TABLE 3.3.3.1
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenals*	Lesions	Spinal cord
Aorta	Liver with gallbladder*	Spleen*
Brain*	Lungs*	Stomach
Cecum	Mesenteric lymph node	Testes with epididymides*
Colon	Ovaries*	Thymus*
Duodenum	Pancreas	Thyroids with parathyroids*
Esophagus	Pituitary *	Trachea
Femur with bone marrow	Prostate*	Urinary bladder
Heart*	Rectum	Uterus* with cervix and vagina
Ileum	Salivary gland -mandibular*	
Jejunum	Sciatic nerve	
Kidneys*	Seminal vesicles*	

*Organ weighed

Results

There were no drug-related clinical signs. Survival rates after 26 weeks of treatment were 100, 100, 100, 100, and 93% (14/15) for the males and 93, 93, 87 (13/15), 93, and 100% for the females in the 0, 100, 300, and 1000 mg/kg/day OM groups and the positive control group, respectively. A single death was observed with the administration of cresidine during week 26. This male died as a result of invasive transitional cell carcinoma; multiple metastases were observed in the lung. There were no unscheduled deaths in the satellite groups. There were sporadic increases and decreases in body weight and food consumption but no consistent and pronounced differences in body weight or food consumption between OM and vehicle treated animals (Fig. 3.3.3.1). In contrast, *p*-cresidine-treated males showed a consistently lower mean body weight ($p < 0.05$ for weeks 7-13 and 19-27, average weight 33% lower than control), and food consumption ($p < 0.05$ for weeks 1, 3-10, 12, 13 and 15-25, average consumption 17% lower than control). There were no treatment-related effects on hematology parameters in mice treated with the test substance. Mice treated with *p*-cresidine showed significantly lower mean values for red blood cells, hemoglobin, and hematocrit ($p < 0.05$).

There were no remarkable differences in organ weights between the control and OM treated groups except for decreases in absolute and relative thyroid/parathyroid weights (32%) for the high dose male group. The *p*-cresidine treated male group showed statistically significant decreases in both absolute and relative seminal vesicle and thyroid/parathyroid weights, and absolute kidney weights and significantly increased absolute brain and spleen weights. Spleen might have been enlarged as a compensatory response to decreased hematological values observed in these animals. The sponsor did not examine the spleens from these animals microscopically.

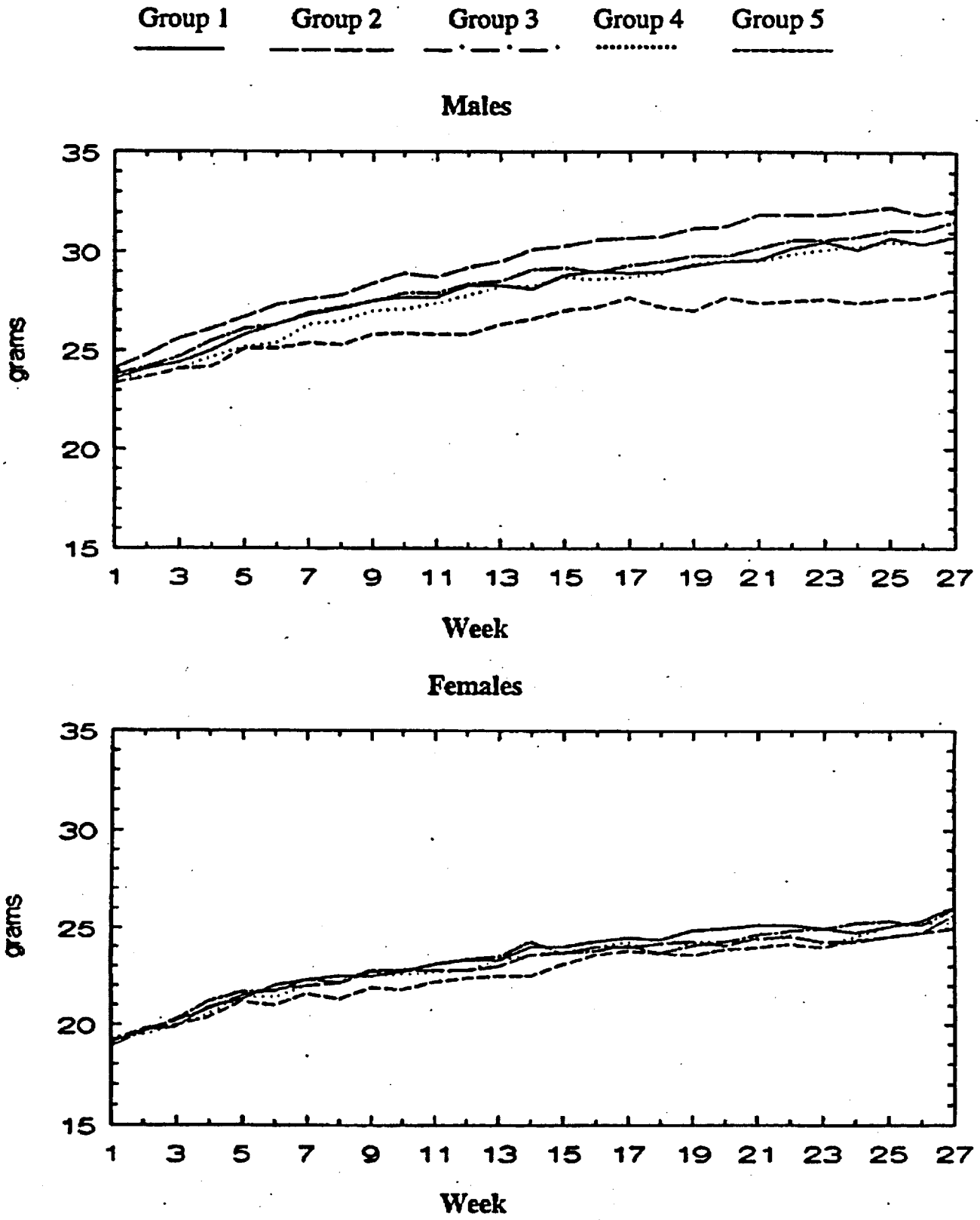

Fig. 3.3.3.1.: Mean body weights for mice treated with olmesartan medoxomil for 26 weeks

Gross pathology findings were restricted to a mass in the duodenum and in the urinary bladder of one male each that received *p*-cresidine. There were no macroscopic pathology findings in olmesartan-treated animals.

Non-neoplastic microscopic findings considered to be related to treatment with the test agent were limited to the kidneys (Table 3.3.3.2). Minimal to slight renal tubular regeneration was observed in all groups ($p < 0.05$) that received olmesartan medoxomil. The incidence and severity of this finding was most prominent in the high dose males. Minimal glomerulopathy, characterized primarily by thickening of the mesangium, occurred in some of the mice receiving 1000 mg/kg/day ($p < 0.05$ for trend). This was considered to be a secondary effect of the

TABLE 3.3.3.2
CARCINOGENICITY STUDY IN P53(+/-) MICE. INCIDENCE AND SEVERITY OF SELECTED MICROSCOPIC OBSERVATIONS OF KIDNEY

		Number of animals affected									
Sex		Male					Female				
Group[a]		1	2	3	4	5	1	2	3	4	5
Number examined:	Grade[b]	**15**	**15**	**15**	**15**	**1**	**15**	**15**	**15**	**15**	**0**
Not remarkable:	↓	**13**	**2**	**0**	**0**	**0**	**14**	**0**	**0**	**0**	**0**
--Tubule, regeneration	->	13	4	2	1	0	14	2	2	4	0
	1>	0	9	12	9	0	1	12	12	8	0
	2>	1	2	1	5	0	0	1	1	3	0
	4>	1	0	0	0	1	0	0	0	0	0
	TL>	15	15	15	15	1	15	15	15	15	0
	MN>	0.4	0.9	0.9	1.3	4.0	0.1	0.9	0.9	0.9	0.0
--Glomerulopathy	->	15	15	15	10	1	15	15	15	12	0
	1>	0	0	0	5	0	0	0	0	3	0
	TL>	15	15	15	15	1	15	15	15	15	0
	MN>	0	0	0	0.3	0	0	0	0	0.2	0.0
--Hypertrophy/hypercellularity, JGA	->	15	6	3	0	1	15	0	1	0	0
	1>	0	9	12	2	0	0	15	11	3	0
	2>	0	0	0	9	0	0	0	3	7	0
	3>	0	0	0	4	0	0	0	0	5	0
	TL>	15	15	15	15	1	15	15	15	15	0
	MN>	0.0	0.6	0.8	2.1	0.0	0.0	1.0	1.1	2.1	0.0

[a]: Group 1 = vehicle control, Group 2 = 100 mg/kg/day, Group 3 = 300 mg/kg/day ; Group 4 = 1000 mg/kg/day olmesartan; Group 5 = 400 mg/kg/day *p*-cresidine

[b]: Grades for severity:

-> finding not present
1> minimal – the least amount of change that can be observed with the light microscope
2> slight – less than average amount of change, but readily discernible as abnormal.
3> Moderate – the average amount of change that is expected for a lesion
4> Moderately severe (marked) – a marked amount of change with possible loss of function of the affected cells or organs
5> Severe

TL>: total; MN>: Mean

juxtaglomerular change that sometimes appeared to extend into the glomerulus. Minimal to slight hypertrophy/hypercellularity of the JGA was observed in most of the animals in low and mid dose groups, and minimal to moderate hypertrophy/hypercellularity was observed in all mice treated with 1000 mg/kg/day. It may be noted that the latter changes in the kidneys were infrequently observed in control animals. No remarkable microscopic changes were noted in any other organs in animals treated with olmesartan medoxomil.

Neoplastic microscopic findings: No drug-induced effects on number of tumor-bearing animals, number of animals bearing benign tumors, number of animals bearing malignant tumors or number of animals bearing multiple tumors were apparent in either sex of p53(+/-) mice that were killed or died during the treatment period, or killed at term (Table 3.3.3.3). The FDA statistician assigned 0.05 as the critical p- value for statistical significance for all comparisons. The sponsor did not perform statistical analysis on tumor findings since the incidence difference between groups was less than 2. A number of spontaneously occurring incidental neoplasms were noted in all groups including the vehicle control and these do not appear to have any relationship to treatment. Malignant lymphoma (lymphocytic) occurred in one female in each of the three olmesartan-treated groups and not in the concurrent vehicle treated group. Leukemia (granulocytic) occurred in a low dose female and a mid dose male and again was absent in the concurrent vehicle control group. Thus, hematological neoplasia was present in all treated groups (0/30, 2/30, 2/30and 1/30 in vehicle control, low, mid and high dose groups, respectively). The FDA analysis showed no statistically significant tumor findings in drug-treated animals of either sex. Subcutaneous fibrosarcoma was present in females, one each in the control, low and high dose groups (Table 3.3.3.4). The sponsor reports the incidences of lymphoma and leukemia in several published studies of various duration in p53(+/-), heterozygous (+/-) and wild type (+/+) mice (Table 3.3.3.5). On the basis of this published historical control data, it was concluded by the sponsor that OM demonstrated no carcinogenic potential in this transgenic mouse model.

