	1	2	56.7	462	1	74.7	395
	28	1	49.2	375	0.5	38.9	401
	91	2	53.6	360	1	64.2	329
C. Cilexetil/HCTZ 4/30	1	2	169.4	1174	2	115.7	756
	28	1	83.2	818	1	54.8	370
	91	2	187.1	1877	2	86.1	624

Table 49. Toxicokinetics of HCTZ After Oral Administration of HCTZ or Candesartan Cilexetil/HCTZ to Dogs.

Dose Group mg/kg	Dosing Day	Males (n=3)			Females (n=3)		
		Tmax (hr)	Cmax (µg/ml)	AUC_{0-24} (µg.hr/ml)	Tmax (hr)	Cmax (µg/ml)	AUC_{0-24} (µg.hr/ml)
HCTZ 30	1	2	4.48	30.8	2	5.32	31.0
	28	2	5.36	33.4	2	7.05	38.9
	91	2	4.69	30.6	2	6.83	37.1
C.Cilexetil/HCTZ 4/30	1	4	4.65	35.2	2	5.84	38.8
	28	2	3.91	29.0	2	5.05	30.1
	91	2	8.37	46.0	2	3.65	16.8

REPRODUCTIVE TOXICOLOGY

Embryo-Fetal Developmental Toxicity Study in Mice (Vol. 16, pg 10)

Study Facility:
Study No: 1991/TE
Study Dates: 2/24/95-6/26/95

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Animals: Presumed pregnant Jcl:ICR mice (28.0-39.2 gm at time of mating). The animals were housed individually and fed *(ad libitum)* a solid diet (CE-2,

Drug Administration: Candesartan cilexetil (Lot # M464-032) and HCTZ (Lot # M373-001) were suspended in aqueous 5% gum arabic suspension and administered orally by gavage from day 6 to day 15 of gestation.

Dose Levels: The experimental groups were as follows:

Treatment Group (Main Study)	Dose Level, mg/kg Candesartan cilexetil/HCTZ	# Pregnant Mice
Control (vehicle)	0/0	19
HCTZ	0/10	20
Candesartan cilexetil/HCTZ	10/10	20
Candesartan cilexetil/HCTZ	100/10	21
Candesartan cilexetil/HCTZ	1000/10	21
(PK Satellite Study)		
HCTZ	0/10	39
Candesartan cilexetil/HCTZ	10/10	39
Candesartan cilexetil/HCTZ	100/10	39
Candesartan cilexetil/HCTZ	1000/10	39

Observations/Measurements: All dams were observed daily for mortality and clinical signs of toxicity. Body weights of dams were measured on days 0, 6, 8, 10, 12, 14, 16 and 18 of gestation. Food consumption by each dam was determined on days 0, 6, 8, 10, 12, 14 and 16 of gestation. On day 18 of gestation, all dams from the main study were killed by cervical dislocation and the uterus, ovaries and other internal organs examined macroscopically. The numbers of corpora lutea, implants, resorptions, placental remnants, dead fetuses and live fetuses were recorded. The sex ratio (number of males X 100/number of males and females) was determined. The live fetuses were examined for external abnormalities. Visceral examinations were conducted on approximately one-half of the live fetuses from the control, HCTZ and high-dose (1000/10 mg/kg) candesartan cilexetil/HCTZ groups; skeletal examinations were performed on the remaining fetuses from these groups. Venous blood samples were obtained from the vena cava of satellite animals at 0.25, 0.5 1, 2, 6 and 24 hours after dosing on days 6 and 15 of gestation (3 dams/sampling time/group) for measurement of plasma concentrations of HCTZ candesartan.

Results

Survival and Clinical Signs

No dams died during treatment and no clinical signs of toxicity were observed.

Body Weight

Body weights among candesartan cilexetil/HCTZ and HCTZ treated groups were comparable to those of vehicle control (Table 50).

Table 50. Maternal Mean Body Weight (gm).

Treatment Group	Dose mg/kg	Gestation Day			
		GD 0	GD 6	GD 12	GD 18
Vehicle Control	0	34.7	36.2	43.4	63.4
HCTZ	10	33.9	35.1	42.6	62.6
Candesartan cilexetil/HCTZ	10/10	34.2	35.3	42.2	63.3
	100/10	34.5	36.0	42.1	62.8
	1000/10	34.6	36.0	41.6	63.2

Food Consumption

Food consumption among treated groups was comparable to that of control.

Cesarean Section Results

There were no significant differences in the numbers of corpora lutea, implants, or live fetuses, and no significant differences in fetal body weight or sex ratio between the control group and any other group (Table 51).

Table 51. Cesarean Section Results.

Parameter	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	100/10	1000/10
Corpora Lutea, mean #	16.2	15.6	15.4	15.6	15.7
Implants, mean #	14.4	14.3	14.7	14.4	14.5
Pre-implantation loss, %	10.0	8.0	4.5	7.9	8.5
Post-implantation loss, %	6.7	7.0	6.2	7.8	6.6
Live Fetuses, mean #	13.4	13.3	13.8	13.3	13.7
Sex Ratio, M/M+F (%)	54.8	50.2	53.2	45.9	49.7
Fetal Weight, mean gm					
Male	1.53	1.49	1.50	1.52	1.49
Female	1.48	1.43	1.43	1.47	1.42

External, Visceral and Skeletal Examination of Fetuses

In the external examination of live fetuses, open eyelids, cleft palate and accessory digits (forelimb) were seen sporadically in all groups including the control group. No significant differences in the frequencies of external abnormalities between treated and control groups were detected (Table 52).

Table 52. External Examination Results[a]

Observation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	100/10	1000/10
# Fetuses Examined (# litters)	254 (19)	266 (20)	275 (20)	279 (21)	301 (22)
Abnormalities (overall), mean %	1.1	1.0	2.5	2.7	5.1
Open eyelids, mean %	0.4	0.0	1.1	2.3	1.7
Cleft palate, mean %	0.7	0.3	1.4	0.0	1.5
Accessory digit (forelimb), mean %	0.0	0.4	0.0	0.0	1.9

[a] Individual types of abnormalities with infrequent occurrences not included in the list. Mean % values are for fetuses.

In the visceral examination, there were no significant differences in overall or individual abnormalities between treated and control groups. The most frequent visceral variation (seen in all groups) was left umbilical artery (Table 53).

Table 53 Visceral Examination Results[a]

Obse.vation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	100/10	1000/10
# Fetuses Examined (# litters)	138 (19)	140 (20)	149 (20)	148 (21)	164 (22)
Abnormalities (overall), mean %	0.0	0.6	0.0	0.0	0.0
Ventricular septal defect, mean %	0.0	0.6	0.0	0.0	0.0
Variations (overall), mean %	12.4	20.3	13.2	19.3	24.5
Left umbilical artery, mean %	12.4	20.3	13.2	19.3	24.5

[a] Mean % values are for fetuses.

In the skeletal examination, some kinds of malformations or variations were observed at low frequencies in all groups, but there were no significant differences in the frequencies of skeletal abnormalities or variations between treated and control groups (Table 54).

Table 54 Skeletal Examination Results[a]

Observation	Treatment Group, mg/kg		
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ 1000/10
# Fetuses Examined (# litters)	116 (19)	126 (20)	137 (22)
Abnormalities (overall), mean %	0.0	3.0	3.1
Decreased # of lumbar vertebrae, %	0.0	3.0	3.1
Variations (overall), mean %	36.2	41.0	23.7
Bifurcation of cervical vert., %	5.3	12.5	4.5
Cervical rib, %	23.0	22.1	10.8
Lumbar rib, %	9.6	14.0	5.2
Accessory sternebrae, %	1.1	3.2	0.0

[a] Individual types of abnormalities with infrequent occurrences not included in the list. Mean % values are for fetuses.

Toxicokinetics

Plasma concentrations and AUCs of candesartan on day 6 and day 15 of gestation increased dose-dependently after administration of candesartan cilexetil (Table 55). There were no differences in the Cmax and AUC of HCTZ when given in combination with candesartan cilexetil (Table 56).

Table 55. Pharmacokinetics for Candesartan After Oral Candesartan Cilexetil/HCTZ to Mice

Candesartan cilexetil Dose, mg/kg/day	Dosing Day	Tmax (hr)	Cmax (ug/ml)	AUC_{0-24} (ug.hr/ml)
10	GD 6	1.0	1.89	8.9
	GD 15	1.0	1.52	6.4
100	GD 6	1.0	15.7	65.6
	GD 15	0.5	8.32	32.9
1000	GD 6	0.5	31.2	161.7
	GD 15	0.25	27.5	222.8

Table 56. Pharmacokinetics for HCTZ After Oral Candesartan Cilexetil/HCTZ to Mice

Candesartan cilexetil/HCTZ Dose, mg/kg/day	Dosing Day	Tmax (hr)	Cmax (ug/ml)	AUC_{0-24} (ug.hr/ml)
0/10	GD 6	1.0	1.35	6.4
	GD 15	1.0	1.84	6.6
10/10	GD 6	1.0	1.87	7.5
	GD 15	1.0	1.65	9.2
100/10	GD 6	1.0	1.72	8.9
	GD 15	0.5	1.93	8.1
1000/10	GD 6	0.5	1.96	10.0
	GD 15	2.0	2.57	12.4

Embryo-Fetal Developmental Toxicity Study in Rats (Vol. 16, pg 112)

Study Facility:
Study No: 1762TE
Study Dates: Study initiation, 3/07/94: Study termination, not stated.

