

4.2.4. 26-Week Oral Toxicity of OM-HCTZ in Dogs. Vol. 9-11.

This GLP study (Project # 89644, Q A'd Report #APRC 148-135) was conducted by a contract laboratory,

The animals were dosed on September 27, 2000 and necropsied on March 30, 2001.

Key Findings

Two males, one each at ^{dose} 16/10 and 32/20 mg OM-HCTZ/kg/day, were sacrificed moribund, the moribundities attributed to treatment-related renal failure. The predominant histopathological findings (observed at both unscheduled and scheduled sacrifices) were cortical tubular hypertrophy with eosinophilia of the renal tubules. Similar renal changes were observed in dogs given OM or HCTZ alone. A treatment-related, significant increase in BUN was observed in both decedent and surviving males of all dose groups. A NOAEL was not determined. A non-linear increase in C_{max} and AUC values for olmesartan with increasing dose levels of OM-HCTZ was obtained throughout the study.

Methods

Five groups, each consisting of 4 male (8.3 to 10.6 kg) and 4 female (6.7 to 9.5 kg) 5 to 6-month old beagle dogs, were given OM-HCTZ daily, in gelatin capsules, at total (OM + HCTZ) doses of 0, 6.5, 13, 26 or 52 mg/kg/day. These doses correspond to 4/2.5, 8/5, 16/10, and 32/20 mg/kg/day OM/HCTZ. In addition, one group received 32 mg/kg/day OM alone and one group received 20 mg/kg/day HCTZ alone. The control animals received empty gelatin capsules. Animals were housed individually. Food was controlled and was provided to each dog once daily, while water was provided to each dog *ad libitum*.

The doses were selected on the basis of a 5-week dose-range finding study (report #APRC 147-068). That 5-week GLP study was originally intended as a 6-month study; however, due to the toxicity of the doses administered, it was terminated early. Treatment at 500/312.5 mg OM-HCTZ/kg/day was discontinued after 10 doses in males and after 9 doses in females. Dosing at 160/100 and 50/31.25 mg OM-HCTZ/kg/day was discontinued after 10 doses in females and after 11 doses in males. Surviving dogs in these dosage groups were maintained without further treatment until euthanized on day 20 and 21 (females and males, respectively). Animals in control and low dose (16/10 mg OM-HCTZ/kg/day) groups were dosed till euthanized on day 36 and 37 (males and females, respectively). A number of dogs treated with OM-HCTZ were sacrificed moribund or found dead between days 9 and 16, including 2/8, 4/8, and 5/8 dogs in the 50/31.25, 160/100 and 500/312.5 mg OM-HCTZ/kg/day groups, respectively. Deaths were attributed to extensive gastrointestinal hemorrhage and necrosis, in association with marked increases in blood urea nitrogen, creatinine, and bilirubin, and hemorrhagic areas in the lungs.

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. Ophthalmic

examinations were conducted prior to the start of treatment and again before dosing during weeks 13 and 26 of treatment. ECG and indirect b.p. were recorded once prior to the initiation of dosing and in study weeks 13 and 26. Laboratory investigations (hematology, clinical biochemistry, and urinalysis) were done once pretreatment and at treatment weeks 1, 2, 4, 13 and 26. Blood samples were collected from a jugular vein following an overnight period of food deprivation. Urine samples were collected during a 16-hour period of food and water deprivation. For toxicokinetics study, blood samples were collected from the jugular, cephalic or saphenous vein on day 1 (1, 2, 4, 8, and 24 hr after dosing) and during weeks 4 and 26 (predose and 1, 2, 4, 8 and 24 hr after dosing).

At the end of the study, animals were fasted overnight, then anaesthetized and exsanguinated. The necropsy included external examination, weighing of selected organs and histopathological examination of all animals (Table 4.2.4.1). An electron microscopic examination was performed on kidney samples from male dogs given 4/2.5 mg OM-HCTZ/kg/day.

TABLE 4.2.4.1
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

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

*: Organ weighed

Results

There were two treatment-related deaths. One male dog given 16/10 mg/kg/day (#402) became moribund and was euthanized *in extremis* during week 9. This dog showed reduced appetite, body weight loss (1.1 kg), reduced activity, emesis and was cold to touch. Clinical pathology findings were consistent with renal failure and histopathological examination revealed renal lesions consisting of a marked, diffuse tubular basophilia with some tubular dilatation, occasional hypertrophy, and interstitial inflammation. These renal lesions, together with extensive gastric ulceration, were considered to be the cause of death.

A second male, treated at 32/20 mg/kg/day (#501), was sacrificed during week 20. This dog had been on a special diet for renal impairment from week 4 and had displayed reduced activity, thin appearance, reduced fecal output and hunched posture. Clinical pathology data collected prior to

sacrifice indicated renal impairment or renal failure. Histopathological examination revealed the same renal lesions as described above and those were considered the cause of death.

Among surviving dogs, vomiting and soft, liquid feces were observed in all treated groups. There were no effects on body weight, body weight gain, or food consumption for dogs surviving to the end of the treatment period. There were no treatment-related ocular changes and no treatment-related effects were observed on electrocardiograms or indirect mean arterial pressure. Increased urine volume was observed in several animals from all treated groups during weeks 1, 2, and 4 and in a few animals at weeks 13 and 26.

There were no treatment-related effects on hematology parameters in dogs that survived to the end of the study. Among decedents, a male dog treated at 16/10 mg/kg/day and sacrificed during week 9 (#402) had increases in white blood cell count, red cell parameters, fibrinogen and activated partial thromboplastin time, along with a reduced lymphocyte count just prior to sacrifice. Another high dose male euthanized during week 20 (#501), had a slight reduction in red cell parameters just prior to sacrifice.

BUN values were slightly but significantly elevated in males in OM-HCTZ groups and in males receiving HCTZ alone relative to concurrent control and pretreatment values at week 26 (Table 4.2.4.2). The sponsor contends that these values are within the historical control range for the laboratory. At weeks 13 and 26, serum potassium was slightly reduced in the majority of males treated with 26 or 52 mg OM-HCTZ/kg/day or HCTZ alone, and in females from all treated groups except the 32 mg OM/kg/day group. Phosphorus was slightly reduced in the majority of females in all treated groups at weeks 13 and 26 of treatment relative to concurrent control and pretreatment values (Table 4.2.4.2). BUN, creatinine, and total bilirubin were markedly (up to 6-fold) elevated at all weeks of measurement in the dogs that were sacrificed prior to termination.

There were no treatment-related effects on organ weights and no treatment-related gross pathological findings. In dogs that survived to termination, the only treatment-related histopathological findings involved in the kidney. The predominant finding in male dogs was cortical tubular hypertrophy with eosinophilia of the tubular epithelia. This was also observed in female dogs, but at a lower incidence, one dog each in the 26 and 52 mg OM-HCTZ/kg/day groups and the OM group. The severity was greatest in dogs treated with OM-HCTZ at the highest dosage level. It may be noted that 2 high dose OM-HCTZ females and one female receiving HCTZ alone had eosinophilia of the tubular epithelium with no tubular hypertrophy (Table 4.2.4.3). The severity of the renal changes increased with increasing doses of OM-HCTZ. The data suggest that male dogs appear to be more susceptible to the development of renal lesions than are females. A NOAEL could not be established in this study.

TABLE 4.2.4.2
GROUP MEAN VALUES FOR BUN AND CREATININE IN DOGS TREATED ORALLY
WITH OM-HCTZ, OM, OR HCTZ FOR 26 WEEKS (REPORT #APRC 148-135)

Treatment	Total dose (mg/kg/day)	BUN (mg/dl)		Potassium (meq/dl)		Phosphorus (mg/dl)	
		Male	Female	Male	Female	Male	Female
Pretreatment							
Control	0	11.3	12.9	5.15	5.16	8.12	7.98
OM-HCTZ	6.5	10.8	12.6	5.06	5.00	8.33	8.61
	13.0	10.5	13.3	5.41	5.36	8.64	8.50
	26.0	12.4	13.9	5.10	4.96	8.46	8.82
	52.0	12.0	13.0	5.13	4.55	8.47	7.84
OM	32.0	10.6	12.7	5.19	4.57	8.64	8.27
HCTZ	20.0	11.8	13.4	4.87	4.57	8.77	7.97
Week 13							
Control	0	13.7	15.9	4.60	4.62	5.84	5.61
OM-HCTZ	6.5	17.0	16.7	4.41	4.23	5.88	5.88
	13.0	18.7	16.9	4.70	4.36	5.93	5.38
	26.0	19.3	18.6	4.17	3.73*	5.54	4.65*
	52.0	23.5	17.7	3.97	4.07*	6.30	4.99
OM	32.0	12.7	16.2	4.79	4.40	6.33	5.46
HCTZ	20.0	17.8	16.3	4.11	3.73*	6.00	5.05
Week 26							
Control	0	11.9	15.2	4.45	4.57	5.12	4.88
OM-HCTZ	6.5	16.7*	16.0	3.95	3.95*	5.07	4.77
	13.0	17.1*	16.3	4.11	4.05*	5.06	4.56
	26.0	17.5*	17.6	3.51*	3.81*	4.76	4.49
	52.0	16.3	18.5	3.51*	4.20	4.89	4.55
OM	32.0	13.0	18.0	4.48	4.71	5.59	5.33
HCTZ	20.0	16.7*	15.5	3.55*	3.74*	4.93	4.67

Significantly different from control, * : p <0.05, ** : p <0.01 (Dunnett's)

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TABLE 4.2.4.3
INCIDENCE OF HISTOPATHOLOGICAL FINDINGS IN THE KIDNEYS OF DOGS TREATED ORALLY WITH OM-HCTZ, OM, OR HCTZ FOR 26 WEEKS (REPORT #APRC 148-135)

		Control		OM-HCTZ			OM	HCTZ
			6.5	13	26	52	32	20
MALES								
Kidneys	No. Examined	4	4 ^a	4	4	4	4	4
Basophilia, tubular		0	0	1	1	1	0	0
Dilatation, tubular		0	0	0	1	1	0	0
Hypertrophy with eosinophilia		0	3	4	2	4	3	4
Cast, hyaline		0	0	2	1	1	1	0
FEMALES								
Kidneys	No. Examined	4	4	4	4	4	4	4
Hypertrophy with eosinophilia		0	0	0	1	1	1	0
Eosinophilia, tubular		0	0	0	0	2	0	1

^a: Electron microscopic examination of sections of renal cortical tissue from these animals did not reveal any abnormal findings different from those that had been recorded by light microscopic examination.

Toxicokinetic measurements showed to some extent an increase in mean olmesartan and HCTZ plasma concentrations with increasing dose levels of OM-HCTZ. However, the values lacked dose proportionality. Non-linear increases in olmesartan C_{max} and $AUC_{0-tlast}$ were observed throughout the study with increasing dose levels. Additionally, AUC and C_{max} values were similar for males in the top two OM-HCTZ dose groups (16/10 and 32/20 mg OM-HCTZ/kg/day) at weeks 4 and 26. Toxicokinetic data were not generated for HCTZ at all sampling weeks for the two lowest dose levels of OM-HCTZ in males and females and at one or two sampling weeks for the remaining dose levels of OM-HCTZ and HCTZ in females, i.e., concentrations were below the limit of quantitation. The time to reach maximum plasma concentration (t_{max}) ranged from 1 to 4 hours with OM and HCTZ. There was no evidence of accumulation of olmesartan or HCTZ after repeated dosing for 26 weeks. Also, there were no apparent differences between the sexes (Table 4.2.4.4). It appears that concomitant administration of OM and HCTZ did not affect the kinetic behavior of olmesartan or HCTZ.

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TABLE 4.2.4.4
TOXICOKINETIC PARAMETERS (MEAN GROUP VALUES) FOR OLMESARTAN AND HCTZ IN DOGS
TREATED ORALLY WITH OM-HCTZ, OM, OR HCTZ FOR 26 WEEKS (REPORT #APRC 148-135)

Dose (mg/kg/day)	Treatment	Day 1		Week 4		Week 26	
		Cmax	AUC	Cmax	AUC	Cmax	AUC
OLMESARTAN							
Male							
6.5	OM-HCTZ	228	671	85.8	355	200	560
13	OM-HCTZ	350	1221	129	965	364	1176
26	OM-HCTZ	279	902	460	2361	414	1635
52	OM-HCTZ	1304	5124	500	1915	345	1415
32	OM	588	2139	370	2450	497	2249
Female							
6.5	OM-HCTZ	100	299	970	373	133	533
13	OM-HCTZ	276	865	121	416	327	1142
26	OM-HCTZ	534	1756	228	899	310	1739
52	OM-HCTZ	757	2694	790	2971	1971	6121
32	OM	379	1456	519	2162	1055	4709
HCTZ							
Male							
6.5	OM-HCTZ	- ^a	-	-	-	-	-
13	OM-HCTZ	2.41	8.88	-	-	-	-
26	OM-HCTZ	2.92	8.75	1.45	7.49	3.53	10.84
52	OM-HCTZ	4.32	17.18	8.43	106.02	5.96	19.87
20	HCTZ	3.82	16.08	2.91	12.80	3.97	17.27
Female							
6.5	OM-HCTZ	-	-	-	-	-	-
13	OM-HCTZ	-	-	-	-	-	-
26	OM-HCTZ	2.39	7.70	-	-	-	-
52	OM-HCTZ	-	-	4.53	14.24	5.04	19.15
20	HCTZ	6.55	21.14	-	-	4.15	15.48

^a -: Parameters not estimated due to limited data (most values below the limit of quantitation)

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V. GENETIC TOXICOLOGY

5.1. Ames Assay, *In Vitro* Bacterial Test of Olmesartan Medoxomil-HCTZ. Vol 19.

This GLP study (Report #APRC 148-041, Q A'd Study #21698-0-409OECD) was conducted by
between August 29, 2000 and
 January 22, 2001.

Key Findings

Olmesartan Medoxomil-hydrochlorothiazide (OM-HCTZ) was negative in all tester strains both with and without metabolic activation at a total concentration of 5411 (3300 + 2081) µg/plate. However, the top non-cytotoxic concentration, 8125 (5000 + 3125) µg/plate, was reproducibly positive in TA98.

