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*APPLICATION NUMBER:*

**21-532**

**PHARMACOLOGY REVIEW**

## PHARMACOLOGY/TOXICOLOGY REVIEW

NDA NUMBER: 21532

DATE OF ORIGINAL SUBMISSION: August 5, 2002

SPONSOR: Sankyo Pharma Inc.  
780 Third Avenue, 47<sup>th</sup> Floor  
New York, NY 10017

REVIEWER: G. Jagadeesh, Ph.D.

DIVISION: Cardio-Renal Drug Products (HFD-110)

REVIEW COMPLETION DATE: March 27, 2003

DRUG PRODUCT: BENICAR HCT™ (olmesartan medoxomil and hydrochlorothiazide)  
Tablets

PROPOSED INDICATION: Hypertension

### ACTIVE INGREDIENTS

#### Olmesartan Medoxomil

Drug Class: Angiotensin II receptor antagonist

Manufacturer: Sankyo Co., Ltd., Japan

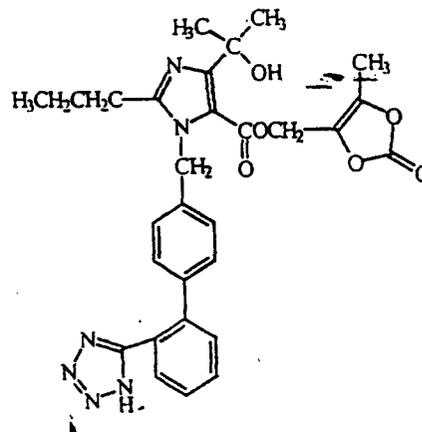
Chemical Name: \_\_\_\_\_

Code Name: CS-866

CAS Registry Number: 144689-63-4

Molecular Formula: C<sub>29</sub>H<sub>30</sub>N<sub>6</sub>O<sub>6</sub>

Molecular Weight: \_\_\_\_\_



Hydrochlorothiazide

Drug Class: Thiazide diuretic

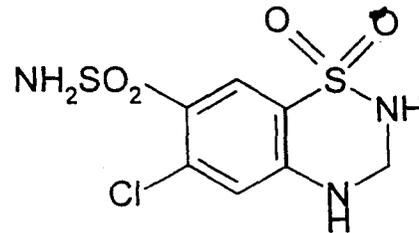
Manufacturer: \_\_\_\_\_

Chemical Name: \_\_\_\_\_

CAS Registry Number: 59-93-5

Molecular Formula: C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>

Molecular Weight: \_\_\_\_\_



RELATED NDAs

- Sankyo NDA 21,286 (Olmesartan Medoxomil for hypertension)
- Merck NDA 20,387 (Losartan-HCTZ for hypertension)
- BMS NDA 20,758 (Irbesartan-HCTZ for hypertension)
- Boehringer NDA 21,162 (Telmisartan-HCTZ for hypertension)

CLINICAL FORMULATION: Olmesartan medoxomil (OM) /hydrochlorothiazide (HCTZ) immediate release film coated tablets will be supplied in dosage strengths: 20/12.5 mg, 40/12.5 mg, \_\_\_\_\_ and 40/25 mg. The products are. The OM-HCTZ formulations are based on the approved OM tablet product formulations and differ from those formulations in that \_\_\_\_\_

TABLE I  
OLMESARTAN MEDOXOMIL (CS-866) -HYDROCHLOROTHIAZIDE FORMULATIONS

Ingredient	20/12.5 mg tablet	40/12.5 mg tablet	40/25 mg tablet
CS-866	30 mg	40 mg	40 mg
Hydrochlorothiazide, Ph. Eur.	12.5 mg	12.5 mg	25 mg
Microcrystalline cellulose, Ph. Eur.			
Lactose, _____ Ph. Eur.			
Hydroxypropyl cellulose, Ph. Eur.			
Magnesium Stearate, Ph. Eur.			
Tablet Core Weight			

ROUTE OF ADMINISTRATION: Oral

PROPOSED DOSAGE REGIMEN: One tablet daily. Dosing should be individualized. Depending on the blood pressure response, the dose may be titrated at intervals of 2-4 weeks.

DISCLAIMER: All tables and graphs are from sponsor's submission unless stated otherwise.

Handwritten notes: HCTZ 12.5, 25, 0.1m, 1.0, 20, 40, with checkmarks.

## *Executive Summary*

### I. Background

Olmesartan medoxomil (OM) is a non-peptidic, orally effective, potent and specific antagonist of angiotensin II, active at the AT<sub>1</sub> receptor. It was approved for the treatment of essential hypertension on April 25, 2002. Hydrochlorothiazide (HCTZ) is a diuretic which inhibits reabsorption of sodium and chloride in the distal convoluted tubule with a concomitant increase in urine volume. HCTZ was approved for the treatment of essential hypertension in mid 1958. Although the mechanism of the antihypertensive effect of thiazides is not fully understood, the effect of HCTZ on blood pressure may be due to a reduction in vascular resistance related to the persistent reduction in sodium with a consequent reduction in plasma volume. The latter effect, however, increases plasma renin activity, resulting in an increase in angiotensin II levels which, in turn, can reduce the antihypertensive effect of the diuretic. Increased levels of angiotensin II also result in increased aldosterone secretion and, consequently, urinary potassium loss. Angiotensin II receptor antagonists, such as OM, by blocking the activation of the AT<sub>1</sub> receptor, reduce these indirect and undesirable effects of HCTZ and, therefore, enhance the antihypertensive effects of HCTZ. The following angiotensin II receptor antagonists, in fixed combination with HCTZ, have been previously approved for the treatment of hypertension: losartan, valsartan, candesartan, irbesartan and telmisartan.

### II. Recommendations

- A. Recommendation on Approvability: Approvable
- B. Recommendations for Additional Nonclinical Studies: None
- C. Recommendations on Labeling

Those sections in the proposed labeling (electronic version dated 7/16/02) that deal with preclinical studies covered by this review are considered satisfactory with the following exception.

Under WARNINGS **Fetal/Neonatal Morbidity and Mortality**, the sponsor's proposed text summarizing the results of studies in mice and rats (page — reads as follows:

“There is no clinical experience with the use of Benicar HCT™ in pregnant women. No teratogenic effects were observed when \_\_\_\_\_ administered to pregnant mice at oral doses up to 1625 mg/kg/day — times the maximum recommended human dose [MRHD] \_\_\_\_\_ on a mg/m<sup>2</sup> basis) or pregnant rats at oral doses up to 1625 mg/kg/day — times the MRHD on a mg/m<sup>2</sup> basis).”

The above text incorrectly expresses the animal doses as multiples of the MRHD and ignores the significant effect of OM-HCTZ on rat fetal weight. The following statement incorporates our recommended changes:

“There is no clinical experience with the use of Benicar HCT™ in pregnant women. No teratogenic effects were observed when 1.6:1 combinations of olmesartan medoxomil and hydrochlorothiazide were administered to pregnant mice at oral doses up to 1625 mg/kg/day (122 times the maximum recommended human dose [MRHD] on a mg/m<sup>2</sup> basis) or pregnant rats at oral doses up to 1625 mg/kg/day (280 times the MRHD on a mg/m<sup>2</sup> basis). In rats, however, fetal body weights at 1625 mg/kg/day (a toxic, sometimes lethal dose in the dams) were significantly lower than control. The no observed effect dose for developmental toxicity in rats, 162.5 mg/kg/day, is about 28 times, on a mg/m<sup>2</sup> basis, the MRHD of Benicar HCT™ (40 mg olmesartan medoxomil /25 mg hydrochlorothiazide /day).”

### III. Summary of Nonclinical Findings

#### A. Brief Overview of Pharmacology

Male spontaneously hypertensive rats were treated orally for 14 days with OM/HCTZ (0.1/10 or 1/10 mg/kg/day), OM (0.1 or 1.0 mg/kg/day) or HCTZ (10 mg/kg/day). The 24-hour mean blood pressure was decreased gradually from the first day of administration with a maximal decrease of about 20% at 1/10 mg OM/HCTZ per kg per day on the fourth day, remaining at this level of blood pressure reduction throughout the remainder of the treatment period. The effect of 0.1/1 mg OM/HCTZ per kg per day was approximately equivalent to that of 1 mg OM/kg/day. HCTZ alone was least effective at lowering blood pressure. The results indicate that concomitant administration of OM and HCTZ has an additive antihypertensive effect.

There are no pharmacokinetic interactions between OM and HCTZ in rats or dogs. Olmesartan was the only metabolite detected after administration of OM with or without HCTZ.

#### B. Brief Overview of Toxicology

The potential toxicity of orally administered OM-HCTZ was evaluated in single- and repeat-dose toxicity studies (up to 26 weeks) in rats and dogs. The genotoxic potential of OM-HCTZ was investigated in two *in vitro* cell lines and in one *in vivo* mouse model. Developmental toxicity was evaluated in pregnant mice and rats.

In single dose toxicity studies no clinical signs were observed in rats or dogs at OM/HCTZ doses up to 2000 mg/kg (1230/770 mg/kg).

The doses of OM-HCTZ encompassed a range of 4.88 to 1625 mg/kg/day (1000 mg OM/625 mg HCTZ/kg/day) in three 26-week oral toxicity studies in rats. Overall body

weight gains were significantly reduced relative to control at doses as low as 48.75 mg/kg/day. Kidney was the main target organ. OM-HCTZ produced a nondose-dependent increase in blood urea nitrogen and (to a lesser extent) creatinine. The mean relative kidney weights were significantly increased. There were histopathological findings in the kidneys of males and females given 48.75 or more mg OM-HCTZ/kg/day. The findings were considered to be part of the spectrum of changes associated with chronic progressive nephropathy in rats. They were generally dose-related and more pronounced in males than females. Chronic progressive nephropathy was also observed in animals treated with 1000 mg OM or 625 mg HCTZ alone, although the severity appeared to be less than that observed with OM-HCTZ. Also reported were isolated incidences of erosion and ulceration of the glandular mucosa of the stomach, observed in males at 16.25 or more and in females at 162.5 or more mg OM-HCTZ/kg/day. A NOAEL could not be established for these effects in rats. Saline supplementation prevented the reduction in body weight gain, reduced the elevation in BUN concentration and eliminated the increase in creatinine. Saline supplementation also decreased the incidence and severity of nephropathy in OM-HCTZ treated rats. However, the incidence of focal ulceration of the glandular gastric mucosa was observed with same frequency in animals given tap water or saline. Systemic exposure to olmesartan and HCTZ increased with increasing doses but in a less than dose-proportional manner. The concomitant administration of OM and HCTZ did not influence the concentration of either olmesartan or HCTZ.

Kidneys and stomach are also target organs for toxicity in dogs. In 5 and 26 week studies, moribundities were attributed to extensive gastrointestinal hemorrhage and necrosis, in association with renal tubule hypertrophy and dilatation. Decedent animals (26 or more mg OM-HCTZ/kg/day) displayed treatment-related significant increases in blood urea nitrogen, creatinine and bilirubin. The predominant histopathological finding (observed at both unscheduled and scheduled sacrifices) was cortical tubular hypertrophy of the renal tubules, the severity of which increased with increasing doses of OM-HCTZ, with males appearing to be more susceptible than females. Similar but less severe renal changes were observed in dogs given OM or HCTZ alone. A NOAEL could not be determined for these effects. There was a non-linear increase in  $C_{max}$  and AUC values for olmesartan with increasing dose levels of OM-HCTZ and there was no accumulation of either drug after repeated administration. Concomitant administration of OM and HCTZ did not affect the kinetic behavior of olmesartan or HCTZ.

OM-HCTZ, OM and HCTZ were negative in all tester strains in the Ames reverse mutation assay both in the presence and absence of metabolic activation (S-9 mix). OM, HCTZ and OM-HCTZ tested positive in the absence (not tested in the presence) of S-9 mix in the *in vitro* chromosomal aberration assay with dose-dependent increases in both structural and numerical (polyploidy) abnormalities observed. These are expected results since OM and HCTZ alone have been reported to induce chromosomal aberrations. Combining OM with HCTZ did not enhance the responses observed with OM or HCTZ alone. The mouse micronucleus test failed to demonstrate a potential for clastogenicity of OM-HCTZ *in vivo*.

OM-HCTZ does not have adverse effects on embryo-fetal development when administered to pregnant mice during organogenesis at doses as high as 1000/625 mg/kg/day. The latter dose is, on a  $\text{mg}/\text{m}^2$  basis, about 122 times the MRHD of OM/HCTZ (40/25 mg/day).