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Animals: Presumed pregnant Jcl:Wistar rats (209-249 gm at time of mating). The animals were housed individually and fed (*ad libitum)* a solid diet (CE-2,).

Drug Administration: Candesartan cilexetil (Lot # M464-035) and HCTZ (Lot # 754648) were suspended in aqueous 5% gum arabic suspension and administered orally by gavage from day 6 to day 20 of gestation. The day on which a copulation plug was found was considered day 0 of gestation.

Dose Levels: The experimental groups were as follows:

Treatment Group	Dose Level, mg/kg Candesartan cilexetil/HCTZ	# Pregnant Rats
Control (vehicle)	0/0	20
HCTZ	0/10	20
Candesartan cilexetil/HCTZ	10/10	19
Candesartan cilexetil/HCTZ	30/10	20
Candesartan cilexetil/HCTZ	100/10	18

Observations/Measurements: All dams were observed daily for mortality and clinical signs of toxicity. Body weights of dams were measured on days 0, 6-18 and 20 of gestation. Individual 24-hr food consumption was determined on days 0, 6, 8, 10, 13, 15 and 18 of gestation. On day 20 of gestation, the dams were killed and the uterus, ovaries and other internal organs examined macroscopically. The numbers of corpora lutea, implants, resorptions, placental remnants, dead fetuses and live fetuses were recorded. The sex ratio (number of males X 100/number of males and females) was determined. Body weight was determined for each live fetus. Approximately one-half of the live fetuses were examined for visceral abnormalities and variations; the remaining half were eviscerated, stained and examined for skeletal abnormalities and variations.

Results

Survival and Clinical Signs
No dams died during treatment and no clinical signs of toxicity were observed.

Body Weight
Significantly lower than control body weights (uncorrected for gravid uterine weight) were noted in dams treated with candesartan cilexetil/HCTZ beginning on day 15 of gestation and continuing to gestation day 20 (Table 57).

Table 57. Maternal Mean Body Weight (gm).

Treatment Group	Dose mg/kg	Gestation Day					
		GD 0	GD 15	GD 16	GD 17	GD 18	GD 20
Vehicle Control	0	228	286	294	305	319	344
HCTZ	10	228	282	291	301	315	346
Candesartan cilexetil/HCTZ	10/10	228	275	**281**	**290**	**300**	328
	30/10	228	**273**	**280**	**289**	**299**	**323**
	100/10	227	275	282	**290**	**300**	**322**

Values in bold type indicate significant differences from vehicle control.

Food Consumption

Food consumption was significantly lower (9% to 14% lower; non-dose related) than control in all candesartan cilexetil/HCTZ treated groups from day 10 to day 18 of gestation.

Cesarean Section Results

There were no significant differences in the numbers of corpora lutea, implants, live fetuses or sex ratio between the control group any other group. Lower than control fetal weight was noted for the 100/10 candesartan cilexetil/HCTZ/kg group (Table 58).

Table 58. Cesarean Section Results.

Parameter	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	30/10	100/10
# of Dams	20	20	19	20	18
Corpora Lutea, mean #	16.0	16.2	16.2	15.8	16.2
Implants, mean #	14.7	15.4	14.8	14.3	14.8
Pre-implantation loss, %	7.7	4.4	8.2	8.5	8.0
Post-implantation loss, %	7.0	5.6	6.9	8.1	8.6
Live Fetuses, mean #	13.8	14.5	13.7	13.2	13.6
Sex Ratio, M/M+F (%)	52.5	50.3	49.3	56.8	51.2
Fetal Weight, mean gm					
Male	2.99	3.01	2.95	2,89	2.78*
Female	2.84	2.79	2.77	2.68	2.63*

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

External, Visceral and Skeletal Examination of Fetuses

No significant differences in the frequencies of external abnormalities between treated and control groups were detected (Table 59).

Table 59. Fetal External Examination Results[a]

Observation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	30/10	100/10
# Fetuses Examined (# litters)	275 (20)	290 (20)	261 (19)	263 (20)	244 (18)
Abnormalities (overall), mean %	0.4	0.0	0.8	0.4	0.0

[a] Mean % values are for fetuses.

In the visceral examination, there were no significant differences in overall or individual abnormalities or variations between treated and control groups. The most frequent visceral abnormality was ventricular septal defect; the most frequent variation observed was left umbilical artery (Table 60).

Table 60 Fetal Visceral Examination Results[a]

Observation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	30/10	100/10
# Fetuses Examined (# litters)	147 (20)	153 (20)	142 (19)	141 (20)	130 (18)
Abnormalities (overall), mean %	2.1	2.4	0.8	3.7	4.5
Ventricular septal defect, mean %	2.0	2.6	0.7	2.8	3.8
Variations (overall), mean %	1.3	0.8	0.0	1.3	0.0
Left umbilical artery, mean %	0.7	0.7	0.0	1.3	0.0

[a] Mean % values are for fetuses.

In the skeletal examination, there were no significant differences in the frequencies of skeletal abnormalities or variations between treated and control groups. Slightly, but statistically significantly, lower than control numbers of ossified sacral-caudal vertebrae in the mid and high dose candesartan cilexetil/HCTZ groups were detected (Table 61).

Table 61 Fetal Skeletal Examination Results[a]

Observation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			10/10	30/10	100/10
# Fetuses Examined (# litters)	128 (20)	137 (20)	119 (19)	122 (20)	114 (18)
Abnormalities (overall), mean %	1.3	0.7	0.0	0.0	0.0
Sternal cleft, %	0.8	0.7	0.0	0.0	0.0
Variations (overall), mean %	2.1	1.5	7.5	2.5	2.7
Dumbbell shaped thoracic vert., %	0.0	0.7	0.8	0.0	0.9
Cervical rib, %	0.8	0.0	1.7	0.0	1.8
Lumbar rib, %	1.6	0.0	0.8	2.5	0.0
Dumbbell shaped lumbar vert, %	0.0	0.7	0.0	0.0	0.0
Ossified sacral-caudal vertebrae, #	6.4	6.5	6.0	5.8*	5.4*

[a] Mean % values are for fetuses.

Embryo-Fetal Developmental Toxicity Study in Rabbits (Vol. 16, pg 223)

Study Facility:
Study No: 2194/TE
Study Dates: Study initiation, 12/14/95; Study termination, not stated.

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Animals: Presumed pregnant Kbl:JW rabbits (2.94-3.65 kg at time of mating) The animals were housed individually and fed (*ad libitum)* a solid diet (LRC-4,).

Drug Administration: Candesartan cilexetil (Lot # M464-034) and HCTZ (Lot # M373-001) were suspended in aqueous 5% gum arabic suspension and administered orally by gavage from day 6 to day 18 of gestation. The day of mating was designated day 0 of gestation.

Dose Levels: The experimental groups were as follows:

Treatment Group	Dose Level, mg/kg Candesartan cilexetil/HCTZ	# Pregnant Rats
Control (vehicle)	0/0	19
HCTZ	0/10	17
Candesartan cilexetil/HCTZ	0.3/10	19
Candesartan cilexetil/HCTZ	1/10	18
Candesartan cilexetil/HCTZ	3/10	18

Note: The highest dose for candesartan cilexetil selected for this study was based on a previous study of candesartan cilexetil (without HCTZ) in pregnant rabbits which showed maternal nephrotoxicity and death at a candesartan cilexetil dose of 3 mg/kg/day. A dose of 10 mg/day of HCTZ was selected because this dose exerted a maximum diuretic effect in rodents.

Observations/Measurements: All dams were observed daily for mortality and clinical signs of toxicity. Body weights of dams were determined on days 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 28 of gestation. Individual 24-hr food consumption was determined on days 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 27 of gestation. Blood samples were taken from the ear vein of each dam 1 hour after dosing on days 6, 13 and 18 of gestation for measurement of plasma concentrations of candesartan and HCTZ. On day 28 of gestation, the dams were killed and the uterus, ovaries and other internal organs examined macroscopically. The numbers of corpora lutea, implants, resorptions, placental remnants, dead fetuses and live fetuses were recorded. The sex ratio (number of males X 100/number of males and females) was determined. Body weight was determined for each live fetus. The live fetuses were examined for external and visceral abnormalities and variations. The fetuses were then eviscerated, stained and examined for skeletal abnormalities and variations. No skeletal examination was performed on the low dose (0.3/10 mg/kg/day) group because no adverse effects on skeletal development were seen in the mid dose group (1/10 mg/kg/day).