Methods

Four *Salmonella typhimurium* strains and one *Escherichia coli* strain were used, with and without metabolic activation. The *S. typhimurium* strains were TA1535, TA1537, TA98 and TA100; the *E. coli* was WP2uvrA. The metabolic activation system was

The positive controls used for various combinations of activation conditions and tester strains are indicated in Table 5.1.1.

TABLE 5.1.1
 POSITIVE CONTROLS

Tester Strain	S9 Mix	Positive Control	Conc. per plate
TA98	+	benzo[a]pyrene	2.5 µg
TA98	-	2-nitrofluorene	1.0 µg
TA100	+	2-aminoanthracene	2.5 µg
TA100	-	sodium azide	2.0 µg
TA1535	+	2-aminoanthracene	2.5 µg
TA1535	-	sodium azide	2.0 µg
TA1537	+	2-aminoanthracene	2.5 µg
TA1537	-	ICR-191	2.0 µg
WP2uvrA	+	2-aminoanthracene	25.0 µg
WP2uvrA	-	4-nitroquinoline-N-oxide	1.0 µg

The highest dose level for the reverse mutation assay was based on the results of a range-finding study that employed ten OM-HCTZ doses ranging from 10.84 to 8125 µg/plate (one plate per dose), both in the presence and absence of S-9 mix. The range-finding study was performed using tester strains TA100 and WP2uvrA. None of the doses resulted in cytotoxicity (no dose-related decrease in the number of revertants and a normal bacterial background lawn). Thus, the highest dose level of OM-HCTZ used in the mutagenicity assay was the same as tested in the

range-finding study. The results of the initial mutagenicity assay were confirmed in an independent experiment. Both experiments were conducted with five doses of the test article in both the presence and absence of S-9 mix along with concurrent vehicle and positive controls using 3 plates per dose. The doses of OM-HCTZ tested were 163 (100+ 63), 541 (333 + 208), 1625 (1000+ 625), 5411 (3330+ 2081) and 8125 (5000+ 3125) µg per plate. The criteria for a positive response were a 2-fold increase above control in number of revertants for tester strains TA98, TA100, and WP2uvrA and a 3-fold increase for tester strains TA1535 and TA1537, as well as a dose-related increase in number of revertants.

Results

In the initial mutagenicity assay, the highest tested concentration of OM-HCTZ (8125 µg/plate), was associated with a 2-fold increase in the number of revertants of *S. typhimurium* strain TA98 both in the absence and presence of metabolic activation and a 2.4-fold increase in revertants of the *E. coli* strain WP2uvrA without metabolic activation (Table 5.1.1). No positive responses were observed in other tester strains, either in the absence or presence of metabolic activation. In the confirmatory assay, the only positive response observed was a 2.3-fold increase in strain TA98 at 8125 µg/plate with metabolic activation (Table 5.1.2). The other positive responses obtained in the initial assay were not reproduced.

TABLE 5.1.1
SUMMARY RESULTS OF THE INITIAL BACTERIAL REVERSION TEST OF OM-HCTZ (CS-866CMB)

TEST ARTICLE ID: CS-866CMB

EXPERIMENT ID: 21698-B1

DATE PLATED: 07-Sep-00

VEHICLE: DMSO

DATE COUNTED: 11-Sep-00

PLATING ALIQUOT: 50 µL

DOSE/PLATE	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACK-GROUND LAWN*
	TA98		TA100		TA1535		TA1537		WP2uvrA		
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: RAT LIVER											
VEHICLE CONTROL	24	6	137	5	11	3	9	5	18	3	1
CS-866CMB (CS-866 + HCTZ)											
163 (100+ 63) µg	32	2	129	21	13	4	10	4	22	8	1
541 (333 + 208) µg	26	5	117	9	12	2	10	3	17	9	1
1625 (1000+ 625) µg	34	1	161	14	13	3	12	4	21	6	1
5411 (3330 + 2081) µg	47	3	167	16	13	3	17	4	31	2	1
8125 (5000 + 3125) µg	48	7	168	30	10	3	15	5	44	7	1
POSITIVE CONTROL**	273	23	810	11	139	21	148	25	176	14	1
MICROSOMES: NONE											
VEHICLE CONTROL	15	2	97	19	18	5	11	1	13	3	1
CS-866CMB (CS-866 + HCTZ)											
163 (100+ 63) µg	14	3	95	4	15	2	7	2	18	4	1
541 (333 + 208) µg	16	9	92	12	16	3	9	6	12	3	1
1625 (1000 + 625) µg	20	5	95	6	8	7	6	4	18	5	1
5411 (3330 + 2081) µg	22	1	119	2	12	2	4	3	18	3	1
8125 (5000 + 3125) µg	30	4	131	23	12	3	7	2	19	2	1
POSITIVE CONTROL***	181	4	656	45	551	27	528	22	147	7	1

** TA98	benzo(a)pyrene	2.5 µg/plate	*** TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2uvrA	2-aminanthracene	25.0 µg/plate	WP2uvrA	4-nitroquinoline-N-oxide	1.0 µg/plate

* Background Lawn Evaluation Codes:

1 = normal	2 = slightly reduced	3 = moderately reduced
4 = extremely reduced	5 = absent	6 = obscured by precipitate
sp = slight precipitate	mp = moderate precipitate (requires hand count)	hp = heavy precipitate (requires hand count)

TABLE 5.1.2
SUMMARY RESULTS OF THE CONFIRMATORY MUTAGENICITY ASSAY OF OM-HCTZ (CS-866CMB)

TEST ARTICLE ID: CS-866CMB

EXPERIMENT ID: 21698-C1

DATE PLATED: 26-Sep-00

VEHICLE: DMSO

DATE COUNTED: 28-Sep-00

PLATING ALIQUOT: 50 µL

DOSE/PLATE	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACK-GROUND LAWN*	
	TA98		TA100		TA1535		TA1537		WP2uvrA			
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.		
MICROSOMES: RAT LIVER												
VEHICLE CONTROL	36	12	147	14	12	7	11	7	20	4	1	
CS-866CMB (CS-866 + HCTZ)												
163 (100 + 63) µg	46	3	135	14	14	5	13	4	24	8	1	
541 (333 + 208) µg	43	8	140	8	11	4	12	3	16	5	1	
1625 (1000 + 625) µg	46	11	168	25	13	2	13	1	23	2	1	
5411 (3330 + 2081) µg	64	2	204	19	15	2	14	3	34	10	1	
8125 (5000 + 3125) µg	82	4	208	20	18	6	19	3	36	5	1	
POSITIVE CONTROL**	305	8	982	41	134	7	137	14	170	36	1	
MICROSOMES: NONE												
VEHICLE CONTROL	19	2	111	11	14	8	10	1	15	2	1	
CS-866CMB (CS-866 + HCTZ)												
163 (100 + 63) µg	23	8	99	4	13	3	8	4	17	3	1	
541 (333 + 208) µg	21	5	111	7	13	6	7	2	14	4	1	
1625 (1000 + 625) µg	32	4	116	9	12	0	8	2	19	3	1	
5411 (3330 + 2081) µg	34	1	120	6	13	4	9	2	22	6	1	
8125 (5000 + 3125) µg	28	5	140	5	15	8	6	2	22	4	1	
POSITIVE CONTROL***	178	13	711	25	642	60	464	65	190	20	1	

** TA98	benzo(a)pyrene	2.5 µg/plate	*** TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2uvrA	2-aminoanthracene	25.0 µg/plate	WP2uvrA	4-nitroquinoline-N-oxide	1.0 µg/plate

* Background Lawn Evaluation Codes:
 1 = normal 2 = slightly reduced 3 = moderately reduced
 4 = extremely reduced 5 = absent 6 = obscured by precipitate
 sp = slight precipitate mp = moderate precipitate (requires hand count) hp = heavy precipitate (requires hand count)

Based on the above results, additional testing was conducted with *S. typhimurium* strain TA98 with OM and HCTZ alone (both initial and confirmatory assays) in the presence and absence of S-9 mix. Both compounds were tested at concentrations of 100, 330, 1000, 3330, and 5000 µg/plate. The revertant numbers did not increase at any of these doses of OM or HCTZ. None of the concentrations was cytotoxic. The positive control compounds caused a significant increase in reverse mutations. In previous studies, OM tested negative in a number of *S. typhimurium* strains including TA98 and *E. coli* strain WP2uvrA in the absence and presence of metabolic activation up to a top concentration of 5000 µg/plate (for details see original review of NDA #21,286). Similarly, HCTZ tested negative in a bacterial reverse mutation assay in the absence and presence of metabolic activation at concentrations ranging from 100 to 10,000 µg/plate (Mortelmans, *et al.*, Environ. Mutagen, 8 (Suppl 7):1-119, 1986).

The sponsor argues that in the OM-HCTZ study, the highest concentration tested, 8125 µg/plate, was substantially in excess (by 62.5%) of the ICH and OECD maximum recommended concentration of 5000 µg/plate. Thus, the sponsor contends that the positive responses that were noted at the highest dose are of questionable biological relevance. However, the sponsor had chosen to include the 8125 (5000 + 3125) µg/plate dose in order to achieve 5000 µg/plate for the OM component. (It should be noted that ICH does not recommend a top concentration for combination products.) The next lower dose of 5411 µg/plate, which is consistent with the ICH and OECD guidelines, exhibited no mutagenic activity.

5.2. In Vitro Chromosomal Aberration Study of Olmesartan Medoxomil-HCTZ in CHL Cells. Vol. 19.

This GLP study (Protocol #00-0037, Q A'd Report #APR 147-081) was conducted by Medicinal Safety Research laboratories, Sankyo, Co., Ltd., Fukuroi, Shizuoka 437-0065, Japan between June 12, 2001 and February 6, 2002. It was designed to determine whether the combination of OM and HCTZ would produce a synergistic effect, as both OM (see original review of NDA #21,286) and HCTZ have been reported to possess clastogenic activity in cell culture models.

Key Findings

Significant increases in cells with structural and numerical (polyploidy) aberrations were observed with OM, HCTZ and OM-HCTZ. Combining OM with HCTZ did not enhance the responses observed with OM or HCTZ alone. No synergism in clastogenic activity was detected between OM and HCTZ in any combination ratio.

Methods

A fibroblastic cell line originating from the lung of a female Chinese hamster (CHL cells) was used. On the day of the experiment, OM and HCTZ were separately dissolved and further diluted with DMSO and mixed with the growth medium. Mitomycin C was used as the positive control. All studies were done in the absence of metabolic activation. Cells were exposed to a medium containing test substance for 6 hours followed by continued culture for an additional 18 hours with a fresh medium excluding the test substance. Colcemide was added into the culture medium 2 hr before the end of incubation to arrest dividing cells in metaphase.

In a preliminary dose-range finding study and cytotoxicity test, the sponsor studied as many as 30 dose combinations of OM and HCTZ (dose ratios of 5/12.5, 10/12.5, 20/12.5, 40/12.5, and 80/12.5). In addition, OM and HCTZ were studied alone (each at 6 different dose levels, Table 5.2.1). Based on the results of the cytotoxicity test (Table 5.2.2), three dose ratios (9 dose combinations) of OM-HCTZ, 3 doses of OM and 3 doses of HCTZ were tested in the full chromosome aberration study (see Table 5.2.3 for details). In judging the results of the observed metaphases, <5% abnormalities was considered "negative", 5% to 10% as "equivocal (+/-)", and >10% as "positive". A gap was not considered as abnormal.

TABLE 5.2.1
CHROMOSOME ABERRATION TEST.
DOSE LEVELS STUDIED IN A DOSE-RANGE FINDING STUDY

CS-866/ HCTZ composition ratio	CS-866 alone	CS-866/HCTZ combination					HCTZ alone
	100/0	80/12.5	40/12.5	20/12.5	10/12.5	5/12.5	0/100
CS-866 (µg/mL)	HCTZ (µg/mL)						
620	0	97	194	388	775	1550	1550
443	N.D.	69	138	277	554	1107	1107
316	0	49	99	198	395	791	791
226	N.D.	35	71	141	282	565	565
161	0	25	50	101	202	403	403
115	N.D.	18	36	72	144	288	288

TABLE 5.2.2
CHROMOSOME ABERRATION TEST.
RESULTS OF CYTOTOXICITY TEST IN DOSE-RANGE FINDING STUDY

Substance	Dose ¹⁾ (µg/mL)	No. of cells (x10 ⁴ cells/mL)			Relative (%) vs. vehicle
		1	2	Average	
Vehicle ¹⁾		96	93	94.5	100.0
Untreated		96	91	93.5	98.9
CS-866 alone	161/0	75	78	76.5	81.0
	316/0	54	48	51.0	54.0
	620/0	40	33	36.5	38.6
	115/18	75	84	79.5	84.1
CS-866/HCTZ ²⁾ 80/12.5	161/25	81	78	79.5	84.1
	226/35	71	65	68.0	72.0
	316/49	45	41	43.0	45.5
	443/69	38	38	37.0	39.2
	620/97	40	35	37.5	39.7
CS-866/HCTZ ²⁾ 40/12.5	115/36	75	69	72.0	76.2
	161/50	60	69	64.5	68.3
	226/71	62	65	63.5	67.2
	316/99	44	46	45.0	47.8
	443/138	38	38	37.0	39.2
CS-866/HCTZ ²⁾ 20/12.5	620/194	38	34	36.0	38.1
	115/72	75	84	79.5	84.1
	161/101	79	74	76.5	81.0
	226/141	57	57	57.0	60.3
	316/198	42	41	41.5	43.9
CS-866/HCTZ ²⁾ 5/12.5	443/277	38	41	39.5	41.8
	620/388	28	29	28.5	30.2
	115/144	78	83	80.5	85.2
	161/202	72	73	72.5	76.7
	226/282	73	73	73.0	77.2
CS-866/HCTZ ²⁾ 10/12.5	316/395	52	50	51.0	54.0
	443/554	41	50	45.5	48.1
	620/775	32	36	35.0	37.0
	115/288	82	77	79.5	84.1
	161/403	75	79	77.0	81.5
CS-866/HCTZ ²⁾ 0/100	226/565	69	59	64.0	67.7
	316/791	45	42	43.5	46.0
	443/1107	31	33	32.0	33.9
	620/1550	14	13	13.5	14.3
	0/288	84	87	85.5	90.5
HCTZ alone	0/403	80	86	73.0	77.2
	0/565	66	67	66.5	70.4
	0/791	68	59	63.5	67.2
	0/1107	51	51	51.0	54.0
	0/1550	34	33	33.5	35.4

1) Vehicle, dimethyl sulfoxide. 2) HCTZ, hydrochlorothiazide. 3) CS-866/HCTZ.

