CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-818

PHARMACOLOGY REVIEW(S)

NOV 5 1997

NDA 20-818 E.A. González Barry, M.S.

EVALUATION OF TOXICITY AND PHARMACOKINETIC DATA

CORRESPONDENCE DATE: 03-28-97
CDER RECEIPT DATE: 03-31-97
DIVISION RECEIPT DATE: 04-01-07
REVIEWER RECEIPT DATE: 04-14-97
REVIEWER START DATE: 06-10-97

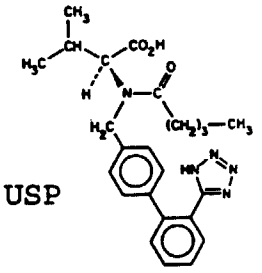
SPONSOR: Novartis Pharm. Corporation, East Hanover, NJ 07936

DRUG: Proprietary Name: Diovan HCT™ (Combination of valsartan* and hydrochlorothiazide, USP).

- Code Numbers for two different drug combination ratios of valsartan and hydrochlorothiazide, respectively: **CGP 63171** (160/12.5mg) and **CGP 63172** (80/12.5).

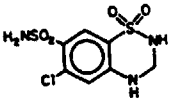
STRUCTURAL FORMULAS OF ACTIVE INGREDIENTS:

N-[*p*-(*o*-1*H*-Tetrazol-5-yl-phenyl)benzyl]-*N*-valeryl-L-valine. *CAS-137862-53-4.*



Valsartan*
Hydrochlorothiazide,USP
MW: 435.5
Code: CGP 48933

2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide *CAS-58-93-5.*



MW: 297.7
Code: SU 5879

PHARMACOLOGIC CLASS: A drug combination of an angiotensin II (**AII**) receptor antagonist specifically active on the **AT1** receptor subtype, and a thiazide diuretic.

PROPOSED THERAPEUTIC INDICATION: Hypertension; however, the combination product is **not** indicated for initial therapy in this disease. **CGP 63172** is indicated for patients whose blood pressure (**BP**) is not adequately controlled with valsartan monotherapy.

FORMULATION/ROUTE OF ADMINISTRATION: Valsartan + hydrochlorothiazide (**HCTZ**) is formulated as a tablet for oral use. The excipients are listed below in a table provided by drug sponsor. All of the excipients have been previously used in approved drugs for oral use.

NDA 20-818 (VALSARTAN + HYDROCHLOROTHIAZIDE), DIOVAN HCTZ ™

TABLE OF CONTENTS

EXCIPIENTS IN ORAL FORMULATION:

Table with list active/inactive ingredients.

Valsartan Hydrochlorothiazide Tablet Final Market Formulation Table

PROPOSED DOSAGE REGIMEN: As a once a day oral dose administration. Doses higher than valsartan 160 mg + HCTZ 25 mg combination have not been studied.

INDs UNDER WHICH NONCLINICAL TRIALS WERE CONDUCTED WITH VALSARTAN ALONE:

RELATED NDAs: ♦ **Valsartan** capsules (**NDA 20-665**) as an angiotensin II receptor antagonist approved 12-23-96 as monotherapy for the treatment of hypertension.

♦ **HCTZ** tablets (**NDA 11-793**) a thiazide diuretic approved in 1959 and widely used alone or in combination with other cardiovascular drugs in the management of hypertension.

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BRIEF INTRODUCTION: Diovan HCT is a proposed combination of the two approved drugs- **valsartan** and **HCTZ** for the oral treatment of hypertension, but not as the first drug of choice for management of the disease.

AII is an octapeptide formed from its precursor decapeptide angiotensin I by the proteolytic action of the angiotensin-converting enzyme (**ACE**). Prevention of the formation of AII via inhibition of ACE is used in the treatment of hypertension and congestive heart failure.

The existence of two AII receptor subtypes (designated as AT_1 and AT_2) have been identified by nonclinical studies in various tissues, both receptor subtypes have been demonstrated in human uterus, adrenal glomerulosa and renal artery, and are differentiated by their sensitivity to different drugs, i.e., AT_1 receptor subtype is sensitive to losartan, and AT_2 receptor subtype is sensitive to CGP 42112A and PD123177*. AT_1 receptors are reported as being responsible for the effects of AII (i.e., vasoconstriction, aldosterone and adrenaline release, water intake and cellular proliferation).

Drug binding studies using rat and marmoset liver membranes indicate that valsartan binds exclusively to the AT_1 receptor subtype. Studies report that since valsartan is a highly specific active antagonist at AT_1 receptor, and as result the drug acts by blocking the vasoconstrictor and aldosterone-secreting effects of AII which aids in the management of hypertension and congestive heart failure.

The diuretic **HCTZ** affects the renal tubular mechanism of electrolyte reabsorption which is used clinically alone or in combination with other antihypertensive drug, also in the management of hypertension.

* Blankley, CJ, et al in **J Med Chem.** 34: 3248-3260, 1991.
Whitebread, SE, et al in **Biochem Biophys Res Commun.** 181: 1365-71

LIST OF NONCLINICAL STUDIES WITH THE DRUG COMBINATION SUBMITTED IN THIS NDA 20-818:

1. PHARMACODYNAMICS

2. TOXICOLOGY

2.1 Acute Toxicity (Single Dose) Studies.
2.1.1 ♦ **Rat** (Test no. 946111)
2.1.2 ♦ **Marmoset** (Test no. 946113)

2.2 Repeat Oral Dose Studies.
2.2.1 ♦ Rat
2.2.1.1-month (Test no. 946114)
2.2.1.2 6-month (Test no. 946116)
2.2.2 ♦ **Marmoset**
2.2.2.1-month (Test no. 946115)
2.2.2.2 6-month (1st study; Test no. 946117)
2.2.2.3 6-month (Follow up study; Test no. 966057)

2.3 Developmental Toxicity. (Seg II)
2.3.1 ♦ **Mouse** (MIN 944119)
2.3.2 ♦ **Rat** (1st study; MIN 944118)
2.2.3 Rat (Follow-up study; MIN 954068)
2.2.4 ♦ **Rabbit** (Dose-range finding study no. 94068)
2.2.5 Rabbit (Follow-up study; MIN 944117)

3. **ADME Studies.**

3.1 ♦ **Rat** (6-month Repeat Dose Studies).
3.1.1 Urinary Excretion of HCTZ (Study No. 1996/096/946116)
3.1.2 Plasma Concentrations of Valsartan (Study No. 1996/156/947907)
3.2 ♦ **Marmoset** (Repeat Dose Studies).
3.2.1 Fourteen-day Study (Plasma Concentrations and Urinary Excretion of HCTZ) (Study No. 1996/087/947909)
3.2.2 Three-month Study (Plasma Concentrations of Valsartan) (Study No. 1995/001/947905)
3.2.3 Six-month Study (Urinary Excretion of HCTZ) (Study No. 1996/132/947908)
3.1.4 Six-month Study (Plasma levels of Valsartan) (Study No. 1996/136/969003)
3.2.5 Six-month Study (Urinary Excretion of HCTZ) (Study No. 1996/154/969003)
3.1.6 Six-month Study (Plasma Concentrations of Valsartan) (Study No. 1996/155/947908)

BRIEF OVERVIEW OF STUDIES REVIEWED: None of the studies submitted in this NDA had been previously reviewed at the IND phase.

1. PHARMACODYNAMICS (V. 9)

Drug sponsor submitted study reports and overall summaries of findings for nonclinical pharmacology studies conducted in its laboratories with **valsartan** or in combination with **HCTZ**.

A total of only 4 nonclinical pharmacology studies were submitted [one **oral** study using spontaneously hypertensive rats (SHR), and two oral studies in marmosets, and one study in SHR given the drug by **subcutaneous** route]. In addition to the study reports, the firm submitted over 15 journal/review articles reporting experiments conducted with **valsartan**, as well as with other angiotensin II antagonists (i.e., losartan, GR 117289 and L-I58,809), ACE inhibitors, and **HCTZ** alone or in combination with other drugs. The articles submitted in the NDA have not been evaluated in this document.

1.1 In Vitro Studies:

No in vitro studies were submitted using the drug combination CGP 63172. Drug sponsor asserted that since the sites of action and receptors are different for each of the drugs in the combination drug CGP 63172 "...in vitro interactions between the two compounds are not expected."

1.2 In Vivo Studies:

1.2.1 **RAT (SHR)**

1.2.1.1 Acute Oral Effects of Valsartan and HCTZ alone or in Combination on Blood Pressure and Heart Rate (HT) in Conscious Spontaneously Hypertensive Rats (SHR). (Study conducted at drug sponsor's labs in Basle, Switzerland. Report no. 61/94, dated: 26-10-94. V.9, p 194)

The purpose of this study was to test the hypothesis that drugs interfering with the renin angiotensin system (RAS) work best in pathophysiologic situations with a stimulated RAS. To test this hypothesis, drug sponsor selected the SHR as the most suitable animal model for hypertension.

Twenty conscious M SHR, with implanted catheter into the left femoral artery for measuring blood pressure (BP), were randomized into 4 groups (n=5 each) and given orally (gavaged drug dissolved in water) the following treatment for 2 days: **I**- solvent control, **II**- valsartan alone 2 mg/kg po once daily; **III**- **HCTZ** alone 10 mg/kg po daily, and by **iv** route- valsartan 2 mg/kg + HCTZ 10 mg/kg daily.

The first oral dose was administered, and this was followed by a second dose 24 hrs later. The doses selected were considered by investigators to be reasonably low so not to miss synergist effect in a still elevated BP in these animals.

Briefly, the effects of the treatments were assessed by comparing the averaged systolic, diastolic, and mean arterial blood pressure (MAP) determined 12 hrs after the second dose of the drugs.

RESULTS

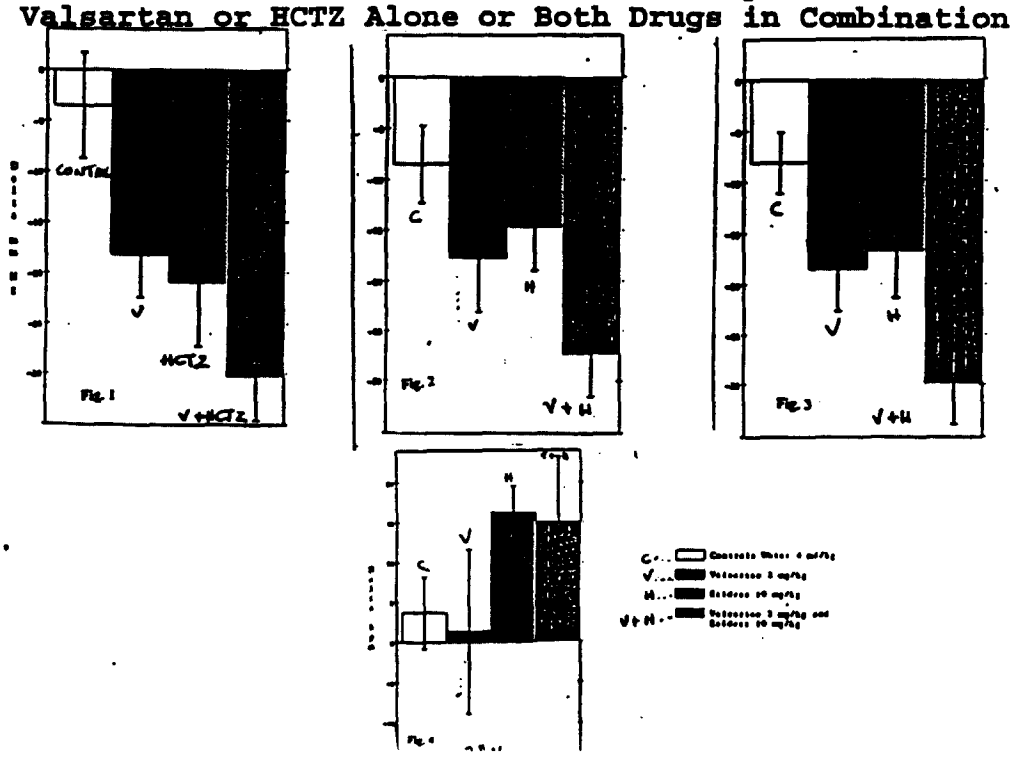
After each treatment, the SHR showed a tendency for their BP to decline during the observed time periods. The effect of the drug combinations was considered by the drug sponsor as being "additive" vs the effects of the individual drugs alone. The drug combination induced a considerable greater hypotensive (↓ mm Hg) effect than after vasaltan or HCTZ monotherapy (Figs. 1, 2, and 3 below).

There were reflex increases in heart rate (HR as BPM) after the drug treatments of HCTZ and the drug combination. (Fig.4)

Drug sponsor considered that although the doses chosen for this study might be considered to be reasonable, it could not be excluded that a combination of different doses would yield a more favorable result. Further, that the lack of a "<u>synergistic effect</u>" could potentially result from a different bioavailability of the compounds when given in combination.

Drug sponsor asserted that in human, a <u>reduced bioavailability</u> has been reported with HCTZ when co-administered with valsartan.

Effects on Mean Arterial Blood Pressure and Heart Rate in Conscious SHR Treated Orally with Valsartan or HCTZ Alone or Both Drugs in Combination.



Conscious SHR: Effects on Mean Blood Pressure, Systolic and Diastolic Blood Pressure and Heart Rate of Oral Doses of Valsartan or HCTZ Alone or Both Drugs in Combination.