TABLE 3.3.3.3
26 WEEK CARCINOGENICITY STUDY IN P53(+/-) MICE. SUMMARY OF NEOPLASTIC FINDINGS*

Dose (mg/kg/day)	Control		100		300		1000		Positive control[1]	
Sex	m	F	m	f	M	f	m	f	m	f
No. of animals examined	15	15	15	15	15	15	15	15	15	15
No. tumor-bearing animals	0	2	0	3	1	1	0	2	1	0
(% incidence)	0	13	0	20	7	7	0	13	7	0
Benign	0	0	0	0	0	0	0	0	0	0
Malignant	0	2	0	3	1	1	0	2	1	0
Benign + Malignant	0	0	0	0	0	0	0	0	0	0
No. primary tumors	0	2	0	3	1	1	0	2	1	0
Benign	0	0	0	0	0	0	0	0	0	0
Malignant	0	2	0	3	1	1	0	2	1	0

*: primary tumors.
1: 400 mg/kg/day p-cresidine.

TABLE 3.3.3.4
26 WEEK CARCINOGENICITY STUDY IN P53(+/-) MICE. INCIDENCE OF PRIMARY NEOPLASMS

ORGAN SYSTEM Organ/Tissue Neoplasm	TD[1]	Control		100		300		1000		Positive control[2]	
		m	f	m	f	M	F	m	f	m	f
		15	15	15	15	15	15	15	15	15	15
HEMATOPOIETIC SYSTEM											
Hematological neoplasia		0	0	0	2	1	1	0	1	0	0
M-leukemia, granulocytic	MA	0	0	0	1	1	0	0	0	0	0
M-lymphoma, lymphocytic	MA	0	0	0	1	0	1	0	1	0	0
INTEGUMENTARY SYSTEM											
Subcutaneous tissue		0	1	0	1	0	0	0	1	0	0
M-fibrosarcoma	MA	0	1	0	1	0	0	0	1	0	0
URINARY SYSTEM											
Urinary bladder		0	0	0	0	0	0	0	0	1	0
M-transitional cell carcinoma	MA	0	0	0	0	0	0	0	0	1	0
BODY CAVITIES											
Cavity, thoracic		0	1	0	0	0	0	0	0	0	0
M-osteosarcoma	MA	0	1	0	0	0	0	0	0	0	0

1: TD (tumor designation): BE = benign, MA = malignant
2: 400 mg/kg/day p-cresidine.
Tumor incidences were not analyzed statistically because the incidence difference between groups was less than 2.

TABLE 3.3.3.5
INCIDENCE OF SPONTANEOUS LEUKEMIA AND LYMPHOMAS IN P53(+/-) MICE

author (study)	tumor	p53+/- male	p53+/- female	wild type male	wild type female	p53-/- male	p53-/- female
Mahler et al (6 month study)	granulocytic leukemia malignant lymphoma	1/108 2/108	0/109 2/109				
Sagartz et al. (26 wk study)	leukemia	0/15	0/15	0/15	0/15		
Finch et al. (6 month study)	lymphoma	8/28-30					
Eastin et al. (24 wk study)	lymphoma	1/15	1/15				
Harvey et al. (spontaneous tumor study)	malignant lymphoma	19/97		5/112		33/60	
Donehower et al. (up to 2 year)	lymphomas	24/184				60/93	

A single transitional cell carcinoma in one male and hyperplasia of the transitional epithelium of the urinary bladder in all other *p*-cresidine treated animals are predictive of ultimate bladder tumorigenicity in these animals (Table 3.3.3.4). The changes were more severe in males than in females. The sponsor argues that multiple carcinomas may not be expected over a 26-week exposure period; however, the sequela of events leading to such a response is evident in all *p*-cresidine treated mice. A 2-year bioassay conducted by the NTP utilized relatively high dietary concentrations (>400 mg/kg/day) of *p*-cresidine and demonstrated a clear positive tumorigenic effect in the bladders of nearly all *p*-cresidine exposed mice. Historical tumor frequency with this agent in 6 twenty six week studies utilizing the p53(+/-) model (400 mg/kg/day *via* gavage, n=15/sex/study) conducted at the contract lab was highly variable and ranged from 0% (0/15) to 33.3% (5/15). Based on the data from several studies, Storer draws the following conclusion (Storer, R: *The p53 workgroup newsletter*, Vol. 1 (1), August 1998, Merck & Co., Inc. West Point, PA). *P*-cresidine appears to be working reliably as a positive control compound for induction of bladder tumors with dietary administration at dose levels from 2500 to 5000 ppm producing higher incidences of lesions than produced by daily gavage dosing in corn oil at 400 mg/kg.

Plasma concentrations of olmesartan showed a non-proportional increase over the dose range studied. Female mice exhibited slightly higher concentrations than male mice. However, the exposure of both male and female animals to test substance was much lower than that observed in the 4-week dose range-finding study (Table 3.3.3.5). In normal healthy male volunteers, 10 daily oral doses of 40 mg olmesartan medoxomil achieved a mean C_{max} of 0.73 µg/ml (study #866-102). Thus, at the high dose of 1000 mg/kg/day in male p53(+/-) mice, systemic exposure to olmesartan (mean concentration determined 2 hr post dose at 26 week, 3.23 µg/ml) was 4.5 times the exposure for humans.

TABLE 3.3.3.5.
PLASMA LEVELS OF OLMESARTAN IN P53(+/-) C57BL/6 MICE
Mean Plasma Concentrations (µg/ml)

Dose (mg/kg/day)	26-Week study		4-week dose range-finding study	
	Male	Female	Male	Female
100	0.72	1.02	2.58	6.63
250			2.13	4.99
300	1.47	1.93		
500			2.38	7.60
1000	3.23	6.63	3.71	12.26

In conclusion, olmesartan medoxomil was not carcinogenic when given to C57BL/6 p53(+/-) heterozygous mice by gavage at doses of 100, 300 or 1000 mg/kg/day. Non-neoplastic changes consisted of renal tubular regeneration and hypertrophy/hypercellularity of the JGA at all dose levels of olmesartan medoxomil. The JGA finding is consistent with the pharmacological site of action of test substance as an angiotensin II receptor blocker. Toxicokinetic analysis in the 26th week of dosing confirmed the exposure to the test substance; however, the exposure was much lower than that observed in a 28 day dose range-finding study. Further, there was no linear relationship between plasma concentration and dose level. The incidence of bladder carcinoma induced by the positive control agent *p*-cresidine was limited to a single male, though there were changes associated with the ultimate tumor phenotype in all *p*-cresidine treated animals.

3.3.4. 26-Week Oral Carcinogenicity Study of Olmesartan Medoxomil in Hras2 Transgenic Mouse (Study #99-B050, 99-0057, Report #APR 147-010, #APRC 147-001 (Toxicokinetics) Vol. 19

This **non-GLP** study was conducted by Medicinal Safety Research Laboratories, Sankyo, Co., Ltd., Fukuroi, Shizuoka-ken, Japan, between July 30, 1999 and April 28, 2000. The study was conducted to assess the potential toxicity and carcinogenic effects of olmesartan medoxomil (OM) when administered orally to Hras2 transgenic mice for 26 weeks.

Male and female CB6F1-TgHras2 mice [redacted] carrying the human prototype c-Ha-ras gene, were approximately 8 weeks old and weighed 20.1-29.1 g (males) or 17.9-24.1 g (females) at the time dosing was initiated. The mice were assigned to three main study groups (15/sex/group) and one satellite (toxicokinetic) group (10/sex/group). Olmesartan medoxomil (lot No. NH207C) was administered in the feed provided to one main study group and the satellite group at a concentration calculated to provide a dose level of 1000 mg/kg/day (Study #99-B050). The dietary mixes were prepared weekly. The mice in the vehicle control group received corn oil by gavage (2 ml/100 g body weight), whereas the mice in the positive control group received a single i.p. injection of 75 mg/kg N-methyl-N-nitrosourea (MNU) (in corn oil, 1 ml/100 g body weight) for 26 weeks. The latter two groups formed a separate protocol (Study #99-0057) that was performed in the same room at the same time and used the same lot of mice as received OM. All animals were housed individually and received food and water *ad libitum*. Both vehicle control and MNU-treated groups received diet in the form of pellets whereas OM-treated animals received powdered diet. Thus, the study lacked dietary control for test substance. The single dose level of OM chosen for this study was based on the results of the 26 week carcinogenicity study in p53(+/-) C57BL/6 mice (section 3.3.3). The sponsor had not submitted a protocol for this study in Hras2 mice.

Observations and Measurements

All animals were observed for clinical signs at least once daily throughout the study. Body weight and food consumption were recorded weekly from a week before the first day of dosing to week 13 and once every 4 weeks thereafter, and on the day of necropsy. Test substance intake was determined for each mouse based on olmesartan concentration in the diet, body weight and food intake. Hematology and clinical chemistry values were determined in all surviving mice on the day after administration of the final dose. The animals were anesthetized with ether and blood samples were collected from the inferior vena cava. The animals were then autopsied. Selected organs from all animals were removed and weighed. Histopathological examination was conducted on all preserved tissues (Table 3.3.4.1) from all main study animals. For toxicokinetics study, blood samples were collected from the inferior vena cava of satellite mice on day 184 (5:00 PM) or day 185 (1:00 AM) for males and day 180 (5:00 PM) or day 181 (1:00 AM) for females. These animals were euthanized and discarded without further examination.