When OM-HCTZ was administered to mated female rats, by gavage, from days 7 to 17 of gestation at oral doses of up to 1000/625 mg/kg/day, two of 25 dams in the 100/62.5 mg/kg/day group and 3 of 32 dams receiving 1000/625 mg/kg/day were found dead 1-3 days before scheduled sacrifice. These rats had lost body weight and had reduced feed consumption 8-10 days prior to death. Most of them were emaciated 2-3 days prior to death. Furthermore, there were adverse necropsy findings such as stomach erosions, hypertrophies of fasciculata and reticularis cells in the adrenal gland, and distal tubule dilatation in the kidney in the deceased rats. Decreases in maternal body weight gain ( $p < 0.05$ ) and food consumption ( $p < 0.05$ ) were observed for rats receiving 30/18.75 mg or more OM/HCTZ per kg per day. There were no significant group differences in the cesarean-sectioning and litter observations except for a decrease in the mean fetal body weights for the 1000/625 mg/kg/day group ( $p < 0.05$ ). Body weight gain, food intake and live fetal body weights were suppressed to a greater extent in the OM-HCTZ group than in the groups receiving OM (1000 mg/kg/day) or HCTZ (625 mg/kg/day) alone. Increases in blood urea nitrogen and creatinine and a decrease in sodium were greater in the 1000/625 mg/kg/day group than in the OM or HCTZ groups. Along with decreases in urinary creatinine and N-acetyl- $\beta$ -glucosaminidase in the high dose combination group, these findings suggest changes in renal function of maternal animals receiving OM-HCTZ. Histopathological examination revealed erosion of the glandular stomach in some combination group animals, which was also observed in the rat repeated dose toxicity study. Combined administration of OM and HCTZ to pregnant rats had greater effects than treatment with OM or HCTZ alone.  $C_{\text{max}}$  and AUC values (dose-related) were higher after 11 days of administration than on the first day of administration in the OM-HCTZ group but not in the OM or HCTZ groups. This accumulation may have been related to the increased renal toxicity observed with the combination. A similar analogy could be drawn from the toxicity observed in the rat repeated dose toxicity study.

Based on these results, the maternal NOAEL of OM-HCTZ is 16.25 (10/6.25) mg/kg/day and the developmental NOAEL is 162.5 (100/62.5) mg/kg/day. These doses are, on a  $\text{mg}/\text{m}^2$  basis, 2.8 and 28 times the MRHD of OM/HCTZ (40/25 mg/day), respectively.

Saline supplementation ameliorated the toxicity of OM-HCTZ as evidenced by reduction in maternal deaths, and in the magnitude of OM-HCTZ associated decreases in food consumption and maternal and fetal body weights. Increases in blood urea nitrogen and creatinine and a decrease in sodium were significantly greater in the non-saline supplemented group than in the saline supplemented group. The incidence and severity of chronic progressive nephropathy were less pronounced in male and totally absent in female rats supplemented with saline. (Similar observations were made in the rat repeated dose toxicity study.) On the other hand, focal ulceration of the glandular gastric mucosa was observed equally in combination groups receiving either water or saline. It is likely

that saline in some way affects renal function, probably by hastening drug excretion, as rats supplemented with saline show marked increases in urine volume and do not show the accumulation of olmesartan and HCTZ that is observed in non-saline supplemented rats.

*In conclusion*, most of the adverse effects seen in the rat general toxicity and reproductive toxicity studies (e.g., reductions in body weight gain and food consumption; increases in BUN and creatinine; gastric irritation and ulceration; and nephropathy) were also observed in the dog repeated dose toxicity study of OM-HCTZ. Taken together, combined administration of OM and HCTZ to pregnant rats and non-pregnant rats and dogs had greater adverse effects than treatment with OM or HCTZ alone, and saline supplementation alleviated most of these effects.

**C. Nonclinical Safety Issues Relevant to Clinical Use**

Although the dog did not tolerate the combination of OM with HCTZ as well as the rat, for both species, the severity of the renal changes (chronic progressive nephropathy) increased with increasing doses of OM-HCTZ, with males appearing to be more susceptible than females. In addition, both rats and dogs have shown low incidences of erosion and ulceration of the glandular mucosa of the stomach. In pregnant rats (but not in non-pregnant rats and dogs), it appears that both OM and HCTZ accumulate with repeated exposure to OM-HCTZ. Benicar HCT™ should be used with caution in patients with severe renal disease and/or gastric ulcers.

**III. Administrative**

A. Reviewer signature: Signed (GJ)

B. Supervisor signature: Concurrence - Signed (CR)

Non-Concurrence - \_\_\_\_\_  
(see memo attached)

C. cc: list:

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## PHARMACOLOGY/TOXICOLOGY REVIEW

### I. PRIMARY PHARMACOLOGY

#### 1.1. Antihypertensive Effect of Olmesartan medoxomil and Hydrochlorothiazide (OM-HCTZ) in Conscious Spontaneously Hypertensive Rats. Vol 4.

This non-GLP study (Study #FBM 00-2214) was conducted by \_\_\_\_\_, between October 10, 2000 and March 4, 2002. The objective of the study was to investigate the antihypertensive effects of olmesartan medoxomil (OM) and hydrochlorothiazide (HCTZ) in spontaneously hypertensive rats (SHRs) when they were individually and concomitantly administered orally for 14 successive days.

#### Methods

A total of 42 male SHRs, 25 weeks old (325-415 gm), were used in the study. The study included 6 groups of 7 animals each. Five weeks before the experiment, each rat was anesthetized and implanted with telemetry transmitters for recording b.p. and heart rate. The drugs were given orally with a disposable syringe via a stomach catheter. OM (lot # OS-001C1) and HCTZ (lot # +HCTMC99L033) were prepared as suspension in \_\_\_\_\_. All animals were initially given vehicle (2 ml/kg), once daily, for 7 successive days (observation period). This was followed by one of six treatments (vehicle, OM: 0.1 or 1 mg/kg; HCTZ: 10 mg/kg; OM-HCTZ: 0.1/10 or 1/10 mg/kg; n=6/treatment) for 14 days. After that, the vehicle was administered to all animals for 5 days (drug-free withdrawal period). The areas under the blood pressure and heart rate curves for 24 hours after administration were calculated and divided by 24 to obtain the 24-hour mean blood pressure or heart rate. Blood samples were collected from the jugular vein 4-5 hr after the administration on the 3<sup>rd</sup> observation day and on the 5<sup>th</sup> and 12<sup>th</sup> drug administration day for determination of plasma renin activity. Urine was collected on the 8<sup>th</sup> day of drug administration. Rats were given 10 ml saline immediately after dosing and were placed in metabolic cages to collect their urine for 5 hr after the administration.

#### Results

All dosing groups demonstrated a decrease in blood pressure from the 1<sup>st</sup> day of the administration period and pressures in treated animals were significantly different from the control group values at all time points (Fig 1.1.1). The mean 24-hour blood pressure in the control group ranged between 163 and 170 mmHg throughout the administration period, whereas the drug-treated groups had blood pressures between 132 and 152 mm Hg. The relative potencies of the various treatments were as follows: OM-HCTZ 1/10 mg/kg > OM-HCTZ 0.1/10 mg/kg > OM 1 mg/kg > OM 0.1 mg/kg > HCTZ. The b.p. lowering effect of OM-HCTZ (0.1/10 mg/kg/day; total dose ~1 mg/kg/day) was approximately equivalent to OM 1 mg/kg/day. This suggests that the antihypertensive effects of OM and HCTZ were additive. Blood pressures in the OM-HCTZ groups remained significantly lower on the first day of the withdrawal period. Blood

pressures of all animals gradually returned to near baseline levels around day 3 to 4 of the withdrawal period. No rebound phenomenon was observed.

Heart rate decreased in the control group gradually from day 7 of the observation period through day 4 of the administration period, after which it leveled off. In the drug treatment groups, the heart rate increased on the first day of drug administration, decreased gradually from the 2<sup>nd</sup> day and remained constant by day 4 of the administration of period.

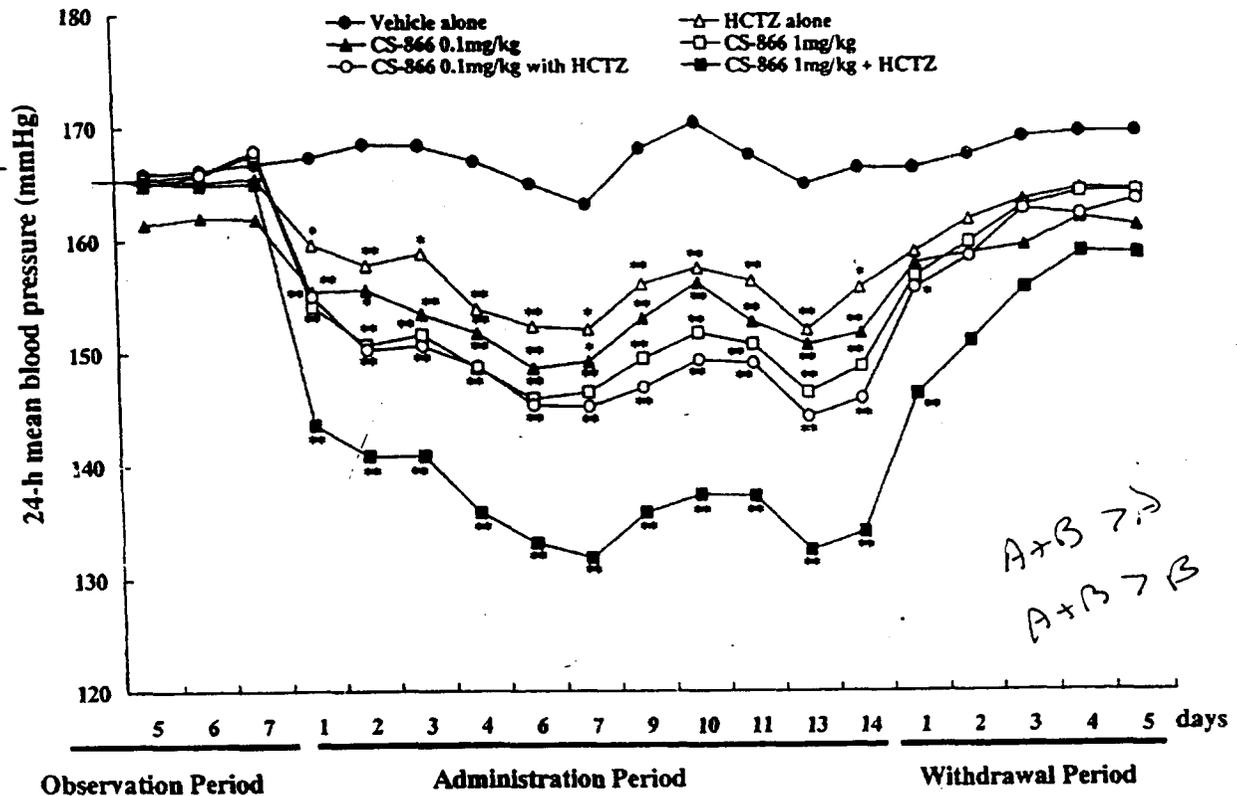


Fig. 1.1.1.: Changes in 24-hr Mean Blood Pressure. (HCTZ is always 10 mg/kg).

\* Statistically significantly different from control group, P<0.05

\*\*Statistically significantly different from control group, P<0.01

A statistically significant increase in plasma renin activity (PRA) relative to control values was noted in rats treated with both doses of OM-HCTZ and 1 mg OM/kg on the 5<sup>th</sup> and 12<sup>th</sup> days of the administration period. The renin activity was higher after the concomitant administration of OM and HCTZ than that after the single drug administration. The difference was more remarkable on the 12<sup>th</sup> day than on the 5<sup>th</sup> day of administration. The increase corresponded to the degree of b.p. reduction (Table 1.1.1). HCTZ and OM 0.1 mg/kg had no effect.

**TABLE 1.1.1**  
**MEAN PLASMA RENIN ACTIVITY (NG/ML/HR) IN CONTROL AND TREATED RATS PRIOR TO**  
**TREATMENT AND DURING THE TREATMENT PERIOD**

Group	Dose (mg/kg/day)	Observation Period		Treatment Period	
		Day 3	Day 5	Day 5	Day 12
Control	0	10.8	8.7		8.0
HCTZ	10.0	7.7	19.7		13.1
OM	0.1	7.7	19.0		18.2
OM	1.0	5.7	30.9*		34.8*
OM -HCTZ	0.1/10	7.7	39.2*		44.3*
OM -HCTZ	1/10	5.8	44.9*		60.0*

\* Statistically significantly different from control (P<0.01)

The 5-hr urine volume and total excretion of sodium and potassium were significantly increased relative to control in the OM-HCTZ and HCTZ groups. Since OM alone had no effect on these parameters, and since the magnitude of the increase was similar in the OM-HCTZ and HCTZ groups, the effect was attributed to the administration of HCTZ.

The study investigators conclude that concomitant administration of OM and HCTZ had an additive antihypertensive effect in SHR. No rebound phenomenon was observed after discontinuation of the treatment. HCTZ but not OM increased urine volume and total excretion of sodium and potassium.

**II. SAFETY PHARMACOLOGY:** No safety pharmacology studies were conducted with OM-HCTZ.

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### III. PHARMACOKINETICS

#### 3.1. Pharmacokinetics of Olmesartan and HCTZ After a Single Oral Administration of Either Drug Alone or in Combination in Male Rats. Vol 4.