Results

Survival and Clinical Signs

Five of the 18 dams in the 1/10 mg/kg/day group died between day 18 and day 26 of gestation. Deaths in these animals were associated with decreases in the number of fecal pellets, stained fur, diarrhea, watery feces and vaginal discharges. In the 3/10 mg/kg/day group, 14 of the 18 dams died between day 14 and day 28 of gestation; deaths in these animals were also associated with decreases in number of fecal pellets, stained fur and diarrhea. Two dams that died in the 1/10 mg/kg/day group and 3 dams that died in the 3/10 mg/kg/day group aborted their litters 1 to 2 days before death.

Body Weight

The dams that died in the 1/10 and 3/10 mg/kg/day groups lost 270-770 gm between commencement of dosing and death. Mean body weights of surviving dams in the 1/10 mg/kg/day group did not differ significantly from control. However, significantly lower than control mean body weights were noted for surviving dams in the 3/10 mg/kg/day group (Table 62).

Table 62. Maternal Mean Body Weight (kg).

Gestation Day	Candesartan Cilexetil/HCTZ Group, mg/kg/day				
	0/0 (Control)	0/10 (HCTZ)	0.3/10	1/10	3/10
0	3.31	3.29	3.27	3.31	3.25
6	3.52	3.52	3.46	3.45	3.40
8	3.53	3.51	3.44	3.44	3.35
10	3.5	3.4	3.48	3.45	**3.32**
12	3.58	3.57	3.49	3.43	**3.28**
14	3.63	3.59	3.52	3.43	**3.26** (17)
16	3.67	3.63	3.57	3.45	**3.22** (15)
18	3.67	3.66	3.60	3.47	**3.23** (12)
19	3.68	3.67	3.61	3.48 (17)	**3.19** (12)
22	3.75	3.74	3.68	3.53 (16)	3.55 (6)
25	3.81	3.78	3.73	3.54 (16)	3.69 (5)
28	3.84	3.79	3.77	3.79 (13)	3.91 (4)

Values in bold type indicate significant differences from vehicle control. Numbers in parentheses indicate the number of surviving dams from which the mean body weight was determined.

Food Consumption

For the dams that died, food consumption was continuously low after the first to middle phase of the dosing period; little to no food was consumed in the week preceding death. No adverse effects on food consumption were noted for dams that survived to cesarean section.

Cesarean Section Results

Among dams surviving to scheduled sacrifice, the numbers of corpora lutea, implants and live fetuses, percent pre and post implantation losses, fetal body weights and sex ratios were similar for treated and control groups (Table 63).

Table 63. Cesarean Section Results.

Parameter	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			0.3/10	1/10	3/10
# of Dams	19	17	19	13	4
Corpora Lutea, mean #	10.1	10.4	10.0	10.8	10.8
Implants, mean #	8.9	7.8	8.5	7.9	9.3
Pre-implantation loss, %	11.8	23.0	14.9	25.2	13.2
Post-implantation loss, %	7.6	5.0	6.6	6.8	9.8
Live Fetuses, mean #	8.3	7.4	7.9	7.3	8.3
Sex Ratio, M/M+F (%)	45.6	44.3	59.9	51.2	45.9
Fetal Weight, mean gm					
Male	37.3	38.6	36.6	38.9	37.7
Female	36.1	38.4	35.9	37.9	36.8

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

External, Visceral and Skeletal Examinations of Fetuses

No significant differences in the frequencies of external abnormalities between treated and control groups were detected (Table 64).

Table 64. Fetal External Examination Results[a]

Observation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			0.3/10	1/10	3/10
# Fetuses Examined (# litters)	157 (19)	126 (17)	151 (19)	95 (13)	33 (4)
Abnormalities (overall), mean %	0.0	0.6	0.6	0.0	0.0

[a] Mean % values are for fetuses.

In visceral examinations, there were no significant differences in overall abnormalities or variations between treated and control groups. Higher than control incidence of small gallbladder was noted in the 3/10 mg/kg/day group. (Table 65).

Table 65 Fetal Visceral Examination Results[a]

Observation	Treatment Group, mg/kg				
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ		
			0.3/10	1/10	3/10
# Fetuses Examined (# litters)	157 (19)	126 (17)	151 (19)	95 (13)	33 (4)
Abnormalities (overall), mean %	0.7	1.3	1.0	0.0	2.5
Small gallbladder	0.0	0.0	0.0	0.0	2.5
Variations (overall), mean %	0.0	0.0	0.6	0.0	0.0

[a] Mean % values are for fetuses.

In skeletal examinations, there were no significant differences in the frequencies of skeletal abnormalities or variations between the treated (mid and high dose) and control groups; the low dose treated group was not evaluated because of the absence of findings in the mid and high dose groups. The incidences of ossified sacral-caudal vertebrae in the mid and high dose candesartan cilexetil/HCTZ groups were comparable to control (Table 66).

Table 66. Fetal Skeletal Examination Results[a]

Observation	Treatment Group, mg/kg			
	Control 0 (vehicle)	HCTZ 10	Candesartan cilexetil/HCTZ	
			1/10	3/10
# Fetuses Examined (# litters)	157 (19)	126 (17)	95 (13)	33 (4)
Abnormalities (overall), mean %	2.1	7.8	0.0	2.5
Variations (overall), mean %	31.1	34.5	29.3	22.5
Ossified sacral-caudal vertebrae, #	19.1	19.1	18.9	18.9

[a] Mean % values are for fetuses.

The no-observed-adverse-effect-level of candesartan cilexetil was 0.3 mg/kg/day for dams when administered concurrently with 10 mg/kg/day of HCTZ. The no-observed-adverse-effect-level of candesartan cilexetil was 1 mg/kg/day for fetuses when co-administered with 10 mg/kg/day of HCTZ.

Plasma Drug Concentrations

Concentrations of candesartan and HCTZ in maternal plasma were measured 1 hour after dosing on day 6, 13 and 18 of gestation. The concentration of candesartan increased with increasing doses. The plasma concentration of HCTZ was not significantly affected by concurrent treatment with candesartan cilexetil (Table 67).

Table 67. Plasma Concentrations of Candesartan and HCTZ 1 Hour After Oral Administration of HCTZ or Candesartan Cilexetil/HCTZ to Pregnant Rabbits.

Treatment Group	Candesartan Concentration , ng/ml		
	GD 6	GD 13	GD18
Candesartan cilexetil/HCTZ, 0.3/10	28.6	23.1	29.2
1/10	84.6	162.2	242.4
3/10	185.6	1320.0	1430.6
	HCTZ Concentration, ug/ml		
	GD 6	GD 13	GD 18
HCTZ, 10	0.89	1.32	1.12
Candesartan cilexetil/HCTZ, 0.3/10	1.09	1.24	1.11
1/10	0.86	1.29	1.49
3/10	1.06	2.70	2.96

GENOTOXICITY

Bacterial Mutagen (Ames) Test of Candesartan Cilexetil/HCTZ (Vol. 16, pg 356)

Study Facility:
Study No: 1806/GE
Study Dates: 6/30/94 – 9/16/94

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Bacterial Strains: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA 1537 and *Escherichia coli* strain WP2uvrA.

Procedure: Candesartan cilexetil (Lot # M464-039) and hydrochlorothiazide (Lot # 754648) were mixed in ratios of 1:2 or 1:4 (anticipated mixture ratios of the clinical product), dissolved in DMSO and added to plates containing the bacterial tester strains in the presence and absence of metabolic activation (liver S-9 fraction obtained from Aroclor 1254-treated rats). In the presence of S-9, doses of each mixture ranged from 313 to 5000 ug/plate, for all bacterial strains. In the absence of S-9, the 1:2 mixture was tested at doses ranging from 39 to 625 ug/plate for strains TA 100 and TA 1537, doses ranging from 78 to 1250 ug/plate for strain TA1535 and doses ranging from 313 to 5000 ug/plate for strains TA 98 and WP2uvrA. The 1:4 mixture was tested in the absence of S-9 at doses ranging from 39 to 625 ug/plate for TA 1537, doses ranging from 78 to 1250 ug/plate for TA 100 and TA 1535 and doses ranging from 313 to 5000 ug/plate for WP2uvrA and TA98. Results from preliminary assays were used to select a range of non-cytotoxic concentrations of candesartan cilexetil/HCTZ mixtures. Sodium azide (AZI, 0.5 ug/plate), N-ethyl-N'-nitro-N-nitosoguanidine (ENNG, 5 ug/plate), 9-aminoacridine (9AA, 80 ug/plate), sodium p-dimethylaminobenzenediazosulfonate (DAPA, 25 ug/plate) and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2, 10 and 40 ng/plate) were used as positive controls in the absence of metabolic activation; 2-aminoanthracene (2AA, 1, 2 and 10 ug/plate), cyclophosphamide (CPP, 200 ug/plate), diethylnitrosamine (DEN, 5 uL/plate) and 3-4 benzpyrene (BP, 5 ug/plate) were used as positive controls in the presence of metabolic activation. DMSO was used as the vehicle control for the test article and for the positive controls AF-2 and 9-AA; distilled water was used as the vehicle control for the positive controls DEN, CPP, ENNG, AZI and DAPA. The mean number of revertant colonies was determined for each concentration (2 plates/conc.) of candesartan cilexetil/HCTZ and for the positive controls in the presence and absence of metabolic activation and compared to the vehicle control (4 plates/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. No statistical analysis was applied.