TABLE 3.3.4.1.
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenals*	Liver *	Sciatic nerve
Aorta[1]	Lungs*	Seminal vesicles
bone & bone marrow (tibia and right femur	Lymph node -right submandibular -mesenteric -left renal	Skin
Brain*	Mammary gland (females only)	Spinal cord (cervical, thoracic, lumbar)
Cecum	Nasal cavity	Spleen*
Colon	Ovaries*	Stomach
Duodenum	Pancreas	Testes*
Eye (bilateral)	Pituitary *	Epididymides (bilateral)
Esophagus	Preputial gland[1]	Thymus*
Gallbladder	Prostate (ventral and dorsal)*	Thyroids with parathyroids
Harderian glands (bilateral)	Rectum	Tongue
Heart*	Salivary glands (bilateral)	Trachea
Ileum		Urinary bladder
Jejunum		Uterus*
Kidneys*		Vagina

*Organ weighed, [1]: for surviving mice only

Results

There were no clinical signs in either the vehicle control or OM-treated groups except for a nodule on the vagina of one control female (day 171 to 183) and a nodule on the pinna of one drug-treated male (day 77 to 89). There was a significantly higher incidence of nodules (at various sites in the body) in the MNU-treated animals, appearing as early as day 53 of treatment. In addition, irregular respiration, decreased feces volume, hypothermia and decreased activity were seen in most of the MNU treated mice. One vehicle control female died on day 94. There were no mortalities in mice treated with OM. In the MNU treated group, all mice died or were euthanized in a moribund condition prior to week 24 (Table 3.3.4.2). Deaths in the MNU treatment group were primarily attributed to malignant lymphoma.

TABLE 3.3.4.2.
26 WEEK CARCINOGENICITY STUDY IN MICE: MORTALITY
Day of death (number of deaths given in parentheses) in MNU treated group

Sex	Moribund	Death
Male	92 (1), 100 (2), 117 (2), 126 (1), 127 (2), 142 (1), 155 (2), 162 (1)	74, 99, 126 (1 each)
Female	78 (1), 115 (1), 122 (2), 123 (1), 124 (3), 129 (2), 138 (1), 157 (2), 158 (1)	108 (1)

The mean body weight of males treated with OM was lower than that of the vehicle control (Fig. 3.3.4.1). Statistically significantly lower mean body weights were observed on days 8-22, day 43 and days 85 to 184 (Table 3.3.4.3). It should be noted that mean body weight for this group was (non significantly) lower than control (~6% lower) prior to initiation of treatment. On the other hand, the mean body weight of females treated with OM was higher than vehicle control on days 22, 43, 50, 71 and 168 (Fig. 3.3.4.2). Body weights of male animals in the positive control group

were similar to those of control group males. Body weights of females in the positive control group were slightly higher than control on days 36 to 71. It may be noted that the OM treated group received their diet in powdered form, while the remaining groups that formed a separate study received the diet in pellet form.

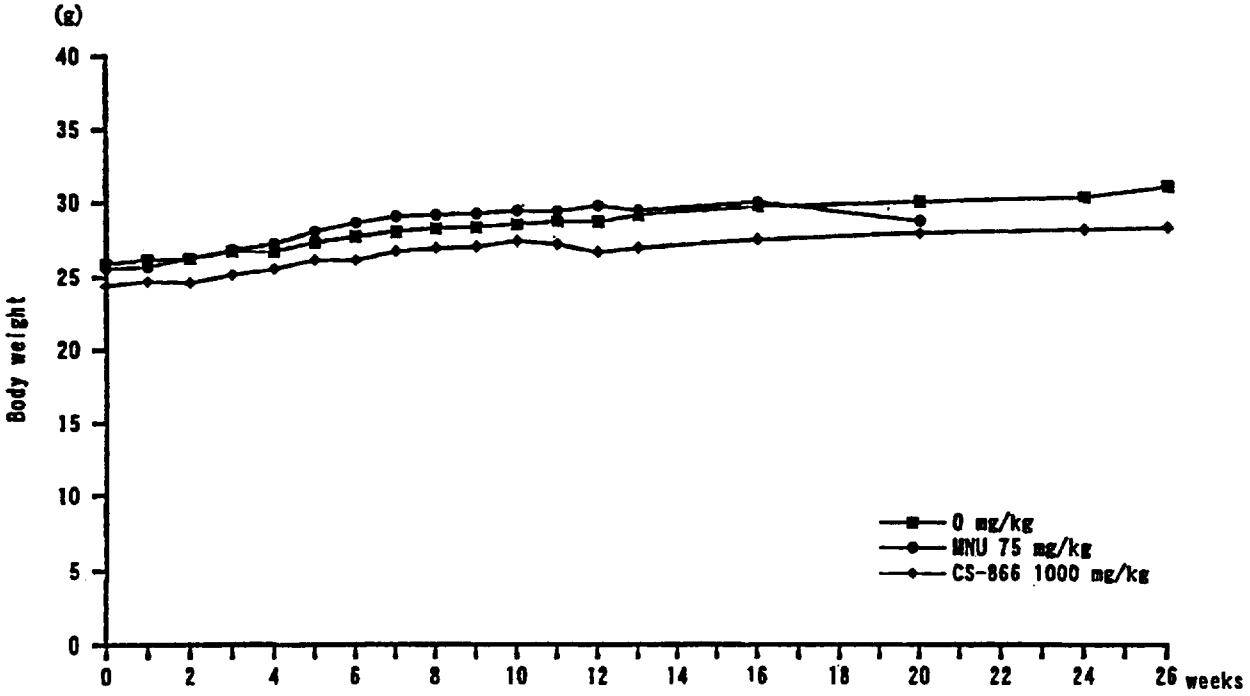

Fig. 3.3.4.1: Body weight changes in male CB6F1-TgHras2 mice treated with olmesartan medoxomil in their diet for 26 weeks

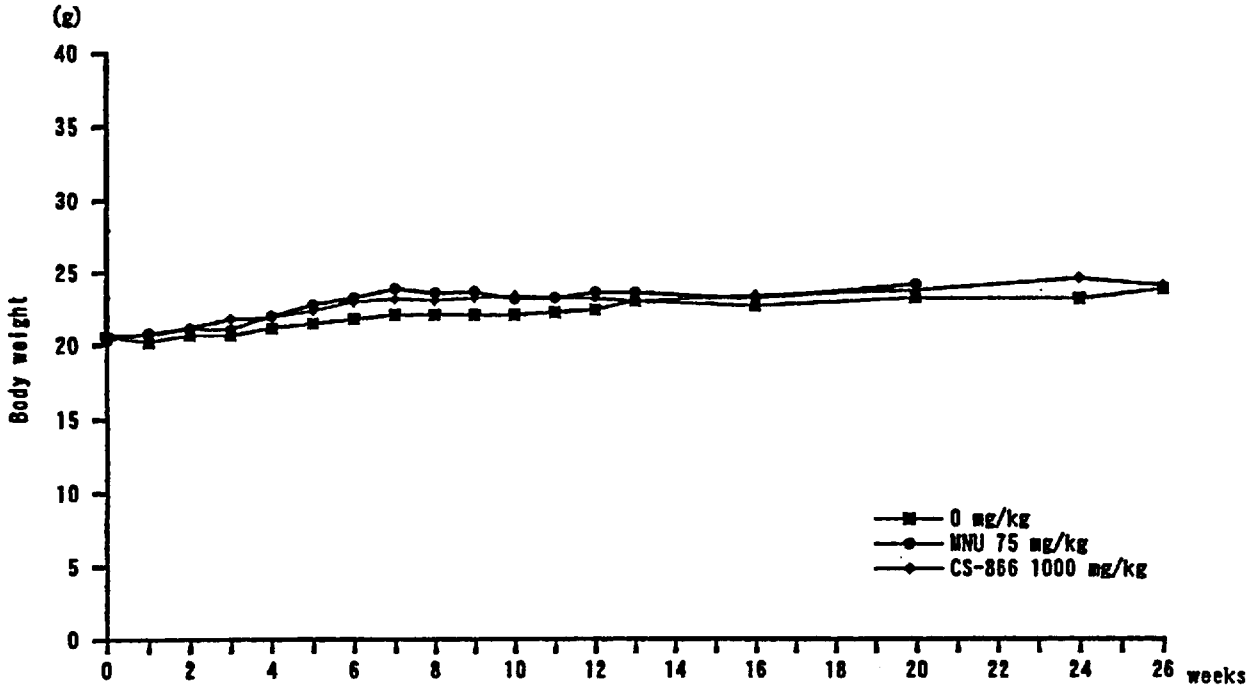

Fig. 3.3.4.2: Body weight changes in female CB6F1-TgHras2 mice treated with olmesartan medoxomil in their diet for 26 weeks

TABLE 3.3.4.3
26 WEEK CARCINOGENICITY STUDY IN MICE: GROUP MEAN BODY WEIGHTS

Study day	Body weight	Dosage (mg/kg/day)					
		Male			Female		
		Control	NMU	OM	Control	NMU	OM
1	B.wt., g	25.9	25.6	24.4	20.5	20.3	20.7
	% diff§		-1.2	-5.7		-1.0	+2.5
8	B.wt., g	26.2	25.7	24.7*	20.2	20.8	20.7
	% diff§		-2.0	-5.7		+3.0	+2.5
15	B.wt., g	26.3	26.3	24.6**	20.7	21.1	21.2
	% diff		-	-6.5		+2.0	+2.4
22	B.wt., g	26.8	26.9	25.2*	20.7	21.1	21.8**
	% diff		-	-6.0		+2.0	+5.3
43	B.wt., g	27.8	28.7	26.2*	21.8	23.3**	23.0*
	% diff		+3.0	-5.8		+6.9	+5.5
50	B.wt., g	28.1	29.1	26.8	22.1	23.9**	23.2*
	% diff		+3.6	-4.6		+8.1	+5.0
71	B.wt., g	28.6	29.5	27.5	22.1	23.2*	23.4*
	% diff		+3.0	-3.9		+5.0	+5.9
85	B.wt., g	28.8	29.9	26.8**	22.5	23.7*	23.3
	% diff		+3.8	-6.9		+5.3	+3.6
92	B.wt., g	29.3	29.6	27.1**	23.1	23.7	23.1
	% diff		+1.0	-7.5		+2.6	0
113	B.wt., g	29.9	30.2	27.7**	22.8	23.3	23.5
	% diff		+1.0	-7.4		+2.2	+3.0
141	B.wt., g	30.3	29.0	28.2*	23.4	24.3	23.9
	% diff		-4.3	-6.9		+3.8	+2.1
169	B.wt., g	30.7		28.5**	23.4		24.8*
	% diff			-7.2			+6.0
184	B.wt., g	31.5		28.7**	24.1		24.3
	% diff			-8.9			+0.8

§: Per cent (%) difference from control body weight
* $p < 0.05$ when compared to control; **: $p < 0.01$ when compared to control

Mean food consumption was statistically significantly greater in both OM and MNU-treated groups than in the vehicle control group. Males receiving MNU had an increased food intake on days 8 to 113 (27 to 57%), while females had an increased intake on days 8 to 71 and days 85 to 141 (21 to 46%). Similarly, males receiving OM had an increased food consumption on days 1 to 141 and day 184 (30 to 77%), and females receiving OM had an increased intake on days 1 to 71 and days 85 to 141 (26 to 61%).