TABLE 5.2.3
CHROMOSOME ABERRATION TEST. CONCENTRATIONS OF OM AND HCTZ TESTED ALONE AND IN COMBINATION IN THE MAIN STUDY

OM alone (µg/ml)	OM - HCTZ ratio			HCTZ alone (µg/ml)
	40/12.5	20/12.5	10/12.5	
	HCTZ			
79	25	49	99	277
158	49	99	198	554
316	99	198	395	1107

Results

Structural and numerical (polyploidy) abnormalities were observed for each combination ratio and for OM and HCTZ alone, with highest incidences at the highest concentrations tested (15.5, 11, 7.5, 15.5 and 7.0 % aberrant cells at the doses of 316/0, 316/99 (ratio 40/12.5), 316/198 (ratio 20/12.5), 316/395 (ratio 10/12.5), and 0/1107 µg OM-HCTZ/ml, respectively, Table 5.2.4). There were no significant findings at doses below the highest concentrations (Fig. 5.2.1). The cytotoxicity at the highest dose for each combination ratio was approximately 50% (Table 5.2.2). Combining OM with HCTZ did not enhance the responses observed with OM or HCTZ alone. No synergism in clastogenic activity was detected between OM and HCTZ in any combination ratio. Incidence of cells with aberrations for vehicle and sham treatment controls was 2.5 and 4%, respectively. Incidence of cells with aberrations for the positive control was 36%.

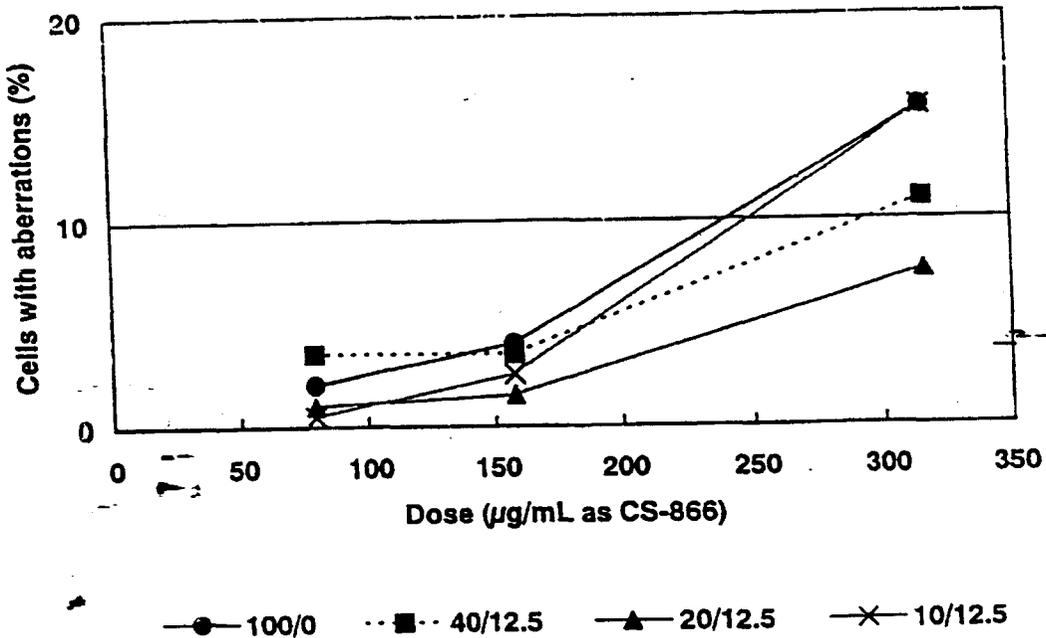


Fig. 5.2.1.: *In vitro* Chromosome Aberration Test of OM-HCTZ in CHL Cells

TABLE 5.2.4
INCIDENCE OF POLYPOIDIES AND CHROMOSOMAL ABERRATIONS IN CHL CELLS TREATED WITH OM AND HCTZ, ALONE OR IN COMBINATION

Substance	Dose ¹⁾ (µg/mL)	No. of cells analyzed	Poly-ploidy (%)	Judge-ment	Structural aberrations (%) ⁴⁾						TA ⁵⁾ (%)	TAG ⁶⁾ (%)	Final judgement ⁷⁾
					gap	ctb	cte	csb	cse	others			
Untreated	100	100	1	-	1	0	0	2	0	1	3	3	-
	100	100	0	-	0	2	0	0	0	3	5	5	-
	200	100	1 (0.5)	-	1 (0.5)	2 (1.0)	0 (0.0)	2 (1.0)	0 (0.0)	4 (2.0)	8 (4.0)	9 (4.5)	-
Vehicle ¹⁾	100	100	0	-	0	1	0	0	0	1	2	2	-
	100	100	0	-	0	0	0	0	0	3	3	3	-
	200	100	0 (0.0)	-	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	5 (2.5)	5 (2.5)	-
CS-866 alone	79/0	100	0	-	1	1	1	0	0	1	3	3	-
		100	1	-	0	0	0	0	0	1	2	2	-
		200	1 (0.5)	-	1 (0.5)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	2 (1.0)	4 (2.0)	5 (2.5)	-
	158/0	100	0	-	0	0	2	1	1	0	2	3	-
		100	1	-	0	0	2	1	1	0	3	3	-
		200	1 (0.5)	-	0 (0.0)	2 (1.0)	2 (1.0)	2 (1.0)	1 (0.5)	2 (1.0)	8 (4.0)	8 (4.0)	-
316/0	100	12	-	0	4	6	8	3	0	17	17	-	
	100	17	-	0	1	6	3	2	3	14	14	-	
	200	29 (14.5)	+	0 (0.0)	5 (2.5)	11 (5.5)	11 (5.5)	5 (2.5)	5 (2.5)	31 (15.5)	31 (15.5)	+	
CS-866/HCTZ ²⁾	79/25	100	0	-	0	0	0	1	1	1	5	5	-
		100	2	-	0	0	0	0	0	2	2	2	-
		200	2 (1.0)	-	0 (0.0)	2 (1.0)	1 (0.5)	1 (0.5)	2 (1.0)	1 (0.5)	7 (3.5)	7 (3.5)	-
	158/49	100	0	-	0	0	1	3	0	1	6	6	-
		100	0	-	0	0	0	2	1	1	5	5	-
		200	0 (0.0)	-	0 (0.0)	0 (0.0)	1 (0.5)	3 (1.5)	2 (1.0)	2 (1.0)	7 (3.5)	7 (3.5)	-
316/99	100	14	-	2	3	2	6	3	2	11	12	-	
	100	18	-	0	1	3	1	1	2	11	11	-	
	200	30 (15.0)	+	2 (1.0)	4 (2.0)	5 (2.5)	7 (3.5)	4 (2.0)	4 (2.0)	22 (11.0)	23 (11.5)	+	
CS-866/HCTZ ³⁾	79/49	100	0	-	1	1	0	1	0	0	3	3	-
		100	0	-	0	0	0	0	0	0	0	0	-
		200	0 (0.0)	-	1 (0.5)	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	2 (1.0)	3 (1.5)	-
	158/99	100	0	-	0	0	0	0	0	1	1	1	-
		100	0	-	0	0	0	0	0	2	2	2	-
		200	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	3 (1.5)	3 (1.5)	-
316/196	100	14	-	1	2	2	1	0	5	8	8	-	
	100	7	-	0	0	3	3	0	0	7	7	-	
	200	21 (10.5)	+	1 (0.5)	2 (1.0)	5 (2.5)	2 (1.0)	3 (1.5)	5 (2.5)	15 (7.5)	15 (7.5)	±	
CS-866/HCTZ ³⁾	79/99	100	0	-	0	0	0	0	0	0	0	0	-
		100	0	-	0	0	0	0	0	0	0	0	-
		200	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	1 (0.5)	-
	158/198	100	2	-	0	1	0	0	1	1	3	3	-
		100	3	-	0	1	1	0	1	0	2	2	-
		200	5 (2.5)	-	0 (0.0)	2 (1.0)	1 (0.5)	0 (0.0)	2 (1.0)	1 (0.5)	5 (2.5)	5 (2.5)	-
316/395	100	20	-	0	8	6	3	3	3	18	18	-	
	100	17	-	0	3	3	3	3	3	13	13	-	
	200	37 (18.5)	+	0 (0.0)	9 (4.5)	8 (4.5)	6 (3.0)	6 (3.0)	6 (3.0)	31 (15.5)	31 (15.5)	+	
HCTZ alone	0/277	100	0	-	0	0	0	0	0	0	0	0	-
		100	0	-	0	0	0	0	0	0	0	0	-
		200	0 (0.0)	-	0 (0.0)	2 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5)	2 (0.5)	-
	0/554	100	0	-	0	0	0	0	0	0	0	0	-
		100	0	-	0	0	0	0	0	1	1	1	-
		200	0 (0.0)	-	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	3 (1.5)	3 (1.5)	-
0/1107	100	20	-	0	1	1	5	4	0	9	9	-	
	100	28	-	0	0	2	0	0	3	5	5	-	
	200	48 (24.0)	++	0 (0.0)	3 (1.5)	3 (1.5)	5 (2.5)	4 (2.0)	3 (1.5)	14 (7.0)	14 (7.0)	±	
Positive MMC	0.1	100	0	-	1	8	24	1	0	2	24	24	++
		100	3	-	5	8	30	1	0	3	38	38	++
		200	0 (0.0)	-	0 (0.0)	10 (5.0)	54 (27.0)	2 (1.0)	0 (0.0)	5 (2.5)	72 (36.0)	75 (37.5)	++

1) Vehicle, dimethyl sulfoxide. 2) HCTZ, hydrochlorothiazide 3) CS-866/HCTZ. 4) ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; others, fragment, multiple or S-pcc. 5) TA, total aberrant cells excluding the gap. 6) TAG, total aberrant cells including the gap. 7) TA < 5%: negative(-), 5% ≤ TA < 10%: equivocal(±), 10% ≤ TA < 20%: positive(+), 20% ≤ TA < 50%: positive(++), 50% ≤ TA: positive(+++).

Previous studies with OM alone (concentrations >124 µg/ml) have documented induction of chromosome aberrations without metabolic activation in a dose-dependent manner (see original review of NDA #21,286). HCTZ has been reported as a possible genotoxicant (IARC monographs on the evaluation of carcinogenic risks to humans No. 50, IARC Press, Lyon, 293-305, 1990). It has been reported that combined treatment with certain genotoxicants increases incidence of chromosome aberration additively or synergistically (Sargent, L. *et al.*, Mutation Res., 224: 79-88, 1989). Though OM and HCTZ are both clastogens, their combination at various composition ratios did not enhance the responses observed with either drug alone. No synergism in clastogenic activity was detected between OM and HCTZ at any combination ratio.

5.3. In Vivo Mouse Micronucleus Assay With Olmesartan Medoxomil-HCTZ. Vol 19

This GLP study (Study #6839-112, Q A'd Report #APRC 147-093) was conducted by _____ between August 24 and September 18, 2001. The study was designed to evaluate OM-HCTZ for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in mouse bone marrow.

Key Findings

Olmesartan medoxomil (OM)-HCTZ was negative in the *in vivo* mouse bone marrow micronucleus test.

Methods

An initial dose range-finding study was carried out in 8-week-old male and female mice of the Crl:CD-1 (ICR) BR strain (from _____). Groups of 3 males and 3 females with weight range of 29.6-35.5 g and 20.7-26.3 g, respectively, were dosed at 812.6 (500 + 312.5), 1625 (1000 + 625), 2438 (1500 + 938) or 3250 (2000 + 1250) mg OM-HCTZ/kg. The test substance was suspended in _____ and administered by oral gavage. No toxic signs were observed and no mortality occurred for two days after dosing. Based on these results, all of the above doses were retained for the micronucleus assay. However, due to an error in preparing the dosing formulation, the highest dose was 3144 (1935 + 1209) and not 3250 mg/kg. It may be noted that the maximum recommended dose according to the OECD guideline is 2000 mg/kg. Due to the absence of differences in sensitivity between male and female mice, only males (6 males/dose) were used in the micronucleus assay. The solvent control was _____ and the positive control was _____ both were administered by gavage.

All animals were examined immediately after dosing, about 1 hr after dosing, and at least daily for the duration of this study. Bone marrow cells were collected from hind limb bones (tibias) 24 hours after dosing for all drug-treatment groups (5/group) and at 48 hours after dosing for the vehicle control and high dose groups (5/group). The extracted bone marrow was processed for an analysis of the frequency of micronucleated polychromatic erythrocytes (PCE), with 2000 PCE scored per animal, and the ratio of PCE to normochromatic erythrocytes (NCE), with at least 500 erythrocytes scored for each animal.

The criteria for a positive response was the detection of a statistically significant increase in micronucleated PCEs for at least one dose level, and a statistically significant dose-related response.

Results

OM-HCTZ was well tolerated and there were no deaths. No statistically significant increases in the frequency of micronucleated PCEs were observed at any dose level or harvest timepoint (Table 5.3.1). The vehicle control group mean (0.4%) was within the historical control range (0

to 0.45%). The doses selected were not cytotoxic to the bone marrow as indicated by the absence of a statistically significant decrease in the PCE:NCE ratio. The positive control, cyclophosphamide, induced a statistically significant increase in the micronucleated PCEs relative to the vehicle control ($2.84 \pm 0.4\%$). It is concluded that OM-HCTZ had no clastogenic activity in this *in vivo* test system under the conditions of the assay. It may be noted that olmesartan medoxomil *per se* has no micronucleus inductivity.