Results: The mixtures of candesartan cilexetil/HCTZ did not cause an increase (equal to or greater than twice 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 (Tables 68-71).

Table 68. Bacterial Mutagen Test (No Metabolic Activation)-1st Test

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate[c]				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water	100 uL	21	114	12	24	10
DMSO	100 uL	31	129	15	24	14
Candesartan cilexetil/HCTZ [a] (1:2 mixture)	39 ug		108			11
	78		119	11		10
	156		121	8		13
	313	22	115	11	24	7
	625	31	92	10	21	8
	1250	25		5	20	
	2500	22			26	
	5000	29			25	
(1:4 mixture)	39					14
	78		128	14		10
	156		125	14		8
	313	27	126	10	27	6
	625	20	100	10	24	3
	1250	16	84	9	24	
	2500	21			28	
	5000	29			31	
DAPA[b]	25 ug	457	838	-	1200	363
AF-2[a]	10 ng	93	450	-	-	-
	40				218	
ENNG[b]	5 ug	-	-			
AZI[b]	-0.5 ug		-	1288	-	-
9AA[a]	80 ug	-	-	-	-	641

[a] DMSO vehicle [b] Water vehicle [c] Values are the mean from 2 plates except for DMSO and water controls in which mean is from 4 plates.

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Table 69. Bacterial Mutagen Test (No Metabolic Activation)- 2nd Test

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate[c]				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water	100 uL	29	149	13	21	11
DMSO	100 uL	330	162	16	21	8
Candesartan cilexetil/HCTZ [a] (1:2 mixture)	39 ug		141			10
	78		149	12		7
	156		119	15		7
	313	34	122	11	20	7
	625	23	95	10	18	5
	1250	28		6	27	
	2500	29			28	
	5000	24			28	
(1:4 mixture)	39					9
	78		152	12		9
	156		152	14		6
	313	27	124	12	26	6
	625	36	107	6	25	7
	1250	30	91	5	20	
	2500	25			22	
	5000	40			32	
DAPA[b]	25 ug	488	912	-	1291	381
AF-2[a]	10 ng	113	432	-	-	-
	40	-	-		230	
ENNG[b]	5 ug	-	-	1670	-	
AZI[b]	0.5 ug		-	397	-	-
9AA[a]	80 ug	-	-	-	-	593

[a] DMSO vehicle [b] Water vehicle [c] Values are the mean from 2 plates except for DMSO and water controls in which mean is from 4 plates.

Table 70. Bacterial Mutagen Test (With Metabolic Activation) 1st Test

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate[c]				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water	100 uL	28	115	11	40	22
DMSO	100 uL	36	121	15	44	17
Candesartan cilexetil/HCTZ [a] (1:2 mixture)	313 ug	27	117	10	43	24
	625	33	120	13	41	24
	1250	29	93	15	48	38
	2500	30	97	8	32	20
	5000	37	113	17	44	16
(1:4 mixture)	313	30	118	13	43	25
	625	32	101	12	50	19
	1250	34	102	11	47	24
	2500	36	98	13	49	24
	5000	31	113	12	52	20
2AA[a]	1 ug	-	755	-	854	-
	2	-	-	271	-	141
	10	935	-	-	-	-
BP[a]	5 ug	-	1018	-	306	173
DEN[b]	5 uL	498	-	-	-	-
CPP[b]	200 ug	-	-	362	-	-

[a] DMSO vehicle [b] Water vehicle [c] Values are the mean from 2 plates except for DMSO and water controls in which mean is from 4 plates.

Table 71. Bacterial Mutagen Test (With Metabolic Activation) 2nd Test

Treatment	Conc/ Plate	Mean # Revertant Colonies/Plate[c]				
		WP2uvrA	TA100	TA1535	TA98	TA1537
Water	100 uL	37	145	14	48	21
DMSO	100 uL	38	164	16	50	21
Candesartan cilexetil/HCTZ [a] (1:2 mixture)	313 ug	29	147	18	51	21
	625	40	128	17	43	26
	1250	36	133	13	45	36
	2500	30	126	12	47	22
	5000	50	145	13	44	21
(1:4 mixture)	313	37	176	16	49	29
	625	44	170	14	45	26
	1250	44	157	14	45	24
	2500	34	128	16	39	32
	5000	42	126	17	55	23
2AA[a]	1 ug	-	739	-	784	-
	2	-	-	270	-	180
	10	1024	-	-	-	-
BP[a]	5 ug	-	1070	-	311	158
DEN[b]	5 uL	532	-	-	-	-
CPP[b]	200 ug	-	-	335	-	-

[a]DMSO vehicle [b] Water vehicle [c] Values are the mean from 2 plates except for DMSO and water controls in which mean is from 4 plates.

Mouse Lymphoma Cell Assay of Candesartan/HCTZ (Vol. 17, pg 41)

Study Facility:
Study No: T3335
Study Dates: 12/21/94 –8/10/95

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

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

Procedure: Candesartan (Lot #M464-R0105) was mixed with HCTZ (Lot #M373-001), dissolved in DMSO and added to mouse lymphoma cell cultures in the presence and absence of metaboiic activation (liver S-9 fraction obtained from Aroclor 1254-treated rats). Results from a preliminary rangefinding assay were used to select a range of concentrations of candesartan and HCTZ that were not markedly cytotoxic. Excessive toxicity (less than 10% relative survival) was observed with candesartan concentrations of 50 ug/mL when combined with varying concentrations of HCTZ. Therefore, a dose of 25 ug candesartan/mL in combination with five doses of HCTZ, ranging from 500 to 2500 ug/mL in the absence of S-9 and from 1000 to 3000 ug/mL in the presence of S-9, was chosen for the definitive study. Cells were incubated with the HCTZ, candesartan/HCTZ, vehicle or positive controls (4-nitroquinoline-1-oxide, 0.05 and 0.1 ug/ml without S-9 and benzo(a)pyrene, 2 and 3 ug/ml with S-9) for 3 hours at 37° C and then the cells were plated to permit cell expression and spontaneous cell mutation. Mutant frequency was defined as the number of mutants per 10^6 viable 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 absence of S-9, statistically significant increases in mutant frequency were obtained with HCTZ alone at doses of 1500 and 2000 ug/mL and a statistically significant positive trend was observed in both experiments. In the presence of S-9, statistically significant increases in mutant frequency were observed with all HCTZ concentrations in Exp. #1 and with 2500 ug HCTZ/mL in Exp. # 2. A linear positive trend was noted for both experiments. In the case of the candesartan/HCTZ combination, statistically significant increases in mutant frequency were noted with all concentrations in the absence of S-9 in Exp. #1 and with concentrations of 1500 and 2500 ug/mL in Exp. #2. In the presence of S-9, statistically significant increases in mutant frequency were observed with concentrations ≥ 1500 ug/ML in Exp. #1 and with doses ≥2500 ug/mL in Exp. #2. A positive linear trend was detected with and without S-9 in both experiments. Thus, statistically significant and dose-related increases in mutant frequency were noted following treatment with HCTZ alone and in combination with candesartan. When compared to HCTZ alone, no obvious synergistic effects were apparent following treatment with the candesartan/HCTZ combination (Tables 72-73)

Table 72. Mouse Lymphoma Cell Assay of HCTZ

Treatment (ug/ml)	S-9	% Cell Survival		Mutant Frequency (#/10^6 cells)	
		Exp #1	Exp #2	Exp. #1	Exp. #2
Vehicle	-	100	100	138	170
HCTZ					
500	-	88	122	190	158
1000	-	95	123	161	183
1500	-	75	98	232*	308*
2000	-	47	42	424*	476*
2500	-	21	11		
3000					
NQO[a]					
0.05	-	-	117	-	365*
0.1	-	-	110	-	529*
Vehicle	+	100	100	115	176
HCTZ					
500	+	ND	78	ND	139
1000	+	102	72	185*	118
1500	+	100	76	197*	172
2000	+	85	62	319*	218
2500	+	57	35	ND	359*
3000		42	35	860*	
Benzo(a)pyrene					
2.0	+	58	76	903*	817*
3.0	+	25	33	1561*	1311*

