CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-093

PHARMACOLOGY/TOXICOLOGY REVIEW

NDA # 21,093

JAN 7 2000

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Anthony G. Proakis, Ph.D.
1/06/00

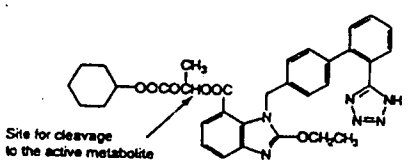
ORIGINAL SUBMISSION DATED: 9/28/99
CENTER RECEIPT DATE: 9/29/99
REVIEWER RECEIPT DATE: 9/29/99

PRODUCT: ATACAND-HCT™ Tablets

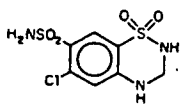
ACTIVE INGREDIENTS: Candesartan cilexetil (TCV-116) and hydrochlorothiazide (HCTZ).

SPONSOR: Astra Merck
725 Chesterbrook Blvd.
Wayne, PA 19087-5677
(610) 695-1000

CHEMISTRY: Candesartan cilexetil (CAS No. 145040-37-5) is described as (±)-1-(cyclohexyloxycarbonyloxy) ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]1H-benzimidazole-7-carboxylate. Its molecular weight is 610.67 and its empirical formula is $C_{33}H_{34}N_6O_6$. Candesartan cilexetil has a chiral center on the ester portion of the molecule and exists as two enantiomers; however, the active drug (candesartan) produced upon hydrolysis is achiral. Hydrochlorothiazide (CAS No. 58-93-5) is described as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. Its molecular weight is 297.72 and its empirical formula is $C_7H_8ClN_3O_4S_2$.



Candesartan cilexetil



Hydrochlorothiazide

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED: [redacted]

PHARMACOLOGICAL CLASS: Angiotensin II Antagonist/Diuretic

PROPOSED INDICATION: Treatment of Hypertension

FORMULATION AND ROUTE OF ADMINISTRATION: ATACAND-HCT is formulated in tablets for oral use containing 16/12.5mg or 32/12.5mg candesartan cilexetil/hydrochlorothiazide per tablet; [redacted]

PROPOSED DOSAGE REGIMEN: The recommended starting dose of ATRACAND-HCT™ is 16/12.5 mg once daily and may be increased to 32/12.5 mg once daily.

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INTRODUCTION

Candesartan cilexetil/hydrochlorothiazide is a combination of two marketed antihypertensive drugs. Candesartan cilexetil (TCV-116) is a prodrug that is rapidly converted during absorption to its active metabolite, candesartan; candesartan exerts its antihypertensive effect via blockade of vascular angiotensin II receptors. The preclinical pharmacology and toxicology of candesartan cilexetil have been reviewed previously under NDA # 20,838. Hydrochlorothiazide (HCTZ), a thiazide diuretic, acts directly on the kidney to increase the excretion of sodium chloride and other electrolytes. The antihypertensive effect of thiazides has been associated with thiazide-induced changes in sodium balance and consequent reduction in extracellular fluid volume. The combination of candesartan cilexetil and hydrochlorothiazide is considered a rational approach to hypertension management by utilizing the antihypertensive properties of both drugs.

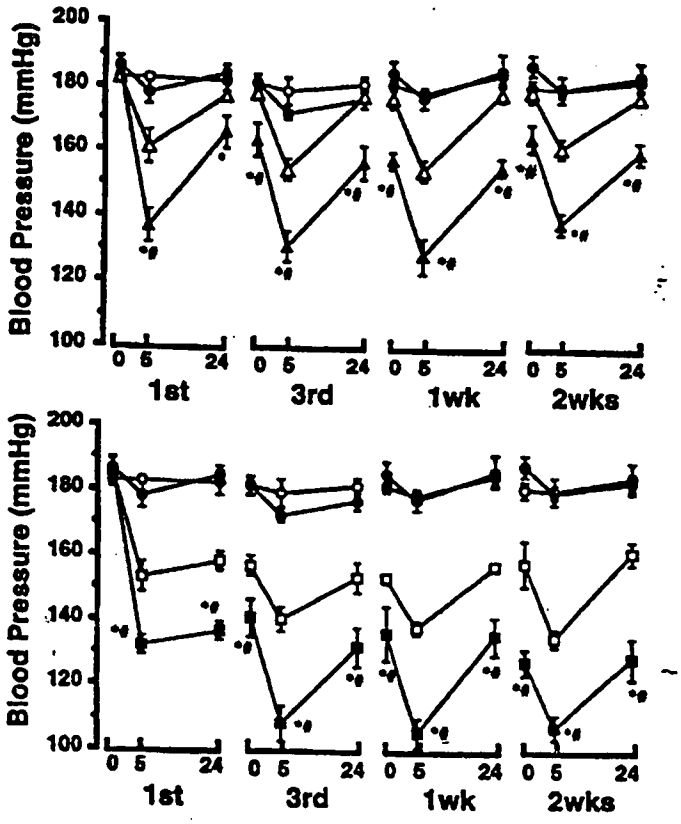
PHARMACODYNAMICS (Vol. 10, pg 10)

Antihypertensive and Diuretic Effects of Candesartan Cilexetil/HCTZ in Spontaneously Hypertensive Rats

Candesartan cilexetil (0.1 and 1 mg/kg), hydrochlorothiazide (HCTZ, 10 mg/kg) and candesartan cilexetil/HCTZ (0.1/10 mg/kg and 1/10 mg/kg) were administered orally to male SHR (20-21 weeks old) once daily for 2 weeks. Systolic blood pressures were measured by the tail cuff method before and 5 hours after drug administration on the 1st, 3rd, 7th and 14th treatment day. Urine was collected for 5 hours after dosing on the 6th and 13th day for measurement of excretion of urine and electrolytes. Blood was collected at time of sacrifice for measurement of plasma renin concentration. HCTZ had little effect on blood pressure (Fig. 1).

The blood pressure was reduced by about 30 mmHg 5 hr after administration of candesartan cilexetil 0.1 mg/kg; the effect disappeared within 24 hours after administration. The combination of candesartan cilexetil/HCTZ, 0.1/10 mg/kg, reduced blood pressure by about 50 and 30 mmHg at 5-hr and 24 hr after administration, respectively. Candesartan cilexetil alone at 1 mg/kg reduced blood pressure by about 30 to 50 mmHg; this dose of candesartan cilexetil, when combined with 10 mg HCTZ /kg, reduced the blood pressure by about 80 mmHg at 5 hours after administration. Candesartan cilexetil, HCTZ and the combination of the two had little effect on the heart rate (Fig. 1).

Figure 1:



Antihypertensive effects of repeated oral administration of TCV-116, hydrochlorothiazide (HCTZ) and a combination of the two in SHR. ○ vehicle, po, ● HCTZ 10 mg/kg,po, △ TCV-116 0.1 mg/kg,po, ▲ TCV-116 0.1 mg/kg,po and HCTZ 10 mg/kg,po, □ TCV-116 1 mg/kg,po, ■ TCV-116 1 mg/kg,po and HCTZ 10 mg/kg,po, n=4. *, # p<0.05, compared with vehicle and TCV-116 treated groups, respectively.

Plasma renin activity (PRA) was increased 5 and 17 times after 2 weeks of treatment with candesartan cilexetil, 0.1 and 1 mg/kg, respectively. HCTZ increased PRA to about twice the control value and the combination of HCTZ and candesartan cilexetil at 0.1 and 1 mg/kg increased PRA about 10 and 100 times that of control, respectively (Table 1).

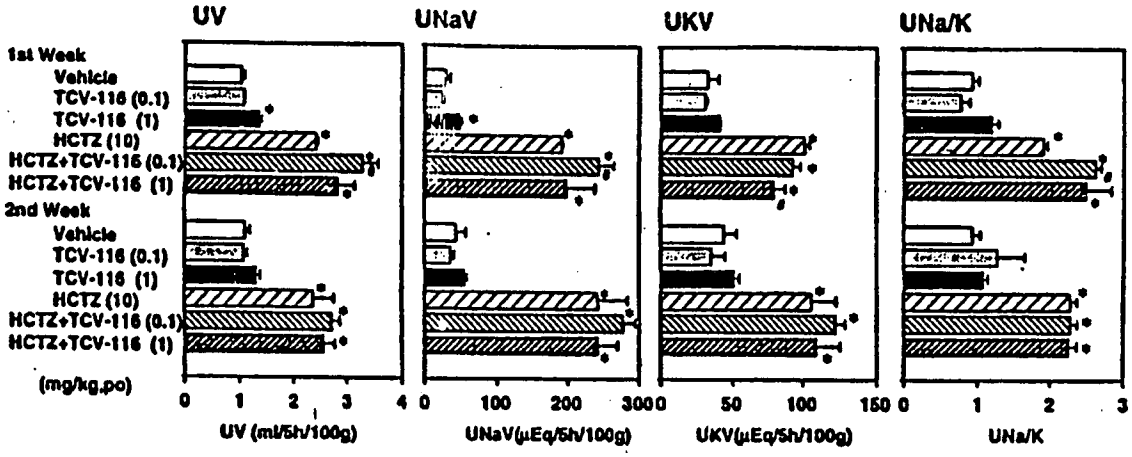
Table 1. Plasma Renin Activity (PRA)

Treatment Group (mg/kg)	PRA (ng AI/ml/h), n=4
Vehicle Control	16.9
Candesartan cilexetil (0.1)	88.7**
Candesartan cilexetil (1)	289.9**
HCTZ (10)	32.1*
Candesartan cilexetil + HCTZ (0.1/10)	182.9**
Candesartan cilexetil + HCTZ (1/10)	1972.9**

*Significantly different from control ($p<0.05$),
** Significantly different from control and HCTZ-treated group ($p<0.01$)

One and two weeks after the initiation of dosing, urine volume was slightly increased above control with candesartan 1 mg/kg. HCTZ markedly increased urinary volume and urinary sodium and potassium excretions. The diuretic and natriuretic effects of HCTZ were intensified by combination with candesartan cilexetil (Fig. 2).

Figure 2



Diuretic effects of repeated oral administration of TCV-116, hydrochlorothiazide (HCTZ) and the combination of the two in spontaneously hypertensive rats. n = 4. *, # p<0.05, compared with vehicle- and HCTZ-treated groups, respectively (Dunnett's test). UV, urinary volume; UNaV, urinary sodium excretion; UKV, urinary potassium excretion; UNa/K, urinary sodium/potassium ratio.

Effect of HCTZ on Candesartan Affinity for AII Receptors

The inhibition of specific binding (i.e., displacement) of [^{125}I]-AII to bovine adrenal cortex AII receptors by candesartan (CV11974) was tested *in vitro* in the presence and absence of 10^{-5} M HCTZ. The inhibition of specific binding of [^{125}I]-AII to bovine adrenal cortical membranes by candesartan was not affected by the presence of 10^{-5} M HCTZ (Table 2).