Valsartan-Eslidrex Studie

		Contr. Verper.	Contr. Behandl.	delta	Valsartan Verper.	Valsartan behandl.	delta	Eslidrex verper.	Eslidrex behandl.	delta	MIX- verper.	MIX behandl.	delta
1	MBP												
2		144.4	142.8	-1.5	155.8	143.1	-12.7	146.3	147.5	1.3	146.9	122.1	-24.8
3		145.0	130.2	-14.8	148.2	138.4	-7.8	157.6	133.6	-24.0	167.1	129.1	-37.9
4		155.0	143.6	-11.4	158.2	128.8	-29.4	149.7	127.7	-22.0	181.0	145.7	-35.4
5		157.9	157.5	-0.4	151.8	135.6	-16.2	175.8	155.2	-20.6	164.0	129.5	-34.5
6		150.0	137.7	-12.3	175.7	149.3	-26.5	179.2	161.2	-18.1	168.1	151.1	-17.0
7													
8	mean	150.5	142.4	-8.1	157.1	138.6	-18.5	161.7	145.0	-16.7	165.4	135.5	-29.9
9	SEM	2.7	4.5	3.0	5.0	3.8	4.1	6.7	6.3	4.6	5.5	5.5	3.9
10													
11	SBP												
12		157.0	161.1	4.1	195.1	173.6	-21.6	186.5	187.4	1.0	187.2	161.7	-25.5
13		176.0	160.7	-15.3	180.0	177.3	-2.6	198.7	172.7	-26.0	203.6	166.1	-37.5
14		167.1	180.1	13.0	182.8	163.0	-19.8	185.8	148.1	-37.7	227.5	194.8	-32.7
15		190.5	180.3	-10.2	197.9	178.7	-19.2	223.0	201.2	-21.8	208.9	169.3	-39.5
16		194.1	185.2	-8.9	213.5	185.3	-28.2	228.1	208.2	-19.8	211.9	195.8	-16.0
17													
18	mean	176.9	173.5	-3.5	193.8	175.6	-18.2	204.4	183.5	-20.9	207.8	177.6	-30.3
19	SEM	7.0	5.2	5.2	6.0	3.7	4.2	9.0	10.7	8.3	6.5	7.3	4.3
20													
21	DBP												
22		138.0	133.6	-4.4	135.7	127.5	-8.2	125.8	127.2	1.4	126.9	102.5	-24.4
23		129.7	115.2	-14.5	124.4	118.8	-5.6	136.9	113.9	-23.0	148.7	110.7	-38.0
24		139.5	125.5	-14.1	143.1	108.8	-34.3	131.8	117.8	-14.0	157.1	121.0	-36.1
25		141.7	146.2	4.5	128.6	114.0	-14.7	152.5	132.4	-20.1	141.8	120.9	-20.8
26		128.0	114.0	-14.0	156.7	131.2	-25.6	154.9	137.7	-17.2	146.4	129.0	-17.4
27													
28	mean	135.4	126.9	-8.5	137.7	120.0	-17.7	140.4	125.8	-14.6	144.2	116.8	-27.4
29	SEM	2.7	6.0	3.8	5.7	4.1	5.4	5.7	4.4	4.3	5.0	4.6	4.1
30													
31	HR												
32		209.0	213.1	4.1	227.3	254.1	26.8	202.6	229.0	26.4	310.0	302.1	-7.9
33		232.0	248.3	16.3	220.6	222.5	2.0	225.3	234.0	8.6	295.7	321.0	25.3
34		253.3	243.0	-10.3	230.4	248.8	18.3	249.0	261.1	12.1	310.7	312.3	1.6
35		180.6	189.5	8.9	309.5	300.5	-9.0	243.6	256.2	12.6	313.1	348.3	35.2
36		208.1	207.5	-0.7	334.8	304.0	-30.8	258.0	279.9	21.9	300.7	322.7	22.0
37													
38	mean	216.6	220.3	3.7	264.5	266.0	1.4	235.7	252.0	16.3	306.0	321.3	15.2
39	SEM	12.3	11.1	4.5	23.9	15.7	10.2	9.9	9.3	3.3	3.3	7.7	8.0

1.2.1.2 Subacute Effects of Valsartan and HCTZ Alone and in Combination on the Blood Pressure and Heart Rate of Conscious-telemetered SH Rats (Study conducted at drug sponsor's labs in Basle, Switzerland. Report no. 96009, dated: 03-10-96. V.9, p 233)

The purpose of this study was to evaluate the effectiveness of HCTZ alone during chronic subcutaneous infusion and when given as adjunctive therapy to valsartan in SHR.

Previous studies with SHR (with indwelling arterial cannulae) showed that **acute** oral doses of HCTZ (3 and 10 mg/kg/day) in combination with a low dose of valsartan (1 and 3 mg/kg/day) reduced BP with an additive effect. The main hypothesis of interest was to investigate "synergy" versus "additivity".

Briefly, 67 conscious-telemetered M SHR (~20-30 wks old) were distributed into 6 groups (n=4/group) and infused with the water vehicle or test drugs alone (i.e., valsartan, benazeprilat, HCTZ) or in combination with HCTZ for 2-wks via subcutaneously (sc) implanted osmotic minipumps.

The drug sponsor reported in detail the materials/methods used to instrument anesthetized SHR with the radiotelemetric implants (implantation operation was performed at least 1-mo prior to experiments), and for recording of physiologic changes. Rats were maintained on a 12-hr light/dark cycle and received standard laboratory rat chow and water ad libitum.

MAP and HR were recorded continuously via a computer, from the unrestrained conscious-telemetered SHR. Values obtained are expressed as group means for each treatment; baseline data were calculated as the average of 3 successive 24 hr values for each rat prior to initiation of the drug infusion. Data were collected at 10 min intervals throughout the day, and 24 hr averages were derived from these values. Data collected were statistically analyzed using methods briefly described and referenced in the NDA.

RESULTS

Reports stated that when the LD and HD HCTZ was infused **sc** in SHR, a significant reduction in MAP was seen during the initial days of the study; the antihypertensive effect diminished with time such that no significant difference was seen between treated vs untreated rats at 2 weeks. At LD HCTZ, but not at the HD, the drug significantly lower HR vs rats treated with the vehicle.

Infusion with CGP 63172 resulted in an enhanced antihypertensive response compared to the individual monotherapies.

When the dose of HCTZ was increased and combined with valsartan, the antihypertensive response was still additive; although there was a greater reduction in BP with the combination, HR was unchanged.

LD HCTZ combined with LD valsartan again induced bradycardia, but valsartan attenuated the reduction in HR. The mechanism responsible for HCTZ induced bradycardia could not be explained by investigators.

Attempts to investigate the specificity of the synergist effect, HCTZ (3 or 10 mg/kg/day) was administered in combination with the ACE inhibitor benazeprilat (1 mg/kg/day). BP was reduced to a greater extent in these rats treated with the combination in which HCTZ was given; however to evoke a greater "additive" effect on BP a HD of HCTZ was required; no consistent tachycardia was reported. Investigators concluded that the effect of this combination was again **additive**.

The investigators considered that coadministration of HCTZ with valsartan (or benazeprilat) "potentiates" the BP lowering effects in conscious SHR. However, responses may can vary in magnitude from "additive to synergistic" depending on the doses used. The greater BP lowering seen in SHR administered the combination of valsartan + HCTZ was not associated with sustained significant increase in HR according to drug sponsor. These findings were considered to be consistent with earlier data demonstrating a similar "potentiation" during coadministration of a diuretic with an ACE inhibitor. The findings suggested that since there were similar results obtained with ACE inhibitors and AT1 receptor antagonists is due to the capacity to which diuretic-induced activation of the RAS occurs.

The table below was prepared by drug sponsor, and edited by this reviewer to illustrate salient findings reported in the study showing the BP values in SHR treated single drugs and in combination with HCTZ.

Baseline BP and HR Values of SHR Treated with Valsartan, Benazeprilat and HCT and in Combination with HCTZ.

Compound-Dose	Baseline Blood Pressure (mmHg)	Baseline Heart Rate (beats/min)	number of animals	AUC (bpm·days)
Vehicle				
0.15 N NaOH	154 ± 4	300 ± 8	6	1 ± 47
Valsartan				
1 mg/kg/d	147 ± 3	293 ± 3	6	84 ± 27
3 mg/kg/d	148 ± 3	288 ± 3	5	103 ± 16*
Benazeprilat				
1 mg/kg/d	147 ± 3	289 ± 3	6	72 ± 31
Hydrochloro-thiazide (HCTZ)				
3 mg/kg/d	150 ± 2	308 ± 5	5	-222 ± 32*
10 mg/kg/d	153 ± 3	299 ± 6	6	9 ± 26
Valsartan + HCTZ				
1+3 mg/kg/d	152 ± 4	308 ± 2	6	-187 ± 44*
1+10 mg/kg/d	153 ± 4	309 ± 4	6	85 ± 30
3+3 mg/kg/d	150 ± 7	308 ± 7	5	-105 ± 78
3+10 mg/kg/d	153 ± 3	306 ± 3	6	276 ± 23*
Benazeprilat + HCTZ				
1+3 mg/kg/d	152 ± 2	303 ± 3	6	162 ± 59*
1+10 mg/kg/d	150 ± 8	300 ± 6	4	111 ± 43

Values represent the mean ± SE for each treatment group. (*) represents a significant difference versus vehicle-treated SHR, where $P<0.05$ using one-way ANOVA followed by Student-Newman-Keuls post-hoc test.

1.2.2 **MARMOSETS**

1.2.2.1 Effects of Prolonged Administration of High Doses of Valsartan on Blood Pressure, Plasma Urea and Creatinine in NA^+ Replete Normotensive Marmosets. (Study conducted at drug sponsor's labs in Basle, Switzerland. Report no. BS 63-1995 dated: 06-10-95. V.9, p 270).

In the toxicology studies, **valsartan** in normotensive rats and marmosets induced nephropathy which was more pronounced in the marmoset at 200 mg/kg and above.

The purpose of this study was to determine the effects of valsartan (up to 200 mg/kg) in Na^+replete normotensive marmosets in the dose range tested in the toxicology studies, and whether the nephropathy reported in toxicology studies could be related to a reduction in systemic BP.

Briefly, in this study, M marmosets (2-8 years) were kept in air conditioned room at 24-27° C and with a 12-hr light/dark cycle. During the study, the animals were maintained on their normal diet with free access to both food and water.

The method used to implant the peritoneal telemetry transmitters was described in detail in the NDA. After the implantation, the marmosets were allowed 4 wks to recover from the surgical intervention. BP and HR were measured, with the implanted pressure telemetry transmitters, from conscious marmosets moving around in their cages. Dosing schedule, body weight and number of animals per dose group are reported below in table prepared by drug sponsor. Unfasted animals were dosed (by gavage) once a day (10 a.m.). Prior to starting treatment, and during the conduct of the study HR and BP were recorded (within 30 min of dosing) on days 1 and 2 of the study, and on the same days of the week for each of the next 4 wks after starting the treatment. Mean values of these parameters were reported for various periods.

Blood samples were collected on day 0 and after 4 wks of treatment for determination of urea and creatinine levels using a photometric method.

RESULTS

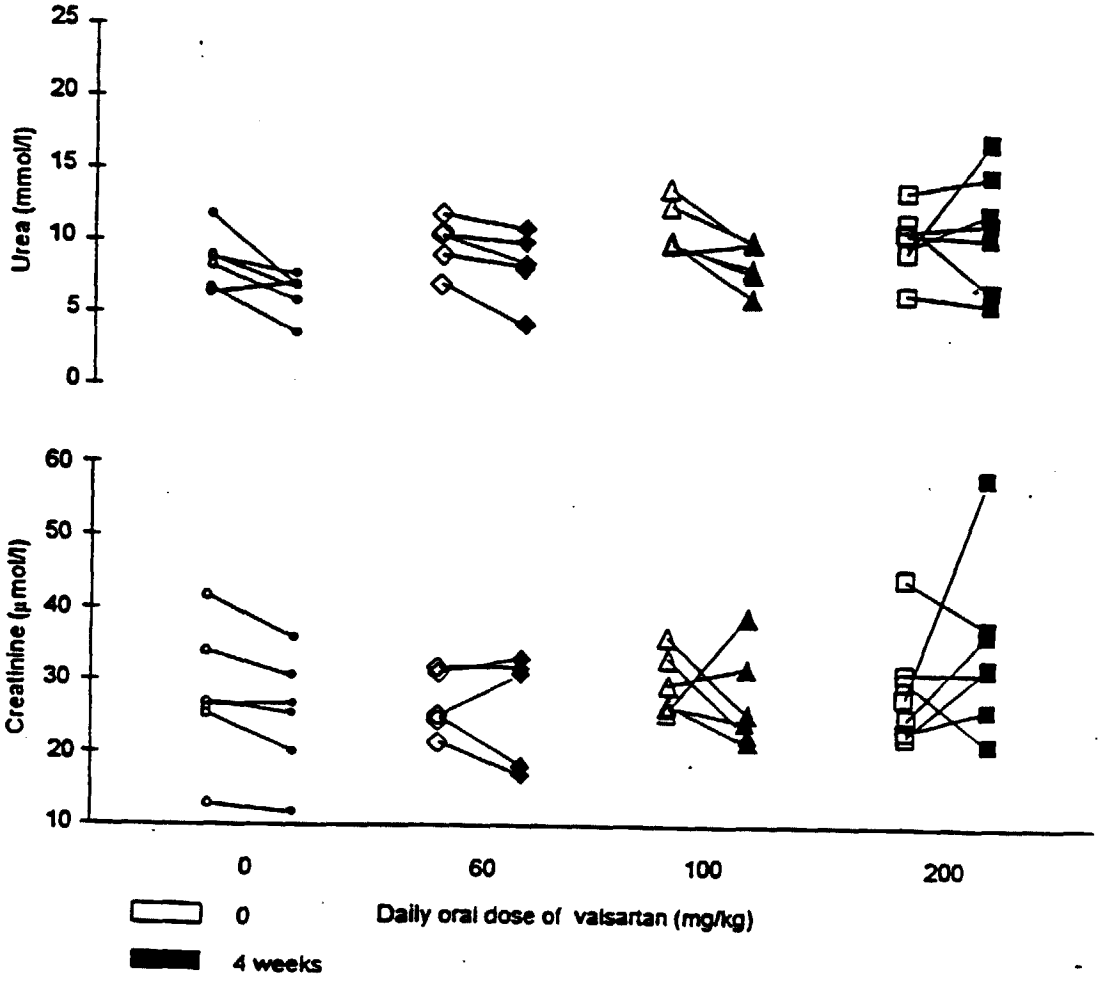
Valsartan alone in the doses of 0, LD-60, MD-120 and HD-200 mg/kg/day induced dose-dependent reductions in both systolic and diastolic BP in normotensive Na^+-replete normotensive marmosets. The initial fall in BP in the first week of treatment was similar for all doses but the duration of the response increased with dose. With all doses BP had almost recovered to pretreatment levels within 24 hrs after dosing.