This non-GLP study (Study #ATR 148-137) was conducted by the Pharmacokinetics Drug Delivery Research Laboratories, Sankyo Co. Ltd., Shinagawa-ku, Tokyo, and Medicinal Safety Research Laboratories, Sankyo Co. Ltd., Fukuroi, Shizuoka 437, Japan, between July 18, 2001 and April 25, 2002. The objective of the study was to investigate the possibility of in vivo drug-drug interaction between olmesartan medoxomil (OM) and hydrochlorothiazide (HCTZ).

#### Methods

Male Wistar-Imamichi rats (7-8 weeks old and weighing 185 to 245 gm) were fasted overnight prior to dosing and were given a single oral dose of [<sup>14</sup>C]OM/HCTZ or OM/[<sup>14</sup>C]HCTZ. Other groups of rats received only [<sup>14</sup>C]OM or [<sup>14</sup>C]HCTZ. OM (labeled and unlabeled) was dissolved in \_\_\_\_\_, and HCTZ (labeled and unlabeled) was dissolved in \_\_\_\_\_ and both were diluted with distilled water to a desired concentration prior to use. Each drug solution was orally administered by stomach tube. The dosing groups were as follows:

Dosing group	[ <sup>14</sup> C]OM	Unlabeled OM	[ <sup>14</sup> C]HCTZ	Unlabeled HCTZ	n
1	1 mg/kg				3
2	1 mg/kg			1 mg/kg	3
3	1 mg/kg			10 mg/kg	3
4			1 mg/kg		4
5		1 mg/kg	1 mg/kg		4
6		10 mg/kg	1 mg/kg		4

The [<sup>14</sup>C]OM (lot No. D-991028) had a specific activity of 1.43 MBq/mg (38.6 μCi/mg) and a radiochemical purity of \_\_\_\_\_. The [<sup>14</sup>C]HCTZ (lot No. CFQ12666) had a specific activity of 1.04 MBq/mg (28.0 μCi/mg) and a radiochemical purity of \_\_\_\_\_. Unlabeled OM (lot No. NH217C) and HCTZ (lot No. HYD0103) were also used in this study.

Blood samples were collected under ether anesthesia from the jugular vein at 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 hr after dosing. Plasma radioactivity was determined by \_\_\_\_\_. Plasma samples were subjected to \_\_\_\_\_ analysis in order to quantify the metabolites associated with each radiolabeled compound.

#### Results

The total radioactivity concentration in the plasma after oral administration of [<sup>14</sup>C]OM with and without non-labeled HCTZ reached maximum between 1 and 1.7 hr. A similar T<sub>max</sub> value was observed with olmesartan, the major metabolite of OM. Olmesartan accounted for about 64% of the total radioactivity (Table 3.1.1). The rest is shared by the parental compound OM and the

conjugate, olmesartan glucuronide. The ~~of~~ of rat plasma after oral administration of [<sup>14</sup>C]OM with and without HCTZ showed two radioactive spots, olmesartan and olmesartan glucuronide. Co-administration of HCTZ had no effect on the metabolism of OM. No metabolites were detected after administration of [<sup>14</sup>C]HCTZ and co-administration of OM had no effect on the metabolism of HCTZ. Statistical analyses furthermore demonstrated that the pharmacokinetic parameters for [<sup>14</sup>C]OM and [<sup>14</sup>C]HCTZ (Table 3.1.2) were not affected by co-administration of HCTZ and OM, respectively. Therefore, it was concluded that there was no drug-drug interaction when OM and HCTZ were co-administered to rats.

**TABLE 3.1.1**  
PHARMACOKINETIC PARAMETERS FOR TOTAL RADIOACTIVITY AND OLMESARTAN (OLM) IN PLASMA AFTER A SINGLE ORAL ADMINISTRATION OF [<sup>14</sup>C]OM (1 MG/KG) IN THE PRESENCE AND ABSENCE OF HCTZ (1 OR 10 MG/KG).

Parameter	<sup>14</sup> C-OM alone (n=3)		<sup>14</sup> C-OM + HCTZ (1 mg/kg) (n=3)		<sup>14</sup> C-OM + HCTZ (10 mg/kg) (n=3)	
	TR	Olm	TR	Olm	TR	Olm
AUC <sub>all</sub> (ng eq.hr/ml)	1718.7	1105.1	1775.4	1086.6	1824.5	1069.4
AUC <sub>last</sub> (ng eq.hr/ml)	1711.4	1100.8	1754.2	1085.5	1812.2	1065.5
t <sub>1/2</sub> (hr)	1.4	1.8	1.6	1.1	1.1	1.1
C <sub>max</sub> (ng eq/mL)	585.1	389.5	588.9	412.7	613.4	392.4
T <sub>max</sub> (hr)	1.0	1.3	1.3	1.3	1.7	1.7
CL/F (ml/hr/kg)	595.0	931.4	577.4	966.0	561.9	856.7

Values are expressed as the mean of 3 rats.  
TR: Total radioactivity

**TABLE 3.1.2**  
PHARMACOKINETIC PARAMETERS FOR RADIOACTIVITY IN PLASMA AFTER A SINGLE ORAL ADMINISTRATION OF [<sup>14</sup>C]HCTZ (1 MG/KG) IN THE PRESENCE AND ABSENCE OF OM (1 OR 10 MG/KG).

Parameter	<sup>14</sup> C-HCTZ alone (n=4)	<sup>14</sup> C-HCTZ + OM (1 mg/kg) (n=4)	<sup>14</sup> C-HCTZ + OM (10 mg/kg) (n=4)
AUC <sub>all</sub> (ng eq.hr/ml)	562.8	696.5	703.8
AUC <sub>last</sub> (ng eq.hr/ml)	562.8	696.5	703.8
t <sub>1/2</sub> (hr)	4.9	5.7	5.7
C <sub>max</sub> (ng eq/ml)	79.4	86.3	82.9
T <sub>max</sub> (hr)	1.0	1.5	1.4
CL/F (ml/hr/kg)	1619.9	1249.4	1219.7

Values are expressed as the mean of 4 rats.

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M. Jagadeesh  
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### 3.2. Interaction in *in vitro* Protein Binding Between Olmesartan and Hydrochlorothiazide. Vol 4.

This non-GLP study (Study #ATR 148-108) was conducted by the Pharmacokinetics Drug Delivery Research Laboratories, Sankyo Co. Ltd., Shinagawa-ku, Tokyo, Japan, between June 12, 2001 and January 30, 2002. The study investigated the possibility of drug-drug interaction between olmesartan, the active metabolite of olmesartan medoxomil (OM) and hydrochlorothiazide (HCTZ) on *in vitro* protein binding using human plasma, human serum albumin (HSA) and human  $\alpha_1$ -acid glycoprotein (HAGP).

#### Methods

$^{14}\text{C}$ -olmesartan was prepared by

\_\_\_\_\_ The radiochemical purity was determined to be \_\_\_\_\_  
by \_\_\_\_\_. The  $^{14}\text{C}$ -HCTZ (lot No. CFQ12666) had a radiochemical  
purity of \_\_\_\_\_. Unlabeled HCTZ (lot No. HYD0103), and olmesartan (lot No. EX-990119)  
were also used in this study.

Individual plasma samples were prepared from a 100 ml sample of blood taken from the brachial vein of each Japanese male volunteer (n=3; 33, 26 and 32 years old). The samples were not combined. The *in vitro* protein binding of  $^{14}\text{C}$ -olmesartan to human plasma, 4% HSA and 0.1% HAGP was determined after incubation at \_\_\_\_\_, at a final concentration of 0.1 and 1  $\mu\text{g}/\text{ml}$  without and with HCTZ (0.1, 1 or 10  $\mu\text{g}/\text{ml}$ ). After the incubation, ultrafiltration was performed and the radioactivity in the filtrate was measured to determine free drug concentration. The binding ratio was calculated as percent of total radioactivity. Similarly, the *in vitro* protein binding of  $^{14}\text{C}$ -HCTZ to human plasma and 4% HSA was determined after incubation at \_\_\_\_\_ at a final concentration of 0.1 and 1  $\mu\text{g}/\text{ml}$  without and with olmesartan (0.1, 1 or 10  $\mu\text{g}/\text{ml}$ ).

#### Results

The mean binding ratios of  $^{14}\text{C}$ -olmesartan at concentrations of 0.1 and 1  $\mu\text{g}/\text{ml}$  in human plasma were 98.9% and 99.1%, respectively, without HCTZ. These ratios did not differ significantly in the presence of unlabeled HCTZ. Furthermore, the binding at 1  $\mu\text{g}/\text{ml}$   $^{14}\text{C}$ -olmesartan to HSA and HAGP was relatively constant with and without HCTZ. There were no statistically significant differences, demonstrating that HCTZ did not affect the plasma protein binding of  $^{14}\text{C}$ -olmesartan (Table 3.2.1).

The effect of non-labeled olmesartan on the binding of  $^{14}\text{C}$ -HCTZ to human plasma at two different concentrations was relatively constant (67% to 69.8%). Additionally, the ratios for binding  $^{14}\text{C}$ -HCTZ to HSA were relatively constant (55.0% to 57.1%) with and without olmesartan. There were no statistically significant differences, demonstrating that olmesartan did not affect the plasma protein binding of  $^{14}\text{C}$ -HCTZ (Table 3.2.2).

Binding of olmesartan  
to HSA

**TABLE 3.2.1**  
EFFECT OF HCTZ ON THE BINDING OF <sup>14</sup>C-OLMESARTAN TO HUMAN PLASMA, 4% HSA AND 0.1% HAGP

**Human plasma**

<sup>14</sup> C-RNH-6270 conc. (µg/ml)	HCTZ conc. (µg/ml)	Binding ratio (%)				
		Subject 1	Subject 2	Subject 3	Mean	S.E.
0.1	0				98.9	0.2
	0.1				99.3	0.0
	1.0				99.1	0.1
	10.0				99.2	0.1
1.0	0				99.1	0.0
	0.1				99.1	0.0
	1.0				99.1	0.1
	10.0				99.1	0.0

**4% HSA**

<sup>14</sup> C-RNH-6270 conc. (µg/ml)	HCTZ conc. (µg/ml)	Binding ratio (%)				
		No. 1	No. 2	No. 3	Mean	S.E.
1.0	0				98.1	0.0
	0.1				98.2	0.0
	1.0				98.1	0.1
	10.0				98.4	0.1

**0.1% human AGP**

<sup>14</sup> C-RNH-6270 conc. (µg/ml)	HCTZ conc. (µg/ml)	Binding ratio (%)				
		No. 1	No. 2	No. 3	Mean	S.E.
1.0	0				73.8	1.7
	0.1				69.5	1.9
	1.0				80.7	3.6
	10.0				79.2	2.9

No significant differences ( $p \geq 0.05$ ) in the binding ratios for groups with or without HCTZ, as determined by the Dunnett's test.

*SE very high?*

**TABLE 3.2.2**  
**EFFECT OF OLMESARTAN ON THE BINDING OF <sup>14</sup>C-HCTZ TO HUMAN PLASMA AND 4% HSA**

**Human plasma**

<sup>14</sup> C-HCTZ conc. (µg/ml)	RNH-6270 conc. (µg/ml)	Binding ratio (%)				
		Subject 1	Subject 2	Subject 3	Mean	S.E.
0.1	0				69.8	1.5
	0.1				67.0	2.7
	1.0				69.7	1.6
	10.0				69.3	1.6
1.0	0				67.5	0.3
	0.1				68.7	0.7
	1.0				68.0	1.4
	10.0				67.7	0.5

**4% HSA**

<sup>14</sup> C-HCTZ conc. (µg/ml)	RNH-6270 conc. (µg/ml)	Binding ratio (%)				
		No. 1	No. 2	No. 3	Mean	S.E.
1.0	0				56.7	1.4
	0.1				57.0	0.6
	1.0				57.1	0.8
	10.0				55.0	1.3

No significant differences ( $p \geq 0.05$ ) in the binding ratios for groups with or without olmesartan, as determined by the Dunnett's test.



#### 4.1. SINGLE-DOSE TOXICITY STUDIES

##### 4.1.1. Acute Oral Toxicity of OM-HCTZ in Rats. Vol. 12

*This GLP study (Project # 89770, Q A'd Report #APRC 148-136) was conducted by a contract laboratory.*

*The animals were dosed on April 18 and necropsied on May 2, 2001.*

##### Key Findings

Oral administration of single doses of OM-HCTZ to rats at (total) doses up to 2000 mg/kg (1230/770 mg/kg) did not result in any significant toxicity.

##### Methods

Three groups, each consisting of five male (141-174 g) and five female (123-146 g) Sprague-Dawley CD (CrI:CD (SD) IGS, ) 6-week-old rats, were given OM-HCTZ at total doses of 0, 1000, or 2000 mg/kg (20 ml/kg). These doses correspond to 615/385 and 1230/770 mg/kg of OM/HCTZ. An additional 4 rats/sex/group were dosed for the evaluation of toxicokinetics. Those animals were fasted overnight and blood samples collected from the orbital sinus two hours after dosing for analysis of olmesartan and HCTZ plasma concentrations. Plasma levels of olmesartan and HCTZ were determined using a validated assay. Following blood sample collection, animals were killed by euthanization and discarded without further examination.