Table 2. Lack of Effect of HCTZ on Candesartan Binding to AII Receptors

Candesartan Conc. (M)	[^{125}]-AII Specific Binding (%) to BAC[a]	
	- HCTZ (10^{-5} M)	+ HCTZ(10^{-5} M)
10^{-10}	90	92
10^{-9}	90	90
10^{-8}	85	81
10^{-7}	42	40
10^{-6}	16	15

[a] BAC= Bovine adrenal cortex membranes

PHARMACOKINETICS/TOXICOKINETICS

Pharmacokinetics and Protein Binding

Pharmacokinetic Interaction Between Candesartan Cilexetil and HCTZ in Rats and Dogs (Vol. 11, pg 168)

Male Jcl:Wistar rats (245-256 gm) and male Beagle dogs (6.5-7.5 kg) were administered candesartan cilexetil (1 mg/kg), hydrochlorothiazide (10 mg/kg) or a combination of candesartan cilexetil/HCTZ (1/10 mg/kg) as suspensions in 5% gum arabic solution. After dosing, serial blood samples were collected for up to 24 hours from the tail vein in rats and cephalic vein in dogs and analyzed for candesartan cilexetil metabolite, candesartan, or hydrochlorothiazide using HPLC techniques.

Pharmacokinetic values for candesartan and HCTZ, when candesartan cilexetil and HCTZ were administered alone or as a combination, are shown in Table 3. After co-administration of candesartan cilexetil and HCTZ to rats, the concentrations of candesartan and HCTZ in the plasma were similar to the concentrations observed after oral dosing of the individual drugs. These results indicate that the pharmacokinetic behavior of candesartan after dosing of candesartan cilexetil is not affected by the coadministration of HCTZ and the reverse is also true.

Table 3. Pharmacokinetics of Candesartan and HCTZ in Rats.

Treatment	Compound Measured	Pharmacokinetic Values (n=3)			
		Tmax, hr	Cmax, µg/ml	$t_{1/2}$, hr	AUC_{0-24}, µg.h/ml
Candesartan cilexetil (1 mg/kg)	Candesartan	1.0	0.399	3.6	2.01
HCTZ (10 mg/kg)	HCTZ	1.0	0.848	2.3	4.09
Candesartan cilexetil /HCTZ (1/10 mg/kg)	Candesartan HCTZ	1.0 1.3	0.344 0.711	3.5 2.4	2.07 3.78

After coadministration of candesartan cilexetil and HCTZ to dogs, the $t_{1/2}$ (4.4 hr) and the AUC (0.055 µg.h/ml) of candesartan in plasma were slightly larger than after dosing with candesartan cilexetil alone (Table 4). Also, the $t_{1/2}$ and the AUC of HCTZ after administration of candesartan cilexetil/HCTZ were smaller than after dosing with HCTZ alone. Thus, in this study, it appears that the elimination of candesartan from the circulation after co-administration tended to be slightly slower than after dosing with candesartan cilexetil alone; the reverse was observed in the elimination of HCTZ, which tended to be eliminated slightly faster than after dosing with HCTZ alone.

Table 4. Pharmacokinetics of Candesartan and HCTZ in Dogs.

Treatment	Compound Measured	Pharmacokinetic Values (n=4)			
		Tmax, hr	Cmax, µg/ml	$t_{1/2}$, hr	AUC_{0-24}, µg.h/ml
Candesartan cilexetil (1 mg/kg)	Candesartan	2.5	0.008	3.5	0.046
HCTZ (10 mg/kg)	HCTZ	3.8	1.224	6.0	14.8
Candesartan cilexetil /HCTZ (1/10 mg/kg)	Candesartan HCTZ	2.3 3.5	0.008 1.327	4.4 4.4	0.055 12.1

Protein Binding to Human Serum Albumin (Vol. 11, pg 153)

In vitro experiments were conducted to determine the effect of HCTZ on the binding of candesartan metabolites, candesartan and M-2, to human serum albumin and the effect of the metabolites on the protein binding of HCTZ. The concentrations of HCTZ, candesartan and M-2 studied were established fron. Cmax levels seen in human serum with the proposed clinical doses of HCTZ and candesartan cilexetil. No interaction between HCTZ and candesartan metabolites, candesartan and M-2, on protein binding was seen (Tables 5-6).

Table 5. Effect of HCTZ on Candesartan* and M-2* Binding to Human Serum Albumin

Compound	HCTZ Conc, µg/ml	Conc. Of Free Candesartan or Metabolite M-2, ng/ml	Relative conc. Of Free Fraction, %
Candesartan	0	1.19	100
	0.2	1.14	96
	2.0	1.13	96
Metabolite M-2	0	1.98	100
	0.2	1.99	100
	2.0	1.95	98

* Candesartan or M-2 concentrations= 0.2 µg/ml

Table 6. Effect of Candesartan and Metabolite M-2 on HCTZ* Binding to Human Serum Albumin

Compound	M-1 or M-2 Conc, µg/ml	Conc. Of Free HCTZ ng/ml	Relative conc. Of Free Fraction, %
Candesartan	0	71.5	100
	0.2	71.4	100
	2.0	71.8	100
Metabolite M-2	0	67.0	100
	0.04	68.2	102
	0.4	68.2	102

* HCTZ concentration = 0.2 µg/ml

Toxicokinetics

When candesartan cilexetil/HCTZ was given to rats in the 4- and 13-week toxicity studies, the Cmax and AUC values for candesartan increased with increasing doses (Tables 7, 8 & 10). Within a given dose group, the Cmax and AUC values for candesartan and HCTZ were similar for males and females, indicating no sex differences in candesartan or HCTZ pharmacokinetics (Tables 7-11). The toxicokinetics of HCTZ were not affected by increasing doses of candesartan cilexetil (Tables 9 &11). Furthermore, both Cmax and AUC values for candesartan and HCTZ determined at the first dosing period for a given dose group were almost equal to those determined near the end of the 4 and 13 week studies, indicating a lack of accumulation of either agent following repeated dosing (Tables 7-11).

Table 7. Toxicokinetics of Candesartan and HCTZ After Administration of Candesartan Cilexetil/HCTZ to Rats. (4-Week Oral Toxicity Study # 1776/SU)

Dose Group, mg/kg	Dosing Day	Candesartan PK in Males (n=3)			Candesartan PK in 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
Candesartan cilexetil/HCTZ, 3/10	1	0.5	0.65	4.4	0.5	0.75	3.9
	24	0.5	0.70	4.9	0.5	0.97	5.3
30/10	1	0.5	9.48	43.0	0.5	11.2	55.6
	24	0.5	8.97	50.5	0.5	8.24	54.3
300/10	1	0.5	31.0	407.5	0.5	38.9	453.5
	24	4.0	30.4	479.5	0.5	38.0	539.7
Candesartan cilexetil, 300	1	0.5	37.8	471.5	2.0	32.8	439.4
	24	2.0	32.6	420.2	0.25	25.5	242.5
Dose Group, mg/kg	Dosing Day	HCTZ PK in Males (n=3)			HCTZ PK in 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
Candesartan cilexetil/HCTZ, 3/10	1	0.25	0.43	3.9	2.0	0.42	2.6
	24	0.25	0.53	3.8	0.25	0.65	4.1
30/10	1	0.25	0.42	3.2	0.25	0.40	3.0
	24	1.0	0.75	5.4	1.0	1.0	5.3
300/10	1	2.0	0.35	3.2	2.0	0.34	2.6
	24	1.0	0.43	4.3	1.0	0.69	6.8
HCTZ, 10	1	2.0	0.51	3.5	2.0	0.52	3.4
	24	0.25	0.61	3.7	2.0	0.67	3.8

Table 8. Toxicokinetics of Candesartan after Administration of Candesartan Cilexetil/HCTZ to Rats. (13-Week Oral Toxicity Study # B-2935)

CC/HCTZ Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0-24\,h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0-24\,h}$ µg.hr/ml
1/10	1	0.5	0.49	1.62	0.5	0.44	1.81
	23	1	0.15	0.61	1	0.12	0.56
	86	1	0.25	0.83	1	0.21	0.75
10/10	1	0.5	2.91	8.83	0.5	2.87	8.69
	23	1	1.28	5.34	1	1.35	5.68
	86	1	2.57	9.35	1	2.58	9.18
100/10	1	0.5	17.40	75.13	0.5	19.54	89.89
	23	2	9.84	44.90	0.5	11.15	46.81
	86	2	16.45	72.75	1	16.36	65.23

Table 9. Toxicokinetics of HCTZ after Administration of Candesartan Cilexetil/HCTZ to Rats. (13-Week Oral Toxicity Study # B-2935)

CC/HCTZ Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0-24\,h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0-24\,hr}$ ug.hr/ml
1/10	1	1	0.450	3.05	1	0.446	2.38
	23	2	0.573	3.08	2	0.500	2.72
	86	2	0.868	4.27	0.25	0.768	4.05
10/10	1	1	0.464	2.66	1	0.471	2.31
	23	2	0.614	3.57	2	0.599	2.64
	86	2	0.931	4.42	1	0.893	3.72
100/10	1	2	0.423	2.69	2	0.470	2.30
	23	2	0.573	3.01	2	0.707	3.78
	86	2	1.010	4.95	1	1.038	3.26

Table 10. Toxicokinetics of Candesartan after Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ to Rats. (13-Week Oral Toxicity Study # B-3156)

Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\ h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\ h}$ ug.hr/ml
Candesartan cilexetil 100	1	0.5	17.9	83.8	0.5	20.3	88.6
	23	1	15.1	62.2	1	14.6	60.4
	86	1	22.1	92.9	1	19.4	72.5
C. cilexetil/HCTZ 100/30	1	0.5	18.5	82.2	1	20.3	89.8
	23	2	15.6	72.0	1	11.7	66.7
	86	1	17.9	85.7	1	15.7	67.9

Table 11. Toxicokinetics of HCTZ after Administration of HCTZ or Candesartan Cilexetil/HCTZ to Rats. (13-Week Oral Toxicity Study # B-3156)

Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\ h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\ h}$ ug.hr/ml
HCTZ 30	1	2	1.98	10.5	1	1.95	10.5
	23	2	2.19	11.9	1	2.17	13.2
	86	2	1.99	11.1	2	2.07	11.3
C. Cilexetil/HCTZ 100/30	1	2	2.38	12.5	2	2.52	11.3
	23	2	2.84	16.6	2	3.40	18.8
	86	1	2.39	11.9	1	2.39	9.5

In the 4-week toxicity study in dogs, the Cmax and AUC values of the parent compound, candesartan cilexetil, after administration of candesartan cilexetil/HCTZ, were comparable to values seen after the administration of candesartan cilexetil alone (Table 12). When candesartan cilexetil/HCTZ was given to dogs in the 4-and 13 week toxicity studies, the Cmax and AUC values for candesartan increased with increasing doses (Tables 13, 15 & 17). At doses (≤ 20/10 mg/kg) of the candesartan cilexetil/HCTZ combination which did not affect survival, HCTZ did not interfere with the kinetics of candesartan, and candesartan cilexetil did not influence the toxicokinetics of HCTZ (Tables 13-18). No apparent sex differences in Cmax and AUC for HCTZ and candesartan were observed (Tables 13-18).