TABLE 5.3.1
IN VIVO MOUSE MICRONUCLEUS ASSAY WITH OLMESARTAN MEDOXOMIL-HCTZ.
SUMMARY OF GROUP MEAN DATA

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL \pm S.E. MALES	RATIO PCE:NCE MEAN \pm S.E. MALES
CONTROLS				
VEHICLE	Vehicle	24 hr	0.06 \pm 0.01	0.46 \pm 0.08
		48 hr	0.10 \pm 0.03	0.38 \pm 0.04
POSITIVE	CP 30.0mg/kg	24 hr	2.84 \pm 0.40*	0.29 \pm 0.01
TEST ARTICLE	500mg/kg	24 hr	0.16 \pm 0.08	0.39 \pm 0.05
		24 hr	0.21 \pm 0.04	0.34 \pm 0.04
		24 hr	0.09 \pm 0.03	0.29 \pm 0.04
		48 hr	0.12 \pm 0.05	0.28 \pm 0.04

* Significantly greater than the corresponding vehicle control, $p < 0.01$.

Vehicle = 0.5% carboxymethylcellulose-sodium solution

CP = Cyclophosphamide

PCE = Polychromatic erythrocyte

NCE = Normochromatic erythrocyte

VI. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

**6.1. Effect of Olmesartan Medoxomil-HCTZ on Embryo-Fetal Development in Mice.
Vol. 21.**

This GLP study (Study #3819-002, QA'd Report #APRC 148-132) was conducted by [redacted] Dosing was initiated on November 16 and completed on November 29, 2000. Animals were sacrificed between November 28 and December 2, 2000. The study investigated the effects of olmesartan medoxomil (OM)-HCTZ on embryo-fetal development.

Key Findings

Oral administration of OM-HCTZ to mated female mice at total doses up to 1625 (1000/625) mg/kg/day on gestational days 6 through 15 did not produce maternal or developmental toxicity.

Methods

Groups of female Crl:CD-1(ICR) BR mice from [redacted] were mated at approximately 10 weeks of age. Those that became pregnant weighed 23-29 gm on day 0 of gestation. Suspensions of OM-HCTZ were prepared in [redacted] and administered orally by gavage (10 ml/kg), once daily, to mated females at total doses of 162.5, 487.5, or 1625 mg OM-HCTZ /kg/day (25 females/group) on gestational days 6 through 15. Similarly treated groups of 6 mated females were used for toxicokinetics analyses on gestation days 6 and 15. These doses correspond to 100/62.5, 300/187.5 and 1000/625 mg OM/HCTZ per kg per day. A control group of 25 pregnant females received the vehicle. Mice were given food and water *ad libitum*. The doses were selected by the sponsor based on previous studies conducted with OM.

Mated mice were observed twice daily, with body weights measured daily, beginning on GD 0. Food consumption was not measured. Blood samples were collected from satellite animals prior to dosing on GD 15 and at 1, 2, 4, 8, and 24 hr after dosing on GDs 6 and 15 (n=3 animals/time point, Table 6.1.1). Blood was collected *via* the orbital sinus.

TABLE 6.1.1
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ ON EMBRYO-FETAL DEVELOPMENT IN MICE.
THE TIME OF BLOOD COLLECTION FOR TOXICOKINETICS EVALUATION

Mice Assigned to Satellite Groups ^a	DG 6 postdosage timepoints					DG 15 postdosage timepoints					
	1 hour	2 hours	4 hours	8 hours	24 hours	Before Dosage	1 hour	2 hours	4 hours	8 hours	24 hours
First three mice	X		X		X		X		X		X
Second three mice		X		X		X		X		X	

a. Based on numerical order of group assignment.

All mice assigned to the main study were sacrificed by carbon dioxide asphyxiation on GD 18 and C-sectioned. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The numbers of corpora lutea, implantations, live fetuses and dead fetuses, and resorptions were recorded. Each fetus was weighed, sexed and examined for gross external alterations.

- Approximately one-half of the fetuses were fixed in Bouin' fluid, preserved in ethanol and examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations (malformations and variations). Mice assigned to toxicokinetic evaluation were sacrificed by carbon dioxide asphyxiation on GD 16. The number and distribution of implantation sites was recorded. The mice were discarded without further evaluation.

Results

All mice assigned to the main study survived until scheduled sacrifice on GD 18. No dams had abnormal clinical signs. In the satellite groups 3 control mice and one each in the low and high dose groups were found dead on GD 6, and one mouse in the mid dosage group was found dead on GD 15. All were pregnant. The cause of death could not be determined but may have been related to blood collection. All other mice survived to scheduled sacrifice. None of the animals showed adverse clinical signs. There were no significant differences in body weights or body weight gains among the groups (both main and satellite animals).

Pregnancy rates were comparable for all groups (22 to 24 mice in each dosage group were pregnant). The litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, total fetal body weights, early and late resorptions, percent dead or resorbed conceptuses, and percent live male fetuses were comparable among the four dosage groups (Table 6.1.2). No dam had a litter consisting of only resorbed conceptuses. There were no treatment-related gross, external, soft tissue, or skeletal malformations or variations in any of the dosage groups.

Mean AUC_{last} of olmesartan generally increased with dosage on both day 6 and day 15 of gestation. Mean AUCs were 1.4 to 1.8 times higher on day 15 than on day 6. After reaching C_{max} ($T_{max} = 1$ hr), plasma olmesartan concentrations declined with time in an apparent multiphasic manner. Concentrations were detectable at the last sampling time ($T_{last} = 24$ hr) for all but animals in the low dosage group on GD 6 ($T_{last} = 8$ hr). C_{max} values for olmesartan were comparable on GD 6 and GD 15. Mean AUC_{last} of HCTZ also increased with dosage on both day 6 and day 15, and were 1.5 to 2.9 times higher on day 15 than on day 6 (Table 6.1.3). T_{max} for HCTZ appeared to be a function of dosage, increasing from 1 hr for the low dosage group to 4 hr for the high dosage group on both GDs 6 and 15. AUC_{last} increased in an apparent dosage proportional manner for both olmesartan and HCTZ on both sampling days.

Based on the results of this study, the maternal and the developmental NOAEL of OM-HCTZ is greater than 1625 mg/kg/day.

TABLE 6.1.1
EMBRYO-FETAL DEVELOPMENT IN MICE. SUMMARY OF CESAREAN-SECTIONING AND LITTER OBSERVATIONS.

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 162.5	III 487.5	IV 1625
MICE TESTED	N	25	25	25	25
PRGNANT	N(%)	24 (96.0)	24 (96.0)	22 (88.0)	23 (92.0)
MICE PREGNANT AND CESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	24	24	22	23
CORPORA LUTEA	MEAN±S.D.	13.1 ± 1.9	13.0 ± 1.3	13.2 ± 2.8	13.2 ± 2.0
IMPLANTATIONS	MEAN±S.D.	12.2 ± 1.5	12.5 ± 1.2	11.6 ± 3.8	12.3 ± 2.5
LITTER SIZES	MEAN±S.D.	11.2 ± 2.2	11.9 ± 1.3	10.8 ± 3.8	11.3 ± 2.4
LIVE FETUSES	N	267	285	235	256
	MEAN±S.D.	11.1 ± 2.1	11.9 ± 1.3	10.7 ± 3.8	11.1 ± 2.2
DEAD FETUSES	N	2	0	2	3
	MEAN±S.D.	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.4	0.1 ± 0.3
RESORPTIONS	MEAN±S.D.	1.0 ± 1.7	0.6 ± 0.8	0.3 ± 1.0	1.1 ± 1.1
EARLY RESORPTIONS	N	14	13	11	12
	MEAN±S.D.	0.5 ± 0.9	0.4 ± 0.7	0.5 ± 0.9	0.5 ± 0.8
LATE RESORPTIONS	N	10	4	7	13
	MEAN±S.D.	0.4 ± 0.7	0.2 ± 0.4	0.3 ± 0.7	0.6 ± 0.8
DAMS WITH ANY RESORPTIONS	N(%)	14 (58.3)	11 (45.8)	12 (54.5)	14 (60.9)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DAMS WITH VIABLE FETUSES	N(%)	24 (100.0)	24 (100.0)	22 (100.0)	23 (100.0)
PLACENTAE APPEARED NORMAL	N(%)	24 (100.0)	24 (100.0)	22 (100.0)	23 (100.0)
LIVE MALE FETUSES	N	145	143	131	127
LIVE MALE FETUSES/LITTER	MEAN±S.D.	55.7 ± 17.2	49.4 ± 19.7	57.5 ± 17.7	48.6 ± 19.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.36 ± 0.21	1.38 ± 0.10	1.46 ± 0.17*	1.38 ± 0.13
MALE FETUSES	MEAN±S.D.	1.39 ± 0.11	1.40 ± 0.10	1.43 ± 0.17*	1.41 ± 0.14
FEMALE FETUSES	MEAN±S.D.	1.31 ± 0.21	1.35 ± 0.10	1.42 ± 0.14 [23]b	1.35 ± 0.13
DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	9.1 ± 10.9	5.0 ± 6.1	8.6 ± 12.2	9.2 ± 7.9

() = NUMBER OF VALUES AVERAGED
 a. Dosage occurred on days 6 through 18 of gestation.
 b. Litter 5633 had no male fetuses.
 c. Litter 5675 had no female fetuses.
 * Significantly different from the vehicle control group value (p<0.05).

TABLE 6.1.4
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ ON EMBRYO-FETAL DEVELOPMENT IN MICE.
TOXICOKINETIC EVALUATIONS.

Group	CS-866CMB Dosage (mg/kg/day)	Day of Presumed Gestation	C _{max} (µg/mL)	T _{max} (h)	T _{1/2last} (h)	AUC _{last} (µg ^h /mL)
RNH-6270						
2	162.5	6	6.95	1	8	22.7
		15	8.22	1	24	40.6
3	487.5	6	10.4	1	24	62.7
		15	6.94	1	24	86.6
4	1625	6	15.3	1	24	95.5
		15	16.1	1	24	133
HCTZ						
2	162.5	6	7.17	1	8	26.8
		15	8.45	1	24	78.6
3	487.5	6	13.1	2	24	121
		15	17.5	4	24	187
4	1625	6	21.3	4	24	162
		15	37.5	4	24	378

TCS-866CMB: OM-HCTZ
 RNH-6270: Olmesartan

APPEARS THIS WAY
 ON ORIGINAL

6.2. Effects of Olmesartan Medoxomil-HCTZ on Embryo-Fetal Development in Rats Vol. 22.

This GLP study (Protocol #3819-003, QA'd Report #APRC 148-133) was conducted by _____ Dosing was initiated on April 9 and completed on April 23, 2001. Animals were sacrificed between April 20 and April 27, 2001. The study investigated the effects of olmesartan medoxomil (OM)-HCTZ on embryo-fetal development.

Key Findings

Oral administration of OM-HCTZ to mated female rats at total doses of up to 1625 (1000/625) mg OM-HCTZ/kg/day on gestational days 7 through 17 produced maternal deaths and adverse clinical effects at 100/62.5 or more mg/kg/day. Significant decreases in body weight gain and food consumption were observed at doses as low as 30/18.75 mg/kg/day. The maternal and developmental NOAELs were, respectively, 10/6.25 and $\geq 1000/625$ mg/kg/day.

Methods

Groups of female CrI:CD®(SD)IGS BR VAF/Plus® rats from _____, were mated at approximately 10 weeks of age. Those that became pregnant weighed 223-257 gm on day 0 of gestation. Suspensions of OM-HCTZ were prepared in _____ and administered orally by gavage (10 ml/kg), once daily, to mated females at total doses (OM + HCTZ) of 4.875, 16.25, 48.75, 162.5 or 1625 mg/kg/day (25 females/group) on gestational days 7 through 17. Similarly treated groups of 6 mated females were used for toxicokinetics analyses on gestation days 7 and 17. These doses, administered at a constant ratio of 1.6:1, correspond to 3/1.875, 10/6.25, 30/18.75, 100/62.5, and 1000/625 mg OM/HCTZ per kg per day. A control group of 25 pregnant females received the vehicle. Rats were given food and water *ad libitum*. The doses were selected on the basis of a previous developmental toxicity study (same strain and same mode of administration) in which a total of 13, 5, and 11 rats were found dead in the 162.5, 487.5, and 1625 mg OM-HCTZ/kg/day groups (n=25 mated rats/group), respectively.

Mated rats were observed twice daily, with body weights measured daily beginning on GD 0. Food consumption was recorded weekly during the pretreatment period and daily thereafter. Blood samples were collected from satellite animals prior to dosing on GD 17 and at 1, 2, 4, 8, and 24 hr after dosing on GDs 7 and 17 (n=3 animals/time point, Table 6.2.1). Blood was collected *via* the orbital sinus.

TABLE 6.2.1
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ ON EMBRYO-FETAL DEVELOPMENT IN RATS.
THE TIME OF BLOOD COLLECTION FOR TOXICOKINETICS EVALUATION

Rats Assigned to Satellite Groups ^a	DG 7 Postdosage timepoints					DG 17 postdosage timepoints					
	1 hour	2 hours	4 hours	8 hours	24 hours	Prior to dosage	1 hour	2 hours	4 hours	8 hours	24 hours
First three rats	X		X		X		X		X		X
Second three rats		X		X		X		X		X	

a. Based on numerical order of group assignment.

All rats assigned to the main study were sacrificed by carbon dioxide asphyxiation on GD 21 and C-sectioned. Gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The numbers of corpora lutea, implantations, live fetuses and dead fetuses, and early and late resorptions were recorded. Each fetus was weighed, sexed and examined for gross external alterations. Approximately one-half of the fetuses were fixed in Bouin's fluid, preserved in ethanol and examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations (malformations and variations). Rats assigned to toxicokinetic evaluation were sacrificed by carbon dioxide asphyxiation on GD 18. Pregnancy status and the uterine contents were recorded. All tissues were discarded without further evaluation.