Main study animals were observed twice daily for 14-days following dosing. Individual body weights were determined before administration and 1, 4, 8, 11 and 15 days after administration. On the final day, animals were fasted overnight, euthanized and exsanguinated. For each animal, necropsy consisted of an external examination, identification of all clinically recorded lesions and a detailed internal examination. Selected organs were dissected free of fat and weighed. All gross abnormalities were examined histopathologically. Remaining tissues were retained in fixative. The following tissues and organs were retained for histopathology examinations.

**TABLE 4.1.1.1.**  
**TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION**

Aorta (thoracic)	Kidneys*	Seminal vesicles
Adrenals*	Lacrimal glands	Skeletal muscle
Bone marrow (sternum)	Liver (sample of 2 lobes)*	Skin (inguinal)
Brain*	Lungs (all lobes)*	Spinal cord (cervical)
Cecum	Lymph nodes (mandibular,	Spleen*
Colon	mesenteric and renal)	Stomach
Duodenum	Mammary gland (inguinal)	Testes*
Epididymides	Optic nerves	Tongue
Esophagus	Ovaries*	Trachea
Eyes	Pancreas	Thymus*
Harderian gland	Pituitary *	Thyroid and parathyroid*
Heart (including section of	Prostate*	Urinary bladder
<del>aorta</del> *)	Rectum	Uterus*
Ileum	Salivary gland	Vagina
Jejunum	Sciatic nerve	

\* : Organ weighed

Results

There were no deaths or treatment-related clinical signs in any dose group. A slight reduction in mean body weight gain was observed for males (35 gm) and females (28 gm) receiving OM-HCTZ at 2000 mg/kg on days 1-4 relative to vehicle control (males 42 gm, females 32 gm). However, these differences are not statistically significant. There were no treatment-related differences in organ weights, gross or histopathological changes.

The plasma concentration of olmesartan and HCTZ observed at 2 hr following oral dosing with OM-HCTZ at 1000 or 2000 mg/kg were generally comparable (Table 4.1.1.2). This suggests a possible saturation of absorption at around 1000 mg/kg/day. Gender differences were not observed.

**TABLE 4.1.1.2**  
**MEAN PLASMA CONCENTRATIONS OF OLMESARTAN AND HCTZ IN RATS TREATED WITH A SINGLE ORAL DOSE OF OM-HCTZ**

Dose (mg/kg)	Olmesartan (µg/ml)		HCTZ ((µg/ml)	
	Male	Female	Male	Female
1000	3.71	2.34	8.57	8.77
2000	3.95	3.78	6.72	10.10

#### 4.1.2. Acute Oral Toxicity of OM-HCTZ in Dogs. Vol. 12.

*This GLP study (Project # 89771, Q A'd Report #APRC 148-137) was conducted by a contract laboratory.*

*The animals were dosed on April 19 and necropsied on May 3, 2001.*

##### Key Findings

Oral administration of single doses of OM-HCTZ to dogs at (total) doses up to 2000 mg/kg (1230/770 mg/kg) did not result in any significant toxicity.

##### Methods

Four groups, each consisting of one male (6.6-7.4 kg) and one female (6.0-6.8 kg) beagle dog, 5-6 months old, were given OM-HCTZ at total doses of 0, 80, 400 or 2000 mg/kg (1/sex/group). These doses correspond to 49.25/30.75, 246/154, and 1230/770 mg/kg OM/HCTZ. Control animals received an equivalent number of empty gelatin capsules. Blood samples were collected from all animals from a jugular vein prior to initiation of dosing and on days 8 and 15 for hematology and serum chemistry analysis. For toxicokinetics study, blood samples were collected from all animals at 1, 2, 4, 8, and 24 hours after dosing from the jugular, cephalic or saphenous veins. Plasma levels of olmesartan and HCTZ were determined using a validated assay.

All animals were observed twice daily for mortality and reaction to treatment for 14 days. Individual body weights were determined before administration and on days 7 and 14. Food consumption was measured prior to treatment and throughout the observation period. Animals were fasted overnight before necropsy. For each animal, necropsy consisted of an external examination, identification of all clinically recorded lesions and a detailed internal examination. Tissues/organs were not weighed. All gross abnormalities were examined histopathologically. Remaining tissues were retained in fixative. Tissues and organs retained for histopathology examinations are given in Table 4.1.2.1.

##### Results

There were no deaths, no treatment-related clinical signs, and no treatment-related effects on body weight, food consumption, or hematology and clinical biochemistry parameters. Both gross and histopathological examinations showed no changes or trends indicative of drug toxicity.

**TABLE 4.1.2.1.**  
**TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION**

Aorta (thoracic)	Jejunum	Sciatic nerve
Adrenals	Kidneys	Skeletal muscle
Bone marrow (sternum)	Liver (sample of 2 lobes)	Skin (inguinal)
Brain	Lungs (all lobes)	Spinal cord (cervical)
Cecum	Lymph nodes (mandibular, mesenteric)	Spleen
Colon	Mammary gland (inguinal)	Stomach
Duodenum	Optic nerves	Testes
Epididymides	Ovaries	Tongue
Esophagus	Pancreas	Trachea
Eyes	Pituitary	Thymus
Gallbladder	Prostate	Thyroid and parathyroid
Heart (including section of aorta)	Rectum	Urinary bladder
Ileum	Salivary gland (submandibular)	Uterus
		Vagina

The systemic exposure to olmesartan and HCTZ in male and female dogs following a single oral dose of OM-HCTZ generally increased in a less than dose-proportional manner, although a more than proportional increase was observed for the olmesartan C<sub>max</sub> in males from 80 to 400 mg/kg and for the HCTZ AUC in males from 400 to 2000 mg/kg. No obvious gender differences were observed for either C<sub>max</sub> or AUC parameters. T<sub>max</sub> values for olmesartan ranged from 1 to 8 hours, with no clear trend with increasing dose level. On the other hand, T<sub>max</sub> values for HCTZ ranged from 1 to 8 hours in males and 1 to 4 hours in females; there was an apparent increase in T<sub>max</sub> with increasing dose (Table 4.1.2.2).

**TABLE 4.1.2.2**  
**TOXICOKINETIC PARAMETERS FOR OLMESARTAN AND HCTZ IN DOGS TREATED WITH A SINGLE ORAL DOSE OF OM-HCTZ**

Dose	OLMESARTAN					
	Males			Females		
	T <sub>max</sub> (hr)	C <sub>max</sub> (µg/ml)	AUC <sub>0-last</sub> (µg.h/ml)	T <sub>max</sub> (hr)	C <sub>max</sub> (µg/ml)	AUC <sub>0-last</sub> (µg.h/ml)
80	8	0.339	3.958	2	0.883	3.071
400	2	2.833	7.601	1	1.688	6.428
2000	4	6.428	40.845	2	6.206	25.789
Dose	HCTZ					
	Males			Females		
	T <sub>max</sub> (hr)	C <sub>max</sub> (µg/ml)	AUC <sub>0-last</sub> (µg.h/ml)	T <sub>max</sub> (hr)	C <sub>max</sub> (µg/ml)	AUC <sub>0-last</sub> (µg.h/ml)
80	-	-	-	1	5.71	18.3
400	1	8.19	38.0	2	12.50	61.0
2000	8	26.10	277.0	4	13.80	98.7

- No parameters were estimated due to limited data

AUC<sub>0-last</sub>: area under the plasma concentration vs. time curve from 0 hr to the last quantifiable value calculated by the trapezoidal method.

## 4.2. REPEAT-DOSE TOXICITY STUDIES

### 4.2.1. 26-Week Oral Toxicity of OM-HCTZ in Rats. Vol. 7-8.

*This GLP study (Project # 89499, Q A'd Report #APRC 148-134) was conducted by a contract laboratory.*

*The animals were dosed on June 21, 2000 and the last necropsy was done on December 22, 2000.*

#### Key Findings

The kidney seems to be the prime target organ for toxicity. Treatment-related increases in BUN and (to lesser degree) creatinine were observed in rats receiving 162.5 or more mg OM-HCTZ/kg/day and BUN was also increased in males given 1000 mg OM/kg/day. At terminal sacrifice, the mean relative kidney weights were significantly increased in all drug treated groups. The microscopic changes observed in the kidney were considered to be part of the spectrum of changes associated with chronic progressive nephropathy in rats. Additionally, isolated incidences of erosion and ulceration of the glandular mucosa of the stomach were observed in all OM-HCTZ and OM-treated groups.

#### Methods

Male and female Sprague-Dawley CD (CrI:CD (SD) BR IGS) rats were approximately 7 weeks old and weighed 186-283 g (males) or 143-240 g (females) at the start of the study. OM-HCTZ was given by oral gavage at total doses of 0, 162.5, 487.5, or 1625 mg/kg/day (15/sex/dose). These doses correspond to 100/62.5, 300/187.5 and 1000/625 mg OM/HCTZ per kg per day. In addition, one group of rats was treated with 1000 mg OM/kg/day and a second with 625 mg HCTZ/kg/day (15/sex/group). The control animals (15/sex/group) received the vehicle (10 ml/kg body weight). Twelve additional (satellite) animals/sex/group were used for toxicokinetic study. Animals were housed individually. Food and water was provided to each rat *ad libitum* unless otherwise specified. The doses were selected by the contract laboratory on the advice of the sponsor. No further details in the current submission.

#### Observations and Measurements

All animals were observed twice daily for mortality and clinical signs. Body weight and food consumption were recorded a week before treatment and then at weekly intervals for all animals. Data for satellite animals were retained but are not reported. Urine samples were collected from all animals in the main study group during treatment weeks 13 and 26. Ophthalmic examinations were conducted on all animals prior to the start of treatment and again on main study animals before dosing during weeks 13 and 26 of treatment. Blood samples for hematology and clinical biochemistry were collected (from the animals in the main study groups only) from the abdominal aorta (under isoflurane anesthesia) at the end of the dosing period. Animals were fasted overnight prior to blood sampling. For toxicokinetics study, blood samples were collected from satellite animals prior to dosing and at 1, 2, 4, 8, and 24 hr after dosing on day 1 and during

weeks 4 and 25. Blood was collected from a jugular vein (3 animals/sex/time point). All main study animals were fasted overnight, anaesthetized with isoflurane and exsanguinated. The necropsy included external examination, weighing of selected organs and sampling of tissues for histopathological examination (Table 4.2.1.1). All tissues from all animals of the control group, the 1625 mg/kg/day OM-HCTZ group, the OM group, the HCTZ group, and all animals that died or were sacrificed moribund, were examined histopathologically. Additionally, all gross lesions, adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid lobes/parathyroids, and uterus were examined for animals treated with 162.5 and 487.5 mg OM-HCTZ/kg/day. The tissues and/organs retained after completion of necropsy are given in Table 4.2.1.1.

**TABLE 4.2.1.1**  
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Aorta (thoracic)	Kidneys*	Seminal vesicles
Adrenals*	Lacrimal glands	Skeletal muscle
Bone marrow (sternum)	Liver (sample of 2 lobes)*	Skin (inguinal)
Brain*	Lungs (all lobes)*	Spinal cord (cervical)
Cecum	Lymph nodes (mandibular, mesenteric and renal)	Spleen*
Colon	Mammary gland (inguinal)	Stomach
Duodenum	Optic nerves	Testes*
Epididymides	Ovaries*	Tongue
Esophagus	Pancreas	Trachea
Eyes	Pituitary *	Thymus*
Harderian gland	Prostate*	Thyroid and parathyroids*
Heart (including section of aorta)*	Rectum	Urinary bladder
Ileum	Salivary gland	Uterus*
Jejunum	Sciatic nerve	Vagina

\*: Organ weighed

## Results

One male receiving 162.5 mg OM-HCTZ/kg/day was sacrificed moribund on day 83. Clinical signs at the time of euthanasia included head tilt, abnormal gait, severely swollen hindlimbs, reduced activity and dehydration. Hematology and clinical biochemistry tests prior to death indicated a marked increase in total white blood cell count and marked increases in blood urea nitrogen and total bilirubin. Pathologically, a massive bilateral chronic suppurative inflammation of the tarsal joint was observed, the cause of which was not determined. In the absence of other pathological findings, the testing laboratory considered this an incidental finding and unrelated to treatment with OM-HCTZ.

One female receiving 487.5 mg OM-HCTZ/kg/day was found dead on day 96. Clinical signs prior to death included thin appearance, dehydration, reduced activity, weak appearance, cold to touch, reduced muscle tone, lateral recumbancy, and red staining of the fur of the muzzle. The death of this animal was attributed to severe inflammation of the heart, the cause of which was not determined. In the absence of similar histopathological findings in other animals, the death was considered as incidental and unrelated to treatment with OM-HCTZ. In addition, a female in

the 1000 mg OM/kg/day satellite group died during blood sampling on day 28. The cause of death was attributed to the sampling procedure.

There were no treatment-related clinical signs in animals that survived to the termination of the study. Overall body weight gain was reduced (>10%) nondose-dependently in all OM-HCTZ groups relative to controls. The data was statistically significant for low dose groups for the first 10 weeks, mid and high dose males and females for up to 20 and 13 weeks of treatment, respectively (Table, 4.2.1.2, Fig. 4.2.1.1).