Table 12. Toxicokinetics of Candesartan Cilexetil After Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ to Dogs. (4-Week Oral Toxicity Study # 1777/SU)

Dose Group, mg/kg	Dosing Day	Males (n=3)			Females (n=3)		
		Tmax (hr)	Cmax (µg/ml)	$AUC_{0\text{-}24}$ (µg.hr/ml)	Tmax (hr)	Cmax (µg/ml)	$AUC_{0\text{-}24}$ (µg.hr/ml)
Candesartan cilexetil/HCTZ,							
4/10	1	1	0.001	0.00	24	0.001	0.02
20/10	1	2	0.022	0.05	1	0.023	0.05
100/10	1	0.5	0.082	0.10	0.5	0.085	0.23
Candesartan cilexetil,							
4	1	0.5	0.003	0.03	8	0.002	0.03
20	1	2	0.035	0.08	1	0.022	0.06
100	1	1	0.082	0.21	1	0.097	0.23

Table 13. Toxicokinetics of Candesartan After Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ in Dogs. (4-Week Oral Toxicity Study # 1777/SU)

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)
Candesartan cilexetil/HCTZ,							
4/10	1	1	0.13	0.9	2	0.15	1.3
	23	1	0.17	1.1	1	0.27	2.1
20/10	1	2	0.56	3.5	1	0.58	3.9
	23	1	1.3	7.0	1	0.90	5.0
100/10	1	1	1.0	6.0	2	1.2	9.0
	23	4	2.7*	21*	-**	-**	-**
Candesartan cilexetil,							
4	1	1	0.13	0.9	0.5	0.12	0.5
	23	1	0.16	1.1	1	0.31	1.4
20	1	1	0.42	2.8	1	0.70	3.0
	23	2	1.1	6.0	1	1.4	9.0
100	1	1	1.4	8.0	2	1.7	8.0
	23	2	1.2	7.0	4	2.5	19.0

* Two surviving males. ** No surviving females.

Table 14. Toxicokinetics of HCTZ After Administration of HCTZ or Candesartan Cilexetil/HCTZ in Dogs. (4-Week Oral Toxicity Study # 1777/SU)

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
Candesartan cilexetil/HCTZ,							
0/10	1	2	4.5	20	1	5.8	28
	23	2	4.3	19	1	5.9	24
4/10	1	1	3.4	14	1	5.1	18
	23	1	3.7	16	1	5.1	17
20/10	1	1	3.9	18	1	3.5	17
	23	2	4.5	21	1	6.0	20
100/10	1	1	4.1	19	2	5.6	24
	23	2	6.8*	90*	-**	-**	-**

* Values from 2 surviving males. ** No values; no surviving females.

Table 15. Toxicokinetics of Candesartan After Administration of Candesartan Cilexetil/HCTZ in Dogs. (13-Week Oral Toxicity Study #B-2936).

C. Cilexetil/HCTZ Dose 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)
0.8/10	1	1	23.2	170	2	14.9	141
	28	4	18.9	214	1	13.9	93
	91	1	21.5	245	4	21.5	167
4/10	1	1	95.5	674	1	206.6	995
	28	2	86.3	580	2	136.1	1448
	91	1	99.9	1031	1	161.3	1219
20/10	1	1	575.7	3292	2	364.5	3572
	28	0.5	367.7	3019	4	702.2	10197
	91	1	625.1	5055	4	353.4[a]	3417[a]

[a] value from one surviving female

Table 16. Toxicokinetics of HCTZ After Oral Administration of Candesartan Cilexetil/HCTZ to Dogs. (13-Week Oral Toxicity Study # B-2936)

C. Cilexetil/HCTZ 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)
0.8/10	1	2	2.95	15.2	1	3.99	17.4
	28	2	2.96	14.6	2	2.72	15.9
	91	2	2.45	12.1	2	3.51	15.4
4/10	1	2	3.66	16.2	1	3.51	15.3
	28	2	2.67	13.0	2	2.68	13.3
	91	1	3.19	14.6	1	4.17	15.9
20/10	1	2	3.65	14.7	2	2.03	14.5
	28	1	2.37	12.5	4	7.98	94.0
	91	1	4.25	14.7	2	3.00[a]	15.5[a]

[a] Value from one surviving female

Table 17. Toxicokinetics of Candesartan After Oral Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ to Dogs. (13-Week Oral Toxicity Study # B-3156)

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 18. Toxicokinetics of HCTZ After Oral Administration of HCTZ or Candesartan Cilexetil/HCTZ to Dogs. (13-Week Oral Toxicity Study # B-3157)

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

SINGLE DOSE TOXICITY

Single Oral Dose Toxicity Study in Rats (Vol. 11, pg 124)

Study Facility: Takeda Chemical Industries, Ltd., Osaka, Japan
Study No: 1753/AC
Study Date: Initiation of dosing- 2/23/94.

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

Animals: Male and female F344/Jcl rats (M=108-124 gm; F=89-105 gm). Animals were housed 5/sex/cage and fed (*ad libitum*) a solid diet (CE-2, [redacted]

Drug Administration: Candesartan cilexetil (Lot #M464-035) and hydrochlorothiazide (Lot # M373-001) were suspended in 5% gum arabic aqueous solution and administered orally as a single dose by gavage.

Dose Levels:

Treatment Group	#/Sex/Group	Oral Dose (mg/kg)
Vehicle (5% gum arabic soln.)	5	20 ml/kg
HCTZ	5	1000
Candesartan cilexetil	5	2000
Candesartan cilexetil/HCTZ	5	1000/1000
Candesartan cilexetil/HCTZ	5	2000/1000

Observations/Measurements: Animals were observed for mortality and clinical signs of toxicity frequently during the first 6 hours after dosing and then once or twice daily during the remainder of the 14-day observation period. Body weights were measured prior to dosing and 2, 6, 9 and 14 days after dosing. At the end of the observation period, the animals were sacrificed and examined macroscopically for external and visceral lesions.

Results

Mortality and Clinical Signs
No animals died during the study and no clinical signs of toxicity were noted among treated or control animals.

Body Weight
Body weight was unaffected by drug treatment.

Macroscopic Examination
At necropsy no external or visceral abnormalities were observed.

REPEATED DOSE TOXICITY

4-Week Oral Dose Toxicity Study of Candesartan cilexetil/HCTZ in Rats (Vol. 11, pg 148)

Study Facility: Takeda Chemical Industries, Ltd., Osaka, Japan
Study No: 1776/SU
Study Date: Initiation of Dosing 5/27/94

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

Animals: Male and female F344/DuCrj rats (M=108-125 gm; F=87-99 gm). The animals were housed individually and fed (*ad libitum*) a solid diet (CE-2,

Drug Administration: Candesartan cilexetil (Lot #M464-039) and hydrochlorothiazide (Lot # M373-001) were suspended in 5% gum arabic aqueous solution and administered orally by gavage daily for 4 weeks.

Dose Levels:

Main Study

Treatment Group	#/Sex/Group	Oral Dose (mg/kg/day)
Vehicle (5% gum arabic soln.)	10	10 ml/kg
HCTZ	10	10
Candesartan cilexetil	10	300
Candesartan cilexetil/HCTZ	10	3/10
Candesartan cilexetil/HCTZ	10	30/10
Candesartan cilexetil/HCTZ	10	300/10

An additional 4/sex/dose group were used for determination of plasma drug concentrations of candesartan and HCTZ.

Observations/Measurements: All animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were measured prior to treatment, twice weekly during the dosing period and at necropsy. Food consumption was measured weekly. Ophthalmoscopic examinations were performed for 5 animals of each sex prior to treatment and on day 22 of the dosing period. Urine output and water intake were measured for a 24-hr period for 5/sex/group on treatment day 27. Urinalysis was performed on samples of urine collected. After the last dose, the animals were anesthetized and blood withdrawn from the abdominal aorta for hematology and blood chemistry analyses. Animals were examined for gross pathology and sections of tissues from major organs were examined for histopathology (Appendix A). Venous blood samples were obtained from animals in the satellite groups after the 1st and 24th dose (at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after dosing) for determination of plasma drug concentrations.

Results:

Mortality and Clinical Signs

No animals died during the study and no clinical signs of toxicity were associated with treatment.

Body Weight, Food Consumption, Water Intake and Urine Output

Lower than control body weights and food consumption were noted in rats treated with 300 mg candesartan cilexetil/kg and with all doses of candesartan cilexetil/HCTZ beginning on treatment day 7. Both water intake and urine output were increased in groups treated with 300 mg candesartan cilexetil, 30/10 and 300/10 mg candesartan cilexetil/HCTZ /kg (Table 19).

Table 19. Mean Body Weight, Food Consumption, Water Intake and Urine Output

Parameter	Sex	Control Value	% Difference From Control Value				
			HCTZ-10	C-300	C/HCTZ 3/10	C/HCTZ 30/10	C/HCTZ 300/10
Terminal Mean Body Weight, gm	M	220	-5.9	-12.3*	12.3*	-20.5*	-17.7*
	F	138	-2.2	-8.7*	-9.4*	-13.8*	-18.1*
Food Consumption gm/rat/wk	M	85	-5.9	-15.3*	-8.2*	-16.5*	-18.8*
	F	64	-6.3	-13.8*	-10.9*	-18.8*	-17.2*
Water Intake ml/day	M	21	-4.8	+47.6*	+9.6	+47.6*	+57.1*
	F	14	+21.4	+64.3*	+28.6	+71.4*	+71.4*
Urine Output gm/day	M	10.7	+1.9	+68.2*	+17.7	+79.4*	+118.7*
	F	6.1	+32.7	+103.3*	+73.8*	+141.0*	+163.9*

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

Ophthalmology

Ophthalmoscopic examinations revealed no treatment related abnormalities.

Hematology and Clinical Chemistry

Lower than control hematologic values were observed in males and females treated with 300 mg candesartan cilexetil and 30/10 and 300/10 candesartan cilexetil/HCTZ. Blood urea nitrogen, creatinine and alkaline phosphatase (ALP) were significantly higher than control in males and females treated with 300 mg candesartan cilexetil/kg and with mid and high doses of candesartan cilexetil/HCTZ. Lower than control plasma levels of aspartate aminotransferase and alanine aminotransferase were noted in male rats treated with 300 mg candesartan cilexetil/kg and 30/10 and 300/10 mg candesartan cilexetil/HCTZ (Table 20).

Table 20. Hematology and Blood Chemistry Findings

Parameter	Sex	Control Value	% Difference From Control Value				
			HCTZ-10	C-300	C/HCTZ 3/10	C/HCTZ 30/10	C/HCTZ 300/10
Hematology							
RBC Count, X $10^4/\mu l$	M	903	+1.0	-13.6*	-0.9	-12.2*	-16.4*
	F	891	+2.7	-14.5*	-0.9	-10.1*	-11.4*
Hematocrit, %	M	49.7	0.0	12.9*	-2.0	-13.1*	-17.1*
	F	48.7	+1.4	15.8*	-0.8	-12.5*	-14.4*
Hemoglobin, g%	M	16.2	0.0	11.7*	-0.6	-11.7*	-15.4*
	F	16.2	1.2	14.8*	0.0	-30.6*	-33.9*
Blood Chemistry							
Urea Nitrogen	M	18.0	+8.3	+231*	+48.3	+398*	+507*
	F	18.8	+11.2	+249*	+63.8	+413*	+547*
Creatinine	M	0.5	0.0	+20.0	0.0	+20.0	+60.0*
	F	0.5	0.0	+20.0	0.0	+40.0*	+80.0*
ALP	M	465	+1.9	+7.7	+10.1*	+21.7*	+25.6*
	F	367	+10.1	+11.4	+25.6*	+37.6*	+31.6*
AST	M	57	+8.8	-12.3	0.0	-10.5*	-10.5*
	F	60	+11.7*	-5.0	+1.6	-3.3	-3.3
ALT	M	31	+3.2	-19.4*	-9.7*	-22.6*	-25.8*
	F	27	+7.4	0.0	-3.7	-11.1	-3.7

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

Organ Weights

Absolute heart weights in candesartan cilexetil and candesartan cilexetil/HCTZ treated animals were significantly lower than control. Relative heart weights were significantly lower than control among candesartan cilexetil-treated males and females but only in candesartan cilexetil/HCTZ-treated females. Higher than control relative adrenal gland weights were noted in candesartan cilexetil and candesartan cilexetil/HCTZ treated animals; the differences seen in absolute weights did not achieve statistical significance (Table 21).