Changes in hematological parameters included statistically significant decreases (8 to 14%) in erythrocytic parameters (RBC, hemoglobin, hematocrit) for males and females receiving OM. Mean MCH and MCHC values were slightly (3-4%) but significantly increased for females treated with OM. A moderate (6%) but significant increase in platelet count was noted in males. Statistically significant increases were observed for WBC (69-150%), absolute neutrophil count (42 to 56%) and absolute lymphocyte count (70 to 166%) in males and females receiving test substance (Table 3.3.4.4). All of these increases in hematology values are relative to concurrent control.

TABLE 3.3.4.4
EFFECT OF OLMESARTAN MEDOXOMIL ON HEMATOLOGY PARAMETERS IN 26 WEEK CARCINOGENICITY STUDY IN HRAS2 TRANSGENIC MOUSE

Group	sex	RBC M/μl	HgB (g/dl)	HT (vol%)	MCH Pg	MCHC %	Platelets k/μl	WBC k/μl	ANE k/μl	ALY k/μl
Vehicle	M	10.3	15.4	49.9	15.0	30.9	1130	1.2	0.33	0.79
	F	10.0	14.9	49.0	15.0	30.5	1024	1.3	0.34	0.94
Olmstn	M	9.4** (-9)	14.2** (-8)	45.8** (-8)	15.1	30.9	1202* (6)	3.0** (150)	0.75** (42)	2.10** (166)
	F	8.6** (-14)	13.4** (-10)	42.8** (-13)	15.6** (4)	31.3** (3)	1073	2.2** (69)	0.53** (56)	1.60** (70)

* : $p < 0.05$; ** : $p < 0.01$ when compared with vehicle control
Numbers in parentheses indicate % deviation from concurrent vehicle control
HgB: hemoglobin; HT: hematocrit; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; ANE: absolute neutrophil count; ALY: absolute lymphocyte count

Clinical chemistry findings included statistically significant ($p < 0.05$) increases in alkaline phosphatase, blood urea nitrogen and creatinine in males and females receiving OM. Higher than control levels ($p < 0.05$) of aspartate aminotransferase and alanine aminotransferase were observed in male mice dosed with the OM (Table 3.3.4.5).

TABLE 3.3.4.5
EFFECT OF OLMESARTAN MEDOXOMIL ON CLINICAL CHEMISTRY PARAMETERS IN 26 WEEK CARCINOGENICITY STUDY IN HRAS2 TRANSGENIC MOUSE

Group	sex	ALP		BUN		Creatinine		AST		ALT	
		u/l	Δ%	mg/dl	Δ%	mg/dl	Δ%	u/l	Δ%	u/l	Δ%
Vehicle	M	233		24.60		0.08		49.90		22.60	
	F	387		21.76		0.07		58.30		29.10	
Olmstn	M	267**	15	45.86**	86	0.09	13	57.0*	14	28.7*	27
	F	464**	20	31.50**	45	0.09**	20	65.7	13	26.9	-8

*: $p < 0.05$; **: < 0.01 when compared with control
Δ%: deviation from concurrent vehicle control

The most notable organ weight findings in OM treated mice were decreases in heart and thymus weights, statistically significant relative to concurrent control, irrespective of whether the comparison was done on an absolute or relative basis. The largest differences from control weight occurred for thymus (50% absolute, 47% relative for female; 30% absolute, 23% relative for males). For heart, a larger effect was seen in males (21% absolute, 13% relative) than in females (11% absolute, 12% relative). Statistically significant increases from concurrent control liver weights were seen in both males (9% absolute, 20% relative) and females (14% absolute, 12% relative). Adrenal weights for OM-treated males were significantly lower ($p < 0.01$) than control when compared on an absolute (25%) or relative (17%) basis. Kidney weights of OM

treated female rats were statistically significantly higher than concurrent control on an absolute (12%) and relative (10.5%) basis (Table 3.3.4.6).

TABLE 3.3.4.6
EFFECT OF OLMESARTAN MEDOXOMIL ON ORGAN WEIGHTS AFTER 26 WEEKS OF DOSING IN HRAS2 TRANSGENIC MOUSE

Group	sex	THYMUS		HEART		LIVER		ADRENALS		KIDNEY	
		Abs[1]	Rel[2]	Abs[1]	Rel[2]	Abs[1]	Rel[2]	Abs[1]	Rel[2]	Abs[1]	Rel[2]
Control	M	33.99	107.85	0.17	0.53	1.42	4.51	2.35	7.45	0.28	0.90
	F	25.32	105.59	0.13	0.55	1.22	5.08	3.91	16.28	0.19	0.80
Olmesartan	M	23.71**	83.15**	0.13**	0.46**	1.55**	5.43**	1.77**	6.25*	0.28	0.97*
	F	37.85**	154.89**	0.12**	0.49**	1.39**	5.70**	4.27	17.60	0.21**	0.88**

[1]: absolute weight in gm except for thymus and adrenal (mg)
[2]: relative to the final body weight in gm % except for thymus and adrenal (mg%)
*: $p < 0.05$; **: <0.01 when compared with control

The female mouse from the control group that died during the dosing period exhibited swelling of the spleen and lymph node and one nodule each in the thymus, the uterus and the lung. There were no macroscopic abnormalities that occurred at a higher than vehicle control incidence in the mice treated with OM which survived the scheduled dosing period. For the positive control group in which all animals died or were sacrificed moribund during the dosing period, the incidence of nodules in the forestomach, jejunum, thymus, and skin, swellings in the spleen and lymph node, and hydrothorax in the thoracic cavity were significantly higher than in the concurrent control group (Table 3.3.4.7).

TABLE 3.3.4.7
INCIDENCE OF MACROSCOPIC FINDINGS AFTER 26 WEEKS OF DOSING IN HRAS2 TRANSGENIC MOUSE

Macroscopic finding	Group 1 (Control		Group 2 (NMU)		Group 3 (Olmesartan)	
	M	F	M	F	M	F
Forestomach, nodule	2	1	15**	15**	1	0
Jejunum, nodule	0	0	1	5*	0	0
Spleen, mottle	2	2	0	4	0	3
Swelling	0	1	5*	9**	0	0
Thymus, nodule	0	1	10**	10**	0	0
Lymph node, swelling	0	1	5*	5	0	1
Submandibular lymph node swelling	0	1	4*	4	0	0
Renal lymph node swelling	0	1	5*	7*	0	0
Mesenteric lymph node swelling	0	1	4*	7*	0	0
Skin nodule	0	1	0	7*	0	0
Kidney, discoloration	0	0	3	3	0	0
Mottle	0	0	2	2	0	0
Nodule	0	0	1	1	0	0
Thoracic cavity, hydrothorax	0	0	7**	6**	0	0

*: $p < 0.05$, **: $p < 0.01$

Non-neoplastic histopathology considered to be related to treatment consisted of mild juxtaglomerular (JG) cell hyperplasia in the kidneys of all mice receiving OM. None of the mice in the vehicle or positive control group showed JG cell hyperplasia in the kidney. The finding is consistent with the site of action of olmesartan. Additionally, an OM treated female exhibited focal nephropathy and dilatation of the pelvic space. Other findings of interest in this group were increased incidences of dilatation of the perpetual gland in males (7 *versus* 4 in the vehicle control, $p > 0.05$)) and cystic endometrial hyperplasia of the uterus in females (4 *versus* 3 in the vehicle control group, $p > 0.05$).

Neoplastic histopathology findings: The FDA statistician assigned 0.05 as the critical p- value for statistical significance for all comparisons. Tumor findings in the drug treated mice were limited to alveolar bronchiolar adenoma in the lung (2 males and 2 females) and squamous cell papilloma in the forestomach (1 male). There were no significant differences between the incidences of neoplastic lesions or pre-neoplastic proliferative changes in the OM and vehicle control groups (Tables 3.3.4.8 and Table 3.3.4.9). Malignant lymphomas, squamous cell papillomas and carcinomas in the forestomach, and hemangio-tumors were observed at a higher than vehicle control incidence in positive control mice. One vehicle control female mouse that died on day 94 exhibited malignant lymphoma in the spleen, thymus and lymph node, and an alveolar/bronchiolar adenoma in the lungs.

TABLE 3.3.4.8
CARCINOGENICITY STUDY IN HRAS2 MICE. SUMMARY OF NEOPLASTIC FINDINGS

Dose (mg/kg/day	0 Corn oil		75 MNU		1000 OM	
Sex	m	f	m	f	m	f
No. of animals examined	**15**	**15**	**15**	**15**	**15**	**15**
No. tumor-bearing animals	3	4	15[1)]	15[1)]	2	2
(% incidence)	20	27	100	100	13	13
Benign	2	2	1	0	2	2
Malignant	1	0	2	0	0	0
Benign + Malignant	0	2	12[1)]	15[1)]	0	0
No. with multiple tumors	0	2	12[1)]	15[1)]	1	0
(% incidence)	0	13	80	100	7	0
No. primary tumors	3	7	35	60	3	2
Benign	2	5	17	37	3	2
Malignant	1	2	18	23	0	0