TABLE 4.2.1.2  
26-WEEK TOXICITY STUDY IN RATS: GROUP MEAN BODY WEIGHTS

SEX GROUP	MALES						FEMALES					
	1	2	3	4	5	6	1	2	3	4	5	6
WEEK -3	148.3	148.0	142.7	145.1	145.6	145.6	130.5	126.7	126.1	128.0	126.3	131.3
WEEK -2	193.1	189.3	186.2	186.4	187.7	189.1	158.7	154.1	154.4	156.3	153.3	160.3
WEEK -1	247.9	242.1	237.3	237.6	241.7	246.1	177.9	172.6	173.6	176.8	174.5	181.1
WEEK 1	304.6	288.1	279.1 B	276.1 B	290.1	297.1	197.3	182.4 A	181.9 A	188.5	195.6	200.6
WEEK 2	342.7	314.9 A	305.7 B	306.4 B	322.5	336.1	215.6	196.3 A	194.1 B	198.3 A	208.7	217.3
WEEK 3	375.2	339.9 A	331.6 B	333.7 B	351.7	369.9	224.3	208.5	204.7 A	208.1	221.3	230.1
WEEK 4	403.1	368.8 A	355.1 B	362.9 A	378.3	399.9	236.3	216.9 A	213.7 A	216.8 A	229.1	241.4
WEEK 5	428.4	390.3 A	374.0 B	377.1 B	396.4	423.3	247.4	229.3	224.8 A	227.3 A	240.7	254.5
WEEK 6	446.1	404.1 A	385.8 B	390.7 B	417.4	440.7	259.0	237.6	228.9 B	233.6 A	250.2	263.7
WEEK 7	466.3	419.9 A	403.6 B	406.8 B	434.0	454.2	261.4	240.4	235.1 A	239.3	255.7	270.2
WEEK 8	480.6	434.1 A	419.6 B	421.5 B	451.3	473.1	268.3	244.6 A	241.4 A	247.4	260.8	273.7
WEEK 9	494.4	444.5 A	431.9 B	434.9 B	464.2	486.0	279.7	250.9 A	247.7 B	253.9 A	267.9	281.9
WEEK 10	504.9	457.1	442.5 B	443.8 A	475.9	494.5	284.3	255.0 A	253.1 A	259.7	275.5	289.4
WEEK 11	513.6	467.7	452.6 A	453.3 A	485.6	506.5	288.8	265.1	258.2 A	266.5	282.0	287.6
WEEK 12	523.3	480.3	462.5 A	465.1 A	495.7	517.5	292.3	268.2	263.3 A	274.0	288.3	298.9
WEEK 13	524.2	480.1	461.9 A	459.5 A	499.2	520.9	290.3	263.9	258.7 A	272.0	287.3	296.4
WEEK 14	535.1	493.5	470.7 A	470.7 A	510.3	533.5	300.0	272.3	272.4	280.4	294.0	301.4
WEEK 15	548.9	509.4	486.4 A	481.5 A	522.2	542.6	306.9	279.3	279.1	285.1	299.1	308.9
WEEK 16	565.3	520.2	495.9 A	493.1 A	535.1	553.7	310.7	283.3	284.1	290.0	303.6	313.1
WEEK 17	563.5	531.1	503.1 A	501.3 A	541.7	561.1	313.3	286.5	288.9	295.5	307.9	318.5
WEEK 18	570.7	538.4	509.0 A	505.3 A	542.7	569.7	319.1	293.6	292.6	300.2	313.6	324.9
WEEK 19	577.6	547.1	516.9 A	514.0 A	556.5	580.9	320.7	297.0	295.2	304.8	317.7	325.7
WEEK 20	584.1	554.3	523.0 A	521.7 A	559.5	589.3	324.3	299.0	296.8	307.8	319.4	329.7
WEEK 21	589.5	562.6	530.7	528.9	569.9	596.3	327.4	304.1	303.1	312.6	324.0	333.4
WEEK 22	593.9	566.1	537.1	535.6	576.7	601.7	327.3	306.3	306.0	315.9	326.9	335.5
WEEK 23	598.8	572.2	543.0	537.0	582.3	605.9	330.1	310.2	308.8	320.2	327.7	340.7
WEEK 24	603.9	578.7	548.1	543.0	588.8	611.3	331.3	315.5	310.9	323.3	332.5	343.0
WEEK 25	610.1	588.9	553.8	551.0	589.3	620.6	336.3	315.1	311.4	327.3	335.7	344.3
WEEK 26	603.6	577.4	543.6	543.1	589.0	616.2	334.7	312.5	311.5	324.0	331.3	340.3

Significantly different from (Group 1)

A: p < 0.05

B: p < 0.01 (Dunnett's)

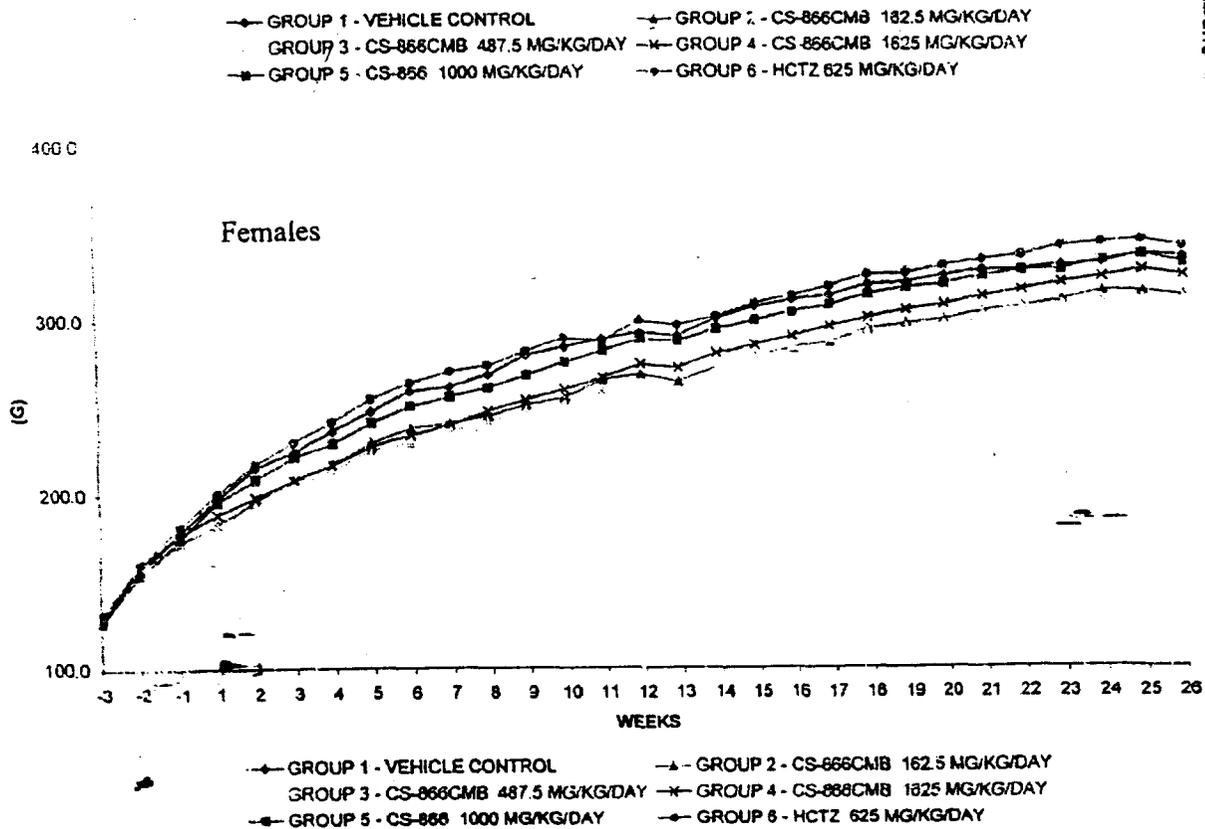
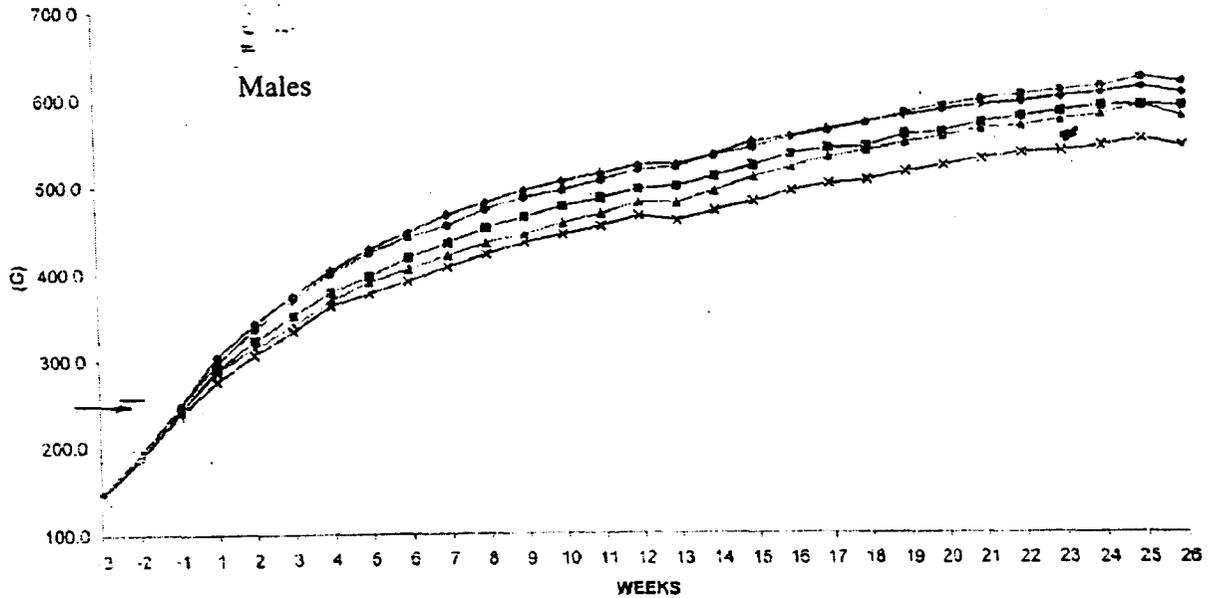


Fig. 4.2.1.i: 26-Week Oral Toxicity Study in Rats. Group Mean Body Weights in Males (top) and Females (bottom). S-866CMB: OM-HCTZ

Mean weekly food consumption was significantly but non-dose-dependently reduced (11-20%) in all OM-HCTZ groups relative to concurrent controls during the second and third weeks of treatment. Additionally, males at 487.5 and 1625 mg OM-HCTZ/kg/day showed continued decrease in food consumption (10-13%,  $p < 0.05$ ) for the following 4 weeks (up to 7<sup>th</sup> week of treatment). Ocular changes consisting of dilatation of the retinal vessels were noted at week 26 in all OM-HCTZ groups and rats receiving 1000 mg OM/kg/day. This finding was observed in approximately half of the animals in all of the OM-HCTZ groups and in approximately 27% of the rats treated with OM. This was observed in both males and females and usually occurred bilaterally. This change did not occur in any of the rats in the control or HCTZ groups. There were no other treatment-related ophthalmological changes.

Group mean RBC, hemoglobin and hematocrit were significantly decreased (6 to 14%) in males and females in all OM-HCTZ groups and male rats treated with OM. The decrease was dose-dependent with respect to males. Since individual values were within historical ranges, findings were considered by the contract laboratory to be of doubtful toxicological significance. There were no other treatment-related changes in hematology parameters.

Treatment-related (but non-dose dependent) increases in blood urea nitrogen (up to 2.8-fold) and creatinine (up to 1.4-fold) were observed in all groups of OM-HCTZ treated rats. Only BUN was increased in males given OM (Table 4.2.1.1). Other differences in group mean values included increased phosphorus (males and females in all OM-HCTZ groups) and slightly reduced total protein (males at 487.5 mg or more OM-HCTZ/kg/day and males in the OM group), although individual values were generally within the range of historical controls. With the exception of an increased urine volume in several males treated with OM-HCTZ (487.5 mg/kg/day and above) there were no treatment-related effects on urinalysis parameters.