Table 21. Organ Weights

Organ	Sex	Mean Organ Weights Among Treatment Groups					
		Vehicle	HCTZ-10	C-300	C/HCTZ 3/10	C/HCTZ 30/10	C/HCTZ 300/10
Heart							
Absolute wt., g	M	0.66	0.63	0.51**	0.55**	0.49**	0.49**
	F	0.46	0.45	0.37**	0.37**	0.35**	0.33**
Relative wt, g/kg	M	0.33	0.33	0.29**	0.31	0.31	0.30
	F	0.36	0.36	0.33**	0.32**	0.33**	0.32**
Adrenal Glands							
Absolute wt., mg	M	38.7	38.8	44.2**	40.3	38.2	41.6
	F	45.8	45.5	45.8	46.8	42.7	39.4*
Relative wt., mg/kg	M	19.2	20.6	25.4**	23.0**	24.3**	25.6**
	F	35.6	36.5	40.1*	40.5*	39.4*	38.1

Significantly different from control value * ($p<0.05$) ** ($p<0.01$)

Macroscopic and Microscopic Pathology

Gross examination revealed no treatment-related external or visceral abnormalities. Histopathologic examination showed a higher than control incidence of basophilic renal tubules in rats treated with 300 mg candesartan cilexetil/kg and all doses of candesartan cilexetil/HCTZ. Hypertrophy of the J-G cells of the kidney was noted in rats treated with candesartan cilexetil and 30/10 and 300/10 mg candesartan cilexetil/HCTZ. Higher than control incidences of atrophy of the adrenal zona glomerulosa was seen in males and females treated with candesartan cilexetil and 30/10 and 300/10 mg candesartan cilexetil/HCTZ (Table 22).

Table 22. Histopathology Findings

Histopathology Finding	Sex	Lesion Incidence (# rats with lesion/#rats examined)					
		Vehicle	HCTZ-10	C-300	C/HCTZ 3/10	C/HCTZ 30/10	C/HCTZ 300/10
Kidney							
Basophilic Renal Tubule	M	1/10	1/10	7/10	2/10	6/10	10/10
	F	3/10	3/10	5/10	4/10	3/10	8/10
J-G Cell Hypertrophy	M	0/10	0/10	9/10	6/10	10/10	10/10
	F	0/10	0/10	9/10	8/10	9/10	10/10
Adrenal Gland							
Zona glomerulosa atrophy	M	0/10	0/10	10/10	0/10	6/10	8/10
	F	0/10	0/10	10/10	2/10	10/10	10/10

Toxicokinetics

No pharmacokinetic interactions between candesartan cilexetil, and HCTZ were observed when the Cmax and AUC for candesartan or HCTZ in the 300/10 candesartan cilexetil/HCTZ group was compared with the values in the candesartan cilexetil 300 mg/kg or HCTZ 10 mg/kg groups. The Cmax and AUC parameters of candesartan and HCTZ after 24 dosing days were substantially the same as after the 1st dose (Table 23).

Table 23. Toxicokinetics of Candesartan and HCTZ After Administration of Candesartan Cilexetil/HCTZ to Rats.

Dose Group, mg/kg	Dosing Day	Candesartan PK in Males (n=3)			Candesartan PK in 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
Candesartan cilexetil/HCTZ,							
3/10	1	0.5	0.65	4.4	0.5	0.75	3.9
	24	0.5	0.70	4.9	0.5	0.97	5.3
30/10	1	0.5	9.48	43.0	0.5	11.2	55.6
	24	0.5	8.97	50.5	0.5	8.24	54.3
300/10	1	0.5	31.0	407.5	0.5	38.9	453.5
	24	4.0	30.4	479.5	0.5	38.0	539.7
Candesartan cilexetil, 300	1	0.5	37.8	471.5	2.0	32.8	439.4
	24	2.0	32.6	420.2	0.25	25.5	242.5
Dose Group, mg/kg	Dosing Day	HCTZ PK in Males (n=3)			HCTZ PK in 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
Candesartan cilexetil/HCTZ,							
3/10	1	0.25	0.43	3.9	2.0	0.42	2.6
	24	0.25	0.53	3.8	0.25	0.65	4.1
30/10	1	0.25	0.42	3.2	0.25	0.40	3.0
	24	1.0	0.75	5.4	1.0	1.0	5.3
300/10	1	2.0	0.35	3.2	2.0	0.34	2.6
	24	1.0	0.43	4.3	1.0	0.69	6.8
HCTZ, 10	1	2.0	0.51	3.5	2.0	0.52	3.4
	24	0.25	0.61	3.7	2.0	0.67	3.8

13-Week Oral Dose Toxicity Study of Candesartan cilexetil/HCTZ in Rats (Vol. 13, pg 10)

Study Facility: [redacted]
Study No: B-2935
Study Date: Initiation of dosing- 4/10/95; Necropsy- 7/11/95

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

Animals: Male and female F344/DuCrj rats (M=121-136gm; F=97-107 gm). The animals were housed individually and fed (*ad libitum*) a solid diet (CRF-1 [redacted]).

Drug Administration: Candesartan cilexetil (Lot #M464-043) and hydrochlorothiazide (Lot # M373-001) were suspended in 5% gum arabic aqueous solution and administered orally by gavage daily for 13 weeks.

Dose Levels:

Main Study

Treatment Group	#/Sex/Group	Oral Dose (mg/kg/day)
Vehicle (5% gum arabic soln.)	10	10 ml/kg
Candesartan cilexetil/HCTZ	10	1/10
Candesartan cilexetil/HCTZ	10	10/10
Candesartan cilexetil/HCTZ	10	100/10

An additional 4 rats/sex/dose group were used for determination of plasma drug concentrations of candesartan and HCTZ.

Observations/Measurements: All animals were observed 2 to 3 times daily for mortality and clinical signs of toxicity. Body weights were measured prior to treatment, twice weekly during the dosing period and at necropsy. Food consumption was measured weekly. Ophthalmoscopic examinations were performed for 5 animals of each sex prior to treatment and during week 13 of the dosing period. Urine was collected from 5/sex/group for a 24-hr period during week 5 and week 13 of the dosing period; urinalysis was performed on samples of urine collected.. At the time of necropsy, the animals were anesthetized and blood withdrawn from the abdominal aorta for hematology and blood chemistry analyses. Animals were examined for gross pathology, major organs removed and weighed and sections from major organs and tissues were prepared for microscopic examination. Of these, all organs and tissues from the control and high dose groups and kidneys, adrenals and stomach (in which drug-related effects were suspected) from the low and middle dose groups were examined histopathologically (Appendix A). Venous blood samples were obtained from animals in the satellite groups after the 1st and 24th dose (at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after dosing) for determination of plasma candesartan and HCTZ concentrations.

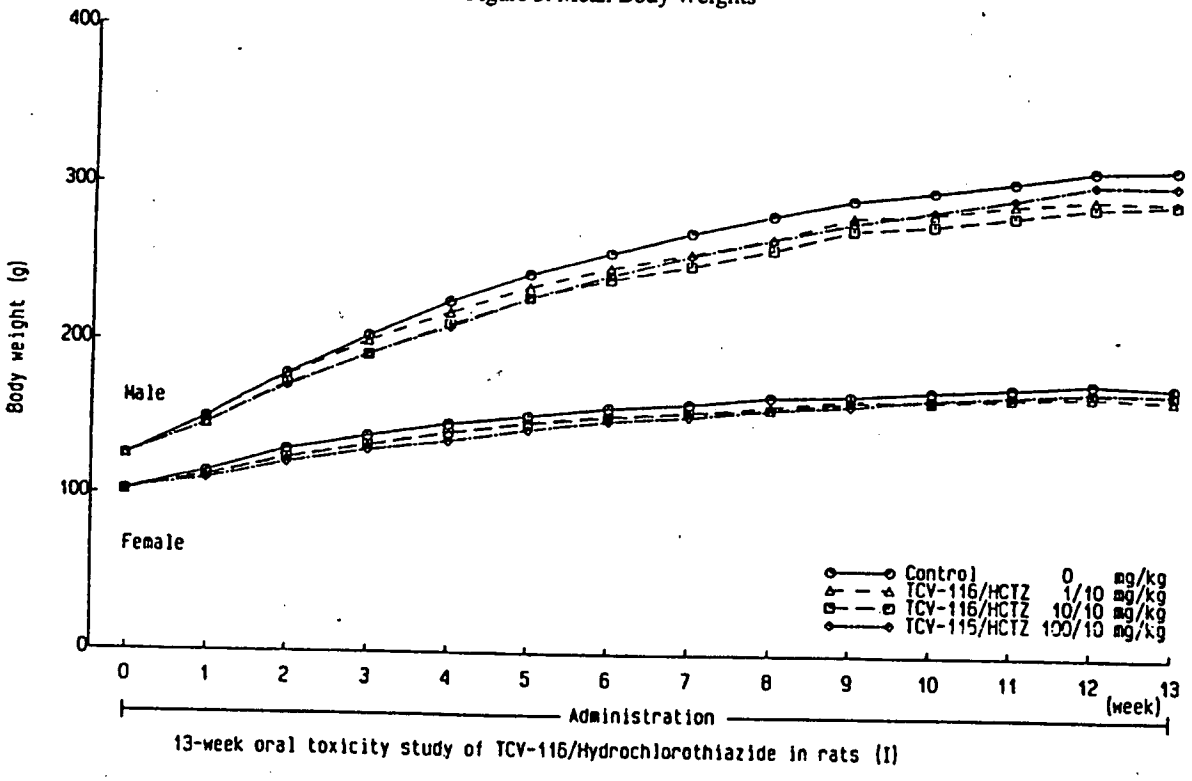
Results:

Mortality and Clinical Signs
No deaths were observed in any of the test groups and no clinical signs of toxicity were detected.

Body Weight and Food Consumption

Lower (up to 8% in males and up to 7% in females) than control mean body weights were noted among candesartan cilexetil/HCTZ groups on various treatment days beginning day 7 in females and day 10 in males. For males, the body weight decrements were larger at the mid-dose than at the high dose; body weight decrements among treated females appeared to be comparable for all treated dose groups (Fig 3). Except on isolated treatment days in which lower than control food consumption was noted (day 7 in high dose males and days 7 and 14 in high dose females) food consumption among treated animals was comparable to control.

Figure 3. Mean Body Weights



Ophthalmology

Ophthalmic examinations showed no treatment-related abnormalities.

Urinalysis

Greater than control urine volume was noted in treated males and females from all dose groups. The increased urine output was generally dose-dependent and was accompanied by increased excretion of electrolytes. The increased urine output among treated animals was associated with higher than control water intake (Table 24).