At the subsequent weeks, BP continued to recover within 24 hr after dosing with the LD, whereas it remained lowered for 24 hr with the MD and HD.

Diastolic BP was lowered to levels as low as 45 mm Hg during the night after a 200 mg/kg dose, and plasma urea and creatinine were increased. Initial increases in HR after dosing on the first 2 days of the study, were attenuated in the following weeks. This HD dose of valsartan increased plasma urea and creatinine and was also was associated with tubular lesions as had been observed in the toxicology studies. However, no data were provided on the marmosets with renal histopathology, but a figure prepared by drug sponsor appears below showing the levels of urea/creatinine.

Drug sponsor asserts that these findings supports the proposition that the renal pathology may be due in part to a reduction in renal perfusion as a consequence of the large reduction in systemic BP. Drug sponsor did not rule out that a complete blockage of the intrarenal RAS may also contribute to the nephropathy.

Plasma urea and creatinine concentrations before and after 4 weeks of treatment of normotensive, sodium-replete marmosets with vehicle or increasing doses of valsartan (60, 120 or 200 mg/kg per day)



1.2.2.2 Effects of Prolonged Administration of Valsartan Alone or in Combination with the diuretic HCTZ on BP and Plasma Urea and Creatinine in NA$^+$replete Normotensive Marmosets. (Study conducted at drug sponsor's labs in Basle, Switzerland. Report **No. BS 64-1995** dated: 24-10-96. V.9, p 252).

The purpose of this study in Na$^+$-replete normotensive marmosets was to evaluate the effects of the combination of valsartan with HCTZ (ratio 3:1) on BP.

Both sexes of marmosets (2-8 y) were kept in single or pairs of the same sex in air conditioned room at 24-27° C and in a 12-hr light/dark cycle. Marmosets were maintained on their normal diet with free access to both food and water during the study.

Briefly, as in the previously described study with marmosets, BP and HR were measured by telemetry (implanted with pressure telemetry transmitters) in conscious and moving around in their cages. Method used to implant the peritoneal telemetry transmitters was described in the NDA and animals were allowed 4 wks to recover after the surgical intervention.

Nonfasted marmosets were dosed once daily for 4 weeks with valsartan 3 mg/kg/day, and HCTZ 1 or 37.5 mg/kg/day by gavage. Number of animals, drugs/dosing schedule and effect on body weight after 4-wks of treatment appear below under **RESULTS** in table below prepared by drug sponsor.

HR, and systolic/diastolic BP (calculated in 1 hr periods; changes were calculated using each animal as its own control) were recorded continuously on days 1 and 2 in the week prior to start dosing, and on the same days in each of the 4 weeks after starting the treatment. Measurements of plasma urea and creatinine concentrations were collected on day 0, and after 2 or 4 weeks of treatment.

RESULTS

Briefly, at the highest dose of the combination (120:37.5 valsatan: HCTZ), two animals lost weight and 1 marmoset died during the 2nd week of treatment (the cause of death or signs before death were not clearly described and the remaining animals in this group were terminated.

In these Na$^+$replete normotensive marmosets, there was no significant reduction in BP with 4 wks treatment of valsartan alone (3 mg/kg/day) or with HCTZ alone (1 mg/kg/day or 37.5 mg/kg/day).

The first dose of the fixed-combination (3:1 mg/kg; valsartan: HCTZ) induced a significant fall in BP. Greater reductions in both systolic and diastolic BP were induced with increased doses of the

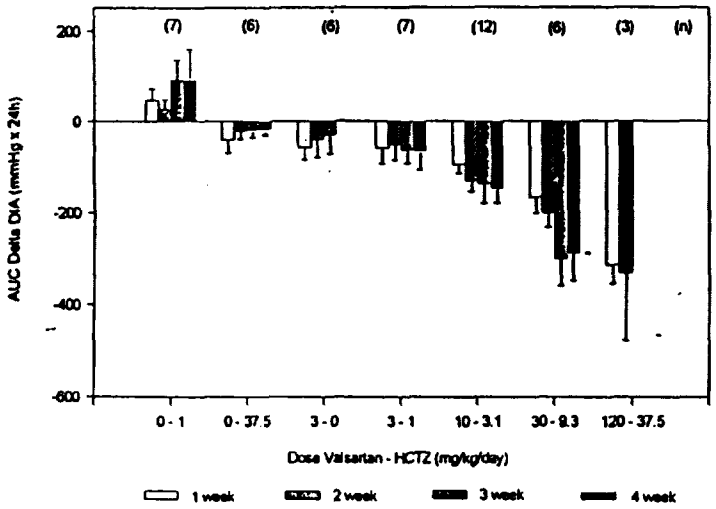
drug combination, and the magnitude and duration of the response was dose-dependent.

Plasma levels of bold creatinine showed considerable variation from animal to animal and no consistent effect was reported. Plasma levels of urea were less variable and were elevated above baseline in the 2 surviving animals receiving the HD (120:37.5 mg/kg; valsartan: HCTZ) of the drug combination. The dose-dependent increases in HR noted after dosing on the first days of treatment were attenuated in the following weeks of the study.

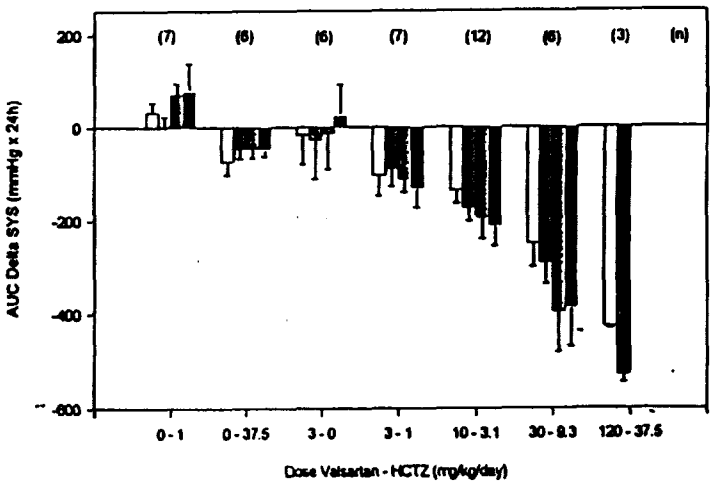
Drug sponsor considered that the continuous reductions in systolic and diastolic BP induced after doses of the fixed combination of valsartan and HCTZ and the consequent renal ischemia may be responsible for the renal lesions as were observed in the 6-mo oral toxicology studies.

The effects of valsartan or HCTZ alone or increasing doses of the combination of both drugs on body weight, AUC, systolic and diastolic BP and plasma concentrations of creatinine and urea are summarized in figures and table below (following 2 pages) prepared by the drug sponsor.

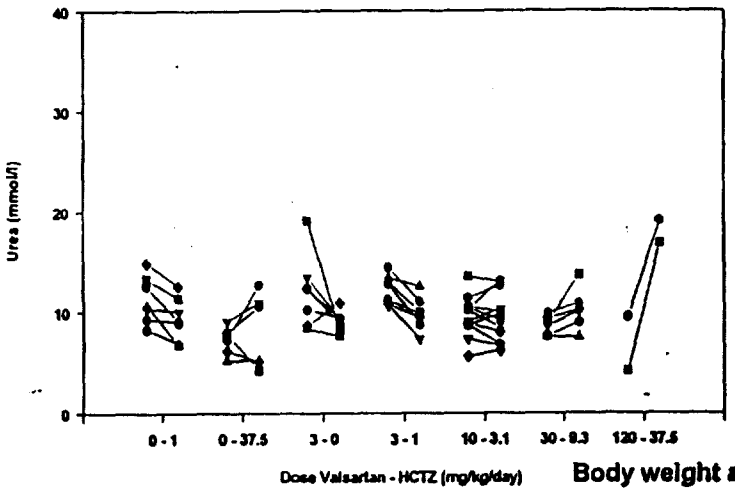
Area under the curve (AUC) for diastolic blood pressure after treatment of normotensive, sodium-replete marmosets with HCTZ or valsartan alone or increasing doses of the combination valsartan/HCTZ (3/1, 10/3.1, 30/9.3 or 120/37.5 mg/kg per day) over 4 weeks



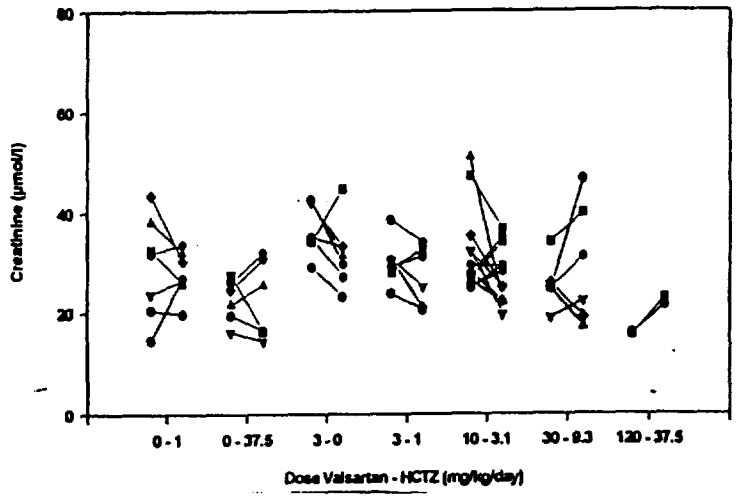
Area under the curve (AUC) for systolic blood pressure after treatment of normotensive, sodium-replete marmosets with HCTZ or valsartan alone or increasing doses of the combination valsartan/HCTZ (3/1, 10/3.1, 30/9.3 or 120/37.5 mg/kg per day) over 4 weeks



Plasma urea concentrations before and after 4 weeks of treatment of normotensive, sodium-replete marmosets with HCTZ or valsartan alone or increasing doses of the combination valsartan/HCTZ (3/1, 10/3.1, 30/9.3 or 120/37.5 mg/kg per day) over 4 weeks



Plasma creatinine concentrations before and after 4 weeks of treatment of normotensive, sodium-replete marmosets with HCTZ or valsartan alone or increasing doses of the combination valsartan/HCTZ (3/1, 10/3.1, 30/9.3 or 120/37.5 mg/kg per day) over 4 weeks



Body weight after daily oral administration of HCTZ or valsartan alone or the combination of HCTZ/valsartan over 4 weeks to normotensive, sodium-replete marmosets

(mean ± SEM)

	Initial weight (g)	After 4 weeks treatment	*n*
HCTZ 1 mg/kg/day	410 ± 21	411 ± 20	7
HCTZ 37.5 mg/kg/day	394 ± 14	400 ± 13	6
valsartan 3 mg/kg/day	379 ± 17	385 ± 20	7
HCTZ/valsartan (mg/kg/day)			
1/3	382 ± 16	383 ± 16	7
3.1/10	401 ± 16	402 ± 15	12
9.3/30	392 ± 18	384 ± 21	6
37.5/120	356 ± 2	*334 ± 30	3

* After 2 weeks treatment

2. TOXICOLOGY (Vs. 10-16)

2.1 Oral Acute Toxicity Studies were conducted with CGP 63172 (16:5 wt:wt ratio in the combination of valsartan- Lot 800393 + HCTZ- Lot 800188) given by gavage to two animal species [rodent: Sprague-Dawley rats, and non-rodent: marmosets (Callithrix jacchus)]. All summary tables used in this **TOXICOLOGY** section were prepared by drug sponsor.

♦ 2.1.1 **Rat** : CGP 63172 Acute Oral Toxicity Study (**Test No. 946111.** Report No. 044/94/SL of a OECD GLP study conducted at drug sponsor's laboratories in the UK started on 10-08-94 and necropsy date of 30-08-94. v.10. p. 63).

The objective of this study was to determine acute oral (gavage) toxicity of CGP 63172 using 5M/5F rats after a single dose of 2000 mg/kg, and to assess the recovery of toxic signs after a 14-day observation period. No control rats were used.

RESULTS

No rats died. Rats were sacrificed on day 15 of the study, and no gross abnormalities were detected at necropsy, thus no tissues were examined microscopically. Based on these findings, drug sponsor concluded that in the absence of toxic effects of the drug treatment, the acute oral toxic dose of CGP 63172 in rat may be expected to be above 2000 mg/kg.