TABLE 4.2.1.3  
GROUP MEAN VALUES FOR BUN AND CREATININE IN RATS TREATED ORALLY  
WITH OM-HCTZ, OM, OR HCTZ FOR 26 WEEKS (REPORT #APRC 148-134)

Treatment	Dose (mg/kg/day)	BUN (mg/dl)		Creatinine (mg/dl)	
		Male	Female	Male	Female
Control	—	13.8	14.5	0.7	0.7
OM-HCTZ	162.5	44.5*	46.4*	0.9*	1.0*
	487.5	51.8*	42.2*	0.9*	0.9*
	1625	47.8*	44.6*	0.9*	1.0*
OM	1000	27.1*	16.7	0.7	0.8
HCTZ	625	14.8	14.6	0.7	0.7

\* Significantly different from control,  $p < 0.01$

Statistically significant increases in relative (but not absolute) kidney weights were observed in all groups of OM-HCTZ-treated animals. Histopathologically, treatment-related changes were observed in the kidneys of males and females treated with OM-HCTZ and were characterized as variable thickening of the tubular basement membrane and, infrequently, of the Bowman's capsular membrane, with or without tubular epithelial basophilia. These changes were mostly graded minimal to slight in severity and were considered to be part of the spectrum of changes

associated with chronic progressive nephropathy in rats. Similar changes were observed in animals treated with OM or HCTZ alone, although the severity appeared to be less than that observed with OM-HCTZ. Females showed a lower incidence than males (Table 4.2.1.4).

**TABLE 4.2.1.4**  
SUMMARY OF INCIDENCE AND SEVERITY OF CHRONIC PROGRESSIVE NEPHROPATHY IN RATS  
TREATED WITH OM-HCTZ, OM, OR HCTZ FOR 26 WEEKS (REPORT #APRC 148-134)

		OM-HCTZ				OM	HCTZ
		Control	162.5	487.5	1625	1000	625
<b>Kidney: Males</b>	No.	15	15	15	15	15	15
Chronic progressive nephropathy	Grade 1	2	10	7	6	9	5
	Grade 2	0	5	6	9	3	3
	Grade 3	0	0	2	0	0	0
Total No./Tissues Affected		2	15	15	15	12	8
Average Grade/Tissues Affected		1.0	1.3	1.7	1.6	1.2	1.4
<b>Kidney: Females</b>	No.	15	15	15	15	15	15
Chronic progressive nephropathy	Grade 1	0	10	10	10	6	2
	Grade 2	0	4	5	5	0	0
	Grade 3	0	0	0	0	0	0
Total No./Tissues Affected		0	14	15	15	6	2
Average Grade/Tissues Affected		0	1.3	1.3	1.3	1.0	1.0

On gross examination, dark foci were observed in the stomachs of males and females in all OM-HCTZ groups and in the OM group, with a higher incidence observed for males.

Microscopically, erosion of the glandular mucosa of the stomach was observed in one male and one female in the 162.5 mg/kg/day OM-HCTZ group, three males in the 1625 mg/kg/day OM-HCTZ group, and one male in the OM group. Ulceration of the glandular mucosa of the stomach was observed in one male and one female in the 487.5 mg/kg/day OM-HCTZ group.

C<sub>max</sub> and AUC<sub>0-*t*last</sub> for olmesartan and HCTZ were dose-related. For the most part, these values increased with increasing doses of OM-HCTZ, but in a less than dose-proportional manner. C<sub>max</sub> and AUC<sub>0-*t*last</sub> values for olmesartan after administration of 1000 mg OM/kg/day alone were similar to those observed after administration of 1625 mg OM-HCTZ/kg/day. C<sub>max</sub> values for HCTZ after administration of 625 mg HCTZ/kg/day alone were generally consistent with the C<sub>max</sub> values observed after administration of 1625 mg OM-HCTZ/kg/day. However, AUC<sub>0-*t*last</sub> values tended to be lower when HCTZ was administered alone in comparison to those observed after administration of 1625 mg OM-HCTZ/kg/day. T<sub>max</sub> values for olmesartan generally ranged from 1 to 2 hours post-dose and T<sub>max</sub> values for HCTZ ranged from 1 to 4 hours. There were no substantial gender differences in these parameters and no apparent trend toward increased values was observed after repeated administration (Table 4.2.1.5).

**TABLE 4.2.1.5**  
**TOXIGOKINETIC PARAMETERS FOR OLMESARTAN AND HCTZ IN RATS**  
**TREATED ORALLY WITH OM-HCTZ FOR 26 WEEKS (REPORT #APRC 148-134)**

Dosage (mg/kg/day)	Males					Females				
	OM-HCTZ		OM	HCTZ		OM-HCTZ		OM	HCTZ	
	162.5	487.5	1625	1000	625	162.5	487.5	1625	1000	625
<b>1. C<sub>max</sub> (µg/ml)</b>										
<b>OM</b>										
Day 1	1.32	1.97	1.75	1.52		2.15	1.31	2.17	1.85	
Week 4	1.40	3.05	2.97	4.94		1.38	1.83	2.22	2.92	
Week 25	1.58	1.92	2.97	2.38		2.30	2.83	4.05	2.75	
<b>HCTZ</b>										
Day 1	4.25	7.08	10.8		6.61	4.97	4.23	6.29		6.16
Week 4	5.93	5.60	9.36		9.68	6.05	6.42	8.37		5.67
Week 25	4.57	5.44	6.81		3.95	6.60	6.65	7.11		5.67
<b>2. AUC<sub>0-12hr</sub> (µg.hr/ml)</b>										
<b>OM</b>										
Day 1	9.37	14.5	20.4	14.5		6.30	12.6	23.6	20.2	
Week 4	8.83	18.3	42.6	22.0		6.29	14.4	19.0	17.5	
Week 25	8.11	14.6	18.9	16.8		13.6	22.0	38.0	28.9	
<b>HCTZ</b>										
Day 1	20.6	33.5	80.0		34.0	16.1	23.1	59.3		33.5
Week 4	32.3	36.9	96.6		51.2	18.7	47.7	49.6		42.3
Week 25	16.1	33.7	50.0		40.8	13.8	34.3	48.2		45.8

In conclusion, daily oral administration of OM-HCTZ to the rat at dose levels of 162.5, 487.5 and 1625 mg/kg/day for 26 weeks resulted in histopathological changes in the kidneys (chronic progressive nephropathy) at all dose levels. Similar changes were also observed in animals given either 1000 mg OM/kg/day or 625 mg HCTZ/kg/day, although at lower incidence and severity. In addition, isolated incidences of erosion and ulceration of the glandular mucosa of the stomach were observed in all OM-HCTZ and OM-treated groups. A NOAEL was not determined in this study.

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#### 4.2.2. 26-Week Oral Toxicity of OM-HCTZ in Rats. Vol. 13-14.

This GLP study (Project # 89945, Q A'd Report #APRC 148-139) was conducted by a contract laboratory,

with the objective of finding a no effect dose for the adverse effects observed in the previous 26 week study (Project # 89499). The animals were dosed on July 18, 2001 and necropsied on January 18, 2002.

##### Key Findings

As in the previous study, the kidney seems to be the prime target organ for toxicity (even at much reduced dosage). Dose-dependent increases in BUN were observed in all treated male groups and in females given 48.75 mg OM-HCTZ/kg/day. The microscopic changes observed in the kidney were considered to be part of the spectrum of changes associated with chronic progressive nephropathy in rats. Additionally, isolated incidences of mild gastric mucosal inflammation (irritation) were observed in males at 16.25 or 48.75 mg/kg/day.

##### Methods

Male and female Sprague-Dawley CD (CrI:CD (SD) BR IGS) rats were approximately 7 weeks old and weighed 201-270 g (males) or 151-200 g (females) at the start of the study. OM-HCTZ was given by oral gavage at total doses of 0, 4.88, 16.25, or 48.75 mg/kg/day (15/sex/dose). These doses correspond to 3/1.88, 10/6.25, and 30/18.75 mg/kg/day OM/HCTZ. The control animals (15/sex/group) received the vehicle (5 ml/kg body weight). Twelve additional satellite animals/sex/ group were used for toxicokinetic study. Animals were housed individually. Food and water was provided to each rat *ad libitum* unless otherwise specified.

The doses were selected on the basis of a previous 26-week oral toxicity study (same rat strain and same mode of administration) which did not establish a NOAEL (report #APRC 148-134, section 4.2.1). In that study, overall body weight gain was reduced (>10%) nondose-dependently in all OM-HCTZ groups (100/62.5, 300/187.5, and 1000/625 mg/kg/day) compared to controls for most part of the study. Mean weekly food consumption was slightly but significantly reduced in all OM-HCTZ groups during the second and third weeks of treatment. Treatment-related increases in blood urea nitrogen and creatinine were observed in all groups of OM-HCTZ treated rats and BUN was increased in males given OM. Histopathologically, treatment-related changes were observed in the kidneys (chronic progressive nephropathy) of males and females treated with OM-HCTZ, OM or HCTZ.

##### Observations and Measurements

All animals were observed twice daily for mortality and clinical signs. Body weight and food consumption were recorded a week before treatment and then at weekly intervals for all animals. Data for satellite animals are not reported. Urine samples were collected from all animals in the main study group during weeks 13 and 26 treatment. Ophthalmic examination was conducted on all animals prior to the start of treatment and again before dosing on main study animals during weeks 13 and 26 of treatment. Blood samples for hematology and clinical biochemistry were



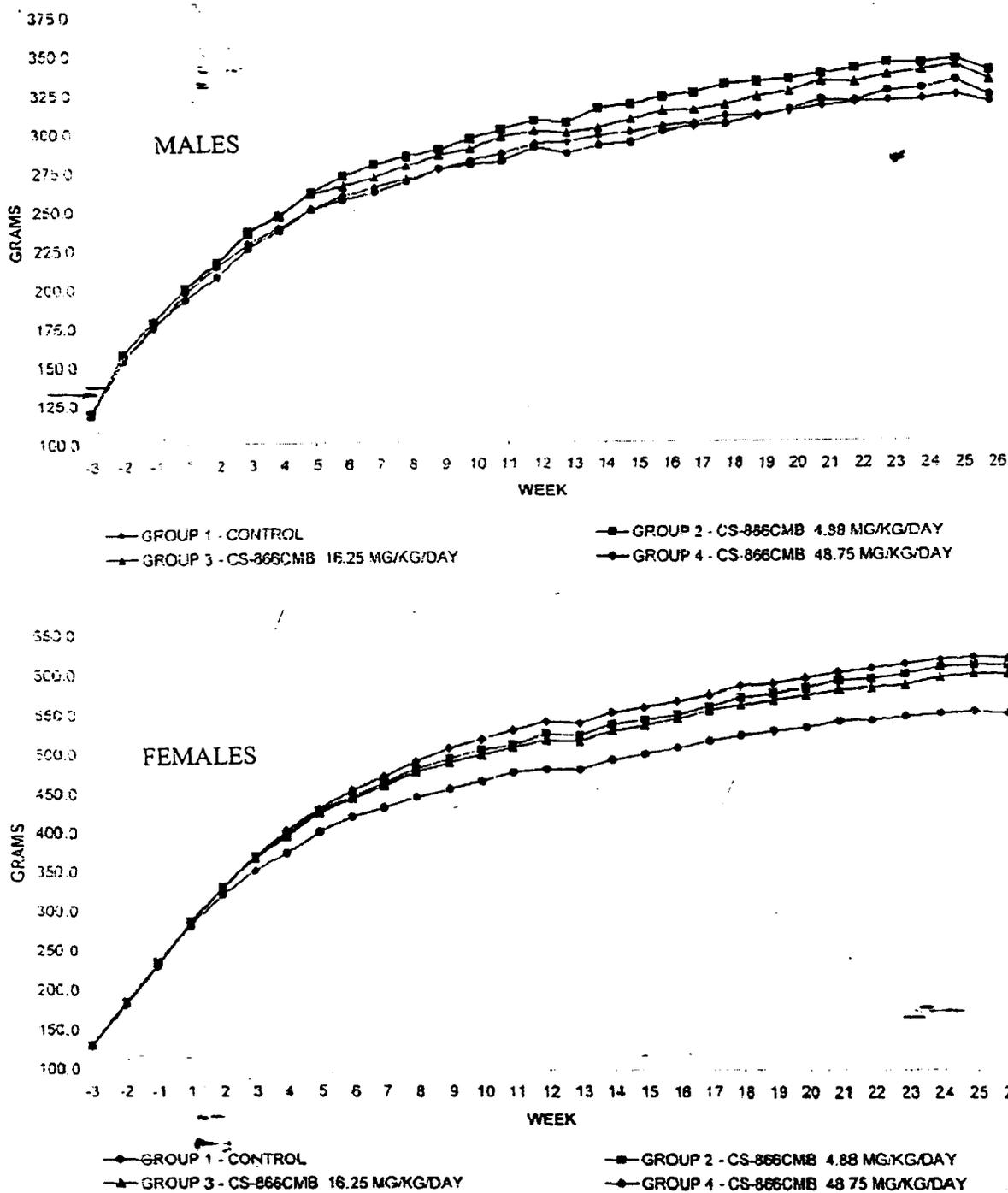


Fig. 4.2.2.1.: 26-Week Oral Toxicity Study in Rats. Group Mean Body Weights in Males (top) and Females (bottom). CS-866CMB: OM-HCTZ