Table 24. Water Intake and Urinalysis

Parameter	Sex	Treatment Week 5				Treatment Week 13			
		Control 0	C/Hctz 1/10	C/Hctz 10/10	C/Hctz 100/10	Control 0	C/Hctz 1/10	C/Hctz 10/10	C/Hctz 100/10
Water Intake, ml/rat/24hr	M	25	26	29	37*	18	23*	29*	29
	F	27	28	32	28	22	24	25	24
Urine Volume, ml/24 hrs	M	[illegible]	10.7*	11.2*	12.6*	4.8	10.0*	14.0*	13.6*
	F	7.6	11.7	12.7*	11.4	7.3	11.5*	10.7	9.7
Sodium, mEq/24 hrs	M	1.07	1.77*	1.54	0.96	0.88	1.32	1.45	1.26
	F	1.44	1.64	1.50	0.85	1.16	1.56	0.88	1.11
Potassium, mEq/24 hrs	M	1.76	2.66*	2.44*	1.86	1.41	2.04	2.46*	2.33*
	F	2.42	2.44	2.42	1.65	2.06	2.23	1.76	1.75
Chloride, mEq/24 hrs	M	1.38	2.25*	1.91	1.30	1.13	1.68	1.81*	1.73*
	F	1.74	2.17	1.91	1.14	1.49	1.85	1.27	1.39

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

Hematology and Clinical Chemistry

Lower than control RBC counts, hemoglobin, hematocrit were noted in the mid and high dose males and/or females treated with candesartan cilexetil/HCTZ. Prothrombin and activated partial thromboplastin times in the 100/10 mg/kg/day male and female groups were significantly shortened compared to control (Table 25).

Table 25. Hematology Findings

Parameter	Sex	Dose Group			
		Control 0	C/Hctz 1/10	C/Hctz 10/10	C/Hctz 100/10
RBC, $x10^4/mm^3$	M	936	951	912	868*
	F	871	884	823*	805*
Hemoglobin, g/dl	M	16.7	16.8	16.4	15.8*
	F	16.9	17.0	16.0*	15.8*
Hematocrit, %	M	48	49	47	45*
	F	48	49	45*	45*
Mean Corpuscular Volume, μ^3	M	51.2	51.2	51.6	52.0*
	F	54.9	54.8	55.2	55.7*
Mean Corpuscular hemoglobin, pg	M	17.8	17.6	17.9	18.2*
	F	19.3	19.2	19.4	19.6
Prothrombin Time, sec	M	11.8	12.0	12.0	11.1*
	F	11.3	11.3	11.0	10.7*
Activated Partial Thromboplastin Time, sec	M	18.5	17.8	17.4	15.4*
	F	15.2	15.1	14.2*	13.8*

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

Significantly higher than control levels of blood urea nitrogen were observed in the 10/10 and 100/10 mg/kg/day male and female groups and significantly higher than control levels of creatinine were see in the 100/10 mg/kg/day male and female group. Lower than control levels of serum sodium, calcium and chloride were seen among treated male and female groups. Treated males and females also showed higher than control levels of inorganic phosphate and alkaline phosphatase activity (Table 25).

Table 25. Blood Chemistry Findings

Parameter	Sex	Dose Group			
		Control 0	C/Hctz 1/10	C/Hctz 10/10	C/Hctz 100/10
BUN, mg/dl	M	16	17	29*	49*
	F	19	20	37*	64*
Creatinine, mg/dl	M	0.65	0.61	0.66	0.76*
	F	0.63	0.62	0.65	0.71*
Glucose, mg/dl	M	139	136	139	137
	F	114	119	123*	124*
Sodium, mEq/l	M	146	144*	144*	144*
	F	145	142	142*	142*
Chloride, mEq/l	M	113	110*	110*	110*
	F	115	112*	112*	112*
Calcium, mg/dl	M	9.5	9.4	9.3*	9.3*
	F	9.1	9.1	8.8*	8.8*
Inorganic Phosphorus, mg/dl	M	6.2	6.3	7.0	7.7*
	F	5.2	5.9	6.5*	7.4*
ALP, IU/l	M	287	303	330*	306
	F	210	249*	255*	235*

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

Gross Pathology and Organ Weights

Macroscopic examination revealed white patches (bilateral) on the kidneys of 3 males in the 100/10 mg/kg/day group. Absolute and/or relative mean weights of a number or organs from treated animals differed from those of vehicle control. The most notable were lower than control absolute and relative heart weights and higher than control absolute and relative kidney weights for all male and female treated groups. (Table 26).

TABLE 4.119
Candesartan Cilexetil Formulations

Batch Numbers		Z5427061	Z5428041 Z5428061 Z5428062 Z5428071 Z5428083/ H1155-01-01-04 Z5428091/ H1155-01-01-03	Z5429041 Z5429061 Z5429062 Z5429071 Z5429081/ H1156-01-01-02 Z5429082 Z5429091/ H1156-01-01-03 Z5429101/ H1156-01-01-04 Z5429111/ H1156-01-01-05 Z5429121/ H1156-01-01-06 Z5429122/ H1156-01-01-08 Z5429131/ H1156-01-01-07 Z5429132/ H1156-01-01-10	Z542E081	Z542B021 Z542B031	H1191-01-01-03 H1191-01-01-04 H1191-01-01-09
Name of Ingredient	Function of Ingredient	Formula (mg/tablet)†	Formula (mg/tablet)†	Formula (mg/tablet)†	Formula (mg/tablet)†*	Formula (mg/tablet)†	Formula (mg/tablet)‡
	Active						
	Filler						
	Disintegrant						
	Lubricant						
	Binder						
	Lubricant						
	Disintegrant						
	Colorant						
	Colorant						

† Manufactured by Takeda, Japan for clinical supplies

‡ Manufactured by Astra Sweden for clinical supplies

* Two tablets (16 mg total) of this formulation were encapsulated and backfilled

and used in Study EC408

Table 26. Organ Weights

Parameter	Sex	Dose Group			
		Control 0	C/Hctz 1/10	C/Hctz 10/10	C/Hctz 100/10
Heart	M				
Absolute wt., gm		0.86	0.74*	0.70*	0.72*
Relative wt., %		0.29	0.27*	0.26	0.25*
Lung					
Absolute wt., gm		0.93	0.93	0.90	0.93
Relative wt., %		0.31	0.34*	0.33*	0.32
Liver					
Absolute wt., gm		7.49	6.89*	6.82*	7.22
Relative wt., %		2.50	2.49	2.48	2.51
Kidneys					
Absolute wt., gm		1.74	1.80	1.89*	2.04*
Relative wt., %		0.58	0.65	0.69*	0.71*
Adrenals					
Absolute wt., mg		47	50	49	50
Relative wt., %		16	18*	18*	17*
Testes					
Absolute wt., gm		2.80	2.83	2.77	2.72
Relative wt., %		0.94	1.02*	1.01*	0.95
Seminal Vesicles					
Absolute wt., gm		0.78	0.92*	0.85	0.89*
Relative wt., %		0.26	0.34*	0.31*	0.31*
Heart	F				
Absolute wt., gm		0.54	0.46*	0.49*	0.48*
Relative wt., %		0.33	0.30*	0.30*	0.30*
Lung					
Absolute wt., gm		0.69	0.66	0.67	0.66*
Relative wt., %		0.42	0.43	0.42	0.41
Liver					
Absolute wt., gm		3.65	3.49	3.60	3.77
Relative wt., %		2.21	2.23	2.26	2.37*
Kidneys					
Absolute wt., gm		1.10	1.16*	1.23*	1.30*
Relative wt., %		0.66	0.74*	0.78*	0.82*
Adrenals					
Absolute wt., mg		46	50	46	47
Relative wt., %		28	32*	29	29

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

Histopathology

Drug-related histopathology was noted in the kidneys, adrenals and stomach of males and females (Table 27).

Table 27. Histopathology Findings

Parameter	Sex	Dose Group Incidence (# affected/# examined)			
		Control 0	C/Hctz 1/10	C/Hctz 10/10	C/Hctz 100/10
Kidneys	M				
Basophilic renal tubules, moderate or severe		0/10	0/10	**1/10**	**3/10**
J-G cell hypertrophy		0/10	**9/10**	**10/10**	**10/10**
Intimal proliferation of interlobular arteries		0/10	0/10	**1/10**	**4/10**
Calcification of renal papilla		1/10	**3/10**	**3/10**	1/10
Adrenals					
Atrophy of zona glomerulosa		0/10	0/10	0/10	**10/10**
Stomach					
Erosion of glandular stomach mucosa		1/10	0/10	**1/10**	0/10
Kidneys	F				
Basophilic renal tubules		0/10	**2/10**	**8/10**	**10/10**
J-G cell hypertrophy		0/10	**9/10**	**10/10**	**10/10**
Intimal proliferation of interlobular arteries		0/10	0/10	0/10	**7/10**
Calcification of renal papilla		1/10	**2/10**	**5/10**	**4/10**
Adrenals					
Atrophy of zona glomerulosa		0/10	0/10	0/10	**10/10**
Stomach					
Erosion of glandular stomach mucosa		0/10	0/10	0/10	**2/10**

Bold values indicate an incidence rate higher than control

Toxicokinetics

Cmax and $AUC_{0\text{-}24h}$ of candesartan increased with increasing doses of candesartan cilexetil/HCTZ. Within a given dose group, the Cmax and $AUC_{0\text{-}24h}$ values for candesartan were similar for males and females indicating no sex differences in candesartan pharmacokinetics. In addition, the Cmax and $AUC_{0\text{-}24h}$ values for HCTZ were nearly equal regardless of the dose levels of candesartan cilexetil. Although the Cmax and $AUC_{0\text{-}24h}$ values for HCTZ tended to be higher in week 13 than in week 1, no evidence of candesartan accumulation due to repeated dosing was detected (Tables 28 and 29).

Table 28. Toxicokinetics of Candesartan after Administration of Candesartan Cilexetil/HCTZ to Rats.

CC/HCTZ Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\,h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\,h}$ ug.hr/ml
1/10	1	0.5	0.49	1.62	0.5	0.44	1.81
	23	1	0.15	0.61	1	0.12	0.56
	86	1	0.25	0.83	1	0.21	0.75
10/10	1	0.5	2.91	8.83	0.5	2.87	8.69
	23	1	1.28	5.34	1	1.35	5.68
	86	1	2.57	9.35	1	2.58	9.18
100/10	1	0.5	17.40	75.13	0.5	19.54	89.89
	23	2	9.84	44.90	0.5	11.15	46.81
	86	2	16.45	72.75	1	16.36	65.23

Table 29. Toxicokinetics of HCTZ after Administration of Candesartan Cilexetil/HCTZ to Rats.

CC/HCTZ Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0-24\ h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0-24\ hr}$ ug.hr/ml
1/10	1	1	0.450	3.05	1	0.446	2.38
	23	2	0.573	3.08	2	0.500	2.72
	86	2	0.868	4.27	0.25	0.768	4.05
10/10	1	1	0.464	2.66	1	0.471	2.31
	23	2	0.614	3.57	2	0.599	2.64
	86	2	0.931	4.42	1	0.893	3.72
100/10	1	2	0.423	2.69	2	0.470	2.30
	23	2	0.573	3.01	2	0.707	3.78
	86	2	1.010	4.95	1	1.038	3.26

13-Week Oral Dose Toxicity Study of Candesartan cilexetil/HCTZ in Rats (Vol. 14, pg 10)

Note: The purpose of this 13-week oral dose toxicity study was to assess the toxicity of candesartan cilexetil/HCTZ in rats at a dose of HCTZ of 30 mg/kg/day, which was 3 times the dose level used in the previous study (Study # B-2935). Only one dose level (100/30 mg/kg/day) of the candesartan cilexetil/HCTZ combination was administered and used to compare any observed tonicities with those elicited by 100/10 mg/kg candesartan cilexetil/HCTZ in the previous study.