The synoptic table below, prepared by drug sponsor, shows the study protocol/methods/doses and remarkable findings of this **single oral dose study in rats.**

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS
Acute toxicity; Sprague-Dawley rats (Tif:RAIf [SPF]); ive/sex/group, approximately 10 weeks of age and weighing 211 to 230 g males) or 164 to 175 g (females) at dose administration.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800393) and HCT (Lot 800188), administered orally by gavage, as a suspension in 0.5% w/v CMC and 0.5% v/v Tween 80, at a single dose of 1523.8:476.2 mg/kg (dose volume of 10 ml/kg). Necropsies were performed 14 days after dosing.	Ciba Pharmaceuticals, Stamford Lodge, United Kingdom C Hankinson Report no. 044/94/SL 04-Nov-94	There were no treatment-related mortalities, clinical signs, body weight or food consumpti effects, or macroscopic abnormalities.

2.1.2 Marmoset : CGP 63172 Acute Oral Toxicity Study (**Test No. 946113**). (Report No. 046/94/SL dated 11-11-94 of OECD GLP study conducted at drug sponsor's laboratories in the UK started on 10-08-94 and necropsy date of 05-09-94. v.10. p. 96)

The objective of this study was to determine a no-effect level (NOEL) of single oral (gavage) dose of CGP 63172 (80:25 wt/wt combination of valsartan + HCTZ) at 420 or 1000 mg/kg in 2M/1F marmosets (~2 kg). Animals were observed for 2 days, and no control marmosets were used.

The marmosets were sacrificed/necropsied 14 days after the first drug dose. Some 40 organ/tissues were collected for fixation/histopathologic processing but no organs were weighed.

RESULTS

CGP 63172 appeared to be well tolerated by the marmosets at a single dose of 1000 mg/kg po. Except for emesis after the first dose of the drug, no other toxic signs were reported. Based on this single sign, the NOEL in these marmosets could be considered to be below 1000 mg/kg po, and the acute oral toxic dose above 1000 mg/kg CGP 63172.

At sacrifice, no treatment related clinical sign/gross abnormalities were reported at necropsy, thus no tissues were examined microscopically.

The synoptic table below, prepared by drug sponsor, shows the study protocol/methods/doses/toxicologic remarkable findings in the **single dose study in marmosets** was prepared by drug sponsor.

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS
Acute toxicity; marmosets (*Callithrix jacchus*); one male or one male and one female, approximately 23 or 24 months (males) or 33 months (female) of age and weighing 346 or 386 g (males) or 468 g (female) at dose administration.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800393) and HCT (Lot 800188), administered orally by gavage, as a suspension in 0.5% w/v CMC and 0.5% v/v Tween 80, to one male marmoset at a single dose of 320:100 mg/kg and one marmoset/sex at a single dose of 761.9:238.1 mg/kg (dose volume of 10 ml/kg). Necropsies were performed 14 days after dosing.	Ciba Pharmaceuticals, Stamford Lodge, United Kingdom CL Turner Report no. 046/94/SL 11-Nov-94	There were no mortalities, clinical signs, b weight or food consumption effects, or macroscopic abnormalities.

♦ 2.2 **Repeat Oral Dose Studies** were conducted with CGP 63172 (16:5 wt:wt ratio of the combination of valsartan- Lot 800194 + HCTZ- Lot 800188), and HCTZ alone.

Drugs were given by gavage to two animal species (**rodent** and **non-rodent**) as were used in the single dose studies:
Sprague-Dawley **rats** and **marmosets** (Callithrix jacchus).

2.2.1 ♦ **Rat** [Sprague Dawley derived Tif:RAIf (SPF)]

2.2.1. **1-month Rat**: CGP 63172 Repeat Dose Toxicity Study (**No. 946114**). (Report No. 052/94/SL dated 08-02-95 of OECD GLP study conducted at drug sponsor's laboratories in the UK started on 09-09-94 and date of last necropsy date of 12-10-94. v.10.)

This rat study was to determine the subacute oral toxicity of CGP 63172 given as daily doses for 1-mo, and to select dose levels for a 26-week repeat dose study in the same species.

Briefly, rats were assigned to 5 different groups using computer generated random numbers, and acclimatized to laboratory conditions for 12 days prior to treatment. Rats were identified with ear tags/tail tatoos. During the study, animals were housed (5 per cage) in a room at ~21° C., and allowed free access to commercial irradiated diet and filtered water.

Protocol called for daily examination for mortality, clinical signs pre- and post- dosing, body weight (means weekly), and water/food consumption (mean values), and at pre-test and week 4 of study for-ECG, hearing/eye tests, clinical chemistry (16 parameters), hematology (13 parameters), and urinalysis (18 parameters). After the last drug administration, all surviving rats were sacrificed and necropsied. Some 12 organs were weighed and over 40 tissues were collected/fixed for microscopic examinations. Deviations from the protocol were reported (i.e., non readable ECG results), but none of the deviations were considered by drug sponsor to have influenced the study outcome. Statistical analyses were performed on the data collected, however trend analysis was not possible due to incomparability of Group 5 (HCTZ treatment alone) with the other drug treated groups.

Toxicokinetics were not performed.

The table below shows rats dosing schedule followed in this study.

	Group 1	Group 2	Group 3	Group 4	Group 5
	0.5% w/v CMC with 0.5% v/v Tween 80				
Dose (mg/kg)	0	65.625	262.5	787.5	187.5 (SU 5879 only)
Volume (ml/kg)	10	10	10	10	10
No. of animals	5♂+5♀	5♂+5♀	5♂+5♀	5♂+5♀	5♂+5♀
Duration of treatment	4 weeks	4 weeks	4 weeks	4 weeks	4 weeks

RESULTS:

No rats died during the study. No remarkable clinical signs of toxicity were reported.

Examinations of rats at week 4 showed that those treated with **HCTZ** alone were reported as showing increase in blood urea levels when compared to vehicle control.

Some rats treated with at various levels of **CGP 63172** occasionally showed slight to moderate decreases in body weight/food consumption, and increases in water consumption vs controls. Clinical chemistry, hematology, or urinalysis were considered by drug sponsor as unremarkably affected at various dose levels of CGP 63172. However, some clinical chemistry reported in this 1-mo study included marked increases in plasma urea, Mg^{++}; slight decreases in K^{+}, protein and cholesterol vs controls.

Rats treated with **HCTZ** alone also showed similar changes as those reported for **CGP 63172** (i.e., moderate increases in urea, increases in cholesterol, and decreases in K^{+}, total protein concentration in M rats). The CGP 63172 treated rats urinalysis showed decreases in specific gravity and increases in Na^{+} and K^{+} output.

Rats were sacrificed one day after the last dosing. No gross abnormalities were reported at necropsy, and no tissues were examined microscopically.

The table below is a synopsis of the study protocol/methods/doses/ toxicologic findings by the drug sponsor on this **1-mo rat study**.

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS
Subchronic toxicity, 1-month oral; Sprague-Dawley rats (Tif: RAIf [SPF]); five/sex/group, approximately 6 weeks of age and weighing 151 to 190 g (males) or 134 to 164 g (females) at study initiation.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800194) and HCT (Lot 800188), administered orally by gavage, as suspensions in 0.5% w/v CMC and 0.5% v/v Tween 80, at daily doses of 0, 50:15.625, 200:62.5 or 600:187.5 mg/kg for at least 4 consecutive weeks (dose volume of 10 ml/kg). Another group of rats received HCT alone at a daily dose of 187.5 mg/kg. Necropsy was conducted at the end of the dosing period.	Ciba Pharmaceuticals, Stamford Lodge, United Kingdom C Hankinson Report no. 052/94/SL 08-Feb-95	0:187.5 mg/kg (HCT suspension only): Moderate ↑ in water consumption (M,F); moderate ↑ in plasma urea (M,F); slight ↓ in plasma triglycerides (M,F); slight ↑ in plasma cholesterol (F); slight ↓ in plasma potassium (F); slight ↓ in total plasma protein (M); slight ↑ in urinary potassium (F); slight ↑ in urinary sodium (M); slight ↓ in urinary specific gravity (M,F); slight to moderate ↑ in urine volume (M,F); slight ↑ in absolute and relative kidney weights (M). ≥ 50:15.625 mg/kg: Slight to severe ↑ in water consumption (M,F); slight to severe ↑ in plasma urea (M,F); slight ↑ in urinary potassium (M,F); slight ↑ in urinary sodium (M). ≥ 200:62.5 mg/kg: Slight to moderate ↓ in body weight gain (M); slight ↓ in food consumption (M); moderate to severe ↑ in plasma potassium (M); moderate to severe ↑ in plasma magnesium (M); slight to moderate ↓ in hemoglobin, hematocrit and total red blood cell count (F); slight ↑ in urinary sodium (F); slight ↓ in urinary specific gravity (M); slight to moderate ↑ in urine volume (M,F).

2.2.1.2 **6-month Rat** Repeat Dose Toxicity with CGP 63172 (80:25 ratio of valsartan:HCTZ) (**Study No. 946116**. Report No. 037/95/SL dated 13-12-95 of OECD GLP study conducted at drug sponsor's laboratories in the UK started on 28-10-94 and date of last necropsy date of 01-06-95. v.11).

This 6-mo rat study was to determine the chronic toxicity of **CGP 63172** or **HCTZ**, and recovery from toxic effects after 1 month without drug treatment.

Doses (selected from the 1-mo study in same strain of rats) were given as daily for 7 days a week appear in a table below.

	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	CGP 63 172	CGP 63 172	CGP 63 172	SU 5879
Dosage (mg/kg)*	See ***	39.375	131.25	393.75	93.75
Volume (ml/kg)	10	10	10	10	10
Main group	20 ♂ + 20 ♀	20 ♂ + 20 ♀	20 ♂ + 20 ♀	20 ♂ + 20 ♀	20 ♂ + 20 ♀
Recovery **	5 ♂ + 5 ♀	5 ♂ + 5 ♀	5 ♂ + 5 ♀	5 ♂ + 5 ♀	5 ♂ + 5 ♀
Duration of treatment (weeks)	26	26	26	26	26

* The dosages for groups 2, 3 and 4 related to 30, 100 and 300 mg/kg of CGP 48 933, respectively. Group 5 received a similar dose of SU 5879 to that administered to the group 4 rats. Dosages given are nominal concentrations.

** Recovery animals were allowed a recovery period of one month (28 days) without treatment.

*** 0.5% w/v aqueous CMC containing 0.5% w/v aqueous Polysorbate 80

Rats (5 of the same sex/per cage) were assigned to the 5 groups using computer generated random numbers, and were acclimatized to laboratory conditions for 13 days. The initial body weight of the rats on first day of dosing ranged from 151 to 183 g. Rats were identified with ear tags/tail tatoos. During the study, animals were housed in a room at ~10-23° C. and commercially irradiated diet/filtered tap water were freely available.

Study protocol called for daily examination for mortality/clinical signs during pre-/post test, and during recovery period from treatment, body weight (mean values reported weekly), food consumption (mean values extrapolated per rat/day), and water consumption (groups means reported at protocol designated time intervals).

Other observations included ECG (5M/5F from control/HD groups on week 12), and at ~ weeks 12, 26, 30 and during recovery period-hearing/eye tests, as well as clinical chemistry (17 parameters), hematology (13 parameters), and urinalysis (18 parameters).

After the last drug administration, all surviving rats (except for recovery period animals) were sacrificed/necropsied (also those found dead). Some 11 organs were weighed, and over 40 tissues were collected/fixed for microscopic examinations.

Deviations from the protocol were reported (i.e., incidences of dosage administration errors, changes in room temperatures, ECG examination of in rats that had been sedated, and ECG traces unreported), but no deviations were considered by the drug sponsor to have influenced the outcome of the study.

Statistical analyses on data collected were performed to show the presence or absence of drug effect. According to drug sponsor, trend comparisons were not done because of the incomparability of Group 5 (**HCTZ** treatment alone) with the other drug treated groups.

Toxicokinetic analysis (5M/5F per group) was performed on day 1 and during week 26 of study. During drug sampling period, the rat's diet was changed to ground form, and food consumption was not recorded.

RESULTS:

Mortality (found dead and killed in extremis) and terminal signs reported during the 6-mo study are reported below in table edited by reviewer.

Summary of unscheduled deaths

Factors Contributing to Death	Group	1		2		3		4		5	
	Dosage mg/kg	0		39.375		131.25		393.75		93.75 SU 5879	
	Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Suspected maladministration of dosage									2		
Animal sedated for ECG Examination								2			
Cystitis/pyelonephritis found at necropsy					1						4
Caecal ulcer/ peritonitis found at microscopy								2	1		
No indications as to cause of death								1	1		

Briefly, notable signs of toxicity reported other than mortality included changes in clinical chemistry as dose-related increases in blood urea, electrolytes (Mg^{++}, K^{+}), creatinine (after 13 wks of drug treatment), enzyme levels (aspartate aminotransferase and alanine aminotransferase) and cholesterol; the levels of triglycerides were reduced after 13 weeks in some rats. Remarkable hematologic changes in **CGP 63172** treated rats included slight to moderate reduction in Hgb levels, RBC and Hct in some rats

Dose-related increases in urinary volume (associated with decreases in specific gravity) and increases in total electrolyte excretion were reported which returned to normal during recovery period.

The report shows that some of the above mentioned parameters returned to normal during the recovery period.