TABLE 4.2.2.2  
26 WEEK TOXICITY STUDY IN RATS: GROUP MEAN BODY WEIGHTS

GROUP 1 CONTROL				GROUP 3 CS-866CMB 16.25 MG/KG/DAY				
GROUP 2 CS-866CMB 4.78 MG/KG/DAY				GROUP 4 CS-866CMB 48.75 MG/KG/DAY				
M A L E S				SEX	F E M A L E S			
1	2	3	4	GROUP	1	2	3	4
128.3	130.9	129.8	129.2	WEEK -3	119.1	120.2	120.7	118.6
180.8	184.9	183.5	183.2	WEEK -2	154.0	158.3	157.7	153.7
231.1	236.1	235.4	235.0	WEEK -1	175.1	179.5	180.1	177.1
285.6	287.5	284.5	282.2	WEEK 1	197.4	200.8	200.5	193.5
331.3	333.0	330.4	322.4	WEEK 2	214.5	217.5	216.5	208.0
371.3	370.5	367.8	352.3	WEEK 3	229.0	236.7	235.9	226.3
404.3	400.4	397.5	375.7	WEEK 4	239.3	246.7	247.9	237.3
431.7	429.1	426.1	402.2	WEEK 5	251.0	261.9	260.9	250.7
456.4	448.8	445.5	421.5	WEEK 6	259.5	272.0	265.8	256.9
472.5	464.6	461.5	433.5	WEEK 7	265.0	279.5	271.5	261.7
493.0	483.3	478.6	447.5 A	WEEK 8	270.3	284.5	278.5	268.5
510.1	496.3	491.3	458.0 A	WEEK 9	276.0	289.1	285.3	276.5
520.8	508.1	501.3	467.8 A	WEEK 10	281.7	295.4	289.1	279.2
532.1	514.2	510.9	478.9 A	WEEK 11	286.2	301.5	296.7	281.5
543.5	527.5	520.1	483.1 A	WEEK 12	292.4	307.2	300.4	289.9
541.3	525.5	518.7	482.3 A	WEEK 13	293.6	306.1	299.7	286.1
555.2	539.9	531.3	495.4 A	WEEK 14	297.1	314.9	302.3	291.1
562.6	546.3	538.8	503.5 A	WEEK 15	300.0	317.8	308.0	293.4
570.8	552.9	547.8	511.0 A	WEEK 16	303.5	322.6	313.3	300.1
578.5	563.9	558.7	520.3 A	WEEK 17	305.4	325.1	314.1	303.8
589.6	575.3	565.9	526.3 A	WEEK 18	309.7	329.9	316.6	304.6
592.7	580.8	572.3	531.9 A	WEEK 19	310.6	331.5	322.1	309.2
599.4	587.1	578.3	536.5 A	WEEK 20	312.7	333.1	325.3	313.6
607.5	597.1	585.7	545.5 A	WEEK 21	315.9	336.7	331.9	319.8
612.3	598.7	587.9	545.9 A	WEEK 22	318.3	340.0	331.1	319.2
618.5	605.5	591.9	551.9 A	WEEK 23	319.4	343.4	335.6	325.4
624.2	614.7	601.4	555.7 A	WEEK 24	320.3	343.0	338.6	327.1
627.9	617.7	606.3	559.3 A	WEEK 25	322.9	345.7	341.9	332.1
626.4	617.5	605.8	556.0 A	WEEK 26	318.3	338.7	332.6	322.7

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: A -  $p < 0.05$  (DUNNETT)

CS-866CMB: OM-HCTZ

Among *red blood cell indices*, RBC, hematocrit, and reticulocyte count in the high dose male group were statistically significantly decreased relative to the control group (0.5% to 4%) and a nondose-dependent reduced reticulocyte count was observed in all female dose groups (20%-35%,  $p < 0.05$ ). Since the decreases are within the historical control range, the sponsor considers them to be of no toxicological significance.

Drug-related *biochemical changes* included dose-related increases ( $p < 0.05$ ) in BUN in all treated male groups (22 to 185%) and in females at 30/18.75 mg/kg/day (50%). There were no treatment-related changes in creatinine levels. Serum phosphorus was increased ( $p < 0.05$ ) in both male (18%) and female (10%) groups at 30/18.75 mg/kg/day. Additionally, a slight but significant ( $p < 0.01$ ) increase (9%) in serum potassium was observed in high dose males.

There were no treatment-related effects on organ weights. On *macroscopic examination*, dark foci were observed in the stomachs of males at 3/1.88 or more mg/kg/day. *Histopathological* correlates of this finding, consisting of minimal to mild gastric mucosal inflammation (irritation), were observed in a few males at 16.25 or 48.75 mg/kg/day. There were several treatment-related microscopic findings in the kidney. Males and females treated with 48.75 mg OM-HCTZ/kg/day had variable thickening of the tubular basement membrane, and, infrequently, of the Bowman's capsular membrane, with or without renal tubular epithelial basophilia, indicative of tubular regeneration. These changes were graded minimal to slight in severity and were considered by

the sponsor to be part of the spectrum of changes associated with chronic progressive nephropathy (CPN). The males were more affected than the females (Table 4.2.2.3). Similar renal changes were observed in rats treated with OM alone (NDA 21,286 review). This study also failed to establish a NOAEL.

**TABLE 4.2.2.3**  
**GROUP INCIDENCE OF KIDNEY HISTOPATHOLOGICAL FINDINGS IN**  
**RATS TREATED ORALLY WITH OM-HCTZ FOR 26 WEEKS**

		OM-HCTZ			
		Control	4.88	16.25	48.75
<b>Males</b>	No.	15	15	15	15
1. Chronic progressive nephropathy	Grade 1	1	3	0	10
	Grade 2	0	0	0	1
Total No./Tissues Affected		1	3	0	11
Average Grade/Tissues Affected		1.0	1.0	0	1.09
2. Tubular Basophilia		2	1	3	1
<b>Females</b>	No.	15	15	15	15
1. Chronic progressive nephropathy	Grade 1	0	0	0	3
	Grade 2	0	0	0	0
Total No./Tissues Affected		0	0	0	3
Average Grade/Tissues Affected		0	0	0	1
2. Tubular Basophilia		2	2	1	8

The plasma concentrations of olmesartan and HCTZ were below the limit of detection in most groups, on most collection days. Though dose linearity for olmesartan was observed for mid and high dose groups, a meaningful conclusion could not be drawn from the insufficient data (Table 4.2.2.4).

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**TABLE 4.2.2.4**  
**TOXICOKINETIC PARAMETERS FOR OLMESARTAN AND HCTZ IN RATS**  
**TREATED ORALLY WITH OM-HCTZ FOR 26 WEEKS (REPORT #APRC 148-139)**

TOXICOKINETICS OF RNH-6270 AND HCTZ							89945
	CS-866CMB (mg/kg)						
	Males			Females			
	4.88	16.25	48.75	4.88	16.25	48.75	
<b>C<sub>max</sub> (µg/ml)</b>							
RNH-6270							
Day 1*	a	a	0.687	a	a	0.865	
Week 4*	a	0.309	0.900	a	a	0.713	
Week 26**	0.252	0.598	0.782	a	0.483	1.68	
HCTZ							
Day 1	a	a	a	a	a	a	
week 4	a	a	a	a	a	a	
week 26	a	a	2.66	a	a	a	
<b>AUC<sub>(0-last)</sub> (µg.hr/ml)</b>							
RNH-6270							
Day 1	a	a	2.12	a	a	2.42	
week 4	a	0.903	3.02	a	a	2.47	
week 26	0.834	1.34	3.42	a	1.37	5.18	
HCTZ							
Day 1	a	a	a	a	a	a	
week 4	a	a	a	a	a	a	
week 26	a	a	7.86	a	a	a	

a = could not be determined; \* = — \*\* = —

CS-866CMB: OM-HCTZ

#### 4.2.3. 26-Week Oral Toxicity of OM-HCTZ in Rats. Saline Supplementation Study. Vol. 15-16.

This GLP study (Project # 89964, Q A'd Report #APRC 148-140) was conducted by a contract laboratory,

to determine whether saline supplementation ameliorated the adverse effects of OM-HCTZ on the kidney. The animals were initially dosed on July 31, 2001 and were necropsied on January 30, 2002.

##### Key Findings

Reduced body weight gains were noted for the entire treatment period for OM-HCTZ treated females and for study weeks 4 to 12 for OM-HCTZ treated males. Saline supplementation ameliorated these effects on body weight gain, completely prevented an elevation in BUN concentration in treated females and reduced a similar elevation by about 50% in treated males (compared to respective treated groups given tap water). Microscopic examination revealed treatment-related renal tubular changes in treated rats, which were more pronounced and frequent in animals given tap water than those given saline. Isolated occurrences of ulceration of the glandular mucosa of the stomach were observed in treated males given saline or water.

##### Methods

Male and female Sprague-Dawley CD (CrI:CD (SD) BR IGS) rats were approximately 6 weeks old and weighed 148-207 g (males) or 116-162 g (females) at the start of the study. OM-HCTZ was given by oral gavage at a total dose 162.5 (100/62.5) mg/kg/day for 26 weeks to two groups of rats (15/sex/group) with one group given tap water and the other saline. Control animals were given either tap water or saline (15/sex/group). Additional (satellite) animals were treated with OM-HCTZ (tap water or saline, 12/sex/group) for toxicokinetics analyses. Animals were housed individually. Food and water was provided to each rat *ad libitum* unless otherwise specified.

In two previous 26-week studies in rats (sections 4.2.1 and 4.2.2), one conducted at doses of 162.5 to 1625 mg OM-HCTZ/kg/day, the other at doses of 4.88 to 48.75 mg/kg/day, the kidney was the target organ for toxicity and both studies failed to establish a NOAEL. In addition, a previous study with OM alone, administered for 28 days to rats, had demonstrated that saline supplementation reduced the renal toxicity of OM. The dose level chosen for the current study has been shown to produce histopathological and biochemical changes in the kidney.

##### Observations and Measurements

All animals were observed twice daily for mortality and clinical signs. Body weight and food consumption were recorded a week before treatment and then at weekly intervals. Data for satellite animals were retained but are not reported in the current submission. Urine samples were collected from all animals in the main study groups during treatment weeks 13 and 26. Ophthalmic examinations were conducted on all animals prior to the start of treatment and again

on main study animals before dosing during weeks 13 and 26 of treatment. Blood samples for hematology and clinical biochemistry were collected (from the animals in the main study groups only) from the abdominal aorta (under isoflurane anesthesia) at the end of the dosing period. Animals were fasted overnight prior to blood sampling. For toxicokinetics study, blood samples were collected from satellite animals prior to dosing and at 1, 2, 4, 8, and 24 hr after dosing on day 1 and during weeks 4 and 26. Blood was collected from a jugular vein (3 animals/sex/time point).

All main study animals were fasted overnight, anaesthetized with isoflurane and exsanguinated. The necropsy included external examination, weighing of selected organs and histopathological examination of the adrenals, eyes, heart, kidneys and stomach of all animals. Additionally, a microscopic peer review of the histopathology findings was performed by \_\_\_\_\_

The following tissues and/organs were retained after completion of necropsy (Table 4.2.3.1).

TABLE 4.2.3.1.  
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Aorta (thoracic)	Kidneys*	Seminal vesicles
Adrenals*	Lacrimal glands	Skeletal muscle
Bone marrow (sternum)	Liver (sample of 2 lobes)*	Skin (inguinal)
Brain*	Lungs (all lobes)*	Spinal cord (cervical)
Cecum	Lymph nodes (mandibular, mesenteric and renal)	Spleen*
Colon	Mammary gland (inguinal)	Stomach
Duodenum	Optic nerves	Testes*
Epididymides	Ovaries*	Tongue
Esophagus	Pancreas	Trachea
Eyes	Pituitary *	Thymus*
Harderian gland	Prostate*	Thyroid and parathyroids*
Heart (including section of aorta)*	Rectum	Urinary bladder
Ileum	Salivary gland	Uterus*
Jejunum	Sciatic nerve	Vagina

\*: Organ weighed

## Results

There were no treatment-related clinical signs in any of the groups. Two OM-HCTZ treated male rats, one each from toxicology and toxicokinetics groups, were euthanized *in extremis* or died on day 157 and 177, respectively. The cause of death was considered incidental and unrelated to treatment. Reduced *body weight* gains ( $p < 0.05$ ) were observed in females treated with OM-HCTZ and given tap water compared to the control groups and the OM-HCTZ group given saline for the entire treatment period (Fig. 4.2.2.1, Table 4.2.2.2). Males treated with OM-HCTZ showed decreased body weight gains ( $p < 0.05$ ) relative to respective controls for study weeks 4 to 12 (Fig. 4.2.2.2, Table 4.2.2.2). There were no treatment-related effects on food consumption, ophthalmological findings and urinalysis parameters.