Results

In the main study, two rats in the 162.5 mg/kg/day dosage group (dam # 4413 and dam # 4419) and one rat in the 1625 mg/kg/day dosage group (dam # 4427) died prior to study termination. The deaths in the 162.5 mg/kg/day group were considered to be treatment-related; the death in the 1625 mg/kg/day group was attributed to an intubation error. All other rats survived until scheduled sacrifice. Clinical observations for both rats that died on the 162.5 mg/kg/day dosage regimen (dam #4413 died on GD 19 and dam #4419 on GD 20) included emaciation 3 days prior to death and chromorhinorrhea and soft or liquid feces a day before death. Both rats lost weight after GD 9 and feed consumption values were reduced 8-10 days before death. All tissues examined appeared normal. Dam #4413 had 14 fetuses. Half of the fetuses examined for visceral alterations had slight dilation of the lateral and third ventricles of the brain and 4 fetuses had undescended testes. All 7 fetuses examined for skeletal alterations had non ossified pubes and ischia. Dam #4419 had 15 fetuses and one early resorption. Three of the 7 fetuses examined for visceral alterations had undescended testes. All 8 fetuses examined for skeletal alterations had non ossified pubes and ischia. The lone high dose dam that was found dead (dam # 4427 died on GD 18) exhibited chromorhinorrhea and excess salivation on GDs 14-17, dried, red perioral substances on GDs 15-17, soft or liquid feces on GDs 16 and 17, and emaciation on GD 17. The rat lost body weight after GD 13 and food consumption values were reduced after GD 12. Necropsy revealed a perforation of the esophagus (suggesting an intubation error), multiple black erosions in the fundic and cardiac mucosa of the stomach and dark areas in the small intestine. The litter consisted of 17 late resorptions.

In the satellite groups of rats, a low dose rat was found dead on GD 17, approximately 1 hr after drug administration. Necropsy of this rat revealed a perforation of the trachea; all other tissues appeared normal. The death was attributed to an intubation accident.

A significant decrease in maternal body weight gain ($p < 0.05$) relative to the control group was observed from day 10 to day 16 of gestation in the groups receiving 48.75 or more mg/kg/day (Fig. 6.2.1). Body weight gains for the entire dosing period were significantly reduced ($p < 0.05$) for the top three dosage groups and body weight gains for the entire gestation period were decreased in the 162.5 and 1625 mg/kg/day groups ($p < 0.05$, 17 and 18% reduction compared to control values, respectively, Table 6.2.2).

MATERNAL BODY WEIGHT

Figure 1

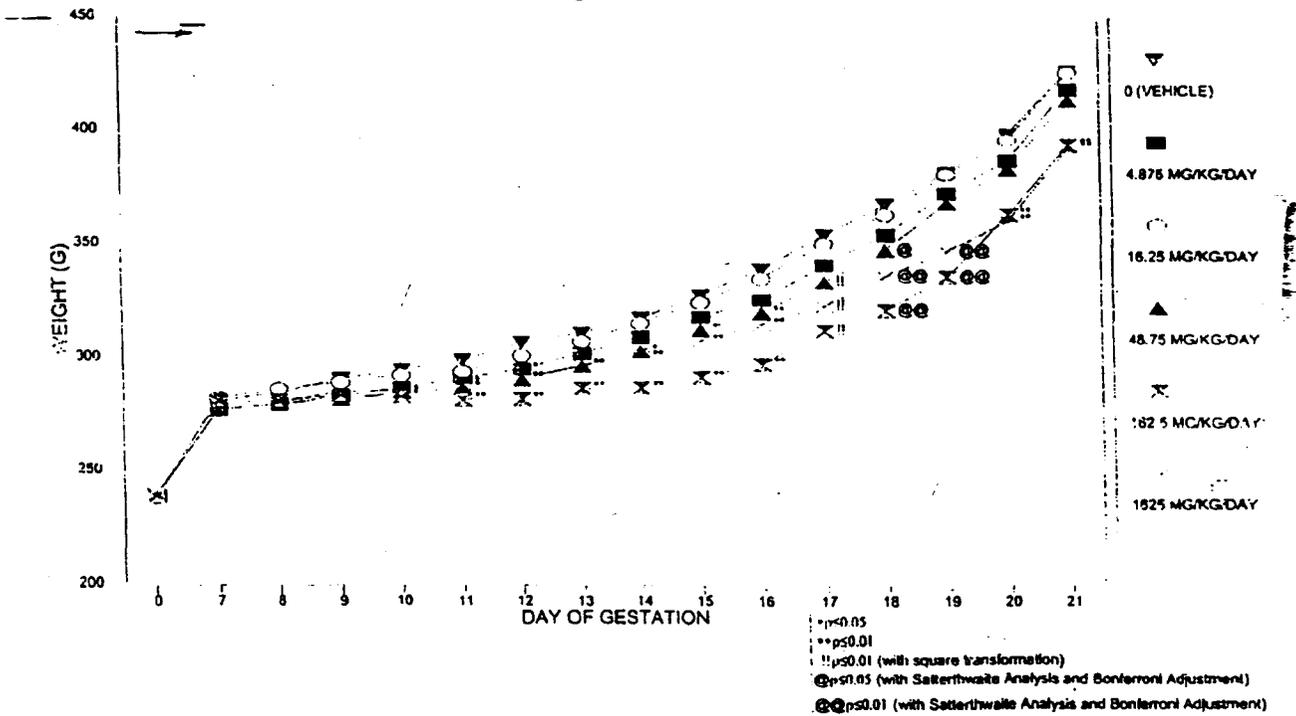


Fig. 6.2.1.: Effect of Olmesartan Medoxomil-HCTZ on Maternal Body Weight.

Absolute (gm/day) and relative (gm/kg/day) food consumption values were reduced in a somewhat dose-dependent manner from day 7 to day 10 of gestation in the groups receiving 48.75 or more mg/kg/day and from day 10 to day 12 of gestation in the groups receiving 162.5 and 1625 mg/kg/day ($p < 0.05$). Furthermore, food consumption was significantly decreased ($p < 0.05$) for the entire gestation period for the top two dosage groups (Table 6.2.3).

TABLE 6.2.2
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ ON EMBRYO-FETAL DEVELOPMENT IN RATS. MATERNAL BODY WEIGHTS - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 4.975	III 16.25	IV 40.75	V 162.5	VI 1625
RATS TESTED	N	25	25	25	25	25	25
PREGNANT	N	24	25	23	24	25	25
MATERNAL BODY WEIGHT (G)							
DAY 0	MEAN±S.D.	239.1 ± 8.7	239.1 ± 8.7	238.6 ± 8.8	238.9 ± 8.3	238.8 ± 9.0	239.6 ± 8.9
DAY 7	MEAN±S.D.	281.4 ± 12.4	277.6 ± 14.2	282.5 ± 13.2	277.3 ± 16.1	281.3 ± 13.1	281.0 ± 12.9
DAY 8	MEAN±S.D.	284.6 ± 15.3	279.4 ± 13.3	286.4 ± 14.9	279.8 ± 16.7	281.2 ± 14.5	280.1 ± 12.7
DAY 9	MEAN±S.D.	290.9 ± 14.3	283.1 ± 13.7	289.5 ± 15.7	281.8 ± 17.8	283.4 ± 15.6	285.2 ± 12.4
DAY 10	MEAN±S.D.	294.8 ± 12.7	286.8 ± 15.7	292.5 ± 16.0	284.6 ± 18.9*	282.9 ± 14.6*	285.6 ± 13.9
DAY 11	MEAN±S.D.	299.2 ± 15.3	291.2 ± 15.0	294.1 ± 17.0	285.7 ± 17.1*	281.7 ± 15.4**	288.5 ± 13.2'
DAY 12	MEAN±S.D.	306.8 ± 17.4	295.2 ± 16.7*	301.1 ± 17.1	290.4 ± 19.7**	282.0 ± 16.3**	291.0 ± 14.0**
DAY 13	MEAN±S.D.	310.9 ± 15.1	301.9 ± 15.9	307.4 ± 18.1	296.5 ± 19.0**	286.6 ± 17.2**	296.6 ± 14.6**
DAY 14	MEAN±S.D.	317.4 ± 15.5	309.2 ± 16.6	315.1 ± 19.8	303.0 ± 19.3*	286.8 ± 22.2**	300.2 ± 15.6**
DAY 15	MEAN±S.D.	327.3 ± 14.8	317.8 ± 17.0	324.4 ± 19.3	312.0 ± 19.4*	291.3 ± 27.2**	307.4 ± 17.8**
DAY 16	MEAN±S.D.	338.9 ± 15.5	325.5 ± 17.8	335.0 ± 20.4	319.8 ± 22.2**	297.3 ± 30.0**	314.7 ± 20.7**
DAY 17	MEAN±S.D.	354.0 ± 19.5	340.9 ± 20.1	350.2 ± 20.7	333.4 ± 23.7!!	312.1 ± 37.6!!!	322.9 ± 23.7!!!
DAY 18	MEAN±S.D.	367.6 ± 18.7	354.3 ± 18.8	363.2 ± 22.7	347.5 ± 24.8**	321.1 ± 41.2**	336.0 ± 18.7**
DAY 19	MEAN±S.D.	381.4 ± 20.2	372.3 ± 19.4	380.7 ± 24.3	368.0 ± 26.4	335.6 ± 47.7**	346.9 ± 20.7**
DAY 20	MEAN±S.D.	397.8 ± 23.5	386.8 ± 19.6	395.7 ± 27.0	383.1 ± 28.2	363.1 ± 29.2**	360.4 ± 23.4**
DAY 21	MEAN±S.D.	425.8 ± 27.8	418.0 ± 21.1	425.6 ± 27.9	413.2 ± 31.9	393.6 ± 31.1**	392.2 ± 33.2**

DAY = DAY OF GESTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on days 7 through 17 of gestation.

b. Excludes a value that appeared incorrectly recorded.

c. Excludes values for dams that were found dead.

* Significantly different from the vehicle control group value (p<0.05).

** Significantly different from the vehicle control group value (p<0.01).

! Significantly different from the vehicle control group value (p<0.01) with Square transformation.

! Significantly different from the vehicle control group value (p<0.05) with Satterthwaite Analysis and Bonferroni Adjustment.

!! Significantly different from the vehicle control group value (p<0.01) with Satterthwaite Analysis and Bonferroni Adjustment.

TABLE 6.2.3
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ ON EMBRYO-FETAL DEVELOPMENT IN RATS. MATERNAL RELATIVE FOOD CONSUMPTION
-- SUMMARY

DOSAGE GROUP		I	II	III	IV	V	VI
DOSAGE (NG/KG/DAY) ^a		0 (VEHICLE)	4.875	16.25	48.75	162.5	1625
RATS TESTED	N	25	25	25	25	25	25
PREGNANT	N	24	25	23	24	25	25
MATERNAL FEED CONSUMPTION (G/KG/DAY)							
DAYS 0 - 7	MEAN _± S.D.	87.4 ± 8.6	85.3 ± 7.7	87.6 ± 6.3	86.9 ± 8.5	89.4 ± 8.2	87.0 ± 6.7
DAYS 7 - 10	MEAN _± S.D.	87.2 ± 7.4 { 21}b	81.9 ± 6.3	80.4 ± 6.0*	79.1 ± 10.0** { 23}b	78.4 ± 11.2**	77.3 ± 7.5**
DAYS 10 - 12	MEAN _± S.D.	83.5 ± 7.2 { 23}b	82.8 ± 6.9	78.6 ± 7.2	78.8 ± 9.2	70.5 ± 11.6**	72.1 ± 10.8** { 24}b
DAYS 12 - 15	MEAN _± S.D.	80.8 ± 15.3 { 22}b	79.2 ± 6.6	83.1 ± 8.1	79.1 ± 8.2	68.5 ± 17.3 { 23}b	72.6 ± 13.1 { 24}b
DAYS 15 - 18	MEAN _± S.D.	84.7 ± 9.8	81.2 ± 4.5	80.0 ± 6.8	78.8 ± 6.9	71.2 ± 18.3 [⊙] { 24}c	67.4 ± 9.0 ^{⊙⊙} { 24}d
DAYS 7 - 18	MEAN _± S.D.	84.4 ± 5.2	80.9 ± 3.8 [⊙]	80.5 ± 4.3 [⊙]	78.8 ± 6.0 ^{⊙⊙}	71.2 ± 13.1 ^{⊙⊙} { 24}d	72.4 ± 6.2 ^{⊙⊙} { 24}d
DAYS 18 - 21	MEAN _± S.D.	71.7 ± 9.3	74.0 ± 7.3	69.8 ± 7.6	74.3 ± 7.0	71.3 ± 8.4 { 23}d	68.2 ± 15.8 { 22}b,d,e
DAYS 7 - 21	MEAN _± S.D.	81.1 ± 5.4	78.9 ± 3.6	77.5 ± 4.1†	77.4 ± 5.2†	72.9 ± 8.2†† { 23}d	71.9 ± 6.4†† { 22}b,d,e
DAYS 0 - 21	MEAN _± S.D.	78.1 ± 4.4	76.6 ± 2.9	76.1 ± 3.4	76.3 ± 4.6	74.7 ± 5.9* { 23}d	73.1 ± 4.7** { 22}b,d,e

DAYS - DAYS OF GESTATION

{ } = NUMBER OF VALUES AVERAGED

- a. Dosage occurred on days 7 through 17 of gestation.
- b. Excludes values that were associated with spillage.
- c. Excludes a value (body weight appeared incorrectly recorded).
- d. Excludes values for dams that were found dead.
- e. Excludes a value that was not recorded.
- * Significantly different from the vehicle control group value (p<0.05).
- ** Significantly different from the vehicle control group value (p<0.01).
- † Significantly different from the vehicle control group value (p<0.05) with Square transformation.
- †† Significantly different from the vehicle control group value (p<0.01) with Square transformation.
- ⊙ Significantly different from the vehicle control group value (p<0.05) with Satterthwaite Analysis and Bonferroni Adjustment.
- ⊙⊙ Significantly different from the vehicle control group value (p<0.01) with Satterthwaite Analysis and Bonferroni Adjustment.