The most prominent macroscopic findings in the rats treated with CGP 63172 that died or were sacrificed in extremis were purulent hemorrhagic cystitis with marked bilateral pyelonephritis (1 LD F); hemorrhagic fluid in stomach/intestines and ulcer in caecum (1 HD M); severe peritonitis and caecal ulcer (1 HD F); ulceration of the forestomach, marked peritonitis and severe purulent prostatitis (1 HD M). Two F rats treated with HCTZ showed bilateral pyelonephritis and 1 F showed purulent hemorrhagic cystitis.

The most remarkable microscopic finding considered treatment related were reported for the kidneys. Tubular basophilia was reported in rats of both sexes from all drug treated groups; the severity of the condition was greater in M rats than in F. This histopathologic change was accompanied by thickening of the basement membranes and occasionally a low grade lymphoid cell infiltration. Drug sponsor suggested that this histopathologic finding could reflect an exacerbation of the background spontaneous nephropathy due to an increased renal demand by both components of CGP 63172. The incidence and severity of **tubular basophilia** are detailed below in tables (edited by reviewer) provided in the NDA. Tables shows that during recovery period, the kidney lesions were **not** reversible.

Males – Treatment Period (Week 26)

Dosage	Control	39.375 mg/kg	131.25 mg/kg	393.75 mg/kg	93.75 mg/kg SU 5879
Number of Animals	20	20	20	19	20
Grading					
1	6	8	13	10	12
2			4	6	
3				2	

Females – Treatment Period (Week 26)

Dosage	Control	39.375 mg/kg	131.25 mg/kg	393.75 mg/kg	93.75 mg/kg SU 5879
Number of Animals	20	19	20	16	16
Grading					
1	8	4	15	9	8
2					
3					

Males – Recovery Period (Week 30)

Dosage	Control	39.375 mg/kg	131.25 mg/kg	393.75 mg/kg	93.75 mg/kg SU 5879
Number of Animals	5	5	5	3	5
Grading					
1	3	2	4	3	1
2					
3					

Females – Recovery Period (Week 30)

Dosage	Control	39.375 mg/kg	131.25 mg/kg	393.75 mg/kg	93.75 mg/kg SU 5879
Number of Animals	5	5	5	5	5
Grading					
1	2	2	4	4	1
2					
3					

Key: Grading

1 Minimal
2 Slight
3 Moderate

Recovery Period Findings:

"Recovery rats" (5M, 5F/per group), starting at wk 26 of study, were allowed a recovery period of 28 days without treatment. Drug sponsor reported that "all the parameters in all treatment groups had returned to normal". However, histopathologic changes related to renal tubular basophilia were still evident.

Drug sponsor considered the effects reported in rats treated with doses of CGP 63172 ranging from of 39.375 up to 393.75 mg/kg/day for 6 months to be dosage-related pharmacologic changes and that when the animals were allowed to recover for 1 month without drug treatment that all parameters had returned to normal levels.

The following table is a synopsis prepared by drug sponsor (edited by reviewer) of the rat 6-mo repeat dose study with CGP 63172 (**Study No. 946116**).

Summary of toxicology findings

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS	
Chronic toxicity, 6-month oral; Sprague-Dawley rats (Tif: RAIf [SPF]); 25/sex/group, approximately 8 weeks of age at study initiation and weighing 177 to 227 g (males) or 151 to 183 g (females) at allocation.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800194) and HCT (Lot 800188), administered orally by gavage, as suspensions in 0.5% w/v CMC and 0.5% v/v Tween 80, at daily doses of 0, 30:9.375, 100:31.25 or 300:93.75 mg/kg for 6 consecutive months followed by an approximate 1-month recovery period (dose volume of 10 ml/kg). Another group of rats received HCT alone at a daily dose of 93.75 mg/kg. Necropsies were conducted upon death, at the end of the treatment period, or following the approximate 1-month recovery period.	Ciba Pharmaceuticals, Stamford Lodge, United Kingdom C Hankinson Report no. 037/95/SL 13-Dec-95	0:93.75 mg/kg (HCT suspension only): Slight to marked ↓ in body weight gain (M); minimal to slight ↓ in food consumption (M); slight to marked ↑ in plasma urea (M,F); slight ↑ in plasma sodium (M); slight ↓ in urinary sodium (M,F); slight ↓ in urinary potassium (M); minimal ↓ in heart weights (M,F); minimal to slight ↑ in renal tubular basophilia (M). ≥ 30:9.375 mg/kg: Slight to marked ↓ in body weight gain (M); minimal to slight ↓ in food consumption (M); slight to marked ↑ in plasma urea (M,F); slight to moderate ↓ in hemoglobin, hematocrit and total red blood cell count (M,F); slight to marked ↑ in urine volume and slight ↓ in urinary specific gravity (M,F); minimal ↓ in heart weights (M,F). 100:31.25 mg/kg: Slight to moderate ↑ in urinary sodium (M); minimal to slight ↑ in renal tubular basophilia (F).	≥ 100:31.25 mg/kg: Slight to moderate ↑ in wa consumption (M); slight to marked ↑ in plasma magnesium (M,F); red cell morphology change (M,F); slight to moderate ↑ in urinary sodium (I slight to moderate ↑ in urinary potassium (M,F minimal to slight ↑ in renal tubular basophilia (300:93.75 mg/kg: Salivation (M); slight to moderate ↑ in water consumption (F); minimal ↑ in plasma creatinine (M,F); slight ↑ in plasm potassium (M); slight ↑ in plasma phosphate (M,F); slight ↑ in plasma cholesterol and ↓ in plasma triglycerides (M); slight ↑ in reticulocyti count (M,F). In general, females appeared to be slightly less affected than males. With the exception of the microscopic renal change, all findings were at least partially reversible following the approximate 1-month recovery period.

2.2.2 **Marmoset** (Callithrix jacchus).

2.2.2.1 **1-month** Marmoset Repeat Dose Toxicity with CGP 63172 (**Study No. 946115**. Report No. 058/94/SL dated 20-03-95 of OECD GLP study conducted at drug sponsor's laboratories in the UK started on 31-08-94 and date of last necropsy date of 14-10-94. v.13).

The objective of this study was to determine the oral (gavage) subchronic toxicity of CGP 63172 in marmosets treated daily, 7 days a week for 1-mo.

Briefly, marmosets were assigned to 5 different treatment groups, and were acclimatized to laboratory conditions for 2 weeks prior to the start of study. The following table shows the study design and dosing schedule for the marmosets.

	Group 1	Group 2	Group 3	Group 4	Group 5
Test article	Control	CGP 63 172	CGP 63 172	CGP 63 172	SU 5879
Dose (mg/kg)	0	39.375	157.5	525	125
Volume (ml/kg)	10	10	10	10	10
No. of animals	3♂+3♀	3♂+3♀	3♂+3♀	3♂+3♀	3♂+3♀
Duration of treatment	28 days	28 days	28 days	28 days	28 days

During the study, marmosets were housed individual or single-sex-pair cages and kept in air conditioned rooms at ~27° C. with artificial lights for 12 hrs/12 hrs in dim lights. Animals were identified with inner thigh tatoos. The initial weight of the animals (aged 20-42 months) ranged from 278 up to 581 g.

Marmosets were fed twice a day. The menu, diet and method of food preparation were described in detail in the NDA. Filtered water was freely available. The study protocol called for daily examinations for mortality, pre- and post- test dosing clinical signs, body weight (reported twice weekly), food consumption (reported weekly), water consumption (mean intake per cage at predetermined intervals, but individual data were not available). At pre-test and week 4 of study, the following examinations were performed: hearing/eye tests, ECG (lead II), clinical chemistry (16 parameters), hematology (13 parameters), and urinalysis (18 parameters).

After the last day of treatment, all surviving marmosets were sacrificed, and necropsied (including those animals found dead). Some 13 organs were weighed (not from animals found dead), and over 40 tissues were collected/fixed for microscopic examinations.

Deviations from the protocol were reported (i.e., changes in room temperature, ECG recorded at the wrong frequency, mistakes in recording body weights, and other deviations), but none of the deviations were considered by drug sponsor/peer review to have affected the integrity or interpretation of the study data.

Statistical analyses were performed on the data collected, however trend test was not suitable for Group 5 (HCTZ treatment alone).

Toxicokinetics were not performed. Blood and urine levels of these drugs were reported in a 6-mo pharmacokinetic study with marmosets. (Study No. 94-7908, below.)

RESULTS

Two F HD **CGP 63172** marmosets were sacrificed on days 6 and 19 because of deteriorating conditions and severe clinical signs; the remaining F in this group were found dead on day 11 of study. Clinical signs before death included loss in body weight, vomiting, dark/black diarrhea, and 1 F showed buccal ulcerations.

Remarkable signs in these marmosets after treatment with CGP 63172 was vomiting; MD animals, HD F, and only 1 LD F vomited up to 2 hrs after dosing. Two F treated with **HCTZ** also vomited twice during the first week of treatment.

Marked body weight loss was mainly reported for all HD F and minimal weight losses were reported for the LD animals and for the HCTZ group.

Compared to controls, at all doses of CGP 63172 were associated with increases in plasma levels of urea, creatinine, and Mg^{++} and slightly reduced levels of Na^{+}; the HCTZ group showed increase in urea and slight decrease in K^{+}.

No treatment related abnormalities were reported for ECG tracings for CGP 63172 or HCTZ at ~ 4 hrs after dosing on the 4th week of treatment.

Marmosets were sacrificed on 1 day after the last dosing. No remarkable changes were reported for organs weights.

No gross abnormalities were reported at necropsy that were considered related to the treatment by drug sponsor.

Remarkable microscopic findings reported in tissues of animals treated with CGP 63172 revealed treatment related changes in the kidney and stomach. Both at MD and HD renal tubular basophilia and cortical mineral deposition were seen in some animals and less clearly on tubular dilatation in F.

The following table was submitted by the drug sponsor showing renal changes in the marmosets treated with the LD and MD CGP 63172.

Dosage	39.375		157.5	
Sex	Male	Female	Male	Female
Renal Tubular basophilia		1/3	1/3	1/3
Cortical tubule mineral deposition	1/3		1/3	1/3

Unilateral minimal cortical tubular mineralization was seen in the 1M at LD, and 1M/1F at HD CGP 63172. However, drug sponsor asserted that the renal pathology is considered to be possibly related to altered tubular physiologic function and "not to represent an overt toxicologic effect" of the drug.

Although drug sponsor asserts that renal pathology reported in these marmosets is to be considered possible related to altered tubular physiologic function, the data reported strongly suggest that administration of CGP 63172 at dosage of 525 mg/kg may be causally related to the renal changes and early death of all F marmosets.

HD F showed gastric degenerative effects characterized by ulceration, submucosal inflammation and focal purulent crypts.

The table on the following page is a synopsis prepared by drug sponsor of this **1-mo repeat dose study in marmosets.** Drug sponsor's original conclusions on the study below were later amended (June 1995) to include minor renal changes (unilateral cortical mineralization and bilateral tubular basophilia) described above.

CGP 63172: 1-Month Oral Toxicity Study In Marmosets
(Synoptic table prepared by drug sponsor.)

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS	FINDINGS AND/OR COMMENTS
Subchronic toxicity, 1-month oral; marmosets (*Callithrix jacchus*); three/sex/group, approximately 20 to 27 months (males) or 20 to 42 months (females) of age and weighing 307 to 581 g (males) or 278 to 510 g (females) at study initiation.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800194) and HCT (Lot 800188), administered orally by gavage, as suspensions in 0.5% w/v CMC and 0.5% v/v Tween 80, at daily doses of 0, 30:9.375, 120:37.5 or 400:125 mg/kg for 28 consecutive days (dose volume of 10 ml/kg). Another group of marmosets received HCT alone at a daily dose of 125 mg/kg. Necropsies were conducted upon death, moribund sacrifice or at the end of the treatment period.	Ciba Pharmaceuticals, Stamford Lodge, United Kingdom P McKenna Report no. 058/94/SL 20-Mar-95	0:125 mg/kg (HCT suspension only): Vomiting (primarily during the first week) (F); slight ↑ in plasma urea (M,F); slight ↑ in plasma creatinine (M); moderate ↓ in plasma potassium (M); slight ↓ in plasma sodium (M,F); slight ↑ in plasma fibrinogen (M); renal tubular basophilia (M,F). 30:9.375 mg/kg: Moderate to marked ↑ in plasma urea (M,F); slight to marked ↑ in plasma creatinine (F); slight ↓ in plasma sodium (F); moderate ↑ in plasma magnesium (F); slight to moderate ↑ in plasma phosphate (M); renal tubular basophilia (F). ≥ 30:9.375 mg/kg: Vomiting (primarily during the first week) (F); slight ↓ in plasma sodium (M); renal cortical mineralization (M). 120:37.5 mg/kg: Moderate to marked ↑ in plasma urea (M,F); slight to marked ↑ in plasma creatinine (F); slight ↓ in plasma sodium (F); moderate ↑ in plasma magnesium (F); renal tubular basophilia (F).	≥ 120:37.5 mg/kg: Vomiting (primarily during the first week) (M); slight ↓ in food consumption and slight to marked ↓ in body weight (M,F); slight to marked ↑ in plasma creatinine (M); slight ↓ in plasma potassium (M); moderate ↑ in plasma magnesium (M); slight ↑ in plasma fibrinogen (M); renal tubular basophilia (M); renal cortical mineralization (F). 400:125 mg/kg: Mortality/moribundity and associated antemortem clinical signs [vomiting, dark/black diarrhea and buccal ulceration] (F); moderate to marked ↑ in plasma urea (M); renal tubular dilatation (F); gastric irritation and degenerative effects (ulceration, submucosal inflammation, and focal purulent crypts in duodenum and stomach) (F).