[1)] Statistically significant increase ($p<0.01$) compared to control group

TABLE 3.3.4.9

26 WEEK CARCINOGENICITY STUDY IN HRAS2 MICE. INCIDENCE OF PRIMARY NEOPLASMS

ORGAN SYSTEM / Organ/Tissue / Neoplasm	TD	Dose level (mg/kg/day)					
		Corn oil		MNU		Olmesartan	
		15M	15F	15M	15F	15M	15F
DIGESTIVE SYSTEM							
Forestomach (n)		15	15	15	15	15	15
Squamous cell papilloma	BE	1	1	13[1)]	13[1)]	1	0
Squamous cell carcinoma	MA	1	0	2	4[2)]	0	0
Duodenum (n)		15	15	14	15	15	15
Adenoma	BE	0	0	0	1	0	0
Jejunum (n)		15	15	15	15	15	15
Adenoma	BE	0	0	0	1	0	0
Adenocarcinoma	MA	0	0	1	4[2)]	0	0
Colon (n)		15	15	15	15	15	15
Adenoma	BE	0	0	1	0	0	0
Adenocarcinoma	MA	0	0	1	0	0	0
Rectum (n)		15	15	15	15	15	15
Adenoma	BE	0	0	0	2	0	0
Adenocarcinoma	MA	0	0	1	0	0	0
HEMATOPOIETIC SYSTEM							
Spleen (n)		15	15	15	15	15	15
Hemangiosarcoma	MA	0	1	0	0	0	0
Generalized tumors (n)		15	15	15	15	15	15
Malignant lymphoma	MA	0	1	13[1)]	13[1)]	0	0
INTEGUMENTARY SYSTEM							
Skin (n)		15	15	15	15	15	15
Squamous cell papilloma	BE	0	1	0	7[2)]	0	0
Subcutis (n)		15	15	15	15	15	15
Hemangiosarcoma	MA	0	0	0	1	0	0
REPRODUCTIVE SYSTEM							
Uterus (n)			15		15		15
Hemangioma	BE		0		1		0
Hemangiosarcoma	MA		0		1		0
RESPIRATORY SYSTEM							
Trachea (n)		15	15	15	15	15	15
Papilloma	BE	0	0	1	0	0	0
Lung (n)		15	15	15	15	15	15
Alveolar/Bronchiolar adenoma	BE	0	2	1	3	2	2
SPECIAL SENSE ORGANS							
Harderian gland (n)		15	15	15	15	15	15
Adenoma	BE	0	1	1	3	0	0
URINARY SYSTEM							
Kidney (n)		15	15	15	15	15	15
Tubular cell adenoma	BE	0	0	0	1	0	0
Urethra (n)		0	0	0	5	0	0
Transitional cell papilloma	BE	0	0	0	5[2)]	0	0
BODY CAVITIES							
Abdominal cavity* (n)		1	0	0	0	0	0
Adenoma	BE	1	0	0	0	0	0

Statistically significant increase compared to control group: 1) : ($p<0.01$); 2) : ($p<0.05$)

TD : Tumor designation, BE: Benign, MA: Malignant

* : Organ or tissue that was examined due to abnormal macroscopic finding

(n) :The numbers in each row for an organ or tissue indicate the number of animals examined

The plasma concentrations of olmesartan determined after 6 months of treatment at two different times of day (5 PM and 1AM of next day) were not significantly different from each other. Female mice exhibited slightly higher plasma concentrations of olmesartan than male mice. However, the exposure of both male and female animals to test substance was much lower than that observed in a 14-day study (study not evaluated) in which mice of the same strain received a single 1000 mg/kg/day dose of olmesartan in the diet (Table 3.3.4.10). In normal healthy male volunteers, 10 daily oral doses of 40 mg olmesartan medoxomil achieved a mean AUC of 4.37 μg.h/ml (study #866-102). Based on mean AUC values, the systemic exposure at the dose of 1000 mg/kg/day in male Hras2 transgenic mice (47.4 μg.h/ml) exceeds the human AUC by a factor of 10.9.

TABLE 3.3.4.10.
PLASMA LEVELS OF OLMESARTAN IN HRAS2 TRANSGENIC MOUSE

Sex	26-Week Study[1]			2-Week Study*	
	Mean Plasma Concentrations (μg/ml)		AUC_{0-24} μg.h/ml	Mean Plasma Concentrations (μg/ml)	
Interval	5 PM	1 AM		Day 4	Day 14
Male	2.03	1.92	47.4	4.43	4.42
Female	2.60	2.80	64.8	5.22	6.13

[1] Blood sampled between days 180 and 184 of dosing
* Report #APRC 147-005, study not evaluated

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3.4. ***Mutagenicity Studies***

3.4.1. Ames Assay. *In Vitro* Bacterial Test of Olmesartan Medoxomil (Report #TR 138-181, Study #92-0004) Vol 17

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between December 19, 1991 and June 15, 1992.

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 a highly UV-sensitive clone, WP2uvrA. The metabolic activation system was S9, prepared from the liver of a male Sprague-Dawley rat given two inducers, phenobarbital and 5,6-benzoflavine. Olmesartan medoxomil (OM) was dissolved in dimethylsulfoxide (DMSO) and used in final concentrations of 5%, 2%, 1%, 0.5%, and 0.2%.

A dose range-finding study, used to determine the highest dose level for the reverse mutation study, employed OM (lot #4) doses of 5 to 5000 μg/plate. These doses resulted in 10% to 50% inhibition of bacterial growth either in the presence or absence of the S9 mixture. Based on this, the reverse mutation assay was carried out using 5000 μg/plate as the highest dose. For each dose, 2 plates were used. The validity of the test system was confirmed by the results obtained with positive control agents for each bacterial strain. The criteria for a positive result was a dose-dependent increase in revertant colony count reaching 2 or more times the corresponding solvent control count; 3 or more times the corresponding solvent control count in the case of TA1535 and TA1537. Statistical analyses were not carried out.

There was a dose-related trend toward an increase in the number of revertants with the TA1537, TA98 and WP2uvrA strains; these trends were not affected by metabolic activation. The revertant numbers of TA1537 at 5000 μg/plate in the direct system and TA1535 at 2000 μg/plate in the activation system were 2.5- and 2-fold higher, respectively, than the corresponding vehicle control counts (Table 3.4.1.1, Fig. 3.4.1.1). However, such increases are not considered evidence of mutagenicity based on the sponsor's criteria for a positive response with these strains.

It is concluded that olmesartan medoxomil tested negative in the reverse mutation assay.

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TABLE 3.4.1.1
RESULTS OF THE REVERSION STUDY (REPORT #TR 138-181).

S9 Mix	Dose µg/plate	Revertant Number (Colony Number/Plate)									
		Base Change Type						Frame-shift Type			
		TA100		TA1535		WP2uvrA		TA98		TA1537	
-	Vehicle	74,93 100,108	(94)	2,4 4,8	(5)	11,12 14,16	(13)	8,9 10,12	(10)	2,3 5,5	(4)
	OM 200	96,97 99,101	(98)	6,6 7,9	(7)	12,14 15,15	(14)	9,10 11,11	(10)	2,4 7,8	(5)
	500	88,102 110,125	(106)	4,4 6,8	(6)	8,14 14,18	(14)	10,10 13,14	(12)	2,4 5,6	(4)
	1000	89,99 106,118	(103)	5,6 7,9	(7)	11,13 16,18	(15)	10,10 11,14	(11)	3,5 6,7	(5)
	2000	95,98 106,113	(103)	4,5 5,8	(6)	18,19 19,23	(20)	9,10 14,20	(13)	3,4 8,9	(6)
	5000	104,110 120,126	(115)	5,6 8,9	(7)	19,20 23,25	(22)	13,18 19,21	(18)	8,8 11,11	(10)
+	Vehicle	93,94 106,112	(101)	3,4 6,8	(5)	13,15 20,20	(17)	12,14 17,18	(15)	5,5 6,7	(6)
	OM 200	89,94 100,112	(99)	5,6 7,7	(6)	16,17 19,2	(19)	10,14 17,17	(15)	4,9 11,21	(9)
	500	97,108 112,118	(109)	5,10 10,11	(9)	19,23 31,34	(27)	14,15 19,21	(17)	7,9 12,13	(10)
	1000	98,106 113,119	(109)	6,7 8,9	(8)	15,18 19,20	(18)	16,17 18,21	(18)	5,6 9,10	(8)
	2000	88,90 100,130	(102)	5,6 14,15	(10)	18,26 27,32	(26)	11,18 24,33	(22)	9,9 11,13	(11)
	5000	98,109 114,137	(115)	8,9 9,10	(9)	26,29 30,40	(31)	17,24 28,33	(26)	9,10 11,14	(11)
Positive controls											
-	Name	AF2		SAZ		AF2		AF2		ICR	
	Dose	0.01 µg/plate		0.5 µg/plate		0.01 µg/plate		0.1 µg/plate		1 µg/plate	
	Colony No/plate	411,427 441,471	(438)	437,476 504,523	(485)	117,118 130,133	(125)	271,308 337,339	(314)	85,86 86,98	(89)
+	Name	BaP		2AA		2AA		BaP		2AA	
	Dose	5 µg/plate		2 µg/plate		20 µg/plate		5 µg/plate		2 µg/plate	
	Colony No/plate	555,569 702,771	(649)	60,70 75,75	(70)	197,264 282,297	(260)	132,142 162,177	(153)	72,80 89,89	(83)

Values in the parentheses are the mean colony numbers.
Vehicle control: DMSO
OM: Olmesartan medoxomil
Abbreviations in the positive controls
AF2: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide
SAZ: sodium azide
ICR: ICR-191
2AA: 2-aminoanthracene
BaP: benzo(a)pyrene