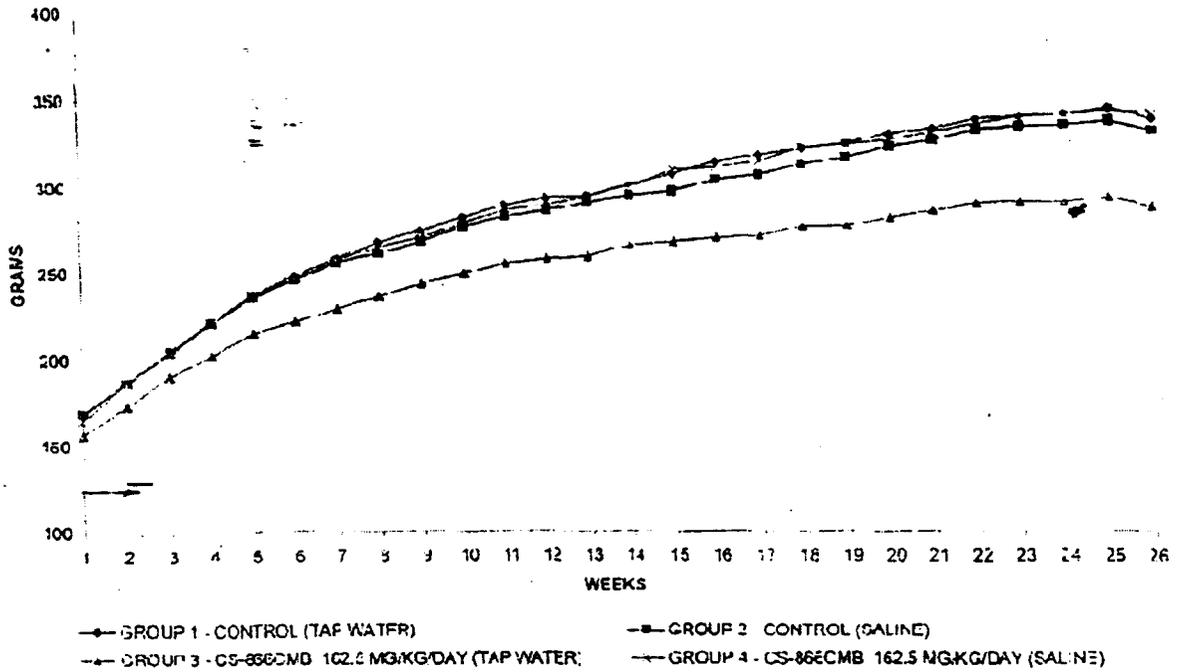


Fig. 4.2.2.1.: 26-Week Oral Toxicity Study in Rats Supplemented With Saline. Group Mean Body Weights of Females. CS-866CMB: OM-HCTZ.

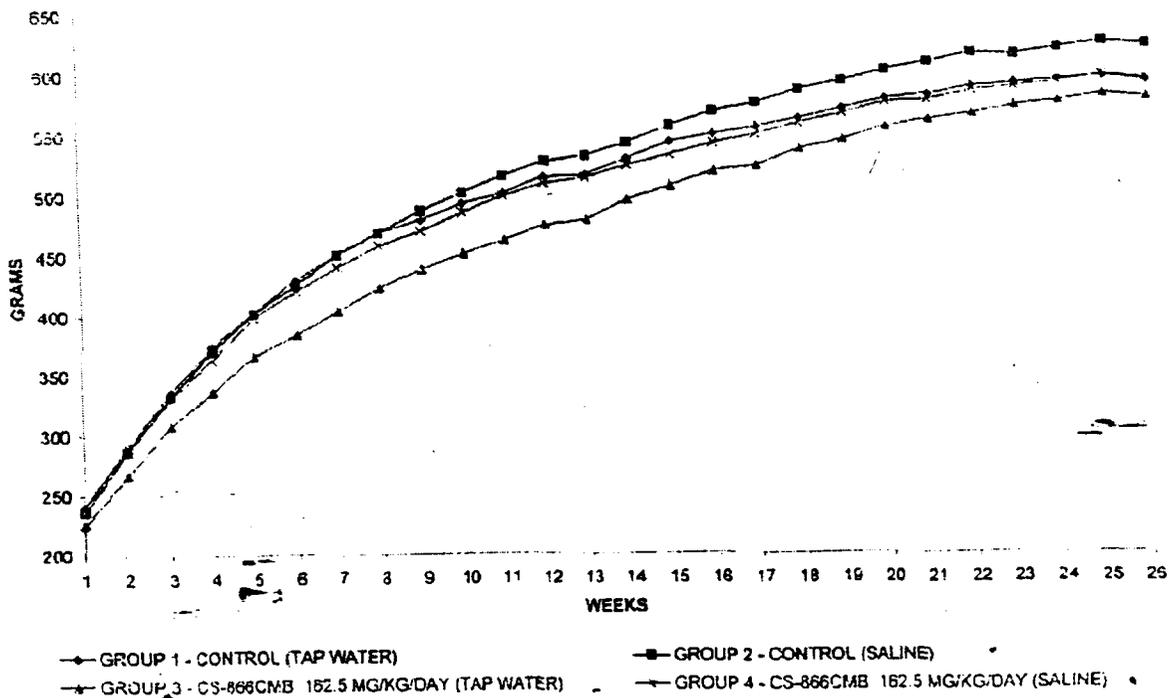


Fig. 4.2.2.2.: 26-Week Oral Toxicity Study in Rats Supplemented With Saline. Group Mean Body Weights of Males. CS-866CMB: OM-HCTZ.

TABLE 4.2.2.2  
26 WEEK TOXICITY STUDY IN RATS SUPPLEMENTED WITH SALINE: GROUP MEAN BODY WEIGHTS

GROUP 1 CONTROL (TAP WATER)				GROUP 3 CS-866CMB 162.5 MG/KG/DAY (TAP WATER)				
GROUP 2 CONTROL (SALINE)				GROUP 4 CS-866CMB 162.5 MG/KG/DAY (SALINE)				
M A L E S				SEX	F E M A L E S			
1	2	3	4	GROUP	1	2	3	4
130.9	130.3	132.2	130.2	WEEK -2	107.4	108.8	107.0	109.9
187.4	181.7	183.2	182.9	WEEK -1 A	143.9	142.8	136.1	139.7
241.3	236.7	225.9	237.3	WEEK 1 B	168.1	169.1	156.5	164.6
290.5	286.6	267.1	286.1	WEEK 2 A	187.1	186.7	173.0	185.9
336.3	332.5	308.6	331.4	WEEK 3	205.3	204.2	190.9GJ	206.0
375.1	371.3	336.8	363.4	A WEEK 4	222.3	221.5	202.5HK	220.7
403.4	402.4	366.7	398.7	WEEK 5	237.7	236.4	215.9HK	237.5
430.3	425.6	384.9	420.2	A WEEK 6	249.1	246.5	222.3JL	247.0
451.3	450.9	403.7	440.3	A WEEK 7	258.9	256.3	229.6JL	257.4
469.3	469.2	423.5	458.4	A WEEK 8	268.1	261.6	236.6JK	265.5
479.9	487.3	439.0	470.6	A WEEK 9	275.1	268.5	244.1JK	270.7
493.5	502.6	451.9	485.9	A WEEK 10	282.5	276.8	249.7JK	278.9
502.6	516.3	462.7	499.7	A WEEK 11	289.4	282.8	255.5HK	286.5
515.9	528.5	475.7	509.7	A WEEK 12	293.7	286.3	258.2HK	289.5
517.5	533.2	479.7	515.1	WEEK 13	294.7	290.8	259.7HK	294.3

GROUP 1 CONTROL (TAP WATER)				GROUP 3 CS-866CMB 162.5 MG/KG/DAY (TAP WATER)				
GROUP 2 CONTROL (SALINE)				GROUP 4 CS-866CMB 162.5 MG/KG/DAY (SALINE)				
M A L E S				SEX	F E M A L E S			
1	2	3	4	GROUP	1	2	3	4
530.4	543.7	496.3	524.6	WEEK 14	301.1	294.9	266.4HK	300.3
544.7	557.4	508.1	533.9	WEEK 15	307.4	297.3	268.3HL	309.6
550.6	569.2	520.5	543.2	WEEK 16	314.9	304.2	270.5JK	311.1
556.5	576.1	524.7	550.2	WEEK 17	318.7	307.3	271.9JL	314.9
563.3	587.5	538.7	559.4	WEEK 18	322.0	313.0	276.5JL	322.5
571.6	594.7	546.1	567.3	WEEK 19	325.1	316.9	277.2JL	324.4
579.7	603.4	556.5	576.5	WEEK 20	330.4	322.8	281.9JL	326.8
583.3	609.9	562.3	578.3	WEEK 21	332.9	326.6	286.0JL	331.1
589.7	617.5	567.3	586.1	WEEK 22	338.4	332.6	290.3JK	335.9
592.4	616.2	573.6	589.5	WEEK 23	340.0	334.3	290.9JL	339.4
594.9	621.5	577.4	593.1	WEEK 24	341.4	334.9	290.7JL	341.1
597.9	626.2	583.3	598.5	WEEK 25	344.9	337.1	293.4JL	343.6
594.6	624.4	581.0	596.2	WEEK 26	338.5	332.0	288.3JL	341.7

Significant differences between controls and CS-866CMB (OM-HCTZ) groups: A: p <0.05, B: p <0.01

Significantly different from group 1: G: p <0.05, H: p <0.01, I: p <0.001

Significantly different from group 4: J: p <0.05, K: p <0.01, L: p <0.001

Red blood cell indices (RBC, hemoglobin and hematocrit) decreased (4.5 to 5.6%) significantly in treated females (tap water and saline) relative to respective concurrent controls. Since the individual values for treated animals were within the range of values observed in concurrent controls, the sponsor does not attach much significance to the small changes noted in treated females.

Drug-related *biochemical changes* were restricted to only one parameter, BUN. A statistically significant increase in BUN was observed in treated males (tap water, 172%; saline, 91%) relative to respective concurrent controls. The mean values for treated females relative to concurrent control were elevated only in the group given tap water (105%).

There were no treatment-related effects on organ weights or gross pathological findings in any group.

*Histopathologically*, treatment-related renal tubular changes were observed in both males and females. The changes were more pronounced and frequent in animals given tap water than in those receiving saline and were more severe in males compared to females. The changes seen in the tubules were described as a nephropathy and were graded minimal to slight in severity (Table 4.2.2.3). The tubular changes, according to the sponsor, were reminiscent of the early stages of chronic progressive nephropathy, which commonly develops as an age-related phenomenon in rats but without the presence of intratubular hyaline casts. The findings were considered to be related to treatment with OM-HCTZ. Focal ulceration of the glandular gastric mucosa was observed in 3 treated males, one in the water group and two in the saline group. A possible relationship with treatment could not be excluded. There were no other treatment-related histopathological findings.

**TABLE 4.2.2.3**  
**INCIDENCE OF HISTOPATHOLOGICAL FINDINGS IN KIDNEYS OF CONTROL RATS AND RATS TREATED WITH 162.5 MG/KG/DAY OM-HCTZ FOR 26-WEEKS (WITH TAP WATER OR SALINE)**

	Males				Females				
	Control		OM-HCTZ		Control		OM-HCTZ		
	Water	Saline	Water	Saline	Water	Saline	Water	Saline	
No. Examined	15	15	15	15	15	15	15	15	
<b>Kidney Findings</b>									
Nephropathy	Grade 1	0	0	8	6	0	1	6	0
	Grade 2	0	0	5	0	0	0	0	0
Total No./Tissues Affected		0	0	13	6	0	1	6	0
Average Grade		0	0	1.38	1.0	0	1.0	1.0	0

Toxicokinetics: Following repeated dosing, predose levels of olmesartan were measurable in the majority of animals in weeks 4 and 26. However, predose levels of HCTZ were below the limit of quantitation for all animals at both weeks of measurement. There were no apparent differences in toxicokinetic parameters between the treated groups receiving tap water and saline. Furthermore, toxicokinetic parameters were comparable between males and females for both olmesartan and HCTZ. Observed Tmax values for olmesartan and HCTZ were in the range of 1 to 2 hours. There was no evidence of accumulation of olmesartan or HCTZ after repeated dosing for 26 weeks (Table 4.2.2.4).

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**TABLE 4.2.2.4**  
**TOXICOKINETIC PARAMETERS FOR OLMESARTAN AND HCTZ IN RATS TREATED WITH 162.5**  
**MG/KG/DAY OM-HCTZ FOR 26-WEEKS (WITH TAP WATER OR SALINE)**

	Males		Females	
	Water	Saline	Water	Saline
<b>C<sub>max</sub> (µg/ml)</b>				
<b>OLMESARTAN</b>				
Day 1	1.97	2.40	2.23	2.67
Week 4	2.35	1.54	1.58	1.24
Week 26	1.96	2.21	2.78	1.86
<b>HCTZ</b>				
Day 1	4.56	BLQ <sup>a</sup>	BLQ	3.77
Week 4	7.31	4.48	4.73	2.99
Week 26	5.25	4.25	4.38	3.71
<b>AUC<sub>0-24hr</sub> (µg.hr/ml)</b>				
<b>OLMESARTAN</b>				
Day 1	8.49	10.9	8.69	8.44
Week 4	10.8	6.47	5.31	5.41
Week 26	7.42	12.4	12.9	12.3
<b>HCTZ</b>				
Day 1	20.8	- <sup>b</sup>	- <sup>b</sup>	15.6
Week 4	32.4	14.1	19.5	15.5
Week 26	25.0	16.1	17.2	17.3

<sup>a</sup> BLQ: below the limit of quantitation

<sup>b</sup> -: value not calculated

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