2.2.2.2 **6-month Marmoset** Repeat Dose Toxicity with CGP 63172 (**Study No. 946117**. Report No. 038/95/SL dated 16-02-96 of OECD GLP study conducted at drug sponsor's laboratories in the UK started on 21-11-94 and date of last necropsy date of 21-07-95. v.14).

This study was conducted to determine the oral toxicity of CGP 63172 during, and after daily administration for 6-months, and to assess the recovery from any toxicologic effects after 1-mo without treatment.

The selection of dosages was based on the results of a 1-mo study in marmosets summarized above in which 525 mg/kg CGP 63172 caused histopathologic changes in the kidney and early death of the F marmosets. (See Study No. 946115 above.)

	Control*	CGP 63 172				SU 5679
Group number	1	2	3	4	5[c]	6
Dosage (mg/kg)	0	39.375	78.75	157.5	315 → 157.5	75
Volume (mL/kg)	10	10	10	10	10	10
Main group	4 m + 4 f	4 m + 4 f	4 m + 4 f	4 m + 4 f	6 m + 6 f	2 m + 2 f
Recovery**	2 m + 2 f	2 m + 2 f	2 m + 2 f	2 m + 2 f		2 m + 2 f
Duration of treatment	182 days	182 days	182 days	182 days	17 and 160 days[d]	182 days

* 0.5% w/v aqueous carboxymethylcellulose containing 0.5% w/v polysorbate 80.

** Recovery groups were allowed a recovery period of 28 days without treatment.

c Dosage was reduced to 157.5 mg/kg from Day 22.

d Dosing suspended between Days 18 and 22.

The marmosets were acclimatized to laboratory conditions for a minimum of 4 weeks prior to the start of study.

Briefly, during the study, marmosets were housed individual or single-sex-pair cages and kept in air conditioned rooms at 23 to 27° C. with artificial lights for 12 hrs/dim lights for 12 hrs. The method of animal identification as well as their initial body weights/ages were within the same range as in the 1-mo study. As in previous studies, animals were also fed twice a day and the diet/menu/method of food preparation were fully described.

From day 20 of study (except for 3 days) marmosets received bottled electrolyte (electrolyte composition of supplement could not be found), and mineral supplements Water, purified by filtration, was freely available to the marmosets.

The study protocol called for daily dosing, examination for mortality, pre- and post- test dosing clinical signs, body weight/food consumption (reported weekly), water/electrolyte consumption (mean intake per animal calculated at protocol required time intervals), and at pre-test and week 25 of study hearing/eye tests, and ECG (wk 25 traces from lead II). Clinical chemistry (16 parameters), hematology (13 parameters), and urinalysis (18 parameters) were determined pretest and weeks 13, 26 and during recovery period on week 30.

One day after the last drug dose or after the end of the recovery period, all marmosets were sacrificed and necropsied (also those found dead). Some 12 organs were weighed (not from animals found dead or sacrificed moribund), and over 40 tissues were collected/fixed for microscopic examinations.

Deviations from the protocol were reported (i.e., 1 MD animal was not dosed 1 day because it was recovery from surgery after amputating a damaged digit; incorrect doses; skipped dosing because of severe clinical signs; urine samples lost; stability of formulated test article not analyzed, changes in room humidity/temperature range, and other deviations). Drug sponsor stated that the deviations from the protocol did not affect the integrity of the study.

Statistical analyses were performed on the data collected. All methods used were described in the NDA.

Toxicokinetics were **not** performed.

RESULTS

No control or **HCTZ** treated marmosets died. Mortality among the **CGP 63172** treated animals was reported as follows:

Group	CGP 63 172	Number of deaths	
		Males	Females
2	39.375 mg/kg	0	1
3	78.85 mg/kg	0	1
4	157.5 mg/kg	1	0
5	315 -> 157.5 mg/kg	1	5

One HD F was found dead, and 1M/2F from the same group were killed on days 14 and 18 of study. Following these deaths it was decided to suspend this HD treatment because of excessive loss of weight. After the animals recovered, they were started on day 22 of study on the new dose reduced from 315 mg/kg to 157.5 mg/kg/day CGP 63172. However, 2 additional F at this new reduced dose were sacrificed in extremis on days 43, and 64 of study. Killed in extremis were 1 MD-2 M / 1 MD-1 F on day 21, and 1 LD F on day 103.

Remarkable clinical signs in this 6-mo study included loss in body weight, vomiting (within 15 min at the HD), diarrhea and buccal ulcerations; similar findings were reported in the 1-mo study.

There was initial body weight losses (for the first 4 wks of study) in all treated animals; F being more sensitive to the weight loss. HD F continued to lose weight until week 14, after which their weight gain was similar to controls. The **HCTZ** group lost weight

during the first 2 weeks of the study but subsequently their weights were within the range of the controls.

Overall, drug sponsor reported that food consumption remained similar for control and LD F, and the HCTZ treated animals. The water/electrolyte solution treated animals showed a much higher food consumption than controls during week 4.

Clinical chemistry in drug treated animals showed dose-related increases in plasma levels of urea, creatinine (except for LD M), and Mg^{++}. Similar findings were also reported in the 1-mo study with CGP 63172. All these reported clinical chemistry changes were reversible during the 1-mo recovery period.

In hematology, only moderate reductions in erythrocyte parameters in some animals were reported.

Urinalysis showed a trend toward increase in volume and decrease in specific gravity with an increase in Na^{+} excretion.

Marmosets sacrificed on 1 day after the last dosing showed no remarkable changes in organ weights except for a reduced but reversible liver and kidney weights in HCTZ treated animals.

No remarkable gross abnormalities were reported at necropsy in LD CGP 63172; animals treated with the higher doses of the drug combination showed gastrointestinal tract lesions (e.g., cecal, colonic discoloration, erosion and red fluid content). Kidney gross changes were predominant in CGP 63172 treated animals; these gross changes included arterial/arteriolar hypertrophy and tubular nephropathy and tubular mineralization.

Histologic changes included both glomerular arterial and arteriolar hypertrophy as well as arteriolar mural proliferation, interstitial nephritis, tubular mineralization (also seen in some marmosets treated for 1-mo only), and tubular basophilia or nephropathy (increased population of nuclei within the cellular wall of proximal convoluted tubules*).

Six marmosets treated with CGP 63172 had kidneys with focal tubular epithelial hyperplastic lesions; none of these lesions were reported for controls or HCTZ treated animals.

Several other histopathologic lesions in separate individual animals from different treatment groups were reported (e.g. for CGP 63172 treated animals these included chronic pericarditis in F; purulent gall bladder inflammation, hepatic centriacinar eosinophilia, an focal inflammation with necrosis, and others).

* Firm reported this pattern of nephropathy has been observed in control marmosets in an unrelated study, however; no bibliographic reference was provided.

Drug sponsor considered these vascular changes to be related to the pharmacologic action of CGP 63172, with the tubular changes as secondary effects of altered tubular function caused by reduced renal perfusion.

CGP 63172 : Synopsis prepared by drug sponsor of 1st the 6-Month Oral Toxicity Study In Marmosets (Test No. 946117) (V.1, pp 102-3.)

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS	
Chronic toxicity, 6-month oral; marmosets (*Callithrix jacchus*); four or six/ sex/group, approximately 19 to 39 months (males) or 19 to 47 months (females) of age and weighing 297 to 520 g (males) or 291 to 631 g (females) at study initiation.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800194) and HCT (Lot 800188), administered orally by gavage, as suspensions in 0.5% w/v CMC and 0.5% v/v Tween 80, at daily doses of 0, 30:9.375, 60:18.75, 120:37.5 or 240:75→ 120:37.5 mg/kg for at least 6 consecutive months followed by an approximate 1-month recovery period (dose volume of 10 ml/kg). Another group of marmosets received HCT alone at a daily dose of 75 mg/kg. Due to mortality/moribundity, severe clinical signs and loss of body weight in the 240:75 mg/kg group, treatment was suspended on days 18 through 21 and the dosage was reduced to 120:37.5 mg/kg on day 22. An electrolyte/mineral supplement (Lectade) and a protein/carbohydrate supplement (Casilan/Farex) were given to all groups to encourage food consumption and sustain nutritional balance from days 20 and 57, respectively. Necropsies were conducted upon death, moribund sacrifice at the end of the treatment period, or following the approximate 1-month recovery period.	Ciba Pharmaceuticals, : Stamford Lodge, United Kingdom P McKenna Report no. 038/95/SL 16-Feb-96	0:75 mg/kg (HCT suspension only): Diarrhea (F); slight to moderate ↓ in hemoglobin, hematocrit and total red blood cell count (M,F); ↓ in liver and kidney weights (M). ≥ 30:9.375 mg/kg: Mortality/moribundity (F); slight to severe vomiting with test article, food and/or blood (M,F); slight to severe soft feces or diarrhea (M,F); mild to moderate ↓ in food consumption (M); mild to severe ↑ in plasma urea (M,F); mild to moderate ↑ in plasma creatinine (F); slight to moderate ↓ in hemoglobin, hematocrit and total red blood cell count (M,F); slight ↓ in urinary specific gravity, and ↑ in urinary volume and sodium (F); gastrointestinal (GI) lesions associated with mortality/moribundity (grossly: gastric/cecal/colonic discoloration, erosions, prolapse and/or discolored/red fluid GI contents; microscopically: gastric mucosal edema/ ulceration, cecal ulceration and/or GI luminal hemorrhage) (F); mild kidney lesions (arterial/ arteriolar hypertrophy, tubular nephropathy and/or renal tubular mineralization) (M,F). ≥ 60:18.75 mg/kg: Mild to moderate ↓ in food consumption (F); mild to moderate ↑ in plasma creatinine (M); mild ↑ in plasma magnesium (M); ulceration/white tip of tongue (F).	120:37.5 mg/kg: Slight to moderate salivatio (F); moderate ↓ in body weights (F); moderat ↑ in Lectade consumption (M,F). ≥ 120:37.5 mg/kg: Mortality/moribundity (M); slight to moderate salivation (M); moderate ↓ body weights (M); mild ↑ in plasma magnesi (F); slight to moderate ↑ in alkaline phosphat (M); slight ↓ in urinary specific gravity, and ↑ urinary volume and sodium (M); ulceration/wi tip of tongue (M); GI lesions associated with mortality/ moribundity (grossly: gastric/cecal/colonic discoloration, erosions, prolapse and/or discolored/red fluid GI conter microscopically: gastric mucosal edema/ulceration, cecal ulceration and/or GI luminal hemorrhage) (M). All changes in surviving animals were reversi following the approximate 1-month recovery period.

2.2.2.3 (Follow up study) **6-month** Marmoset Repeat Dose Toxicity Study with CGP 63172. (**Study No. 966057**. Report dated 25-11-96 of OECD GLP study conducted at drug sponsor's labs in the UK started on 09-02-96 and date of last necropsy date of 28-08-96. v.16.)

The objective of this follow-up study (to the 6-mo study No. 946117 summarized above) was to determine the oral toxicity during/after daily administration of **CGP 631722** also for 6-mo (80:25 combination of valsartan + HCTZ) and, chronic effects of the drug combination at lower dosages.

The selection of dosages was based on the previous 6-mo study summarized above in which a LD of 39.375 mg/kg/day CGP 63172 caused renal changes and early death of 1 F marmoset.

The following table shows marmosets groups and dosing (gavage once a day) schedule. A **HCTZ** group was not included in this study.

	Group 1	Group 2	Group 3	Group 4
	Control	CGP 63 172	CGP 63 172	CGP 63 172
Dosage (mg/kg)	0	3.938	13.125	39.375
Volume (mL/kg)	10	10	10	10
Main group	4 m + 4 f	4 m + 4 f	4 m + 4 f	4 m + 4 f
Duration of treatment	182 days	182 days	182 days	182 days

m Male
f Female
Control animals received the vehicle, 0.5% w/v carboxymethylcellulose with 0.5% v/v polysorbate 80,

Briefly, the marmosets were assigned to 4 different treatment groups, and were acclimatized to laboratory conditions for a minimum of 3 months prior to the start of study. The initial weight at dosing was for M ~282-434 g, and for F ~330-498 g (both of sexes at ages ranging from ~ 1 to ~ 3 years). During the study, marmosets were housed individually or in pairs of the same sex cages, and kept in air conditioned rooms at 23-27° C. with artificial lights for ~12 hrs/dim lights for ~12 hrs (from 06-05-96 until the end of the study); full lighting was provided for 1 further hour to allow for late evening dose applications to other studies in the unit). Marmosets were also identified with inner thigh tatoos, and also fed twice a day (the diet/menu were described in the NDA). Water, (tap or purified?) was freely available to the marmosets. From wks 3-23 marmosets received bottled electrolyte sol. as a mineral supplement.

Study protocol called for daily dose administration, examination for mortality, pre- and post-test dosing clinical signs, body weight/food consumption (group means reported weekly), water/electrolyte consumption (mean intake per animal calculated

daily during weeks 3, 14 and 23). Prior to dosing (pre-test) and on weeks 0, 13 and 25 of study, clinical chemistry (17 parameters), and hematology (12 parameters) were examined. Urine analysis (18 parameters) was performed on weeks 11 and 24 of study.

One day after the last drug dose all marmosets were sacrificed. Twelve (12) organs were weighed, and over 40 tissues were collected/fixed for microscopic examinations. Microscopic examination were performed on all gross lesions and on the kidneys of all marmosets were examined.