Study Facility:
Study No: B-3156
Study Dates: Initiation of dosing 11/20/95; Necropsy 2/20/96

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

Animals: Male and female F344/DuCrj rats (M=116-133gm; F=93-105 gm). The animals were housed individually and fed (*ad libitum*) a solid diet (

Drug Administration: Candesartan cilexetil (Lot #M464-043) and hydrochlorothiazide (Lot # M373-001) were suspended in 5% gum arabic aqueous solution and administered orally by gavage daily for 13 weeks.

Dose Levels:

Main Study

Treatment Group	#/Sex/Group	Oral Dose (mg/kg/day)
Vehicle (5% gum arabic soln.)	10	10 ml/kg
HCTZ	10	30
Candesartan cilexetil	10	100
Candesartan cilexetil/HCTZ	10	100/30

An additional 4 rats/sex/dose group were used for determination of plasma drug concentrations of candesartan and HCTZ.

Observations/Measurements: All animals were observed 2 to 3 times daily for mortality and clinical signs of toxicity. Body weights were measured prior to treatment, twice weekly during

the dosing period and at necropsy. Food consumption was measured weekly. Ophthalmoscopic examinations were performed for 5 animals of each sex prior to treatment and during week 13 of the treatment period. Urinalysis was performed on samples of urine collected from 5 rats/sex/group for 24-hr periods during treatment weeks 5 and 13. At the time of necropsy, the animals were anesthetized and blood withdrawn from the abdominal aorta for hematology and blood chemistry analyses. Animals were examined for gross pathology, major organs removed and weighed, and sections from major organs and tissues (Appendix A) were prepared onto slides and examined histopathologically. Venous blood samples were obtained from animals in the satellite groups after the 1st dose, in week 4 (23rd dose) and week 13 (86th dose) at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after dosing for determination of plasma candesartan and HCTZ concentrations.

Results:

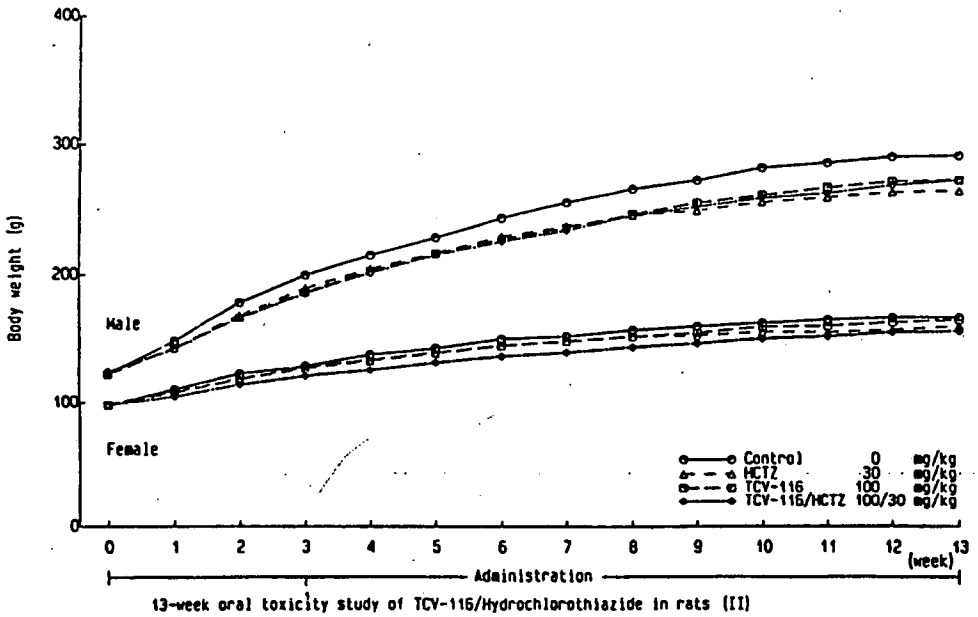
Mortality and Clinical Signs

No deaths were observed in any of the test groups and no clinical signs of toxicity were detected.

Body Weight and Food Consumption

Lower than control body weights (6% lower in males and 7 % lower in females at end of study) were noted among candesartan cilexetil/HCTZ groups on various treatment days beginning day 7 for females and day 10 for males. For candesartan cilexetil treated males, mean body weight at the end of the dosing period was 6^ lower than control; for candesartan cilexetil treated females, mean body weight was comparable to control. For HCTZ-treated males, mean body weight at the end of the dosing period was 9% lower than control; for females, mean body weight was 4 % lower than control (Fig 4). Except on isolated treatment days in which lower than control food consumption was noted (days 14 and 21 for males and days 14 and 28 for females), food consumption among candesartan cilexetil/HCTZ treated animals was comparable to control. Food consumption among HCTZ and candesartan cilexetil treated groups was comparable to control.

Figure 4. Mean Body Weights



Ophthalmology

No treatment-related ocular effects were observed.

Urinalysis

Greater than control urine volume was noted during weeks 5 and 13 in males treated with HCTZ or candesartan cilexetil/HCTZ, and during week 5 in males treated with only candesartan cilexetil. The increased urine output was generally accompanied by increased excretion of electrolytes and higher than control water intake. (Table 30).

Table 30. Water Intake and Urinalysis

Parameter	Sex	Treatment Week 5				Treatment Week 13			
		Control 0	Hctz 30	C. cilexetil 100	C/Hctz 100/30	Control 0	Hctz 30	C. cilexetil 100	C/Hctz 100/30
Water Intake, ml/rat/24hr	M	22	26*	27*	33*	19	26*	24*	34*
	F	23	23	25	29*	23	19	22	28*
Urine Volume, ml/24 hrs	M	4.1	8.6*	8.2*	12.5*	5.1	11.9*	6.9	14.7*
	F	6.2	7.3	7.0	8.5	7.0	6.9	6.5	11.3
Sodium, mEq/24 hrs	M	0.83	1.23*	0.79	0.96	0.85	1.50*	0.82	1.18
	F	1.18	0.93	0.79	0.73	1.13	1.05	0.85	0.84
Potassium, mEq/24 hrs	M	1.33	1.84*	1.46	1.66	1.34	2.15*	1.50	2.01
	F	1.66	1.40	1.28	1.22	1.63	1.54	1.47	1.52
Chloride, mEq/24 hrs	M	1.04	1.59*	1.06	1.24	1.01	1.87*	1.10	1.50
	F	1.40	1.21	0.96	0.97	1.38	1.43	1.13	1.17

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

Hematology and Clinical Chemistry

Lower than control RBC counts, hemoglobin and hematocrit were noted in males and females treated with candesartan or candesartan cilexetil/HCTZ. Shorter than control prothombin time was noted in males treated with candesartan cilexetil/HCTZ and shorter than control activated partial thromboplastin time was noted in females treated with candesartan cilexetil/HCTZ (Table 31).

Table 31. Hematology Findings

Parameter	Sex	Dose Group, mg/kg/day			
		Control 0	Hctz 30	C. cilexetil 100	C/Hctz 100/30
RBC, $x10^4/mm^3$	M	960	994	881*	860*
	F	887	893	832*	812*
Hemoglobin, g/dl	M	17.4	17.9*	16.5*	16.2*
	F	17.1	17.1	16.4*	16.2*
Hematocrit, %	M	49	51*	46*	45*
	F	48	47	45*	45*
Mean Corpuscular Volume, μ^3	M	51.3	51.4	52.8*	52.8*
	F	54.0	53.1*	54.4	55.0*
Mean Corpuscular hemoglobin, pg	M	18.1	18.0	18.7	18.9*
	F	19.3	19.1	19.7	20.0*
Prothrombin Time, sec	M	13.2	14.2	12.4	11.6*
	F	11.3	11.5	11.3	10.6
Activated Partial Thromboplastin Time, sec	M	18.6	20.8	18.3	14.5
	F	15.9	16.3	14.9	13.3*

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

Significantly higher than control levels of blood urea nitrogen were observed in males and females treated with candesartan cilexetil or candesartan cilexetil/HCTZ and significantly higher than control levels of creatinine were see in the candesartan cilexetil/HCTZ male and female groups. Lower than control levels of serum sodium and chloride were seen among male and female groups treated with HCTZ or candesartan cilexetil/HCTZ. Lower than control serum

calcium was seen in females treated with candesartan cilexetil or candesartan cilexetil/HCTZ. Males and females in the candesartan cilexetil/HCTZ group showed higher than control levels of inorganic phosphate and alkaline phosphatase activity (Table 32).

Table 32. Blood Chemistry Findings

Parameter	Sex	Dose Group			
		Control 0	Hctz 30	C. cilexetil 100	C/Hctz 100/30
BUN, mg/dl	M	22	24	33*	69*
	F	23	28	34*	95*
Creatinine, mg/dl	M	0.62	0.63	0.66	0.87*
	F	0.64	0.69	0.65	0.82*
Sodium, mEq/l	M	145	143*	143*	141*
	F	144	142*	141*	141*
Chloride, mEq/l	M	113	107*	112	109*
	F	115	109*	115	111*
Calcium, mg/dl	M	9.6	9.8	9.4	9.4
	F	9.4	9.4	9.2*	9.1*
Inorganic Phosphorus, mg/dl	M	6.0	5.8	6.8	8.0*
	F	5.7	5.6	6.0	7.9*
ALP, IU/l	M	303	308	334	358*
	F	231	270*	232	280*

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

Gross Pathology and Organ Weights

Macroscopic examination revealed white patches (bilateral) on the kidneys of 3 males and dark red spots on the glandular stomach of 2 males in the candesartan cilexetil/HCTZ group. A number of organs from treated animals had absolute and/or relative weights that differed from those of vehicle control (Table 33). The most notable were lower than control absolute and relative heart weights among males and females treated with candesartan cilexetil or candesartan cilexetil/HCTZ and higher than control absolute and relative kidney weights of animals treated with candesartan cilexetil/HCTZ (males and females) or candesartan cilexetil (females).

Table 33: Organ Weights

Parameter	Sex	Dose Group			
		Control 0	Hctz 30	C. cilexetil 100	C/Hctz 100/30
Terminal Body Weight, gm	M	272	245*	254*	253*
Heart					
Absolute wt., gm		0.82	0.72*	0.68*	0.69*
Relative wt., %		0.30	0.29	0.27*	0.27*
Lung					
Absolute wt., gm		0.91	0.88	0.96	0.90
Relative wt., %		0.34	0.36*	0.34	0.36
Liver					
Absolute wt., gm		6.61	5.96*	6.12	6.06*
Relative wt., %		2.43	2.43	2.41	2.39
Kidneys					
Absolute wt., gm		1.72	1.74	1.80	1.96*
Relative wt., %		0.63	0.71*	0.71*	0.78*
Adrenals					
Absolute wt., mg		42	43	44	44
Relative wt., %		16	18*	17	18*
Testes					
Absolute wt., gm		2.84	2.84	2.66	2.81
Relative wt., %		1.04	1.16*	1.06	1.11
Terminal Body Weight, gm	F	156	147*	152	142*
Heart					
Absolute wt., gm		0.55	0.50*	0.47*	0.45*
Relative wt., %		0.35	0.34	0.31*	0.31*
Lung					
Absolute wt., gm		0.66	0.68	0.66	0.64
Relative wt., %		0.42	0.47*	0.43	0.46*
Liver					
Absolute wt., gm		3.61	3.35*	3.59	3.51
Relative wt., %		2.32	2.28	2.36	2.47*
Kidneys					
Absolute wt., gm		1.09	1.11	1.18*	1.28*
Relative wt., %		0.70	0.75*	0.77*	0.90*
Adrenals					
Absolute wt., mg		45	45	44	45
Relative wt., %		29	31	29	32

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

Histopathology

Drug-related histopathology was noted in the kidneys, adrenals and stomach of males and females (Table 34).). The incidence of intimal proliferation of renal interlobular arteries was higher in males and females treated with candesartan cilexetil/HCTZ than after candesartan cilexetil alone indicating that HCTZ appears to increase the susceptibility to the intimal proliferation induced by candesartan cilexetil.