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

* Significantly higher than vehicle control ($p<0.05$)

Table 73. Mouse Lymphoma Cell Assay of Candesartan/HCTZ

Treatment (ug/ml)	S-9	% Cell Survival		Mutant Frequency (#/10^6 cells)	
		Exp #1	Exp #2	Exp. #1	Exp. #2
Vehicle	-	100	100	138	170
Candesartan 25 ug/mL + HCTZ	-				
500	-	97	109	199*	149
1000	-	101	103	221*	173
1500	-	77	78	287*	301*
2000	-	35	36	583*	469*
2500		1	9		
3000					
NQO[a]					
0.05	-	-	117	-	365*
0.1	-	-	110	-	529*
Vehicle	+	100	100	115	176
Candesartan 25 ug/mL + HCTZ	+				
500	+	ND	67	ND	109
1000	+	95	48	153	142
1500	+	85	46	242*	171
2000	+	62	40	320*	218
2500		15	22	790*	410*
3000		11	20		560*
Benzo(a)pyrene					
2.0	+	58	76	903*	817*
3.0	+	25	33	1561*	1311*

[a] 4-nitroquinoline-1-oxide [b] Not determined [c] Not determined due to low cell survival
* Significantly higher than vehicle control ($p<0.05$)

Rat Bone Marrow Chromosomal Aberration Assay (Vol. 17, pg 225)

Study Facility:
Study No: 586/59
Study Dates: 1/25/95 - 3/28/95

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Animals: Male and female F-344 rats (178-212 gm and 134-161 gm, respectively)

Procedure: Candesartan cilexetil (Lot # M464-033) and hydrochlorothiazide (Lot # M373-001) were prepared as a mixture at a 1:2 ratio suspended in 5% gum arabic solution and administered to rats as single oral doses of 0 (vehicle), 250/500, 500/1000 and 1000/2000 mg candesartan cilexetil/HCTZ/kg (15/sex/dose group). Sponsor's selection of the high dose of candesartan cilexetil/HCTZ was based on a preliminary study in 3M and 3F given a single dose of 1000/2000 mg/kg candesartan cilexetil/HCTZ; sponsor states that this dose was well tolerated and showed no signs of severe toxicity but no study results are presented for assessment. An additional 5/sex/group were given the positive control, 40 mg cyclophosphamide/ml in saline. Approximately 2 hours prior to sacrifice, animals were injected intraperitoneally with colchicine

(2 mg/kg) to arrest dividing cells at metaphase. Test article and vehicle control rats were killed in groups of 5M and 5 F at 6, 18 and 30 hours after dosing. Cyclophosphamide-treated animals were killed 24 hours after dosing. Both femurs from each animal were removed and bone marrow was withdrawn by syringe and needle. Bone marrow cells were fixed on slides and stained. The slides were analyzed microscopically for chromosomal aberrations. Fifty cells in metaphase were analyzed from each animal for chromosomal aberrations that included chromosome gaps, deletions and exchanges. The test agent was considered to be positive if (1) a statistically significant increase in the frequency of cells with structural aberrations occurred at one or more dose and/or sampling time, (2) the incidence of cells with aberrations at such data points exceeded the normal range and (3) the increased frequency was dose-related.

Results The frequencies of cells with chromosomal aberrations in rats treated with candesartan cilexetil/HCTZ were similar to and not significantly different from those seen in concurrent vehicle control rats (Tables 74-76). The positive control, cyclophosphamide, produced a significantly higher than control frequency of chromosomal aberrations. Thus, a 1:2 mixture of candesartan cilexetil/HCTZ at doses up to 1000/2000 mg/kg did not induce chromosomal aberrations in bone marrow cells of rats.

Table 74. Rat Bone Marrow Chromosome Aberration Assay- 6 Hour Period

Treatment Group (mg/kg)	# Rats & Sex	# Cells Scored	# Cells with Aberrations Including Gaps
Vehicle	5M	250	2
	5F	250	4
Candesartan cilexetil/HCTZ			
250/500	5M	250	3
	5F	250	4
500/1000	5M	250	1
	5F	250	4
1000/2000	5M	250	0
	5F	250	5
Cyclophosphamide, 40[a]	5M	250	231*
	5F	250	243*

[a] Assay results from 24-hr sampling period. *Significantly different from vehicle ($p<0.001$)

Table 75. Rat Bone Marrow Chromosome Aberration Assay- 18-Hour Period

Treatment Group (mg/kg)	# Rats & Sex	# Cells Scored	# Cells with Aberrations Including Gaps
Vehicle	5M	250	1
	5F	250	2
Candesartan cilexetil/HCTZ			
250/500	5M	250	2
	5F	250	0
500/1000	5M	250	1
	5F	250	1
1000/2000	5M	250	1
	5F	250	5
Cyclophosphamide, 40[a]	5M	250	231*
	5F	250	243*

[a] Assay results from 24-hr sampling period. * Significantly different from vehicle ($p<0.001$)

Table 76. Rat Bone Marrow Chromosome Aberration Assay- 30 Hour Period

Treatment Group (mg/kg)	# Rats & Sex	# Cells Scored	# Cells with Aberrations Including Gaps
Vehicle	5M	250	0
	5F	250	2
Candesartan cilexetil/HCTZ 250/500	5M	250	4
	5F	250	0
500/1000	5M	250	5
	5F	250	4
1000/2000	5M	250	1
	5F	250	0
Cyclophosphamide, 40[a]	5M	250	231*
	5F	250	243*

[a] Assay results from 24-hr sampling period. *Significantly different from vehicle ($p<0.001$)

Micronucleus Test in Mice (Vol. 17, pg 10)

Study Facility: Takeda Chemical Industries, Ltd., Osaka Japan
Study No: 1904/GE
Study Dates: Study initiation: 11/04/94; Study termination: Not stated

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Animals: Male Jcl:ICR mice (6 weeks old)

Procedure: Candesartan cilexetil (Lot # M464-034) and hydrochlorothiazide (Lot # M373-001) were mixed at a ratio of 1:2, suspended in 5% gum arabic solution and administered to male mice (5/dose group) at doses of 0 (vehicle), 250/500, 500/1000 and 1000/2000 mg/kg orally by gavage for 2 consecutive days. The high dose was associated with significantly lower (25%-30%) than control levels of reticulocytes, an indication of bone marrow toxicity, which was used as the basis for high-dose selection. A separate group of mice received the positive control, mitomycin C (2 mg/kg IP). Mice were killed 24 hours after the last treatment, one femur was removed from each animal and bone marrow cells were extracted. The bone marrow cells were smeared on slides, stained and examined for the presence of micronuclei. Two thousand polychromatic erythrocytes/animal were examined. The frequency of micronucleated erythrocytes for each treatment was compared with control. A statistically significant increase above control frequency of micronucleated erythrocytes at any dose of a test agent was regarded as a positive genotoxic response.

Results: Treatment with candesartan cilexetil/HCTZ caused no significant increase above vehicle control in the frequency of micronucleated polychromatic erythrocytes (Table 77). The frequency of micronucleated cells increased above control in the mitomycin-C treated group. Thus, the mixture of candesartan cilexetil/HCTZ was judged to be negative in this genotoxicity assay.

Table 77: Micronucleus Test in Mice

Treatment Group, mg/kg	Route	# Doses	# Mice	% PCE with Micronuclei[a]
Vehicle	PO	2	5	0.12
Candesartan cilexetil/HCTZ				
250/500	PO	2	5	0.20
500/1000	PO	2	5	0.15
1000/2000	PO	2	5	0.10
Mitomycin-C, 2.0	IP	1	5	4.59*

[a] Based on 2000 polychromatic erythrocytes (PCE) per animal.
* Significantly different from vehicle control ($p<0.05$)

Unscheduled DNA Synthesis Assay in Rats (Vol. 17, pg 160)

Study Facility:
Study No.: 586/58
Study Dates: 1/25/95 - 3/10/95

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Animals: Male Fischer F-344 rats (180-241 gm)

Procedure: Candesartan cilexetil (Lot # M464-033) and hydrochlorothiazide (Lot # M373-001) were prepared as a mixture in a 1:2 ratio, suspended in 5% gum arabic solution and administered to rats orally by gavage at doses of 0 (vehicle), 250/500, 500/1000 and 1000/2000 mg/kg (5/group for 2-4 hr sample period and 5/group for 12-14-hr sample period). A preliminary study in 3M and 3F rats showed that a dose of 1000/2000 mg/kg candesartan cilexetil/HCTZ, which was the highest dose tested, produced no clinical signs of toxicity or body weight loss over a 4-day post dose period. According to the sponsor, the lack of toxicity was the basis for the high-dose selection; however, no study results are presented for assessment. Two other groups of 5 rats were administered the positive controls, 2-acetamidofluorene (2-AAF; 75 mg/kg for 12-14 hr assay) and dimethylnitrosamine (DMN; 10 mg/kg for 2-4 hr assay). Animals were killed at 2-4 or 12-14 hours after dosing, the livers removed and treated with collagenase to prepare hepatocyte suspensions. The hepatocytes were incubated with [^{3}H]-thymidine and cell suspensions placed on slides, fixed and examined microscopically for presence of nuclear and cytoplasmic grains per cell. Nuclear and mean cytoplasmic grain counts were then recorded and the net grains/nucleus (NNG) determined; one hundred cells were analyzed per animal. The test agent was considered positive in this assay if at any dose and for either assay period (1) the test article yielded a group mean NNG greater than 0 with 20% or more of cells showing NNG values ≥5 and (2) an increase was seen in both NNG and the percentage of cells in repair.