Statistical analyses were performed on the data collected. All methods used were described in the NDA.

Toxicokinetics were **not** performed.

Deviations from the protocol were reported (i.e., changes in room humidity/temperature range, some animals were offered a protein/carbohydrate supplement to promote weight gain during a few occasions, food/water consumption were not analyzed using trend test, urine samples collection and analyses were not performed for 2 animals). However; drug sponsor stated that the deviations from the protocol did not affect the integrity of the study.

RESULTS

No animals died during the study.

HD marmosets showed a treatment related vomiting, while the incidence for MD and LD F was within the limits of the control animals. Soft feces/diarrhea was noted in treated marmosets during this study, however; drug sponsor considered this effect to be unrelated to the drug treatment.

MD and HD males showed a slight decrease in body weight only during the first weeks of the study when compared to controls.
Urinalysis of 2 HD M showed a minimal increase in Na+ concentration when compared to other doses and control animals.

Hematology showed a slight reductions in Hgb concentration, total red cell counts and Hct, and a slight raise in the percentage reticulocyte counts during week 13 of study; by the end of the study on week 26 the hematologic changes were not apparent.

Remarkable histopathologic changes were reported in the kidneys and adrenals. Remarkable histopathologic changes reported in MD and HD animals included prominent vasculature (afferent arterioles) complicated with tubular nephropathy with intraluminal mineralization in the proximal convoluted tubules. An occasional crystal (compound not reported) within the lumen of the proximal convoluted tubules were reported in 2 HD M. A brown ceroid pigment

in the proximal tubular epithelium (identified in the submission as most probably lipofuscin) was noted in HD M.

Drug sponsor concluded that orally dosing marmosets with 3.938 up to 39.3 mg/kg/day CGP 63172 for six months was associated with so-call "pharmacologically-induced" changes consisting of vomiting, glomerular arteriolar hypertrophy and nephropathy with mineralization in the proximal convoluted tubules mainly at the HD and to a lesser extent at the MD. Drug sponsor did not classify these observed changes as pathologic and drug-induced.

The table below is a synopsis prepared by drug sponsor on this 6-mo follow-up study with CGP 63172 in marmosets.

CGP 63172: FOLLOW-UP 6-MONTH ORAL TOXICITY STUDY IN MARMOSETS (TEST NO. 966057)

Summary of toxicology findings

STUDY TYPE/SPECIES DATA	COMPOUND ID/REGIMEN	SITE, INVESTIGATORS, REPORT/STUDY NO., DATE	FINDINGS AND/OR COMMENTS
Chronic toxicity, 26-week oral; marmosets (*Callithrix jacchus*); four/sex/ group, 14 to 27 months (males) or 21 to 25 months (females) of age and weighing 282 to 434 g (males) or 330 to 498 g (females) at study initiation.	CGP 63172, a 16:5 wt:wt combination of CGP 48933 (Lot 800194) and HCT (Lot 800188), administered orally by gavage, as suspensions in 0.5% w/v CMC and 0.5% v/v Tween 80, at daily doses of 0, 3:0.938, 10:3.125 or 30:9.375 mg/kg for at least 26 consecutive weeks followed by an approximate 1-month recovery period (dose volume of 10 ml/kg). An electrolyte/mineral supplement (Lectade) and a protein/carbohydrate supplement (Casilan/Farex) were given to all groups to encourage food consumption and sustain nutritional balance. Necropsies were conducted at the end of the treatment period.	Ciba-Geigy Limited, Stamford Lodge, United Kingdom P McKenna Report no. 966057 25-Nov-96	3:0.938 mg/kg: No effects. ≥ 10:3.125 mg/kg: Slight ↓ in body weight gain (M); renal glomerular arteriolar hypertrophy and nephropathy with mineralization (M,F). 30:9.375 mg/kg: Vomit with test article (M,F); slight ↓ in erythrocytic parameters (M,F); minim ↑ in urinary sodium (M); crystals within lumen o proximal renal tubules (M).

2.3 **DEVELOPMENTAL STUDIES** (FDA Segment II; Vs. 17-22)

These GLP studies were conducted to evaluate the potential of the drug combination **CGP 63172** (16:5 wt:wt combination of valsartan-Lot 800194, plus HCTZ- Batch 800188 formulated in 3% aq. corn starch), given orally (gavage) to pregnant mice (CD-1), rats (Sprague-Dawley), and rabbits (Hra:NZW-SPF) during the period of organogenesis to induce maternal toxicity, embryo-or fetotoxicity or teratogenicity.

The animal species selected (2 rodents and 1 non-rodent) for the developmental studies were based on the firm's accumulated historical data, and prior experience with these species. The oral route was selected because it is the intended route for humans. The doses of CGP 63172 were selected based on preliminary developmental studies (dose-range finding) conducted in mice/rats/rabbits.

No fertility and early embryonic or prenatal and post-natal development studies were reported with the drug combination.

Drug sponsor reports that there were difficulties formulating suspensions of **valsartan** alone, and of the drug combination **CGP 63172**. The firm then decided that for these nonclinical studies the highest concentration of the drug combination (formulated in 3% corn starch) to be tested would not exceed the ratio of 600:187.5 mg/kg of valsartan and **HCTZ**, respectively.

♦ **2.3.1 MOUSE** (CD-1)

♦ 2.3.1.1 Oral Teratology (Seg II) Study in mouse with the Drug Combination **CGP 63172** (**Study MIN 944119**. US Tox/Path Report # 95064. Study conducted at drug sponsor's labs. from 11-30-94 to 01-08-95 v.17).

The mating period of mice was from 12/15-21/94, and dosing was from 12-22-94 to 01-05-95 on gestation days 6-15; the daily drug treatment was calculated on the most recent body weight recorded (i.e., on gestation days 6, 8 and 12). In life studies were completed on last gestation day-18 (day of necropsy).

The following shows the pregnant mice dosing schedule.

Dosing schedule

Group	Number of female mice	Daily dose (mg/kg)	Dosing volume (ml/kg)	Dosing conc. (mg/ml)	Number of dose days
1 (Control)	28	0	10	0	10
2 (HCT)	28	0:187.5	10	0:18.8	10
3	28	60:18.8	10	6:1.9	10
4	28	200:62.5	10	20:6.3	10
5	28	600:187.5	10	60:18.8	10

The **CGP 63172** doses selected for this study were based on a previously conducted oral developmental study in mice with one of the active ingredients- **valsartan** (at LD-60, MD-200 or HD-600 mg/kg. Valsartan could not be formulated in a way to deliver more than 600 mg/kg in Test No. 936133)*. In that study with valsartan alone, the drug sponsor states that valsartan produced "...no significant findings."

Briefly, during the study, mice were housed in a room at ~73°F, supplied with ~14 hrs of artificial light. Mice were assigned identification numbers/randomized to 5 treatment groups, and mated with stock M mice of the same strain in a 2:1 ratio until a sperm plug was observed (designated as day 0 of pregnancy).

In-life observations included- daily for mortality and clinical signs, and for food consumption/body weight on gestation days 0, 6, 8, 12 and 18. Treated mice received tap water ad libitum; consumption of food/water were monitored for contaminants, and maintained within the laboratories acceptable limits.

All dams were sacrificed on day 18 of pregnancy, and major viscera were examined grossly (including 2 mice found dead considered due to dosing accidents).

Reproductive parameters recorded included combined weight of uterus/ovaries/oviducts for pregnant-full term mice, the numbers of corpora lutea/resorption/implantation sites, dead/live fetuses, and fetal sex ratios and fetal gross visceral/skeletal parameters.

Statistical evaluation was conducted on the above listed parameters (i.e., body weight, food consumption, fetal weight, fetal observations, etc.) to detect differences between vehicle control Group 1 and all other treated groups. Bibliographic references to and explanation of the statistical methods used were provided in the NDA submission.

*

Seg II Study No. 96133 in mice treated with valsartan alone was evaluated in NDA 20-665 (09-30-96) by G. Jagadeesh, Ph.D. His report states that no teratogenic effects were reported at 600 mg/kg/day/10 in non-pregnant mice produced a slight decrease in body weight and no other adverse effects; this same dose given to pregnant mice during the period of organogenesis was not teratogenic. However, regarding this last mentioned study, the reviewer stated that "...but lack of effect may simply reflect the drug's relatively poor bioavailability in the mouse compared to rats and rabbits."

RESULTS

♦ **Mice Maternal Observations:**

Mortality/Clinical signs/Necropsy: No treatment related mortality were reported. Two dams died, reported as caused by dosing accidents. Other than incidental findings, no drug treatment related necropsy findings were reported.

The summary table below of the gestational parameters in this mouse study was provided drug sponsor.

Summary of reproductive parameters

(Mean ± Standard deviation)

Parameters	Dose level (mg/kg/day)				
	Control (0)	0:187.5	60:18.8	200:62.5	600:187.5
Corpora lutea	14.30 ± 2.80 (27)	14.15 ± 1.57 (26)	13.96 ± 2.14 (26)	14.19 ± 2.59 (27)	14.77 ± 2.14 (26)
Number of implantation sites	12.44 ± 2.49 (27)	12.62 ± 1.60 (26)	12.46 ± 2.76 (26)	12.48 ± 2.58 (27)	13.15 ± 1.35 (26)
Number of early resorptions	0.56 ± 0.75 (27)	0.62 ± 0.98 (26)	0.88 ± 1.40 (26)	0.67 ± 0.83 (27)	0.77 ± 0.86 (26)
Number of late resorptions	0.04 ± 0.19 (27)	0.12 ± 0.33 (26)	0.04 ± 0.20 (26)	0.07 ± 0.27 (27)	0.08 ± 0.27 (26)
J number of resorptions	0.59 ± 0.80 (27)	0.73 ± 1.04 (26)	0.92 ± 1.38 (26)	0.74 ± 0.86 (27)	0.85 ± 0.97 (26)
Number of live fetuses	11.78 ± 2.45 (27)	11.73 ± 1.73 (26)	11.38 ± 2.62 (26)	11.59 ± 2.52 (27)	12.31 ± 1.26 (26)
Number of dead fetuses	0.07 ± 0.27 (27)	0.15 ± 0.61 (26)	0.15 ± 0.46 (26)	0.15 ± 0.36 (27)	0.00 ± 0.00 (26)
Postimplantation loss	0.67 ± 0.88 (27)	0.88 ± 1.31 (26)	1.08 ± 1.65 (26)	0.89 ± 0.97 (27)	0.85 ± 0.97 (26)
% postimplantation loss	5.63 ± 7.45 (27)	6.74 ± 10.05 (26)	8.21 ± 11.40 (26)	6.88 ± 7.78 (27)	6.20 ± 6.67 (26)
Fetal sex ratio (% males)	53	54	53	51	49

♦ **Mice Embryo/Fetal Observations:**

Fetal body weight was not remarkably affected by the drug combination or with hydrochlorothiazide alone.

Fetal gross malformations noted in all groups were considered to be incidental and not treatment related by drug sponsor. Cleft palate findings were considered by drug sponsor to fall within the range of historical controls (data not provided), and those published in the literature. (Perrau J. Levels of spontaneous malformations in the CD rat and the CD-1 mouse. Lab Anim Science 1976; v 26 (Part 2):293-300.) N.B. Cleft palate (not dose related) was reported in NDA 20-665 in 1 fetus from dam treated with **valsartan alone** 600

mg/kg/day and 2 fetuses from different litters from dam treated with 200 mg/kg/day.

Hyperextension of the hindlimbs noted in some fetuses from MD/HD dams, and ablepharia/exencephally and syndactyly in some fetuses from the HD dams were not attributed by drug sponsor to treatment with CGP 63172.

- The summary tables below on mouse fetal skeletal malformations and skeletal variations (both selected by reviewer as reported by litter) were prepared by drug sponsor.

CGP 63172: AN ORAL TERATOLOGY (SEGMENT II) STUDY IN MICE (MIN 944119)

Summary of fetal examinations (by litter)

Type/Parameter	Dose Level (mg/kg/day) Control (0)	0.0:187.5	60:18.8	200:62.5	600:187.5
GROSS MALFORMATIONS:					
Ablepharia	0	0	0	0	1
Cleft palate	0	1	1	2	0
Exencephaly	0	0	0	0	1
Hindlimbs hyperextended	3	0	0	2	1
Syndactyly	0	0	0	0	1
No. of Litters with Gross Malformations:	3	1	1	4	3
No. of Litters Examined Grossly:	27	26	26	27	26
VISCERAL VARIATIONS:					
Bladder - enlarged	0	1	0	0	0
Kidney - reduced	0	0	1	0	0
renal papilla short	0	0	0	0	2
. of Litters with Visceral Variations:	0	1	1	0	2
No. of Litters Examined Viscerally:	27	26	26	27	26

Fetal gross examination results

Group Dose (mg/kg)	Dam number	Fetus number	Gross finding
1 (0)	1	5	Hyperextension of left hindlimb
	20	7	Hyperextension of left hindleg
	35	6	Hyperextension of right hindlimb
2 (0:187.5)	56	5	Cleft palate**
		6	Cleft palate*
		7	Cleft palate**
		9	Cleft palate*
3 (60:18.8)	78	6	Cleft palate*
4 (200:62.5)	111	4	Hyperextension of left hindleg
	115	9	Cleft palate*
	125	5	Hyperextension of both hindlimbs
	127	5	Cleft palate**
5 (600:187.5)	152	10	Left forepaw and left hindpaw syndactyly**
	158	11	Ablepharia and exencephaly
	165	3	Hyperextension of left hindlimb

The specific fetal visceral variations (by litter) reported in table above (previous page) such as enlarged bladder, reduced kidney, shortened renal papilla reported by incidence per dam/fetus number, were not considered by the drug sponsor related to the treatment with CGP 63172 or HCTZ.