Table 34. Histopathology Findings

Parameter	Sex	Dose Group Incidence (# affected/# examined)			
		Control 0	Hctz 30	C. cilexetil 100	C/Hctz 100/30
Kidneys	M				
Basophilic renal tubules, moderate or severe		0/10	0/10	1/10	10/10
J-G cell hypertrophy		0/10	0/10	10/10	10/10
Intimal proliferation of interlobular arteries		0/10	0/10	4/10	10/10
Calcification of renal papilla		6/10	4/10	9/10	5/10
Adrenals					
Atrophy of zona glomerulosa		0/10	0/10	10/10	10/10
Stomach					
Erosion of glandular stomach mucosa		0/10	0/10	0/10	2/10
Kidneys	F				
Basophilic renal tubules, moderate or severe		0/10	0/10	0/10	0/10
J-G cell hypertrophy		0/10	0/10	10/10	10/10
Intimal proliferation of interlobular arteries		0/10	0/10	4/10	10/10
Calcification of renal papilla		1/10	1/10	0/10	0/10
Adrenals					
Atrophy of zona glomerulosa		0/10	0/10	10/10	10/10
Stomach					
Erosion of glandular stomach mucosa		0/10	0/10	0/10	2/10

Bold values indicate an incidence rate higher than control

Toxicokinetics

Cmax and $AUC_{0\text{-}24h}$ values for candesartan after candesartan cilexetil/HCTZ administration were similar to those observed after administration of candesartan cilexetil alone. Similarly, Cmax and $AUC_{0\text{-}24h}$ values for HCTZ after administration of candesartan cilexetil/HCTZ were similar to those seen after administration of HCTZ alone. Cmax and $AUC_{0\text{-}24h}$ values for HCTZ or candesartan were similar for males and females indicating no sex differences in candesartan pharmacokinetics. In addition, both Cmax and $AUC_{0\text{-}24h}$ values for candesartan and HCTZ determined at the first dosing were almost equal to those determined in weeks 4 and 13 indicating a lack of drug accumulation following repeated dosing (Tables 35 and 36).

Table 35. Toxicokinetics of Candesartan after Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ to Rats.

Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\,h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\,h}$ ug.hr/ml
Candesartan cilexetil 100	1	0.5	17.9	83.8	0.5	20.3	88.6
	23	1	15.1	62.2	1	14.6	60.4
	86	1	22.1	92.9	1	19.4	72.5
C. cilexetil/HCTZ 100/30	1	0.5	18.5	82.2	1	20.3	89.8
	23	2	15.6	72.0	1	11.7	66.7
	86	1	17.9	85.7	1	15.7	67.9

Table 36. Toxicokinetics of HCTZ after Administration of HCTZ or Candesartan Cilexetil/HCTZ to Rats.

Dose Group mg/kg/day	Dosing day	Male (n=3)			Female (n=3)		
		Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\,h}$ ug.hr/ml	Tmax, hr	Cmax, ug/ml	$AUC_{0\text{-}24\,h}$ ug.hr/ml
HCTZ 30	1	2	1.98	10.5	1	1.95	10.5
	23	2	2.19	11.9	1	2.17	13.2
	86	2	1.99	11.1	2	2.07	11.3
C. Cilexetil/HCTZ 100/30	1	2	2.38	12.5	2	2.52	11.3
	23	2	2.84	16.6	2	3.40	18.8
	86	1	2.39	11.9	1	2.39	9.5

4-Week Oral Dose Toxicity Study in Dogs (Vol: 12, pg 10)

Study Facility: Takeda Chemical Industries, Inc., Osaka, Japan
Study No.: 1777/SU
Study Date: Initiation of Dosing 4/25/94

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

Animals: Male and female Beagle dogs (Males=8.2-11.5 kg; Females = 7.0-9.7 kg). The dogs were housed individually and provided a daily ration of 300 gm of a pellet diet (CD-5,).

Drug Administration: Candesartan cilexetil (Lot # M464-039) and hydrochlorothiazide (Lot # M373-001) were suspended in 5% gum arabic solution and administered to dogs 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
HCTZ	3	10
Candesartan cilexetil	3	4
Candesartan cilexetil	3	20
Candesartan cilexetil	3	100
Candesartan cilexetil/HCTZ	3	4/10
Candesartan cilexetil/HCTZ	3	20/10
Candesartan cilexetil/HCTZ	3	100/10

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. Heart rate was measured prior to dosing and on treatment days 2 or 3 for the control and high dose candesartan cilexetil/HCTZ groups. Ophthalmoscopic examinations were performed on all dogs prior to dosing and on treatment day 14. Water intake and 24 hr-urine output was measured for all dogs pretest and on treatment days 5 or 6 and 19 or 20. Urinalysis was performed. Blood was obtained (from the cephalic vein) from all animals during the pretreatment period and on treatment days 8 and 22 for hematology and blood chemistry analyses. Venous blood samples were also obtained at 0.5, 1, 2, 4, 8 and 24 hours after the 1st and 23rd doses in all groups except the control group for measurement of candesartan cilexetil metabolites, candesartan and M-2, and for HCTZ in those animals which received 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
Two high dose (100/10 mg/kg) candesartan cilexetil/HCTZ males were sacrificed in a moribund state after the 11th and 24th doses and 3 females in the same group were sacrificed after the 10th (2 dogs) and 14th doses. Decreased locomotor activity, lack of food consumption and wasting were observed in those animals that were sacrificed in a moribund state. Dogs receiving the same high dose of candesartan cilexetil, but without HCTZ, were unaffected.

Body Weight
Body weights of the high dose candesartan cilexetil/HCTZ treated animals that were sacrificed prior to study termination were decreased from predose levels (mean decrease of 900 gm for 2 males or 3 females); mean body weights of control and other treated groups showed a slight net gain (30 to 267 gm) between initial and terminal measurements.

Heart Rate and ECG
Heart rate and ECG were unaffected by treatment in surviving animals.

Ophthalmology
Ophthalmic examinations showed no treatment related abnormalities.

Urinalysis
Water intake and urine output were not affected by candesartan cilexetil/HCTZ or HCTZ treatments. Lower than pretreatment excretion of sodium, potassium and chloride was noted in 1 male and 1 female in the 100/10 candesartan cilexetil/HCTZ mg/kg/day group in their moribund state (days 21 and 6, respectively).

Hematology
Slightly lower than control mean RBC counts, hematocrit and hemoglobin values were noted on day 22 in males and, to a lesser degree, in females treated with candesartan cilexetil or candesartan cilexetil/HCTZ (Table 37).

Table 37. Hematology Findings

Parameter	Sex	Dose Group, mg/kg/day							
		Control	Hctz	Candesartan cilexetil			Candesartan cilexetil/Hctz		
		0	10	4	20	100	4/10	20/10	100/10
RBC, $x10^4$/ul	M	687	616	611	627	593	583	509*	674
	F	769	711	754	788	711	729	724	728
Hematocrit, %	M	46.8	41.8	40.7	43.6	40.5	39.6*	34.9*	45.3
	F	52.0	48.6	51.3	51.6	48.3	49.4	49.2	50.3
Hemoglobin, g%	M	14.7	13.7	13.1	14.1	12.9	12.8	11.1*	14.7
	F	17.2	16.1	17.2	16.8	15.4	16.0	16.1	1.4

* Significantly different from control ($p<0.05$). Values are the means from 3 dogs.

Clinical Chemistry
Analysis of blood samples obtained from 5 animals before moribund sacrifice showed increases in urea nitrogen, creatinine, AST, ALT, inorganic phosphorus (5/5 dogs), increases in calcium, bilirubin, total protein, glucose, cholesterol, albumin, LDH (3/5 dogs), increases in triglyceride (2/5 dogs) and decreases in chloride (5/5 dogs), sodium (2/5 dogs) and potassium (1/5 dogs).

Blood chemistry parameters among HCTZ, candesartan cilexetil and candesartan cilexetil/HCTZ treated groups did not significantly differ from those in the control group.

Macroscopic Examination

In animals in the 100/10 mg/kg candesartan cilexetil/HCTZ group that were sacrificed in the moribund state, gross examination showed dark red discoloration of stomach mucosa in all 5 (2males and 3 females), pale discoloration of the kidneys of 1 male and 3 females, dark red discoloration of the cortex of the kidney in 3 females and a pale discolored focus on the tongue of one female.

Histopathology

Microscopic examination of all animals revealed treatment related lesions of the kidney, stomach and tongue (Table 38). Lymphoid depletion in the thymus (1/3M and 3/3F) and mesenteric lymph nodes (1/3F) was noted in animals sacrificed early and was regarded as secondary to the moribund state.

Table 38. Histopathology Findings

Lesion	Sex	Incidence (# affected/# examined)							
		Vehicle	Hctz 10	C 4	C 20	C 100	C/Hctz 4/10	C/Hctz 20/10	C/Hctz 100/10
Kidney									
Renal tubule regeneration*	M	0/3	0/3	0/3	0/3	0/3	0/3	1/3	2/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3
Renal tubule dilatation	M	0/3	0/3	0/3	0/3	0/3	0/3	3/3	3/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3
J-G cell hypertrophy	M	0/3	0/3	1/3	3/3	3/3	3/3	3/3	3/3
	F	0/3	0/3	1/3	2/3	3/3	3/3	3/3	3/3
Stomach									
Erosion or Ulcer	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3
	F	0/3	0/3	0/3	0/3	0/3	1/3	0/3	3/3
Hemorrhage	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3
Calcification	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	1/3	2/3
Tongue									
Ulcer	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3

*Moderate to marked degree of regeneration.

Toxicokinetics

The Cmax and AUC of candesartan cilexetil and metabolite M-1 (candesartan) increased with increasing doses of candesartan cilexetil or candesartan cilexetil/HCTZ (Table 39). The Cmax and AUC for candesartan after the 23rd dose of candesartan cilexetil or candesartan cilexetil/HCTZ tended to be higher than after the 1st dose. HCTZ did not appear to interfere with the pharmacokinetics of candesartan cilexetil. Also, candesartan cilexetil did not influence the pharmacokinetics of HCTZ (Tables 40-41).

Table 39. Toxicokinetics of Candesartan Cilexetil After Administration of Candesartan Cilexetil 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)
Candesartan cilexetil/HCTZ,							
4/10	1	1	0.001	0.00	24	0.001	0.02
20/10	1	2	0.022	0.05	1	0.023	0.05
100/10	1	0.5	0.082	0.10	0.5	0.085	0.23
Candesartan cilexetil,							
4	1	0.5	0.003	0.03	8	0.002	0.03
20	1	2	0.035	0.08	1	0.022	0.06
100	1	1	0.082	0.21	1	0.097	0.23

Table 40. Toxicokinetics of Candesartan After Administration of Candesartan Cilexetil or Candesartan Cilexetil/HCTZ in 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)
Candesartan cilexetil/HCTZ,							
4/10	1	1	0.13	0.9	2	0.15	1.3
	23	1	0.17	1.1	1	0.27	2.1
20/10	1	2	0.56	3.5	1	0.58	3.9
	23	1	1.3	7.0	1	0.90	5.0
100/10	1	1	1.0	6.0	2	1.2	9.0
	23	4	2.7*	21*	-**	-**	-**
Candesartan cilexetil,							
4	1	1	0.13	0.9	0.5	0.12	0.5
	23	1	0.16	1.1	1	0.31	1.4
20	1	1	0.42	2.8	1	0.70	3.0
	23	2	1.1	6.0	1	1.4	9.0
100	1	1	1.4	8.0	2	1.7	8.0
	23	2	1.2	7.0	4	2.5	19.0

* Two surviving males. ** No surviving females.