Results: Treatment with a 1:2 mixture of candesartan cilexetil/HCTZ at doses up to 1000/2000 mg/kg yielded NNG values less than 0, well below the value needed for a positive response (Tables 77-78). The positive controls 2-AAF and DMN induced increases in group mean net

grain count of 5 or more and the increases were noted in 50% or more of cells. Thus, these doses of candesartan cilexetil/HCTZ did not increase unscheduled DNA synthesis in rat hepatocytes.

Table 77. UDS Assay (2-4 Hr. Assay Period)

Treatment Group, mg/kg	Mean NNG	% Cells in Repair[a]
Vehicle	-1.3	0.5
Candesartan cilexetil/HCTZ		
250/500	-1.4	0.2
500/1000	-1.5	0.5
1000/2000	-1.5	0.8
DMN, 10	8.4	74.5

[a] Based on cells with NNG 5

Table 78. UDS Assay (12-14Hr. Assay Period)

Treatment Group, mg/kg	Mean NNG	% Cells in Repair[a]
Vehicle	-1.1	0.8
Candesartan cilexetil/HCTZ		
250/500	-1.4	0.2
500/1000	-1.6	1.2
1000/2000	-1.8	1.2
2-AAF, 75	11.9	88.8

[a] Based on cells with NNG 5

Chromosome Aberration Assay of Candesartan (CV-11974)/HCTZ in Chinese Hamster Lung (CHL) Cells (Vol. 18, pg 11)

Study Facility: [redacted]
Study No.: 586/55-1052
Study Dates: 1/31/95-7/17/96

GLP Compliance: Compliance with GLP regulations attested.
QA Reports: Yes

Cell Culture: Chinese Hamster lung (CHL) cells in culture

Procedure: Candesartan (Lot # M464-R0105) was dissolved in warm physiological saline. Hydrochlorothiazide (Lot # M373-001) was dissolved in DMSO and the solutions were added individually to the cell cultures at a single concentration of 200 ug/ml of candesartan and varying concentrations up to 3000 ug/ml of HCTZ in the presence and absence of metabolic activation with S-9 fraction obtained from livers of Aroclor 1254 treated rats. Appropriate vehicles were used as negative controls under each treatment condition. Methylmethane sulfonate and cyclophosphamide (dissolved in DMSO) were used as positive controls in the absence and presence of S-9, respectively. In one series of experiments, the media containing the drug treatments remained on the cell cultures continuously for 20 or 44 hours, the treatments were removed and the cultures were harvested. In a second series of experiments, cell cultures were

exposed to vehicle or drug treatments for a 2-hour period (pulse), the treatment medium then removed and cultures incubated for a further 18-hours before harvesting. The treatment scheme of the main study was as follows:

Treatment		CV-11974 (200 µg/mL)	S-9	HCTZ (highest concentration, µg/mL)	Duration of treatment (hours)	Harvest time (hours after start of treatment)
Continuous	20+0	-	-	250	20	20
		+	-	250	20	20
	44+0	-	-	250	44	44
		+	-	250	44	44
Pulse	2+18	-	-	3000	2	20
		+	-	3000	2	20
	2+18	-	+	3000	2	20
		+	+	3000	2	20

CV-11974 is candesartan, the active metabolite of candesartan cilexetil

Colchicine was added to the cell cultures 1.5 hrs before harvesting to arrest dividing cells at metaphase. Cells were fixed onto slides and examined for the presence of chromosome aberrations. Approximately 100 metaphases/replicate/treatment group were analyzed for chromosome aberrations. The test article was considered to be positive in this assay if 1) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurred at one or more concentrations and 2) the proportion of cells with structural aberrations at such doses exceeded the normal range.

Results

Treatment of cultures with HCTZ alone or in combination with candesartan in the absence of S-9 for 20 or 44 hours resulted in significant increases in the frequency of cells with structural aberrations. At the 2 highest concentrations (200 and 250 u/ml) all cultures yielded aberrant cells that exceeded the historical negative control range (Tables 79-82).

Treatment of cell cultures with HCTZ alone for 2 hours in the absence of S-9 resulted in frequencies of cells with structural aberrations that were similar to concurrent solvent controls, although one replicate treated with the highest dose (3000 ug/ml) of HCTZ yielded a frequency of aberrant cells that exceeded the normal range (Table 83). When 200 ug/ml candesartan was present with HCTZ, the levels of aberrant cells in cultures treated with the highest HCTZ dose were significantly greater than concurrent solvent control (Table 84). Although a statistically significant increase above control aberrant cell frequency was noted in the presence of candesartan and HCTZ, the overall frequency of aberrant cells in the absence and presence of candesartan was not markedly different. Therefore, the increase in aberrant cell frequency seen with HCTZ in the presence of candesartan may be in part a reflection of the lower concurrent solvent control frequency.

Treatment of CHL cell cultures with HCTZ in the presence of S-9 for 2 hours followed by an 18 hr drug free incubation period resulted in small but statistically significant increases in the frequencies of cells with structural aberrations. Frequencies of aberrant cells at the higher doses marginally exceeded the historical solvent control range. The presence of 200 ug/ml of candesartan caused a slight enhancement of the aberrant cell frequency produced by 3000 ug/ml of HCTZ.

Table 79. Chinese Hamster Lung Assay Results of HCTZ Alone (20 hr Treatment Period) in the Absence of S-9

20+0 hour, - S-9, without CV-11974

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	0	0		3.1
	B	100	1	1		3.2
	Totals	200	1	1		(3.2)
25	A	100	5	1		6.6
	B	100	3	2		1.9
	Totals	200	8	3	NS	(4.3)
50	A	100	1	1		2.5
	B	100	1	1		3.0
	Totals	200	2	2	NS	(2.8)
100	A	100	3	1		2.3
	B	100	3	1		2.4
	Totals	200	6	2	NS	(2.4)
150	A	100	5	4		3.0
	B	100	[7]	[5]		2.2
	Totals	200	12	9	p ≤0.01	(2.6)
200	A	100	[11]	[7]		2.3
	B	100	[7]	[5]		3.1
	Totals	200	18	12	p ≤0.001	(2.7)
250	A	100	[24]	[15]		2.4
	B	100	[18]	[13]		2.3
	Totals	200	42	28	p ≤0.001	(2.4)
MMS, 12.5	A	25	[14]	[5]		
	B	25	[9]	[7]		
	Totals	50	13	12	p ≤0.001	

§ Statistical significance (Appendix 5a)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 80.Chinese Hamster Lung Assay Results of HCTZ + 200 ug Candesartan /ml (20 hr Treatment Period) in the Absence of S-9.

20+0 hour, - S-9, with 200 μg/mL CV-11974

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	5	4		2.1
	B	100	3	1		1.1
	Totals	200	8	5		(1.6)
25	A	100	6	4		2.6
	B	100	2	2		2.9
	Totals	200	8	6	NS	(2.8)
50	A	100	4	2		3.7
	B	100	3	2		2.2
	Totals	200	7	4	NS	(3.0)
100	A	100	4	0		4.2
	B	100	6	1		2.5
	Totals	200	10	1	NS	(3.4)
150	A	100	5	2		3.5
	B	100	8	6		2.9
	Totals	200	13	8	NS	(3.2)
200	A	100	8	5		4.0
	B	100	16	12		2.8
	Totals	200	24	17	p ≤0.01	(3.4)
250	A	100	12	8		2.0
	B	100	20	16		2.6
	Totals	200	32	24	p ≤0.001	(2.3)

§ Statistical significance (Appendix 5a)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 81. Chinese Hamster Lung Assay Results of HCTZ Alone (44 hr Treatment Period) in the Absence of S-9.