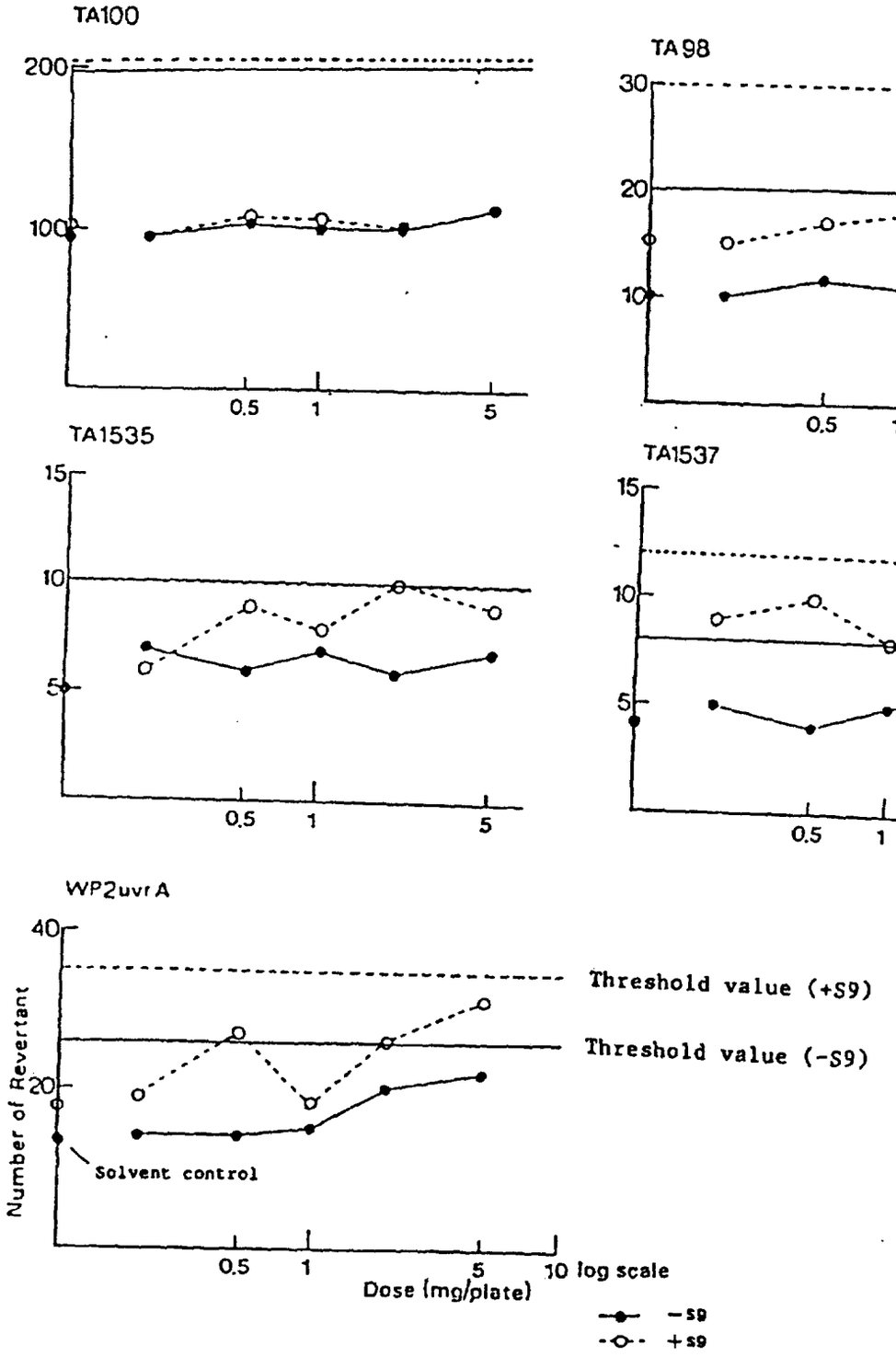

Fig 3.4.1.1.: Reverse mutation study of olmesartan medoxomil

3.4.2. *In Vitro* Bacterial Test. Second study (Report #. [redacted] Study #129-057) Vol. 17

This GLP study is similar to that of previous study (#TR138-181) except that it was conducted by a contract lab [redacted] and the test compound was synthesized by a different method. The study was initiated on October 4 and completed on December 10, 1996.

The experimental design and methodology are essentially similar to that described in the previous section. Olmesartan medoxomil (OM) (lot #NH203C) and positive control compounds (see section 3.4.1) were dissolved in DMSO. Based on the results of the previous study and a dose-finding study, this study was carried out using 5000 μg/plate as the highest dose, and other doses were settled by a serial dilution of the maximum dose with a common ratio of 2. For each dose, 2 plates were used. Test substance was judged as positive when the revertant colony counts exhibited dose-dependency and reached a level at least twice the corresponding solvent control. Statistical analyses were not carried out.

Results: No growth inhibition was observed for any of the tester strains with OM (data not given in the report). The revertant numbers did not increase with dose for any of the tester strains both in the direct and metabolic activation systems. The positive control compounds significantly induced reverse mutation in each strain. The results are summarized in the following pages (Tables 3.4.2.1 and 3.4.2.2).

Based on these results it is concluded that olmesartan medoxomil did not induce gene mutation in bacteria under the conditions of the study.

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TABLE 3.4.2.1
RESULTS OF THE BACTERIAL REVERSION TEST OF OLMESARTAN MEDOXOMIL. DIRECT METHOD

Compound	Dose (μg/plate)	Mean revertant colonies per plate				
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
DMSO a)	0	107	11	21	27	9
CS-866	156	122	11	18	18	10
	313	120	12	16	18	7
	625	119	11	20	18	9
	1250	111	9	20	21	12
	2500	125	11	20	23	12
	5000	123	13	23	21	14
Positive control compound		AF-2	NaN_3	AF-2	AF-2	ACR
Dose (μg/plate)		0.01	0.5	0.01	0.1	80
Mean revertant colonies per plate		665	527	140	416	483

AF-2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide NaN_3 : Sodium azide ACR : 9-Aminoacridine
a): Solvent control

TABLE 3.4.2.2
RESULTS OF THE BACTERIAL REVERSION TEST OF OLMESARTAN MEDOXOMIL. INDIRECT METHOD, +S9 MIX

Compound	Dose (μg/plate)	Mean revertant colonies per plate				
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
DMSO a)	0	115	18	26	28	14
CS-866	156	118	10	25	25	15
	313	109	13	26	32	9
	625	114	17	19	29	8
	1250	120	19	20	27	10
	2500	122	13	25	30	10
	5000	116	17	37	41	15
Positive control compound		2-AA	2-AA	2-AA	2-AA	2-AA
Dose (μg/plate)		1	2	10	0.5	2
Mean revertant colonies per plate		462	288	452	237	143

2-AA : 2-Aminoanthracene
a): Solvent control

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3.4.3. Mutagenicity Studies of Compounds Related to Olmesartan Medoxomil: Gene Mutation Assays of RNH6270 and RNH-8097 in Bacteria (Report #TR 142-075, Study #94-B027). Vol. 18

This non-GLP study was conducted by Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between March 10, 1994 and August 18, 1995. The study investigated the mutagenic effects of RNH-6270 (olmesartan), the active metabolite (de-esterified form) of olmesartan medoxomil (OM) and RNH-8097, the ester side chain of OM.

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 a highly UV-sensitive clone, WP2uvrA. The metabolic activation system was S9, prepared from the livers of 7-week old male Sprague-Dawley rats given two inducers, phenobarbital and 5,6-benzoflavone. Olmesartan (lot #5) and RNH-8097 (lot #A1548-8097) were dissolved in dimethylsulfoxide (DMSO) to a concentration of 50 mg/ml (5%). A dose range-finding study was not performed. Each test substance was tested at the maximal concentration of 5000 μg/plate along with four lower concentrations. In addition to these 5 dose groups, solvent control and positive control groups were included in the test. Two plates containing the medium were used at each dose level for each tester strain. Each tester strain was also treated with an appropriate positive control substance.

TA1535: sodium azide (direct method), 2-aminoanthracene (metabolic activation method)
TA1537: ICR-191 (direct method), 2-aminoanthracene (metabolic activation method)
TA98 and TA100: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (direct method), benzopyrene (metabolic activation method)
WP2*uvr*A: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (direct method), 2-aminoanthracene (metabolic activation method)

Responses were considered positive when the mean number of revertant colonies in a treated group was more than twice that of the solvent control group, and the increase in the number of revertant colonies also showed a dose-dependency. However, for TA1535 and TA1537, the criterion used for a positive response was a 3-fold or greater increase over solvent control colony count. Statistical analyses were not carried out.

Results

No dose-dependent increase in the number of revertant colonies was observed with olmesartan with any tester strain with or without metabolic activation. However, in cultures treated with RNH-8097 in the absence of metabolic activation, a dose-dependent increase in revertant colonies of TA100 was observed. Smaller increases in revertant colonies were seen with tester strains TA1537 and WP2*uvr*A in the absence of metabolic activation (Table 3.4.3.1). In the experiments utilizing metabolic activation, the highest revertant counts were seen at the highest tested dose of RNH-8097 with all tester strains but TA1537. However, all colony counts were less than twice solvent control. In conclusion, mutagenic responses to RNH-8097 were marked in TA100 and weakly positive in TA1537 and WP2*uvr*A.

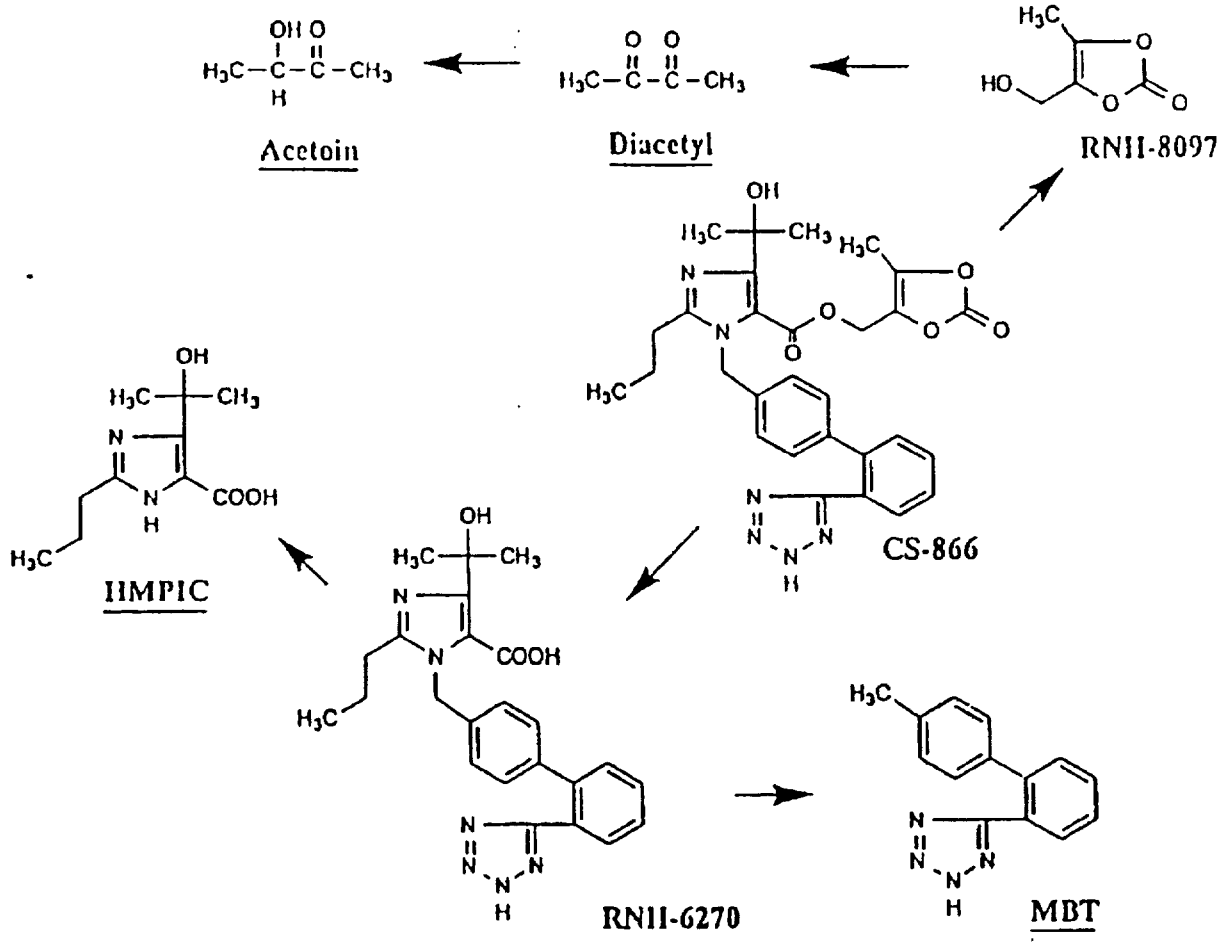
TABLE 3.4.3.1
RESULTS OF REVERSE MUTATION TEST OF RNH-8097