The summary table below, prepared by drug sponsor and edited by reviewer, shows fetal skeletal malformations/variations based on incidence of observations per litter. Significant increases ($p \leq 0.05$) in the incidence of a few skeletal variations were only reported at MD and HD CGP 63172, and for HCTZ (i.e. additional sternebrae and delayed ossification.)

CGP 63172 ; AN ORAL TERATOLOGY (SEGMENT II) STUDY IN MICE (MIN 944119)

SUMMARY OF FETAL EXAMINATION PARAMETERS

(BY LITTER)

TYPE/PARAMETER	DOSE LEVEL (MG/KG/DAY) CONTROL (0)	0;187.5	60;18.8	200;62.5	600;187
SKELETAL MALFORMATION :					
CENTRUM/VERTEBRAE					
AGENESIS	0	0	0	0	1
FUSED	0	0	0	0	1
IRREGULAR SHAPE	0	0	0	0	1
RIBS					
FUSED	0	1	0	0	0
NO. LITTERS WITH SKELETAL MALFORMATIONS:	0	1	0	0	2
NO. LITTERS EXAMINED SKELETALLY	27	26	26	27	26
SKELETAL VARIATION :					
SKULL					
FRONTALS - ADDITIONAL	11	8	9	11	13
FRONTALS - NOT COMPLETELY OSSIFIED	2	2	3	3	5
HYOID - NOT COMPLETELY OSSIFIED	0	1	1	0	1
HYOID - NOT OSSIFIED	0	1	0	3	1
INTERPARIETALS - NOT COMPLETELY OSSIFIED	0	1	1	1	0
OCCIPITALS - NOT COMPLETELY OSSIFIED	9	7	5	2	8
PARIETALS - NOT COMPLETELY OSSIFIED	0	0	1	0	0
SQUAMOSALS - NOT COMPLETELY OSSIFIED	0	1	0	0	0
CENTRUM/VERTEBRAE					
ADDITIONAL	2	2	1	1	2
AGENESIS	0	0	0	2	0
BIFURCATION	3	2	0	2	3
BIPARTITE	0	0	0	0	2
CERVICAL RIB	7	11	11	5	10
FLOATING RUDIMENTARY 14TH RIB	2	1	1	1	1
FULLY FORMED 14TH RIB	11	8	11	18	16
IRREGULAR SHAPE	1	0	0	0	0
NOT COMPLETELY OSSIFIED	9	4	2	1	3
NOT OSSIFIED					
14TH RUDIMENTARY RIB	1	3	0	1	4*
	16	10	18	19	19
STERNEBRAE					
ADDITIONAL	1	1	2	9*	5*
BIPARTITE	1	4	5	0	2
FUSED	1	0	2	0	1
IRREGULAR SHAPE	21	19	20	20	22
NOT COMPLETELY OSSIFIED	6	6	6	8	6
NOT OSSIFIED	0	0	0	0	1
FORELEG/FOREPAW					
DISTAL PHALANGES - NOT COMPLETELY OSSIFIED	2	4	0	1	3
MIDDLE PHALANGES - NOT COMPLETELY OSSIFIED	24	23	21	18	19
MIDDLE PHALANGES - NOT OSSIFIED	13	9	5	5	9
PROXIMAL PHALANGES - NOT COMPLETELY OSSIFIED	8	1	2	0	4
PROXIMAL PHALANGES - NOT OSSIFIED	3	0	1	0	0
HINDLEG/HINDPAW					
CALCANEUS - NOT COMPLETELY OSSIFIED	17	17	12	16	16
CALCANEUS - NOT OSSIFIED	15	9	12	7	14
DISTAL PHALANGES - NOT COMPLETELY OSSIFIED	0	0	0	1	0
METATARSALS - NOT COMPLETELY OSSIFIED	1	0	0	0	0
METATARSALS - NOT OSSIFIED	1	0	0	0	0
MIDDLE PHALANGES - NOT COMPLETELY OSSIFIED	23	25*	22	24	22
MIDDLE PHALANGES - NOT OSSIFIED	19	17	15	15	20
PROXIMAL PHALANGES - NOT COMPLETELY OSSIFIED	14	11	10	9	11
PROXIMAL PHALANGES - NOT OSSIFIED	6	2	2	0	5
NO. LITTERS WITH SKELETAL VARIATIONS:	27	26	26	27	26
NO. LITTERS WITH SKELETAL VARIATIONS (EXCLUDING FOREPAW & HINDPAW):	26	26	26	27	26
NO. LITTERS EXAMINED SKELETALLY	27	26	26	27	26

Data reported indicate that oral doses of CGP 63172 of 78.8 up to doses not higher than 787.5 mg/kg/day or HCTZ alone (187.7 mg/kg/day) to pregnant mice during the period of organogenesis caused no treatment related maternal toxicity, or reproductive parameters, fetal weights and fetal gross, visceral or skeletal development.

The synoptic table below of the mouse developmental study (MIN 94119) with CGP 63172 was prepared by drug sponsor.

Study type/ species data	Compound ID/regimen	Site, investigators, Tox/Path no., date	Findings and/or comments
Segment II; CD-1 mice (Crl: CD-1[ICR] BR), 28 females/ group; approximately eight to nine weeks of age and weighing 22 to 29 g on gestation day 0.	CGP 63172 (16:5 wt: wt combination of CGP 48933 [Lot 800194] and HCT [Batch 800188]) administered orally by gavage, as suspensions in aqueous 3% corn starch, at daily doses of 0, 60:18.8, 200:62.5 or 600: 187.5 mg/kg (dose volume of 10 ml/kg) on gestation days 6 through 15. An additional group received an HCT suspension in aqueous 3% corn starch at a daily dose of 187.5 mg/kg. Females were necropsied upon death or sacrificed on gestation day 18.	Ciba-Geigy Corporation Summit, New Jersey CL Smith and ET Yau T/P no. 95064 14-Nov-95	0:187.5 mg/kg (HCT suspension only): No significant findings. ≥ 60:18.8 mg/kg: No significant findings. There were no treatment-related maternal effects. Reproductive param fetal weights and fetal gross, visceral and skeletal development unaffected by HCT or CGP 63172 administration.

♦ **3.3.2 Rat (Sprague-Dawley)**

3.3.2.1 (1st Study). Oral Teratology (Seg II) Study in Rat with **CGP 63172 (Study MIN 944118**. T/P (US) #95053. GLP Study No. 946121 conducted at drug sponsor's labs started on 11-08-94. v.18-19)

Mating period started on 11-21-94, dosing started 11-28-94 (day 6 of gestation), and ended 12-12-94 (gestation day 15) with in-life phase studies were completed 12-17-94.

The following table shows the rat dosing schedule.

Group	Number of rats Female	Daily dose (mg/kg)	Dosing volume (ml/kg)	Dosing conc. (mg/ml)	Number of dose days
1	26	0	10	0	10
2	26	0:187.5	10	0:18.8	10
3	26	50:15.6	10	5:1.6	10
4	26	200:62.5	10	20:6.3	10
5	26	600:187.5	10	60:18.8	10

To justify the dose selection in the present rat developmental study, drug sponsor reported that a "Seg II study in rat was conducted with **valsartan** alone (Test no. 936133) with doses of 60, 200 and 600 mg/kg/day resulted in reduced maternal body weight gain at ≥200 mg/kg/day, and with reduction in fetal body weight at 600 mg/kg/day." Further, that in a 1-mo repeat dose study with **HCTZ** (187.5 mg/kg) or with the drug combination CGP 63172 (50:15.6 up to 600:187.5 mg/kg/day) resulted in weight gain for F at the highest dose of the combination, and increased water consumption for the HCTZ group. Thus, based on these findings, the selected HD of CGP 63172 was anticipated by the drug sponsor to produce maternal effects, at the MD minimal or no maternal effects, and at LD no effects.

Briefly, drug sponsor reports that the 5 groups of rats in the present developmental study were treated as reported above in dose schedule table. The drugs were given by gavage as suspensions in aqueous 3% corn starch, and the 2 control groups each received the vehicle. Drug doses were calculated on the most recent body weight recorded on gestation days 6, 8 and 12.

For this study, rats were housed in a room at ~73°F, supplied with ~14 hrs of artificial light. Prior to mating, rats were assigned identification numbers, randomized to the 5 treatment groups, and mated with stock male rats of the same strain in a 2:1 ratio until the presence of sperms were observed in vaginal washing; that day

was designated day 0 of gestation. F rats at this time were ~ 12 weeks of age (weighing 216-292 g on gestation day 0.)

In-life observations included- daily for mortality and clinical signs, and for food consumption/body weight on gestation days 0, 6, 8, 12, 16 and 20. The consumption of food/water (tap water ad libitum) were monitored for contaminants, and maintained within the laboratories acceptable limits.

All dams were killed on day 20 of pregnancy, and major viscera of all rats were examined grossly (including those rats found dead).

Reproductive parameters recorded included combined weight of uterus/ovaries/oviducts for pregnant-full term rats, the numbers of corpora lutea/resorption/implantation sites, dead/live fetuses, fetal sex ratios and fetal gross visceral/skeletal parameters. Live fetuses were weighed/sexed/examined grossly and 1/3 placed in Bouin's fixative for visceral examination 2/3 placed in 70% ethanol and later stained with alizarin red S for skeletal examination.

Statistical evaluation was performed on the above listed parameters (i.e., body weight, food consumption, fetal weight, etc.) to detect differences between vehicle control and HCTZ group, and vehicle control vs. drug combination groups. An explanation of the statistical methods used was provided in the NDA.

RESULTS

♦ Rat Maternal Observations (1st Study):

Mortality/Clinical signs/Necropsy: Fourteen (14) dams treated with **CGP 63172** were found dead between days 12-20 of the study. The deaths were as follows: 3/26 were found dead and 1/26 killed by misgavage (on day 11) from the MD-200:62.5 group; 10/26 found dead and 1/26 moribund (and killed) from the HD-600:187.5 group. Incidence of clinical signs noted prior to death are listed below in a table prepared by sponsor.

Incidence of clinical observations

Observation	Dose level (mg/kg)				
	Control (0)	0:187.5	50:15.6	200:62.5	600:187.5
Alopecia	0/26	0/26	1/26	4/26	0/26
Ataxia	0/26	0/26	0/26	1/26	0/26
Chromodacryorrhea	0/26	0/26	0/26	0/26	1/26
Cut	1/26	0/26	0/26	0/26	0/26
Death	0/26	0/26	0/26	4/26*	11/26**
Gasping/labored respiration	0/26	0/26	0/26	1/26	2/26
Lethargy	0/26	0/26	0/26	1/26	2/26
Ptosis	0/26	0/26	0/26	0/26	2/26
Ralse	0/26	0/26	0/26	0/26	1/26
Salivation	0/26	0/26	0/26	0/26	21/26
Stains on fur	0/26	0/26	0/26	3/26	11/26
Stool: decreased	0/26	0/26	0/26	7/26	13/26
diarrhea	0/26	0/26	0/26	1/26	0/26
soft	0/26	0/26	0/26	2/26	11/26
no stool	0/26	0/26	0/26	2/26	2/26
Scab	0/26	1/26	0/26	0/26	1/26
Unthrifty	0/26	0/26	0/26	0/26	7/26

*One attributed to misgavage.

**One sacrificed moribund.

Slight reductions in mean food consumption were reported for the LD and marked reductions during gestation days 6 and 16 with the MD/HD of the drug combination CGP 63172.

Overall, treatment related decreases in mean body weight were observed during dosing period in all drug treated rats including those treated with HCTZ alone. Following cessation of dosing, a rebound effect was indicated by increased bodyweight gain on gestation days 16-20.

Group mean data of gestational parameters and fetal sex ratio are summarized below in table provided by drug sponsor.

Summary of reproductive parameters and fetal sex ratios

Summary of reproductive parameters
(Mean ± SD)

Parameter	Dose level (mg/kg)				
	Control (0)	0:187.5	50:15.6	200:62.5	600:187.5
Number of females mated	26	26	26	26	26
Number of pregnant females	26	25	26	24	24
% of pregnant females	100	96.2	100	92.3	92.3
ber of corpora lutea	19.46 ± 4.27	18.36 ± 2.84	18.15 ± 1.78	18.90 ± 2.91	19.15 ± 2.79
umber of implantation sites	15.69 ± 3.86	16.56 ± 2.74	16.00 ± 2.88	16.67 ± 1.11	17.69 ± 2.21
Number of litters examined	26	25	26	21	13
Number of live fetuses	14.77 ± 3.70	15.32 ± 2.43	14.96 ± 2.90	16.19 ± 1.17	16.85 ± 2.76
Number of early resorptions	0.92 ± 0.93	1.24 ± 1.27	1.04 ± 1.15	0.48 ± 0.68	0.85 ± 0.90
Number of late resorptions	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Number of total resorptions	0.92 ± 0.93	1.24 ± 1.27	1.04 ± 1.15	0.48 ± 0.68	0.85 ± 0.90
Number of dead fetuses	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Postimplantation loss	0.92 ± 0.93	1.24 ± 1.27	1.04 ± 1.15	0.48 ± 0.68	0.85 ± 0.90
% postimplantation loss	5.67 ± 5.66	7.09 ± 6.88	6.69 ± 7.13	2.81 ± 4.01	5.14 ± 5.93
Fetal sex ratio (% males)	49.48	54.05	52.96	49.12	46.12