44+0 hour, - S-9, without CV-11974

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	2	2		4.2
	B	100	4	2		4.7
	Totals	200	6	4		(4.5)
25	A	100	0	0		4.9
	B	100	2	1		4.5
	Totals	200	2	1	NS	(4.7)
50	A	100	2	2		4.0
	B	100	3	1		3.6
	Totals	200	5	3	NS	(3.8)
100	A	100	6	5		4.1
	B	100	1	0		2.7
	Totals	200	7	5	NS	(3.4)
150	A	100	4	0		2.9
	B	100	17	12		3.0
	Totals	200	21	12	p ≤0.05	(3.0)
200	A	100	10	5		1.4
	B	100	9	8		3.1
	Totals	200	19	13	p ≤0.05	(2.3)
250	A	55	10	10		0.6
	B	100	10	7		2.4
	Totals	155	20	17	p ≤0.001	(1.5)

§ Statistical significance (Appendix 5c)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 82. Chinese Hamster Lung Assay Results of HCTZ +200 ug Candesartan/ml (44 hr Exposure Period) in the Absence of S-9.

44+0 hour, - S-9, with 200 μg/mL CV-11974

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	1	1		3.0
	B	100	[8]	[6]		2.2
	Totals	200	9	7		(2.6)
25	A	100	3	2		3.0
	B	100	3	3		3.3
	Totals	200	6	5	NS	(3.2)
50	A	100	2	1		3.1
	B	100	2	1		3.4
	Totals	200	4	2	NS	(3.3)
100	A	100	1	0		2.9
	B	100	1	1		2.6
	Totals	200	2	1	NS	(2.8)
150	A	100	[10]	[7]		2.2
	B	100	[7]	4		1.8
	Totals	200	17	11	NS	(2.0)
200	A	100	[11]	[9]		3.2
	B	100	[18]	[16]		1.6
	Totals	200	29	24	p ≤0.001	(2.4)
250	A	100	[20]	[16]		1.2
	B	90	[21]	[19]		1.3
	Totals	190	41	35	p ≤0.001	(1.3)

§ Statistical significance (Appendix 5c)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 83. Chinese Hamster Lung Assay Results of HCTZ Alone
(2 hr Exposure Period + 18 hr Drug-Free Incubation) in Absence of S-9.

2+18 hour, - S-9, without CV-11974

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Signifi-cance §	Mitotic index (mean)
Solvent	A	100	3	3		6.1
	B	100	1	1		7.2
	Totals	200	4	4		(6.7)
500	A	100	1	0		6.7
	B	100	6	3		6.7
	Totals	200	7	3	NS	(6.7)
1000	A	100	2	2		5.8
	B	100	2	1		7.6
	Totals	200	4	3	NS	(6.7)
1500	A	100	6	5		6.9
	B	100	4	0		6.7
	Totals	200	10	5	NS	(6.8)
2000	A	100	5	3		5.5
	B	100	4	3		6.4
	Totals	200	9	6	NS	(6.0)
2500	A	100	2	0		6.4
	B	100	5	2		8.6
	Totals	200	7	2	NS	(7.5)
3000	A	100	10	8		5.8
	B	100	6	2		6.4
	Totals	200	16	10	NS	(6.1)

§ Statistical significance (Appendix 5d)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 84. Chinese Hamster Lung Assay Results of HCTZ + 200 ug Candesartan/ml (2 hr Exposure Period+ 18 hr Drug-Free Incubation) in Absence of S-9.

2+18 hour, - S-9, with 200 μg/mL CV-11974

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	1	1		6.7
	B	100	1	1		7.2
	Totals	200	2	2		(7.0)
500	A	100	5	4		6.9
	B	100	2	2		7.0
	Totals	200	7	6	NS	(7.0)
1000	A	100	1	0		5.2
	B	100	0	0		7.4
	Totals	200	1	0	NS	(6.3)
1500	A	100	2	0		5.0
	B	100	1	0		6.4
	Totals	200	3	0	NS	(5.7)
2000	A	100	1	0		6.7
	B	100	1	0		7.4
	Totals	200	2	0	NS	(7.1)
2500	A	100	4	3		7.3
	B	100	4	2		6.7
	Totals	200	8	5	NS	(7.0)
3000	A	100	[redacted]	[redacted]		5.5
	B	100	[redacted]	[redacted]		3.0
	Totals	200	20	15	p ≤0.001	(4.3)

§ Statistical significance (Appendix 5d)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 85. Chinese Hamster Lung Assay Results of HCTZ Alone (2 hr Exposure Period+ 18 hr Drug-Free Incubation) in Presence of S-9.

2+18 hour, + S-9, without CV-11974

Treatment (µg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	1	0		4.8
	B	100	5	4		6.3
	Totals	200	6	4		(5.6)
500	A	100	1	1		7.6
	B	100	3	2		5.1
	Totals	200	4	3	NS	(6.4)
1000	A	100	0	0		8.0
	B	100	1	1		6.5
	Totals	200	1	1	NS	(7.3)
1500	A	100	6	5		6.5
	B	100	3	3		5.0
	Totals	200	9	8	NS	(5.8)
2000	A	100	3	3		8.2
	B	100	2	2		6.2
	Totals	200	5	5	NS	(7.2)
2500	A	100	7	[5]		4.4
	B	100	7	[6]		6.2
	Totals	200	14	11	p ≤0.05	(5.3)
3000	A	100	6	5		6.8
	B	100	8	[7]		5.0
	Totals	200	14	12	p ≤0.05	(5.9)
CPA, 12.5	A	25	[6]	[6]		
	B	25	[12]	[9]		
	Totals	50	18	15	p ≤0.001	

§ Statistical significance (Appendix 5b)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table 86. Chinese Hamster Lung Assay Results of HCTZ + 200 ug Candesartan/ml (2 hr Exposure Period + 18 hr Drug-Free Incubation) in Presence of S-9.

2+18 hour, + S-9, with 200 μg/mL CV-11974

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	3	1		7.6
	B	100	1	0		6.9
	Totals	200	4	1		(7.3)
500	A	100	4	4		5.3
	B	100	2	1		7.8
	Totals	200	6	5	NS	(6.6)
1000	A	100	2	2		5.8
	B	100	4	2		5.7
	Totals	200	6	4	NS	(5.8)
1500	A	100	6	5		6.1
	B	100	6	2		6.9
	Totals	200	12	7	p ≤0.05	(6.5)
2000	A	100	3	1		8.8
	B	100	8	6		8.2
	Totals	200	11	7	p ≤0.05	(8.5)
2500	A	100	3	3		5.9
	B	100	5	5		5.6
	Totals	200	8	8	p ≤0.05	(5.8)
3000	A	100	28	23		6.7
	B	100	21	18		4.3
	Totals	200	49	41	p ≤0.001	(5.5)

§ Statistical significance (Appendix 5b)
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

SUMMARY AND EVALUATION

Candesartan cilexetil is an ester prodrug that is rapidly converted *in vivo* to its active metabolite, candesartan. The antihypertensive properties of candesartan cilexetil in experimental models of hypertension in animals have been adequately demonstrated and have been previously reviewed for the ATACAND™ NDA (Review and Evaluation of Pharmacology and Toxicology Data, NDA # 20,838; Review Dated Feb. 17, 1998). The antihypertensive actions of the candesartan cilexetil/HCTZ combination described in this application were assessed in spontaneously hypertensive rats. The oral coadministration of 10 mg/kg of HCTZ with 0.1 or 1.0 mg of candesartan cilexetil/kg reduced systolic blood pressures to levels below those seen with candesartan cilexetil or HCTZ alone. The combination of candesartan cilexetil and HCTZ also increased plasma renin activity to levels greater than those seen with candesartan cilexetil or HCTZ alone. These doses of the combination (0.1/10 and 1/10 mg/kg PO) had minimal effects on heart rate. Under *in vitro* conditions, the presence of HCTZ (10^{-5} M) did not alter candesartan's binding properties to angiotensin II receptors.

In a pharmacokinetic study in rats, the combination of 1 mg candesartan cilexetil/kg with 10 mg HCTZ/kg did not affect the pharmacokinetics (Tmax, Cmax and AUC) of either drug. After coadministration of 1 mg/kg candesartan cilexetil and 10 mg/kg HCTZ to dogs, the elimination of candesartan from the circulation was slightly slower and the elimination of HCTZ was slightly faster than after dosing with candesartan cilexetil or HCTZ alone. However, toxicokinetic studies conducted in rats (doses up to 100 mg/kg candesartan cilexetil and 30 mg/kg HCTZ) and dogs (doses up to 20 mg/kg candesartan cilexetil and 30 mg/kg HCTZ) as components of 4- and 13-week oral repeated-dose toxicity studies showed no pharmacokinetic interactions between HCTZ and candesartan cilexetil. In addition, HCTZ (0.2 ug/ml) had no effect on binding of candesartan to human serum proteins. Similarly, the binding of HCTZ to human serum albumin was not affected by increasing concentrations (0.2 and 2.0 ug/ml) of candesartan.

Administration of single oral doses of up to 2000/1000 mg/kg candesartan cilexetil/HCTZ to male and female rats elicited no mortality and no discernable signs of toxicity.