Pregnancy rates were comparable for all groups (23 to 25 pregnant rats in each dosage group). There were no significant group differences in the mean number of corpora lutea, implantation sites, fetuses or pre- and post-implantation losses. There were no dead fetuses (Table 6.2.4). Fetal body weights in the 1625 mg OM-HCTZ/kg/day dosage group were lower, but not significantly lower, than control. No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were related to treatment with OM-HCTZ.

TABLE 6.2.4
SUMMARY OF CESAREAN-SECTIONING AND LITTER OBSERVATIONS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 4.875	III 16.25	IV 48.75	V 162.5	VI 1625
RATS TESTED	N	25	25	25	25	25	25
PREGNANT FOUND DEAD	N(%) N(%)	24(96.0) 0(0.0)	25(100.0) 0(0.0)	23(92.0) 0(0.0)	24(96.0) 0(0.0)	25(100.0) 2(8.0)	25(100.0) 1(4.0)
RATS PREGNANT AND CESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	24	25	23	24	23	24
CORPORA LUTEA	MEAN±S.D.	18.4 ± 1.8	17.7 ± 2.5	19.5 ± 4.1	18.6 ± 2.9	18.0 ± 2.4	19.0 ± 3.4
IMPLANTATIONS	MEAN±S.D.	15.4 ± 2.4	15.2 ± 1.9	15.0 ± 2.3	15.5 ± 3.0	15.1 ± 2.5	15.9 ± 2.1
LITTER SIZES	MEAN±S.D.	15.2 ± 2.4	14.5 ± 2.3	14.8 ± 2.3	15.2 ± 3.0	14.6 ± 2.3	15.4 ± 1.8
LIVE FETUSES	N MEAN±S.D.	366 15.7 ± 2.4	362 14.5 ± 2.3	341 14.8 ± 2.3	365 15.2 ± 3.0	336 14.6 ± 2.3	371 15.4 ± 1.8
DEAD FETUSES	N	0	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.5	0.7 ± 1.8	0.2 ± 0.5	0.3 ± 0.5	0.5 ± 0.7	0.4 ± 0.8
EARLY RESORPTIONS	N MEAN±S.D.	5 0.2 ± 0.5	17 0.7 ± 1.8	5 0.2 ± 0.5	7 0.3 ± 0.5	10 0.4 ± 0.7	10 0.4 ± 0.8
LATE RESORPTIONS	N MEAN±S.D.	0 0.0 ± 0.0	0 0.0 ± 0.0	0 0.0 ± 0.0	0 0.0 ± 0.0	2 0.1 ± 0.3	1 0.0 ± 0.2
DAMS WITH ANY RESORPTIONS	N(%)	4(16.7)	8(32.0)	4(17.4)	7(29.2)	10(43.5)	8(33.3)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	25(100.0)	23(100.0)	24(100.0)	23(100.0)	24(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	25(100.0)	23(100.0)	24(100.0)	23(100.0)	24(100.0)
LIVE MALE FETUSES	N	211	181	164	189	162	171
LIVE MALE FETUSES/LITTER	MEAN±S.D.	57.1 ± 13.7	49.8 ± 15.4	48.0 ± 13.2	50.8 ± 12.2	48.1 ± 11.0	46.0 ± 13.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.33 ± 0.42	5.39 ± 0.33	5.47 ± 0.30	5.41 ± 0.30	5.22 ± 0.32	5.06 ± 0.70
MALE FETUSES	MEAN±S.D.	5.18 ± 0.42	5.57 ± 0.32	5.61 ± 0.30	5.56 ± 0.33	5.35 ± 0.32	5.22 ± 0.75
FEMALE FETUSES	MEAN±S.D.	5.12 ± 0.45	5.23 ± 0.33	5.33 ± 0.32	5.25 ± 0.33	5.09 ± 0.31	4.89 ± 0.67
RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	1.3 ± 3.2	4.2 ± 10.8	1.5 ± 3.7	2.0 ± 3.2	3.2 ± 4.2	2.7 ± 4.7
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	6(25.0)	8(32.0)	6(26.1)	5(20.8)	6(26.0)	7(29.2)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	7(1.9)	12(3.3)	13(3.4)	7(2.1)	7(2.1)	9(2.4)
FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	2.3 ± 4.5	3.4 ± 5.8	3.9 ± 8.9	1.9 ± 4.2	1.9 ± 3.5	2.3 ± 4.2

a. Dosage occurred on days 7 through 17 of gestation.

Mean plasma concentrations of olmesartan and HCTZ generally increased with dose on both day 7 and 17 of gestation, though not proportionally. The concentrations tended to be higher on day 17 than on day 7, within each group. HCTZ was not detected in plasma at the lowest dosage (Table 6.2.5). Both olmesartan and HCTZ reached the systemic circulation relatively rapidly (T_{max} , 1-2 hr). After reaching C_{max} , the concentration declined with time in an apparent mono-exponential way. T_{last} appeared to be dosage-dependent, ranging from 4 hours at lower doses to 24 hours at the highest dose for both compounds. AUC_{last} increased with dose for both olmesartan and HCTZ on both sampling days in an approximately proportional manner up to 162.5 mg/kg/day. AUC_{last} for both compounds exhibited a much flatter increase from 162.5 mg/kg/day to 1625 mg/kg/day, suggesting possible saturation of absorption processes at the high dose. Olmesartan and HCTZ AUC values on day 17 were higher than on day 7 by approximately 64-133% for olmesartan and 170 to 261% for HCTZ (magnitude of increase unrelated to dose).

TABLE 6.2.5
TOXICOKINETIC PARAMETERS FOR OLMESARTAN AND HCTZ

Group	CS-866CMB Dosage (mg/kg/day)	Day of Presumed Gestation	C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	T_{last} (h)	AUC_{last} ($\mu\text{g}\cdot\text{h/mL}$)	AUC ($\mu\text{g}\cdot\text{h/mL}$)	$T_{1/2}$ (h)
RNH-6270								
II	4.875	7	0.351	1	4	0.814	1.62	3.3
		17	0.445	2	8	1.90	2.10	2.1
III	16.25	7	0.592	1	4	1.69	2.93	2.9
		17	1.74	1	8	5.76	6.01	1.7
IV	48.75	7	1.46	2	8	5.69	5.98	1.7
		17	2.72	2	8	8.95	9.80	2.0
V	162.5	7	2.67	1	24	15.6	15.9	4.3
		17	7.73	2	8	23.5	30.6	3.1
VI	1625	7	5.03	2	24	42.0	42.8	4.3
		17	7.20	1	24	82.8	99.6	9.4
HCTZ								
II	4.875	7	*	*	*	*	*	*
		17	*	*	*	*	*	*
III	16.25	7	0.604	1	4	1.48	2.15	2.1
		17	0.649	2	4	1.90	7.77	9.5
IV	48.75	7	1.47	1	4	3.56	8.01	3.9
		17	1.86	1	8	8.63	9.58	2.3
V	162.5	7	4.28	1	8	16.8	18.8	2.4
		17	5.47	2	8	32.1	50.8	5.2
VI	1625	7	9.26	2	24	89.7	91.3	4.1
		17	8.40	2	24	127	138	6.8

* All mean concentrations were below the quantifiable limit.

† Day 17 values derived after exclusion of 3 concentrations: No. 2985, 2 h and No 2986, pre-dose and 8 h.

RNH-6270: Olmesartan; CS-866CMB: OM-HCTZ

AUC_{last} : Area under the plasma concentration vs. time curve from 0 hr to the last quantifiable value.

AUC: Area under the plasma concentration time curve (not otherwise defined) †

6.3. Effects of Saline Supplementation on EmbryoFetal Toxicity of Olmesartan Medoxomil-HCTZ in Rats. Vol. 20.

This non-GLP study (Protocol #00-B083, Report #APR 148-030) was conducted by Medicinal Safety Research Laboratories, Sankyo Co. Ltd., Fukuroi, Shizuoka 437, Japan, between December 22, 2000 and August 23, 2001. Dates of dosing and necropsy are not given in the submission. The objective of the study was to determine whether saline supplementation would ameliorate the maternal and fetal toxicity observed with OM-HCTZ in a previous study (section 6.2).

Key Findings

Adverse effects on maternal survival, body weight gain and food intake, and on live fetal body weight were greater in the OM-HCTZ treated groups than in the OM or HCTZ treated groups. Increases in blood urea nitrogen and creatinine were also greater in OM-HCTZ treated groups than in OM or HCTZ treated groups, and erosions in the glandular stomach, dilatation of the distal tubules of the kidneys and adrenal hypertrophy were seen only in OM-HCTZ treated groups. All of these OM-HCTZ effects were ameliorated or eliminated by saline supplementation.

Methods

Groups of female Crj:CD®(SD)IGS rats from _____ were mated at approximately 9 weeks of age. Pregnant rats in the toxicity evaluation group weighed 191-253 gm and pregnant rats in the toxicokinetic evaluation group weighed 209-273 gm on day 0 of gestation. Suspensions of OM-HCTZ were prepared in 0.5% CMC-Na and administered orally by stomach tube (10 ml/kg), once daily, to two groups of mated females (7/group) from days 7 to 17 of gestation at a dose of 1000/625 mg OM/HCTZ per kg per day; one group was provided with water and the second group was provided with saline. Additionally, groups of pregnant rats (7/group) were treated with the vehicle, 1000 mg OM/kg/day (lot No. OS-001C), or 625 mg HCTZ/kg/day (lot No. +HCTMC97L040). The animals in these groups received tap water. Satellite groups of rats (5/group) were treated identically for determination of toxicokinetic parameters. Rats were given food and water *ad libitum*.

All pregnant rats were observed daily, with body weights measured on GDs 0, 3, 7, 9, 11, 13, 17, 19 and 21. Food consumption was recorded on the day of body weight measurement except on GD 0. Urine samples from animals in the toxicity evaluation groups were collected for 24 hr on GDs 12, 17, 19, and 21 and analyzed for creatinine, N-acetyl-β-D-glucosaminidase, protein, and sodium. Blood samples were collected from animals in the toxicity evaluation groups from the tail vein on GDs 12, 17, 19, and 20 and examined for BUN, creatinine, and sodium. Blood samples were collected from satellite animals prior to dosing on GDs 17 and at 2, 4, 8, and 24 hr after dosing on GDs 7 and 17 (n=3 animals/time point, Table 6.2.1). Blood was collected from the tail vein.

All rats, including those assigned to the toxicokinetics study, were anesthetized with ether and euthanized by cutting the abdominal aorta on GD 21. They were autopsied and their thoracic and peritoneal organs (including uterus and ovaries) were observed macroscopically. The numbers of corpora lutea, implantations, live fetuses and dead fetuses were recorded. Weights of heart, lung, liver, kidneys and adrenals were obtained for animals in the main study groups only. Histopathological examinations of the heart, lung, liver, kidneys, adrenals, stomach, duodenum, jejunum, ileum, cecum, colon and rectum of all animals were performed. Live fetuses in the toxicity and toxicokinetics evaluation groups were weighed, sexed and examined for external anomalies, including abnormalities in the oral cavity.

Results

Two dams (1F05, 1F03) in the toxicokinetics groups receiving OM-HCTZ (water) died on GDs 20 and 21. Both dams showed smudge around the nose at the time of death, but no abnormal clinical signs were observed until the previous day. No abnormal changes were observed in other groups. A statistically significant decrease ($p < 0.05$, 20% on GD 19) in maternal body weight gain relative to the control group was observed on GDs 15, 17, 19 and 21 in animals receiving OM-HCTZ and given water in both toxicology and toxicokinetic groups (Fig. 6.3.1, Tables 6.3.1. and 6.3.2). A similar decrease was not observed for rats treated with OM-HCTZ and given saline or rats treated with OM alone. However, rats treated with HCTZ had significantly decreased body weight gain on GDs 13, 15 and 17 (11% on GD 17). A similar significant decrease was not observed in the toxicokinetics evaluation group (Table 6.3.2).

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Fig. 6.3.1.: Effect of OM-HCTZ (with and without saline supplementation), OM and HCTZ on maternal body weight. Top: Toxicology evaluation; Bottom: Toxicokinetic evaluation. CS-866: OM; CS-866CMB: OM-HCTZ

TABLE 6.3.1
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION),
OM AND HCTZ ON MATERNAL BODY WEIGHTS (GM) - TOXICOLOGY GROUPS

Group-No. Dose mg/kg		Day of Pregnancy									
		0	3	7	9	11	13	15	17	19	21
01 0.5% CMC	N)	7	7	7	7	7	7	7	7	7	7
	Mean)	223.99	248.57	272.97	285.01	299.56	309.73	324.91	347.51	377.56	413.76
	S.D.)	10.50	12.48	13.12	15.04	17.25	17.41	19.88	24.15	24.00	31.03
02 CS-866 CMB-1625 (Water)	N)	7	7	7	7	7	7	7	7	7	7
	Mean)	235.91	259.83	282.40	284.00	292.54	293.31	297.51	314.49*	330.43*	367.14*
	S.D.)	14.09	15.08	16.71	19.53	20.58	20.67	21.61	21.87	24.37	30.47
03 CS-866 CMB-1625 (Saline)	N)	7	7	7	7	7	7	7	7	7	7
	Mean)	228.53	253.49	278.11	287.19	299.23	304.94	320.77	340.24	372.09	410.41
	S.D.)	17.46	15.10	18.81	19.83	19.73	22.86	26.87	24.36	31.28	31.50
04 CS-866 1000	N)	7	7	7	7	7	7	7	7	7	7
	Mean)	228.51	251.19	272.67	284.37	296.23	300.99	308.29	327.09	356.73	396.01
	S.D.)	16.69	15.28	16.16	17.58	21.74	23.00	21.68	24.13	24.05	21.82
05 HCTZ 625	N)	7	7	7	7	7	7	7	7	7	7
	Mean)	227.16	248.81	270.86	269.80	278.94	280.87*	291.64*	310.67*	344.83	374.77
	S.D.)	10.58	8.83	10.76	14.21	11.47	14.46	21.87	22.46	31.57	39.87

Significantly different from 01 group * P < 0.05 (Dunnett's test)

TABLE 6.3.2
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION),
OM AND HCTZ ON MATERNAL BODY WEIGHTS (GM) - TOXICOKINETICS GROUPS

Group-No. Dose mg/kg		Day of Pregnancy									
		0	3	7	9	11	13	15	17	19	21
01 CS-866 CMB 1625 (Water)	N)	5	5	5	5	5	5	5	5	5	2
	Mean)	235.22	258.72	281.48	275.90	279.20	285.22	290.20	303.82	301.90	369.90
	S.D.)	14.26	16.38	15.49	13.39	11.03	15.40	15.91	18.86	35.19	12.73
02 CS-866 CMB 1625 (Saline)	N)	5	5	5	5	5	5	5	5	5	5
	Mean)	230.02	250.84	272.00	273.96	283.58	298.32	309.98	327.92	356.46	391.54
	S.D.)	26.17	26.04	27.18	27.88	27.76	30.35	28.30	29.97	28.64	28.74
03 CS-866 1000	N)	5	5	5	5	5	5	5	5	5	5
	Mean)	231.40	253.42	275.54	278.14	287.60	298.76	311.20	332.80	351.56	385.40
	S.D.)	12.70	15.49	18.56	20.51	21.99	22.34	24.18	28.77	31.17	27.78
04 HCTZ 625	N)	5	5	5	5	5	5	5	5	5	4
	Mean)	233.58	256.16	277.34	268.60	280.04	290.38	304.42	326.58	348.86	377.75
	S.D.)	10.69	8.41	11.58	10.30	8.68	9.38	11.49	12.39	17.81	11.38

According to this reviewer's calculation (ANOVA, Dunnett's test), body weights for the OM-HCTZ (water) toxicology and toxicokinetics groups on GDs 15, 17, 19, and 21 are significantly different (p < 0.05) from control group (CMC) body weights. A significant difference (p < 0.05) is also noted for the OM (CS-866) toxicokinetics evaluation group on GD 19.