S9 mix	Agent	Dose µg/plate	Numbers of revertants/plate (Average)														
			TA100			TA1535			WP2*uvr*A			TA98			TA1537		
-	Vehicle		110	118	(114)	10	6	(8)	18	14	(16)	12	8	(10)	2	3	(3)
	RNH-8097	200	118	107	(113)	3	7	(5)	20	17	(19)	11	18	(15)	2	4	(3)
		500	138	131	(135)	8	7	(8)	20	23	(22)	8	15	(12)	3	3	(3)
		1000	167	162	(165)	7	4	(6)	29	28	(29)	17	18	(18)	9	8	(9)
		2000	314	275	(295)	10	11	(11)	34	32	(33)	10	7	(9)T	8	6	(7)
		5000	554	561	(558)	13	9	(11)	40	37	(39)						
	Positive control	Agent*	AF2 (0.01)			SAZ (0.5)			AF2 (0.01)			AF2 (0.1)			ICR (1)		
			417	400	(409)	264	253	(259)	172	186	(179)	457	431	(444)	556	573	(565)
+	Vehicle		101	95	(98)	6	5	(6)	25	36	(31)	26	26	(26)	9	7	(8)
	RNH-8097	200	103	110	(107)	4	11	(8)	29	31	(30)	23	23	(23)	4	3	(4)
		500	124	108	(116)	9	3	(6)	22	19	(21)	20	22	(21)	3	9	(6)
		1000	120	107	(114)	8	6	(7)	33	29	(31)	24	18	(21)	7	4	(6)
		2000	141	128	(135)	8	7	(8)	25	42	(34)	21	20	(21)	11	11	(11)
		5000	196	179	(188)	9	10	(10)	40	37	(39)	36	29	(33)	6	11	(9)
	Positive control	Agent*	BaP (5)			2AA (2)			2AA (20)			BaP (5)			2AA (2)		
			873	971	(922)	209	231	(220)	816	877	(847)	201	161	(181)	109	91	(100)

AF2: 2-(2.Furyl)-3-(5-nitro-2-furyl)acrylamide, SAZ: Sodium azide, ICR: ICR-191, BaP: benzo(a)pyrene, 2AA: 2-Aminoanthracene
T: Cytotoxic effects were observed
* : µg/plate

3.4.4. Mutagenicity Studies of Compounds Related to Olmesartan Medoxomil II. Gene Mutation Assays of HMPIC, MBT, Diacetyl and Acetoin in Bacteria (Report #TR 141-145, Study #94-B067). Vol. 17

This non-GLP was study conducted by Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between September 6, 1994 and February 16, 1995. The study investigated the mutagenic effects of metabolites and compounds related to olmesartan medoxomil (CS-866 in the figure). Olmesartan medoxomil (OM) is a prodrug, which is hydrolyzed by aryl esterase (present in mice, rats, dogs and humans) to olmesartan (RNH-6270). The ester side chain, RNH-8097, has not been detected in any species. Diacetyl and acetoin are tautomers and are formed from both RNH-8097 and OM. There is virtually no further metabolism of olmesartan (for details, see ADME section #2.8). HMPIC (5-(1-hydroxy-1-methylethyl)-2-propylimidazole-4-carboxylic acid) and MBT (5-(4'-methylbiphenyl-2-yl) tetrazole) are components included in the structure of olmesartan.



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 a highly UV-sensitive clone, WP2uvrA. The metabolic activation system was S-9 mix, prepared from the livers of 7-week old male Sprague-Dawley rats given two

inducers, phenobarbital and 5,6-benzoflavone. HMPIC (lot number not given), MBT (lot number not given), diacetyl ([redacted] lot #24H3497) and acetoin ([redacted] lot #V4H4056) were dissolved in dimethylsulfoxide (DMSO) to a concentration of 50 mg/ml (5%). A dose range-finding study was not performed. Each test substance was tested at the maximal concentration of 5000 μg/plate along with four lower concentrations (200, 500, 1000 and 2000 μg/plate). In addition to these 5 dose groups, solvent control and positive control groups were included in the test. Two plates containing the medium were used at each dose level for each tester strain. Each tester strain was also treated with an appropriate positive control substance.

TA1535: sodium azide (direct method), 2-aminoanthracene (metabolic activation method)
TA1537: ICR-191 (direct method), 2-aminoanthracene (metabolic activation method)
TA98 and TA100: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (direct method), benzopyrene (metabolic activation method)
WP2*uvr*A: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (direct method), 2-aminoanthracene (metabolic activation method)

Responses were considered positive when the mean number of revertant colonies in a treated group was 2 or more times the solvent control group, and the increase in the number of revertant colonies also showed a dose-dependency. However, for TA1535 and TA1537, the criterion used for a positive response was a 3-fold or greater increase over solvent control colony count. Statistical analyses were not carried out.

Results

No toxicity and no dose-dependent increase in the number of revertant colonies was observed with HMPIC or acetoin. Though MBT showed no positive response in terms of revertants, toxic effects to the testers were observed at all three high doses, more so with the direct method than with the metabolic activation method. In contrast, diacetyl caused a greater than 2-fold increase in revertants relative to vehicle control in tester strains TA100 and WP2uvrA (almost 2-fold in TA98) in the presence of S-9 mix. Additionally, a dose-dependent increase in revertant colonies was observed with all of the above three tester strains (Table 3.4.4.1). The toxic effects for testers were more clearly observed with the direct method than with the metabolic activation method. All of the testers showed expected responses to the corresponding positive controls (marked increase in revertants) with both methods.

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TABLE 3.4.4.1
REVERSE MUTATION TEST OF DIACETYL.

S9 Mix	Agent	Dose *	Number of revertants / plate (Average)				
			TA100	TA1535	WP2uvrA	TA98	TA1537
-	Vehicle		84 109 (97)	8 9 (9)	17 25 (21)	11 12 (12)	2 3 (3)
	Diacetyl	200	151 185 (168)	6 12 (9)	31 43 (37)	4 4 (4) T2	2 6 (4)
		500	158 199 (179)	8 10 (9)	33 43 (38)	0 0 (0) T3	8 8 (8) T2
		1000	114 119 (117)	2 4 (3)	33 45 (39)	0 0 (0) T4	3 3 (3) T3
		2000	0 0 (0) T3	0 0 (0) T3	17 23 (20) T1	0 2 (1) T4	0 0 (0) T3
		5000	0 0 (0) T4	0 0 (0) T4	0 1 (1) T3	0 0 (0) T4	0 0 (0) T4
	Positive Control	Agent(*)	AF2 (0.01)	SAZ (0.5)	AF2 (0.01)	AF2 (0.1)	ICR (1)
			347 360 (354)	211 248 (230)	160 187 (174)	468 499 (484)	388 414 (401)
+	Vehicle		101 122 (112)	6 10 (8)	26 31 (29)	15 21 (18)	3 7 (5)
	Diacetyl	200	144 156 (150)	7 14 (11)	36 45 (41)	32 36 (34)	7 7 (7)
		500	174 224 (199)	9 13 (11)	63 67 (65)	31 35 (33)	6 9 (8)
		1000	232 232 (232)	6 11 (9)	61 61 (61)	32 37 (35)	7 15 (11)
		2000	63 87 (75) T2	3 4 (4) T2	32 40 (36)	2 4 (3) T2	6 9 (8) T2
		5000	0 0 (0) T4	0 0 (0) T4	0 1 (1) T3	0 0 (0) T4	0 0 (0) T4
	Positive Control	Agent(*)	BaP (5)	2AA (2)	2AA (20)	BaP (5)	2AA (2)
			889 1025 (957)	312 315 (314)	895 937 (916)	199 242 (221)	237 265 (251)

* : μg/plate Vehicle : DMSO AF2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
SAZ : Sodium azide ICR : ICR-191 BaP : Benzo(a)pyrene 2AA : 2-Aminoanthracene

TABLE 3.4.4.2
REVERSE MUTATION TEST OF MBT

S9 Mix	Agent	Dose *	Number of revertants / plate (Average)				
			TA100	TA1535	WP2uvrA	TA98	TA1537
-	Vehicle		86 89 (88)	8 10 (9)	17 31 (24)	10 12 (11)	2 5 (4)
	MBT	200	54 66 (60)	0 1 (1)	13 20 (17)	9 9 (9)	0 0 (0)
		500	6 7 (7) T3	0 0 (0)	12 21 (17)	4 10 (7)	0 1 (1)
		1000	9 14 (12) T3	0 0 (0) T3	15 15 (15)	0 0 (0) T2	0 0 (0) T4
		2000	2 4 (3) T3	0 0 (0) T3	15 22 (19)	0 0 (0) T3	0 0 (0) T4
		5000	0 4 (2) T3	0 0 (0) T3	15 21 (18)	0 0 (0) T3	0 0 (0) T4
	Positive Control	Agent(*)	AF2 (0.01)	SAZ (0.5)	AF2 (0.01)	AF2 (0.1)	ICR (1)
			305 311 (308)	190 197 (194)	129 144 (137)	459 463 (461)	111 131 (121)
+	Vehicle		119 121 (120)	3 8 (6)	24 27 (26)	9 15 (12)	7 9 (8)
	MBT	200	101 106 (104)	8 9 (9)	30 40 (35)	13 22 (18)	0 6 (3)
		500	87 90 (89)	3 6 (5)	23 26 (25)	12 15 (14)	3 4 (4)
		1000	44 54 (49)	1 2 (2)	22 29 (26)	8 12 (10)	1 2 (2)
		2000	2 9 (6) T2	0 0 (0)	21 22 (22)	6 8 (7)	0 0 (0) T3
		5000	3 6 (5) T3	0 0 (0) T3	15 17 (16)	0 0 (0)	0 0 (0) T4
	Positive Control	Agent(*)	BaP (5)	2AA (2)	2AA (20)	BaP (5)	2AA (2)
			817 921 (869)	262 282 (272)	626 639 (633)	173 201 (187)	232 250 (241)

* : μg/plate Vehicle : DMSO AF2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
SAZ : Sodium azide ICR : ICR-191 BaP : Benzo(a)pyrene 2AA : 2-Aminoanthracene

3.4.5. Chromosome Aberration Test of Olmesartan Medoxomil in CHL Cells (Report #TR 140-049,Study #93-0032). Vol. 17.

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between February 23 and October 27, 1993.