Repeated oral dose toxicity studies of up to 13 weeks duration were conducted in rats and dogs. Studies in rats of 4 weeks duration at oral candesartan cilexetil/HCTZ doses of 3/10, 30/10 and 300/10 mg/kg/day and 13 weeks duration at oral candesartan cilexetil/HCTZ doses of 1/10, 10/10, 100/10 and 100/30 mg/kg/day were used to evaluate the toxicity from repeated daily administration of the combination. No adverse effects on survival were seen at these dose levels in either the 4- or 13-week studies. Adverse effects on body weight, hematology, blood chemistry, organ weights and histopathology were, however, detected. Lower than control terminal body weight was reported for male and female rats (9% to 21% lower) after 4 weeks at doses of 3/10 to 300/10 mg/kg/day. The magnitude of the terminal body weight decrement was 6% to 8% lower than control for males and females at doses of 10/10 and 100/10 mg/kg/day in the 13-week studies. Erythroid values were lower than control in male and female rats in both the 4- (3/10 and 300/10 mg/kg/day) and 13-week (10/10 and 100/10 mg/kg/day) studies. This effect is common to agents that inhibit renin-angiotensin activity in the kidney which, in turn, leads to reduction of erythropoetin secretion. This effect is limited in degree and, based on previous studies with candesartan cilexetil, is reversible. Other adverse findings (higher than control serum BUN, creatinine and inorganic phosphorus, higher than control kidney weights, and renal histopathology) reflect drug-induced effects on the kidney, the primary target organ of

toxicity. The incidence of renal J-G cell hypertrophy was dose related and attributed to the well recognized pharmacologic consequence of blockade of angiotensin II receptor activity of candesartan. Higher than control incidence of basophilic renal tubules, indicative of renal tubule cell repair or regeneration, was evident at doses ≥10/10 mg/kg/day. The highest no-adverse-effect level (excluding J-G cell hypertrophy) in the 13-week oral toxicity study in rats was 1/10 mg/kg/day.

Toxicity from repeated dosing was also evaluated in dogs treated for 4 weeks at daily oral candesartan cilexetil/HCTZ doses of 4/10, 20/10 and 100/10 mg/kg and for 13 weeks at daily oral doses of 0.8/10, 4/10 and 20/10 mg/kg. Five of six dogs (2M, 3F) were sacrificed in a moribund state prior to the end of the 4-week study and 2/3 females treated with 20/10 mg/kg/day were killed (after about 4-5 weeks of treatment) in a moribund state in the 13-week study. Severe physical deterioration in these dogs was preceded by large body weight decrements and anorexia. Dogs receiving doses that had no adverse effects on survival (0.8/10, 4/10 and 4/30 mg/kg/day) showed no adverse effects on body weight or food consumption, urinalysis, blood chemistry or organ weights. The predominant treatment-related histopathology among animals surviving to study termination was higher than control incidences of hypertrophy of the J-G cells of the kidney (seen at all doses tested). The highest non-toxic dose (excluding J-G cell hypertrophy) of candesartan cilexetil/HCTZ observed in dogs in the 4- and 13-week studies was 4/10 mg/kg/day.

Embryo-fetal toxicity studies were conducted in mice, rats and rabbits. In mice, no maternal toxicity or embryo-fetal developmental toxicities (external, visceral and skeletal abnormalities) were noted at maternal doses up to 1000/10 mg/kg/day of candesartan cilexetil/HCTZ. In rats, significant decrements in body weight were observed through gestation day 20 for dams receiving candesartan cilexetil/HCTZ doses of 30/10 and 100/10 mg/kg/day. However, no embryolethality was observed and no increases above control incidence of external, visceral or skeletal abnormalities were seen at doses up to 100/10 mg/kg/day, the highest dose tested. In rabbits, a dose of 0.3/10 mg/kg/day had no adverse effects on dams and resulted in no embryolethality or developmental toxicities. Also, no embryolethal effects were observed with maternal doses of 1/10 mg/kg/day, although this dose elicited significant maternal toxicity (5/18 dams died). The highest dose tested, 3/10 mg/kg/day, caused a higher incidence of maternal deaths (78%).

Animal doses used in the developmental toxicity studies were compared with the intended human dose on a body surface area basis (Table 87). This approach appears to be the most appropriate for consistency across the 3 animal studies conducted. Although the sponsor derived drug exposure data (Cmax and AUC) in mice, similar information was not obtained for rats or rabbits. In rabbits, plasma drug concentrations were only obtained 1 hour after dosing and these values may not adequately reflect Cmax. In developmental studies in rats, no toxicokinetic data was obtained. Thus, comparisons based on body surface area appear to be more useful, especially for proper labeling of the product, rather than using different methods for each species in comparing animal doses to those in humans.

Table 87. NOAEL for Candesartan Cilexetil/HCTZ.

Species	Maternal NOAEL		Embryo-fetal NOAEL	
	mg/kg/day	mg/m^2/day[a]	mg/kg/day	mg/m^2/day[a]
Mouse	1000/10	3000/30	1000/10	3000/30
Rat	10/10	60/60	100/10	600/60
Rabbit	0.3/10	3.6/120	1/10	12/120
Maximum Recommended Human Dose 32/12.5 mg/day	mg/kg/day	mg/m^2/day	Animal-to-Human Dose Multiple[b]	
			vs. Mouse	vs. Rat / vs. Rabbit
	0.53/0.21	19.6/7.8	153X/3.8X	31X/7.7X / 0.6X/15.4X

[a] Conversion of doses from mg/kg to mg/m^2 assumes a human body weight of 60 kg and uses following conversion factors (k_m): Mouse=3, Rat=6, Rabbit=12, Human=37.

[b] Embryo-fetal NOAEL (mg/m^2/day) used in calculating animal-to-human dose multiples.

Four tests were conducted with candesartan cilexetil/HCTZ and two tests were conducted with candesartan/HCTZ to evaluate the genotoxic potential of candesartan cilexetil or its metabolite, candesartan, when combined with HCTZ. No evidence of genotoxic potential was detected *in vitro* or *in vivo* in tests with candesartan cilexetil/HCTZ (Table 88). In the mouse lymphoma assay which evaluated a fixed concentration of candesartan in conjunction with varying concentrations of HCTZ, positive genotoxicity was detected only when concentrations of HCTZ in the candesartan/HCTZ combinations reached levels at which HCTZ alone was genotoxic. In a similar manner, assessment of a fixed concentration of candesartan with increasing concentrations of HCTZ in the Chinese hamster lung assay showed that a combination of candesartan/HCTZ was not genotoxic until the concentration of HCTZ in the combination reached levels at which HCTZ alone showed positive genotoxic effects.

Table 88. Summary of Genotoxicity Studies

Genotoxicity Assay	S-9	Candesartan cilexetil/HCTZ Concentration/Dose	Activity/Response
Ames Test	-	39-5000 µg/plate	Negative
	+	313-5000 µg/plate	Negative
Rat Bone Marrow Chromosome Aberration Assay		250/500 to 1000/2000 mg/kg	Negative
Mouse Micronucleus Test		250/500 to 1000/2000 mg/kg/2 days	Negative
Rat Unscheduled DNA Synthesis		250/500 to 1000/2000 mg/kg	Negative
	S-9	Candesartan /HCTZ Concentration/Dose	Activity/Response
Mouse Lymphoma Assay	-	25/500 to 25/2000 ug/ml	Positive[a]
	+	25/500 to 25/1000 ug/ml	Negative
	+	25/1500 to 25/3000 ug/ml	Positive[a]
Chinese Hamster Lung Chromosome Aberration Assay	- (20 hr)[c]	200/25 to 200/150 ug/ml	Negative
	-	200/200 & 200/250 ug/ml	Positive[b]
	- (44 hr)[c]	200/25 to 200/150 ug/ml	Negative
	-	200/200 & 200/250 ug/ml	Positive[b]
	- (2 hr + 18 hr)[d]	200/500 to 200/2500 ug/ml	Negative
	-	200/3000 ug/ml	Positive[b]
	+	200/500 to 200/1000 ug/ml	Negative
	+	200/1500 to 200/3000 ug/ml	Positive[b]

[a] HCTZ alone showed a higher than control incidence in mutant frequency (positive)at ≥500 ug/ml without S-9 and higher than control incidence in mutant frequency at ≥1500 ug/ml with S-9.

[b] At concentrations of the combination which have shown higher than control incidence in mutant frequency (positive), companion assays conducted with HCTZ alone have also shown positive genotoxic activity at comparable concentrations

[c] Value represents the total period in which cell were exposed to test substance.

[d] Values represent 2 hr period in which cell were exposed to test agent +18 hr period of drug-free incubation .

3 pages redacted from this section of the approval package consisted of draft labeling

RECOMMENDATION

From a preclinical perspective, this new drug application for ATACAND-HCT (candesartan cilexetil/HCTZ) is approvable with the recommended changes in labeling.

/S/

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