Food consumption was significantly decreased ($p < 0.05$) on GDs 11, 13, 15, 17 and 19 for rats treated with OM-HCTZ and given water (both toxicology and TK groups, Tables 6.3.3. and 6.3.4). Though low values were observed for rats exposed to OM alone on all the above days, statistical significance relative to control ($p < 0.05$) could be achieved only on GDs 15, 17 and 19 for rats in the toxicology group and on GD 19 for rats in the TK group. Food consumption values for other groups were comparable to control values.

TABLE 6.3.3
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION), OM AND HCTZ ON MATERNAL FOOD CONSUMPTION (GM) - TOXICOLOGY GROUPS

Group-No. Dose mg/kg		Day of Pregnancy								8
		3	7	9	11	13	15	17	19	
01 0.5% CMC	N)	7	7	7	7	7	7	7	7	7
	Mean)	23.3	25.4	25.9	27.7	26.6	27.4	29.0	29.6	28.0
	S.D.)	1.7	2.1	2.3	2.9	2.7	3.8	4.2	4.5	4.6
02 CS-866 CMB 1625 (Water)	N)	7	7	7	7	7	7	7	7	7
	Mean)	25.0	27.3	23.6	22.6**	21.9*	20.3**	23.4	22.4**	24.4
	S.D.)	1.6	2.2	2.2	2.8	1.8	2.2	2.6	2.6	6.7
03 CS-866 CMB 1625 (Saline)	N)	7	7	7	7	7	7	7	7	7
	Mean)	24.0	26.6	26.0	26.3	25.1	27.4	28.9	29.3	30.3
	S.D.)	2.0	2.1	2.5	3.2	3.8	4.5	4.4	3.3	3.5
04 CS-866 1000	N)	7	7	7	7	7	7	7	7	7
	Mean)	23.1	25.1	25.6	25.7	23.6	21.7*	22.7*	24.1*	26.0
	S.D.)	1.1	2.0	2.6	2.6	2.0	2.4	5.9	2.8	3.7
05 HCTZ 625	N)	7	7	7	7	7	7	7	7	7
	Mean)	22.9	25.0	24.4	25.0	23.0	24.1	25.4	28.0	27.0
	S.D.)	2.0	0.8	1.0	1.0	3.7	3.8	2.5	3.3	3.8

TABLE 6.3.4
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION), OM AND HCTZ ON MATERNAL FOOD CONSUMPTION (GM) - TOXICOKINETICS GROUPS

Group-No. Dose mg/kg		Day of Pregnancy								8
		3	7	9	11	13	15	17	19	
01 CS-866 CMB 1625 (Water)	N)	5	5	5	5	5	5	5	5	2
	Mean)	24.6	27.0	20.6	19.8	21.2	18.6	21.8	13.6	23.5
	S.D.)	2.4	2.0	0.9	2.2	2.9	3.8	2.5	8.1	3.5
02 CS-866 CMB 1625 (Saline)	N)	5	5	5	5	5	5	5	5	5
	Mean)	22.4	24.6	23.4	24.0	27.4	26.2	28.2	28.6	26.4
	S.D.)	1.1	2.5	3.5	1.9	1.9	1.5	2.4	2.7	2.4
03 CS-866 1000	N)	5	5	5	5	5	5	5	5	5
	Mean)	23.2	24.4	23.2	24.4	25.0	24.8	26.8	23.4	24.2
	S.D.)	3.6	3.4	3.6	2.6	4.3	4.7	5.2	3.7	1.8
04 HCTZ 625	N)	5	5	5	5	5	5	5	5	4
	Mean)	23.2	26.0	22.8	25.4	26.8	26.4	26.6	25.6	27.3
	S.D.)	1.9	2.4	1.6	2.5	3.7	1.5	0.9	3.6	3.9

According to this reviewer's calculation (Dunnett's test), food consumption values for the OM-HCTZ (water) toxicology and TK groups on GDs 11, 13, 15, 17 and 19 are significantly different ($p < 0.05$) from control group values.

Urinalysis revealed a significant ($p < 0.05$) increase in urine volume (63-119%) and sodium excretion (220-597%) for rats treated with OM-HCTZ and given saline and a decrease in sodium excretion (-33%) for the group treated with OM-HCTZ and given water. Values for creatinine and N-acetyl- β -glucosaminidase were significantly decreased ($p < 0.05$, 29-57%) in both groups treated with OM-HCTZ (Table 6.3.5).

TABLE 6.3.5
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION), OM AND HCTZ ON MATERNAL URINALYSIS PARAMETERS - TOXICOLOGY GROUPS

Group-No. Dose mg/kg	Urine volume ml/Day	Day of Pregnancy																																															
		12				17				19				21																																			
		N	Mean	S.D.		N	Mean	S.D.		N	Mean	S.D.		N	Mean	S.D.																																	
01 0.5% CMC		7	30.57	11.90	7	28.00	6.27	7	24.00	15.86	7	18.29	11.67	7	31.706	12.558	7	32.971	6.398	7	42.420	16.998	7	59.583	20.598	7	12.21	3.82	7	11.49	1.52	7	11.70	3.46	7	12.87	3.19	7	82.7	26.7	7	68.4	18.5	7	67.8	20.7	7	52.0	12.7
02 CS-866CMB (Water) 1625		7	30.57	4.04	7	33.86	4.67	7	38.71	8.36	7	22.71	7.70	7	30.629	4.983	7	23.439 #	2.397	7	21.246 **	3.626	7	46.216	16.771	7	8.37 *	2.55	7	5.53 ##	0.61	7	5.03 #	0.83	7	10.16	3.57	7	64.4	13.2	7	46.1 #	3.5	7	36.6	7.3	7	34.3	12.8
03 CS-866CMB (Saline) 1625		6	49.67 **	9.89	7	46.29 **	4.46	7	32.57 **	19.81	7	36.57 #	20.89	6	23.083 *	4.298	7	19.281 ##	3.076	7	19.427 **	6.748	7	31.231	10.976	6	6.02 **	1.37	7	5.99 ##	1.45	7	5.60 #	2.02	7	9.11	3.33	6	264.7 **	42.4	7	258.0	25.2	7	289.6	47.4	7	348.3 #	28.6
04 CS-866 1000		7	24.71	3.64	7	24.57	7.93	7	25.14	6.84	7	13.43	3.95	7	34.916	5.139	7	34.750	12.533	7	34.046	7.529	7	64.804	15.193	7	12.1	1.97	7	10.09	4.80	7	4.93	1.63	7	11.31	3.41	7	84.1	18.6	7	55.7	11.0	7	48.3	8.4	7	43.4	18.8
05 HCTZ 625		7	32.29	6.10	7	25.29	4.75	7	24.43	18.58	7	21.86	18.02	7	29.649	4.96	7	30.596	7.284	7	28.441	13.178	7	54.507	21.562	7	10.24	3.09	7	12.01	3.27	7	14.96	5.83	7	14.70	5.24	7	77.9	20.1	7	60.4	7.9	7	48.4	9.0	7	65.0	13.7

Significantly different from 01 group (control) # $P < 0.05$, ## $P < 0.01$ (Dunnett's rank test)

Significantly different from 01 group (control) * $P < 0.05$, ** $P < 0.01$ (Dunnett's test)

Blood urea nitrogen values were significantly increased in rats treated with OM-HCTZ and given water (153-227%) and rats treated with OM (44-75%); this effect was not observed in rats treated with OM-HCTZ and given saline. Blood creatinine levels were significantly increased on some days of dosing in all groups (13-36%), except for the group treated with OM-HCTZ and given saline. Blood sodium values were decreased in all groups treated with OM, either alone or in combination with HCTZ (~2%) (Table 6.3.6).

TABLE 6.3.6
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION), OM AND HCTZ ON MATERNAL BLOOD CHEMISTRY PARAMETERS - TOXICOLOGY GROUPS

Group-No. Dose mg/kg	UN mg/dL	Day of Pregnancy																																			
		12				17				19				21																							
		N	Mean	S.D.		N	Mean	S.D.		N	Mean	S.D.		N	Mean	S.D.																					
01 0.5% CMC		7	23.39	2.81	7	25.41	3.73	7	24.66	4.05	7	23.53	4.34	7	0.321	0.025	7	0.316	0.017	7	0.386	0.206	7	0.326	0.026	7	143.0	1.5	7	140.3	1.9	7	140.3	1.4	7	141.4	1.1
02 CS-866CMB (Water) 1625		7	65.46 **	7.33	7	83.2 ##	14.41	7	62.46 ##	33.27	7	27.56	4.79	7	0.429 **	0.030	7	0.430 **	0.042	7	0.371	0.080	7	0.307	0.025	7	141.7	1.4	7	137.3 **	2.3	7	137.4 *	0.8	7	138.4 *	2.8
03 CS-866CMB (Saline) 1625		7	29.67	5.05	7	32.66	3.06	7	27.01	2.20	7	24.17	1.66	7	0.346	0.032	7	0.314	0.023	7	0.299	0.020	7	0.306	0.010	7	141.6	2.4	7	137.7 *	1.7	7	138.4	1.6	7	140.0	1.9
04 CS-866 1000		7	32.29	6.88	7	44.44 **	11.52	7	35.41 ##	6.46	7	27.57	5.07	7	0.363 *	0.028	7	0.369 *	0.023	7	0.327	0.020	7	0.337	0.023	7	141.3	1.9	7	138.4	0.8	7	137.7 *	1.5	7	140.1	1.6
05 HCTZ 625		7	29.04	4.36	7	26.80	3.98	7	24.44	1.62	7	23.23	3.41	7	0.361 *	0.028	7	0.336	0.038	7	0.303	0.025	7	0.337	0.021	7	143.1	2.5	7	140.0	1.2	7	141.7	2.8	7	140.9	1.7

Significantly different from control gp ## $P < 0.01$ (Dunnett's rank test); * $P < 0.05$, ** $P < 0.01$ (Dunnett's test)

There were no significant differences between treatment groups and the control group in the numbers of corpora lutea, implantations, dead embryos and fetuses, live fetuses, and sex ratio in both the toxicology and the toxicokinetic satellite groups. However, live male fetal body weights were decreased ($p < 0.05$) in both toxicology and toxicokinetics evaluation groups treated with OM-HCTZ and given water in (Table 6.3.7 and the footnote to the table). No external anomalies were observed in any group.

TABLE 6.3.7
EFFECT OF OLMESARTAN MEDOXOMIL-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION), OM AND HCTZ ON EMBRYO-FETAL DEVELOPMENT IN RATS - TOXICOLOGY GROUPS

Group-No. Dose mg/kg		01 0.5% CMC	02 CS-866 CMB 1625 (Water)	03 CS-866 CMB 1625 (Saline)	04 CS-866 1000	05 HCTZ 625	
No. of animals examined	Total	7	7	7	7	7	
No. of dams with live fetuses	Total	7	7	7	7	6	
No. of corpora lutea	Total	107	109	108	115	107	
	Mean±S.D.	15.3± 0.8	15.6± 2.1	15.4± 2.5	16.4± 1.3	15.3± 3.3	
No. of implants	Total (%)	105 (98.2)	95 (86.6)	100 (93.6)	108 (94.0)	94 (83.7)	
	Mean±S.D.	15.0± 0.6	13.6± 3.8	14.3± 1.4	15.4± 1.1	13.4± 5.9	
No. of dead fetuses	Total (%)	5 (4.8)	4 (3.5)	4 (3.9)	4 (3.7)	4 (16.7)	
	Mean±S.D.	0.7± 1.1	0.6± 0.8	0.6± 0.8	0.6± 0.8	0.6± 0.8	
Dead	Total (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Mean±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Late	Total (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (14.3)	
	Mean±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.1± 0.4	0.1± 0.4	
Early	Total (%)	5 (4.8)	4 (3.5)	4 (3.9)	3 (2.8)	3 (2.4)	
	Mean±S.D.	0.7± 1.1	0.6± 0.8	0.6± 0.8	0.4± 0.5	0.4± 0.8	
Implantation sites	Total (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Mean±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
No. of live fetuses	Total (%)	100 (95.2)	91 (96.5)	96 (96.1)	104 (96.3)	90 (83.3)	
	Mean±S.D.	14.3± 1.4	13.0± 3.4	13.7± 1.4	14.9± 1.3	12.9± 5.9	
Sex ratio Male:Female	Total	48:52	37:54	51:45	57:47	46:44	
	Male %	Mean±S.D.	48.2±11.1	39.3±17.8	52.6± 8.9	55.1±11.2	50.4±12.3
Body weight (g)	Male	Mean±S.D.	5.66±0.36	5.19±0.40*	5.93±0.33	5.55±0.24	5.48±0.25
	Female	Mean±S.D.	5.35±0.30	5.08±0.51	5.60±0.28	5.27±0.36	5.22±0.17
No. of live fetuses with anomalies	Total (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

* Weights of male and female fetuses in the OM-HCTZ (CS-866CMB) toxicokinetics evaluation group that was given water were, respectively, 4.83 ± 0.27 and 4.70 ± 0.21 gm (mean ± S.D.). Both these values are significantly ($p < 0.05$) lower than control (CMC group) values (5.66 ± 0.36 and 5.35 ± 0.30 gm).