Table 41. Toxicokinetics of HCTZ After Administration of HCTZ or Candesartan Cilexetil/HCTZ in 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
Candesartan cilexetil/HCTZ,							
0/10	1	2	4.5	20	1	5.8	28
	23	2	4.3	19	1	5.9	24
4/10	1	1	3.4	14	1	5.1	18
	23	1	3.7	16	1	5.1	17
20/10	1	1	3.9	18	1	3.5	17
	23	2	4.5	21	1	6.0	20
100/10	1	1	4.1	19	2	5.6	24
	23	2	6.8*	90*	-**	-**	-**

* Values from 2 surviving males. ** No values; no surviving females.

13-Week Oral Toxicity Study in Dogs (Vol. 15, pg 10)

Study Facility:
Study No.: B-2936
Study Dates: Initiation of Dosing 4/25/95; Necropsy 7/27/95

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

Animals: Male and female Beagle dogs (Males=8.8-10.4 kg; Females = 8.0-9.1 kg). The dogs were housed individually and provided a daily ration of 300 gm of a pellet diet (CD-5,

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/HCTZ	3	0.8/10
Candesartan cilexetil/HCTZ	3	4/10
Candesartan cilexetil/HCTZ	3	20/10

Note: Selection of high dose based on results of the 4-week study in which a dose of 100/10 candesartan cilexetil/HCTZ was associated with deaths (early sacrifice) of 5/6 dogs.

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. Urine was collected for 22 hours prior to dosing and in months 1, 2 and 3 of the dosing period for urinalysis. Ophthalmoscopic examinations were performed on all dogs prior to dosing 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 from all animals prior to dosing 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 in all groups, except the control group, for measurement of candesartan cilexetil, candesartan and 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) from all dogs were fixed on slides and examined for microscopic pathology.

Results:

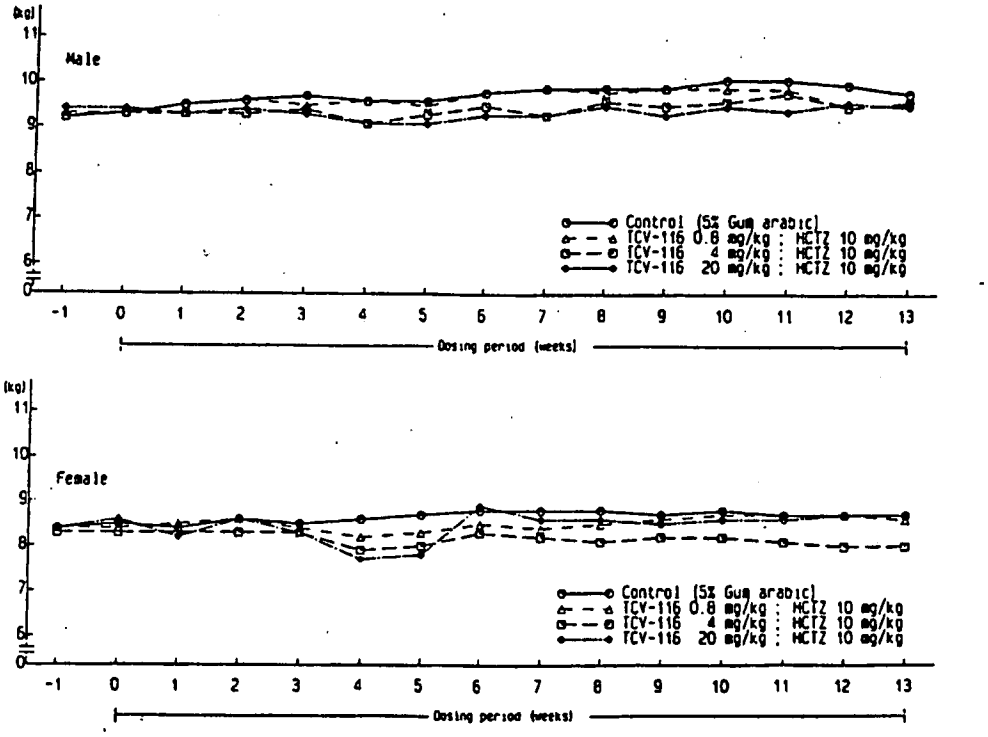
Mortality and Clinical Signs
Two females in the 20/10 mg/kg/day group were killed in a moribund state on dosing days 31 and 38. Prior to sacrifice, the two dogs showed decreased spontaneous movement, hypothermia,

paleness of conjunctival and oral mucosa, constipation and vomiting. No treatment related signs of toxicity were noted in the remaining animals.

Body Weight and Food Consumption and Water Intake

A gradual reduction in body weight was noted in the two animals sacrificed in extremis. No remarkable effects on body weight were noted in animals surviving to study termination (Fig 5). In the dogs sacrificed in extremis, anorexia lasted for approximately 2 weeks before sacrifice. No remarkable effects on food consumption were detected among surviving animals. No treatment related effect on water intake was detected.

Figure 5. Body Weights



Urinalysis

Significantly higher than control sodium excretion and lower than control osmolarity were noted for 0.8/10 and 4/10 mg/kg/day female groups. Slightly, and not significantly, higher than control urine volumes were noted for treated males and females (Table 42). Other urinary parameters among treated groups did not differ from control.

Table 42. Urinalysis Results (Treatment month 3).

Parameter	Sex	Candesartan cilexetil/Hctz Dose Group, mg/kg/day			
		0 (Control)	0.8/10	4/10	20/10
Urine Volume, ml/22hr	M	130	123	227	167
	F	170	227	230	200[a]
Osmolarity, mOs/kg	M	1485	1438	1052	1153
	F	1668	1183*	1105*	811[a]
Sodium, mEq/22hr	M	13.6	8.4	18.5	19.1
	F	14.0	21.0*	21.7*	24.8[a]
Chloride, mEq/22hr	M	19.1	17.0	30.1	24.0
	F	26.9	30.6	29.2	22.6[a]

* Significantly different from control value ($p<0.05$). [a] Value from 1 dog

Hematology

In all treated groups, including the animals killed in extremis, there were no treatment or dose-related hematological effects.

Blood Chemistry

In animals sacrificed in extremis, marked elevations of serum urea nitrogen, creatinine and inorganic phosphate, and decreases in serum sodium and chloride, from predose levels were noted prior to sacrifice. Blood chemistry parameters in treated animals surviving to study termination showed no remarkable differences from pretreatment or concurrent control levels.

Ophthalmology

Ophthalmic examinations revealed no ocular abnormalities in any animal.

Electrocardiography

Electrocardiographic parameters (heart rate and PR, QRS, QT, QTc intervals) among candesartan cilexetil/HCTZ treated groups were similar to control.

Gross Pathology

Paleness of the renal cortex was noted in both females and diffuse erosion of the stomach mucosa and atrophy of the submandibular and mesenteric lymph nodes was noted in one of the females sacrificed in extremis.

Organ Weights

Organ weights of treated animals were comparable to control.

Histopathology

The predominant treatment related histopathological finding among animals surviving to study termination was hypertrophy of the J-G cells of the kidney, a finding in all drug-treated and none of control dogs (Table 43). Tubular dilation was reported only in (2/3) high dose females. Other lesions among treated animals were observed at comparable incidences in control animals. In addition to the renal lesions in the 2 females that were sacrificed in extremis, one of these dogs presented at necropsy with stomach mucosal erosion and hemorrhage, and atrophy of the esophageal, parotid and submandibular glands.

Table 43. Histopathology Findings (# Dogs with Lesion/# Dogs Examined).

Lesion	Sex	Candesartan cilexetil/Hctz Dose Group, mg/kg/day			
		0 (Control)	0.8/10	4/10	20/10
Kidney	M				
Tubular dilatation		0/3	0/3	0/3	0/3
J-G Cell hypertrophy		0/3	**3/3**	**3/3**	**3/3**
Regeneration of renal tubules		2/3	**3/3**	**3/3**	2/3
Kidney	F				
Tubular dilatation		0/3	0/3	0/3	**2/3**
J-G Cell hypertrophy		0/3	**3/3**	**3/3**	**3/3**
Regeneration of renal tubules		2/3	2/3	**3/3**	**3/3**

Values in bold type indicate an incidence larger than control

Toxicokinetics

Plasma concentrations of candesartan increased with increasing doses of candesartan cilexetil/HCTZ. Plasma concentrations of HCTZ exhibited similar peak levels (Cmax values) in

all treated groups. No apparent sex differences in Cmax and AUC values for HCTZ and candesartan were observed (Tables 44-45):

Table 44. Toxicokinetics of Candesartan After Administration of Candesartan Cilexetil/HCTZ in Dogs.

C. Cilexetil/HCTZ Dose 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)
0.8/10	1	1	23.2	170	2	14.9	141
	28	4	18.9	214	1	13.9	93
	91	1	21.5	245	4	21.5	167
4/10	1	1	95.5	674	1	206.6	995
	28	2	86.3	580	2	136.1	1448
	91	1	99.9	1031	1	161.3	1219
20/10	1	1	575.7	3292	2	364.5	3572
	28	0.5	367.7	3019	4	702.2	10197
	91	1	625.1	5055	4	353.4[a]	3417[a]

[a] value from one surviving female

Table 45. Toxicokinetics of HCTZ After Oral Administration of Candesartan Cilexetil/HCTZ to Dogs.

C. Cilexetil/HCTZ 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)
0.8/10	1	2	2.95	15.2	1	3.99	17.4
	28	2	2.96	14.6	2	2.72	15.9
	91	2	2.45	12.1	2	3.51	15.4
4/10	1	2	3.66	16.2	1	3.51	15.3
	28	2	2.67	13.0	2	2.68	13.3
	91	1	3.19	14.6	1	4.17	15.9
20/10	1	2	3.65	14.7	2	2.03	14.5
	28	1	2.37	12.5	4	7.98	94.0
	91	1	4.25	14.7	2	3.00[a]	15.5[a]

[a] Value from one surviving female

13-Week Oral Toxicity Study in Dogs (Vol. 15, pg 223)

Note: The purpose of this additional 13-week oral dose toxicity study was to assess the toxicity of candesartan cilexetil/HCTZ in dogs at a HCTZ dose of 30 mg/kg/day, which was 3 times the dose level used in the previous study (Study B-2936). Only one dose level (4/30 mg/kg/day) of the candesartan cilexetil/HCTZ combination was administered and observed toxicities were compared with effects elicited by the 4/10 mg/kg/day candesartan cilexetil/HCTZ combination in the previous study.

Study Facility:
Study No.: B-3157
Study Dates: Initiation of Dosing 12/20/95; Necropsy 3/22/96

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

Animals: Male and female Beagle dogs (Males 8.3-10.8 kg; Females 7.5-9.9 kg). The dogs were housed individually and provided a daily ration of 300 gm of a pellet diet (CD-5,