At necropsy, absolute and relative heart weights were significantly ($p < 0.05$) lower than control (20% and 10% lower, respectively) and relative adrenal weight was significantly higher than control (20% higher) in animals treated with OM-HCTZ and given water. No significant differences from control were observed in other treated groups.

Microscopic examination revealed erosion in the glandular stomachs of three dams receiving OM-HCTZ (water), two in the toxicology group (#2F01 and #2F03) and one in the TK evaluation group (1F05). The latter and a second animal (1F03) in the same group showed hypertrophy of fasciculata and reticularis cells in the adrenal. Animal #1F05 also had distal tubule dilatation in the kidney. It may be noted that both animals (1F03 and 1F05) in the TK evaluation group had died (on GD 21 and 20, respectively). Given the abnormal histopathological changes, the deaths in the satellite toxicokinetic group could be due to administration of test substance.

The systemic exposure to olmesartan or HCTZ on day 1 (GD 7) after administration of OM-HCTZ was comparable in the groups given water or saline. However, on GD 17, AUC values for olmesartan, and AUC and C_{max} values for HCTZ, were higher in the water group compared to the saline group (Table 6.3.8). Furthermore, AUC and C_{max} values for HCTZ in the OM-HCTZ (water) group on GD 17 were higher than those observed in the group treated with HCTZ alone. The time course for plasma concentration of olmesartan or HCTZ was similar across groups.

TABLE 6.3.8

TOXICOKINETIC PARAMETERS FOR OLMESARTAN AND HCTZ IN PREGNANT RATS TREATED WITH OM-HCTZ (WITH AND WITHOUT SALINE SUPPLEMENTATION), OM OR HCTZ FROM DAYS 7 TO 17 OF GESTATION

Dosage/Gp	GD	C _{max} (µg/ml)	AUC _{0-24h} (µg.hr/ml)	T _{max} (hr)
OLMESARTAN				
OM-HCTZ (water)	Day 7	2.62	30.72	4.0
	Day 17	3.94	49.27	4.4
OM-HCTZ (saline)	Day 7	2.97	35.14	3.2
	Day 17	3.03	28.22	2.0
OM alone	Day 7	3.57	31.10	3.2
	Day 17	2.62	26.18	2.8
HCTZ				
OM-HCTZ (water)	Day 7	9.66	95.08	2.0
	Day 17	21.49	308.04	5.2
OM-HCTZ (saline)	Day 7	11.67	103.42	2.4
	Day 17	10.67	86.51	2.8
HCTZ alone	Day 7	11.48	107.97	3.2
	Day 17	8.64	87.22	2.4

VII. OVERALL SUMMARY AND EVALUATION

Pharmacodynamics

Olmesartan medoxomil (OM) is a non-peptidic, orally effective, potent and specific antagonist of angiotensin II, active at the AT₁ 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 AT₁ 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.

The antihypertensive effect of OM in combination with HCTZ (at dose ratios of 1:10 and 1:100) was investigated in conscious male spontaneously hypertensive rats (SHR) receiving the combination by oral administration for 14 successive days. The 24-hour mean blood pressure was decreased gradually from the first day of administration with a maximal decrease of about 20% at 1 mg OM/10 mg 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 mg OM/10 mg 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. This suggests an additive effect of these drugs when used in combination. No rebound phenomenon was observed after discontinuation of the treatment. A remarkable increase in plasma renin activity was observed in rats treated with OM or OM-HCTZ and the increase corresponded to their antihypertensive effects. Urine volume and excretion of sodium and potassium were increased in the rats treated with HCTZ or OM-HCTZ, effects attributable to the pharmacological activity of HCTZ.

Pharmacokinetics

The pharmacokinetics and metabolism of orally administered ¹⁴C labeled OM and HCTZ were unaffected by co-administration of unlabeled HCTZ and OM (dosage ratios of 1:1, 1:10 and 10:1), respectively. Olmesartan was the only metabolite detected after administration of OM with or without HCTZ. The data from plasma protein binding studies demonstrated that OM and HCTZ did not affect the binding of the other compound to proteins in human plasma. It is concluded that there are no pharmacokinetic interactions between OM and HCTZ. Thus, the ratio of unbound olmesartan to unbound HCTZ in plasma would not be altered by administration of OM-HCTZ.

Toxicology

In all toxicity studies, OM-HCTZ contained OM and HCTZ in combination at a fixed ratio of 1.6:1.0.

Acute Toxicity

Single dose oral toxicity studies were performed with OM-HCTZ in rats and dogs. No clinical signs were observed at total doses up to 2000 mg/kg (1230/770 mg/kg).

Chronic Toxicity

The potential for adverse effects following repeated oral administration of OM-HCTZ, OM, or HCTZ was investigated in the rat and the dog.

Rats

The chronic toxicity of OM-HCTZ was evaluated in rats in gavage studies at doses of up to 1625 mg/kg/day (1000/625 mg OM/HCTZ per kg per day) administered for 26 weeks. There were no treatment-related deaths or clinical signs. Overall body weight gains of males and females were significantly reduced relative to control at doses as low as 48.75 and 162.5 mg OM-HCTZ/kg/day, respectively. The response was nondose-dependent, greater for males than females, and lasting the entire treatment period for males. Mean weekly food consumption was also significantly and nondose-dependently reduced relative to concurrent control for rats given 162.5 or more mg OM-HCTZ/kg/day and the reduction was more severe for males than females. Dilatation of retinal vessels was noted in rats receiving 162.5 or more mg OM-HCTZ/kg/day. None of these effects were observed in animals receiving either OM (1000 mg/kg/day) or HCTZ (625 mg/kg/day) alone.

RBC, hemoglobin, and hematocrit were decreased significantly and dose-dependently relative to control at 162.5 (48.75 for males) or more mg OM-HCTZ and 1000 mg OM (males only)/kg/day. A treatment-related, nondose-dependent increase ($p < 0.05$) in blood urea nitrogen (up to 2.8-fold) was observed in males at doses as low as 4.88 and in females at doses as low as 48.75 mg OM-HCTZ/kg/day. BUN was also increased in males receiving OM alone (1000 mg/kg/day). A significant increase in creatinine was noted in rats receiving 162.5 or more mg OM-HCTZ/kg/day. At the 26 week sacrifice, the mean relative kidney weights were significantly increased at doses of 162.5 or more mg OM-HCTZ/kg/day. Histopathologically, treatment-related changes were observed in the kidneys of males and females given 48.75 or more mg OM-HCTZ/kg/day. They were characterized as variable thickening of the tubular basement membrane and, infrequently, of the Bowman's capsular membrane, with or without tubular epithelial basophilia indicative of tubular degeneration. 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. The renal changes 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. Similar renal changes had been observed in a previous study in rats treated with OM alone at 300 or more mg/kg/day for 3 months (see NDA 21,286 review). Thirteen weeks of dietary administration of HCTZ to Fischer 344/N rats had been shown to induce mineralization of the kidney at doses as low as 250 ppm (Bucher, J.R. *et al.*, J Appl Toxicol., 10: 359, 1990).

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 similar observation was made in one male receiving 1000 mg OM/kg/day. It is not clear from the published literature whether HCTZ has any effect on the stomach. Taken together the combination increases the incidence and/or severity of renal tubular changes and erosion and ulceration of the glandular mucosa of the stomach. A NOAEL for these effects could not be established in rats.

Saline supplementation prevented the reduction in body weight gain, reduced the elevation in BUN concentration and eliminated the increase in creatinine. Renal tubular changes were more pronounced and frequent in animals given tap water than those receiving saline. The incidence of focal ulceration of the glandular gastric mucosa was observed with the 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. There were no substantial gender differences in either C_{max} or AUC values and no accumulation of drug after repeated administration. The coadministration of HCTZ did not influence the concentration of olmesartan; nor did the coadministration of OM influence the concentration of HCTZ. Furthermore, there were no apparent differences in toxicokinetic parameters between the treated groups receiving tap water and those receiving saline.

Dogs

The chronic toxicity of OM-HCTZ was evaluated in dogs at oral doses of 4/2.5, 8/5, 16/10 and 32/20 mg/kg/day administered for 26 weeks. There were no significant differences from control body weight, food consumption, hematology, or organ weights at any dose level. Two males, one at 16/10 and another at 32/20 mg OM/HCTZ/kg/day, were sacrificed moribund, their moribund state attributed to renal failure as they were diagnosed with tubular hypertrophy and dilatation, and marked (up to 6-fold) increases in BUN, creatinine and total bilirubin. Additionally, the lower dose dog had extensive gastric ulceration. The predominant histopathological finding, observed at both unscheduled and scheduled sacrifices, was hypertrophy and eosinophilia of cortical tubular epithelia in the kidney. The severity of the renal changes increased with increasing doses of OM-HCTZ, with males appearing to be more susceptible than females. Similar renal changes were observed in dogs given 32 mg OM/kg/day or 20 mg HCTZ/kg/day. A similar effect was demonstrated for OM in a previously conducted 3 month study at doses as low as 125 mg OM/kg/day (see NDA 21,286 review). BUN values were significantly, but non dose-dependently, elevated in both decedent (up to 6-fold) and surviving males (less than a fold) in all dose groups relative to concurrent control and pretreatment values. Creatinine was elevated in the decedent dogs only (approximately 3-fold). Doses higher than 32/20 mg/kg/day were

aborted after 2 weeks of dosing due to excessive toxicity and deaths. All dogs that died or were sacrificed moribund had increased red blood cell indices; increased BUN and creatinine, increased ALT and AST; and decreased sodium chloride. The rapid deterioration to a moribund state was said to have been due to a compromised circulatory system. Extensive, treatment-related histopathological changes were observed in the kidneys, lungs, heart and GIT. Taken together, these studies suggest that the combination increases renal and gastrointestinal tract toxicity. A NOAEL for these effects has not been established.

A non-linear increase in C_{max} and AUC_{0-1ast} values for olmesartan with increasing dose levels of OM-HCTZ was observed in the 26 week study. There were no substantial gender differences for either C_{max} or AUC values and no accumulation of drug after repeated administration. Concomitant administration of OM and HCTZ did not affect the kinetic behavior of olmesartan.

Genotoxicity

An investigation for evidence of genetic toxicity was conducted with olmesartan-HCTZ, OM and HCTZ. Two *in vitro* studies, an Ames reverse mutation assay and a Chinese hamster lung cell chromosomal aberration assay, and one *in vivo* mouse micronucleus test, were conducted. OM-HCTZ, OM and HCTZ were negative in all tester strains in the Ames reverse mutation assay, both in the presence and in absence of S-9 mix. OM, HCTZ and OM-HCTZ tested positive in the absence of S-9 mix (not tested in the presence of S-9 mix) in the *in vitro* chromosomal aberration assay. Dose-dependent increases in structural and numerical (polyploidy) abnormalities were observed for each of the 3 combination ratios tested: 40/12.5, 20/12.5, 10/12.5. These are expected results since OM (see NDA 21,286 review) and HCTZ (literature review) alone have been reported to induce chromosome aberrations. But combining OM with HCTZ did not enhance the responses observed with OM or HCTZ alone. No synergism in clastogenic activity was detected between OM and HCTZ in any combination ratio. The mouse micronucleus test failed to demonstrate a potential for clastogenicity of OM-HCTZ *in vivo*.

Reprotoxicity

Developmental toxicity studies with OM-HCTZ were conducted in mice and rats at total doses of up to 1625 mg/kg/day (1000/625 mg OM/HCTZ per kg per day).

Mice

Oral administration of OM-HCTZ to mated female mice at doses of 100/62.5, 300/187.5 and 1000/625 mg/kg/day on gestation days 6 through 15 did not produce maternal or developmental toxicity. Thus, OM-HCTZ does not have adverse effects on embryo-fetal development when administered to mice during organogenesis at doses as high as 1000/625 mg/kg/day. The latter dose is, on a mg/m^2 basis, about 122 times the MRHD of OM/HCTZ (40/25 mg/day). C_{max} and AUC values for both olmesartan and HCTZ (measured on gestation days 6 and 15), increased in a dose-related manner, although the increases were less than dose proportional.

Rats

When OM-HCTZ was administered to mated female rats, by gavage, on 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 (1 of 25 from one study and 2 of 7 from another) 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 ($p < 0.05$) in maternal body weight gain and food consumption 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 statistically significant decrease in the mean fetal body weights for the 1000/625 mg/kg/day group. 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. Decreases in urinary creatinine and N-acetyl- β -glucosaminidase in the high dose combination group suggest changes in renal function in maternal animals receiving OM-HCTZ. Histopathological examination revealed erosion in the glandular stomach in some animals in the combination group, 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. Higher C_{max} and AUC values were observed 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. (Values were dose-related on both days.) This accumulation may have been related to the increased renal toxicity (i.e., increased BUN and nephropathy) 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 mg/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 the 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 greater in the non-saline supplemented group than in the saline supplemented group ($p < 0.05$). 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 (e.g., reductions in body weight gain and food consumption; increases in BUN and creatinine; gastric irritation and ulceration; and nephropathy) observed in the rat general toxicity and reproductive toxicity studies 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.

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