Tests employing a Chinese hamster lung (CHL) fibroblast cell line were done for olmesartan with and without metabolic activation using an S9 mixture. The S9 was derived from the liver of a Sprague-Dawley rat (7 weeks of age), which was pretreated with a combination of phenobarbital and 5,6-benzoflavone. Tests conducted without the S9 mixture are considered to have been conducted by the "direct method." Cells were exposed to a medium containing test substance for 24 or 48 hours with the direct method, and for 6 hours (with or without S-9) with the metabolic activation method, followed by continued culture for an additional 18 hours with a fresh medium excluding the test substance and S9 mixture.

In a preliminary dose-finding study, olmesartan medoxomil (OM) (Lot #NH001C3) was dissolved in DMSO and used in doubling dilutions, the highest concentration being 10 mM. Based on the results obtained, a second dose-finding study was done, with scoring of mitotic indices in chromosome preparations of the treated cells. In the second dose finding study, the maximum dose levels were set at 0.625 mM for the 24 hr treatment and 0.313 mM for the 48 hour treatment using the direct method, and 2.5 mM olmesartan using the metabolic activation method (with or without S9 mixture). Appropriate negative and positive control groups were included. The full chromosome aberration study for olmesartan employed the maximum doses derived from the dose-finding study, with three lower dose levels derived by doubling dilution. The highest doses used were 0.446 mM (direct method, 24 h), 0.261 mM (direct method, 48 h), 2.08 mM (activation design, with S9), and 1.11 mM (activation design, without S9). Negative and positive controls and the exposure/incubation schedules were as for the dose-finding study. In judging the results of the observed metaphases, <5% abnormalities was considered "negative", 5% to 10% as "trace positive", and >10% as "positive". According to the OECD guidelines, a test substance is judged positive if there is a concentration-related increase or a reproducible increase in the number of cells with chromosome aberrations.

Results

In the metabolic activation design without the use of S9 mixture, 39.0% and 13.5% structural abnormalities were observed at the doses of 1.11 mM and 0.555 mM, respectively (Table 3.4.5.1 and Figure 3.4.5.1). Of the abnormalities at 1.11 mM, 84.6% and 42.3%, respectively, were of the break and exchange types. A similar pattern was seen at 0.555 mM where 59.3% and 37.0%, respectively, were of the break and exchange types. Additionally, 32% of cells contained numerical abnormalities (polyploidy) only at 0.555 mM. There were no positive results with the direct method (24 h and 48 h) (Table 3.4.5.2 and Figure 3.4.5.1) or when activation with S9 was used. Positive and negative control results except for benzo(a)pyrene fell within historical ranges. Benzo(a)pyrene is not an appropriate positive control for testing in the absence of S-9.

The sponsor contends that the genetic risk with OM is relatively low based on the calculated low TR value (numerical expression for the incidence of the exchange type abnormalities) and high

D_{20} value (the concentration that causes abnormalities in 20% of metaphases). The TR for OM was 26.6 and the D_{20} was 0.39 mg/ml. In contrast, the D_{20} and TR values obtained for the positive controls in this study were 0.000029 mg/ml and 470,000 for mitomycin C, and 0.007 mg/ml and 1,125 for benzo(a)pyrene. In conclusion, OM induced structural and numerical aberrations under test conditions but the action of the test substance may be considered to be weak.

TABLE 3.4.5.1
CHROMOSOME ABERRATION TEST WITH OLMESARTAN MEDOXOMIL.
METABOLIC ACTIVATION METHOD.

Treatment	S9 mix	Dose mM	Cell no.	Polyploidy		Number and ratio (%) of structural abnormality								
					judgement	g	Chromatid type		Chromosome type		Others	Total		judgement
							ctb	cte	csb	cse		+g	-g	
None	+		100 100 200	1 0 1 (0.5)	-	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	-
	-		100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 1 1 (0.5)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 1 1 (0.5)	0 1 1 (0.5)	-
Vehicle (DMSO)	+		100 100 200	0 1 1 (0.5)	-	0 0 0 (0)	0 1 1 (0.5)	1 0 1 (0.5)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	1 1 2 (1.0)	1 1 2 (1.0)	-
	-		100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 1 1 (0.5)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 1 1 (0.5)	0 1 1 (0.5)	-
OM	+	0.520	100 100 200	0 0 0 (0)	-	1 1 2 (1.0)	1 0 1 (0.5)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	2 1 3 (1.5)	1 0 1 (0.5)	-
		1.04	100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	-
		2.08	100 100 200	2 0 2 (1.0)	-	0 0 0 (0)	0 1 1 (0.5)	0 1 1 (0.5)	1 1 2 (1.0)	1 0 1 (0.5)	0 0 0 (0)	2 3 5 (2.5)	2 3 5 (2.5)	-
	-	0.278	100 100 200	0 0 0 (0)	-	1 0 1 (0.5)	0 1 1 (0.5)	0 0 0 (0)	1 0 1 (0.5)	0 1 1 (0.5)	0 0 0 (0)	2 2 4 (2.0)	1 2 3 (1.5)	-
		0.555	100 100 200	31 33 64 (32.0)	++	0 0 0 (0)	1 1 2 (1.0)	4 2 6 (3.0)	9 5 14 (7.0)	4 0 4 (2.0)	1 1 2 (1.0)	18 9 27 (13.5)	18 9 27 (13.5)	+
		1.11	100 100 200	0 0 0 (0)	-	2 0 2 (1.0)	26 30 36 (28.0)	17 16 33 (16.5)	9 1 10 (5.0)	0 0 0 (0)	3 0 3 (1.5)	43 37 80 (40.0)	41 37 78 (39.0)	++
Positive Control BaP	+	0.05 µg/ml	100 100 200	0 0 0 (0)	-	2 0 2 (1.0)	1 8 9 (4.5)	16 20 36 (18.0)	1 0 1 (0.5)	0 1 1 (0.5)	2 0 2 (1.0)	21 23 44 (22.0)	19 23 42 (21.0)	++
	-	0.05 µg/ml	100 100 200	0 0 0 (0)	-	0 0 0 (0)	1 0 1 (0.5)	0 0 0 (0)	1 0 1 (0.5)	0 0 0 (0)	0 0 0 (0)	2 0 2 (1.0)	2 0 2 (1.0)	-

g: gaps; ctb: chromatid breaks; cte: chromatid exchanges; csb: chromosome breaks; cse: chromosome exchanges; others: fragmentations, multiple types; DMSO: dimethylsulfoxide; BaP: benzo(a)pyrene (positive); -: aberration frequency <5% (negative); ±: aberration frequency >5% and <10% (suspect positive); ++: aberration frequency >20% and <50% (positive); +++: aberration frequency >50% (positive)

TABLE 3.4.5.2
CHROMOSOME ABERRATION TEST WITH OLMESARTAN MEDOXOMIL. DIRECT METHOD

Treatment	Duration hr	Dose mM	Cell no.	Polyploidy		Number and ratio (%) of structural abnormality								
					judgement	g	Chromatid type		Chromosome type		Others	Total		judgement
							ctb	cte	csb	cse		+g	-g	
None	24		100 100 200	1 0 1 (0.5)	-	0 0 0 (0)	1 0 1 (0.5)	1 0 1 (0.5)	0 0 0 (0)	0 1 1 (0.5)	0 0 0 (0)	2 1 3 (1.5)	2 1 3 (1.5)	-
	48		100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	-
Vehicle (DMSO)	24		100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	1 1 2 (1.0)	0 0 0 (0)	0 0 0 (0)	1 1 2 (1.0)	1 1 2 (1.0)	-
	48		100 100 200	1 0 1 (0.5)	-	0 1 1 (0.5)	0 1 1 (0.5)	0 2 2 (1.0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	1 3 4 (2.0)	1 2 3 (1.5)	-
OM	24	0.112	100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	-
		0.223	100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 1 1 (0.5)	0 0 0 (0)	0 1 1 (0.5)	0 0 0 (0)	0 0 0 (0)	0 1 1 (0.5)	0 1 1 (0.5)	-
		0.446	100 100 200	3 4 7 (3.5)	-	1 0 1 (0.5)	1 0 1 (0.5)	5 4 9 (4.5)	1 3 4 (2.0)	0 0 0 (0)	0 0 0 (0)	8 6 14 (7.0)	7 6 13 (6.5)	±
	48	0.065 3	100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 0 0 (0)	0 0 0 (0)	1 1 2 (1.0)	0 0 0 (0)	0 0 0 (0)	1 1 2 (1.0)	1 1 2 (1.0)	-
		0.131	100 100 200	0 0 0 (0)	-	1 1 2 (1.0)	0 0 0 (0)	0 1 1 (0.5)	1 0 1 (0.5)	0 0 0 (0)	0 0 0 (0)	2 2 4 (2.0)	1 1 2 (1.0)	-
		0.261	100 100 200	0 0 0 (0)	-	0 0 0 (0)	0 0 0 (0)	1 0 1 (0.5)	2 0 2 (1.0)	0 0 0 (0)	0 0 0 (0)	2 0 2 (1.0)	2 0 2 (1.0)	-
Positive Control MMC	24	0.05 µg/ml	100 100 200	1 0 1 (0.5)	-	0 4 4 (2.0)	10 7 17 (8.5)	29 29 58 (29.0)	1 11 12 (6.0)	3 1 4 (2.0)	0 2 2 (1.0)	39 44 83 (41.5)	39 41 80 (40.0)	++
	48	0.05 µg/ml	100 100 200	0 0 0 (0)	-	0 1 1 (0.5)	21 6 27 (13.5)	46 46 92 (46.0)	3 18 21 (10.5)	4 2 6 (3.0)	4 13 17 (8.5)	59 60 119 (59.5)	59 60 119 (59.5)	+++

g: gaps; ctb: chromatid breaks; cte: chromatid exchanges; csb: chromosome breaks; cse: chromosome exchanges; others: fragmentations, multiple types; DMSO: dimethylsulfoxide; MMC: mitomycin C (positive control); -: aberration frequency <5% (negative); ±: aberration frequency >5% & <10% (suspect positive); ++: aberration frequency >20% and <50% (positive); +++: aberration frequency >50% (positive)

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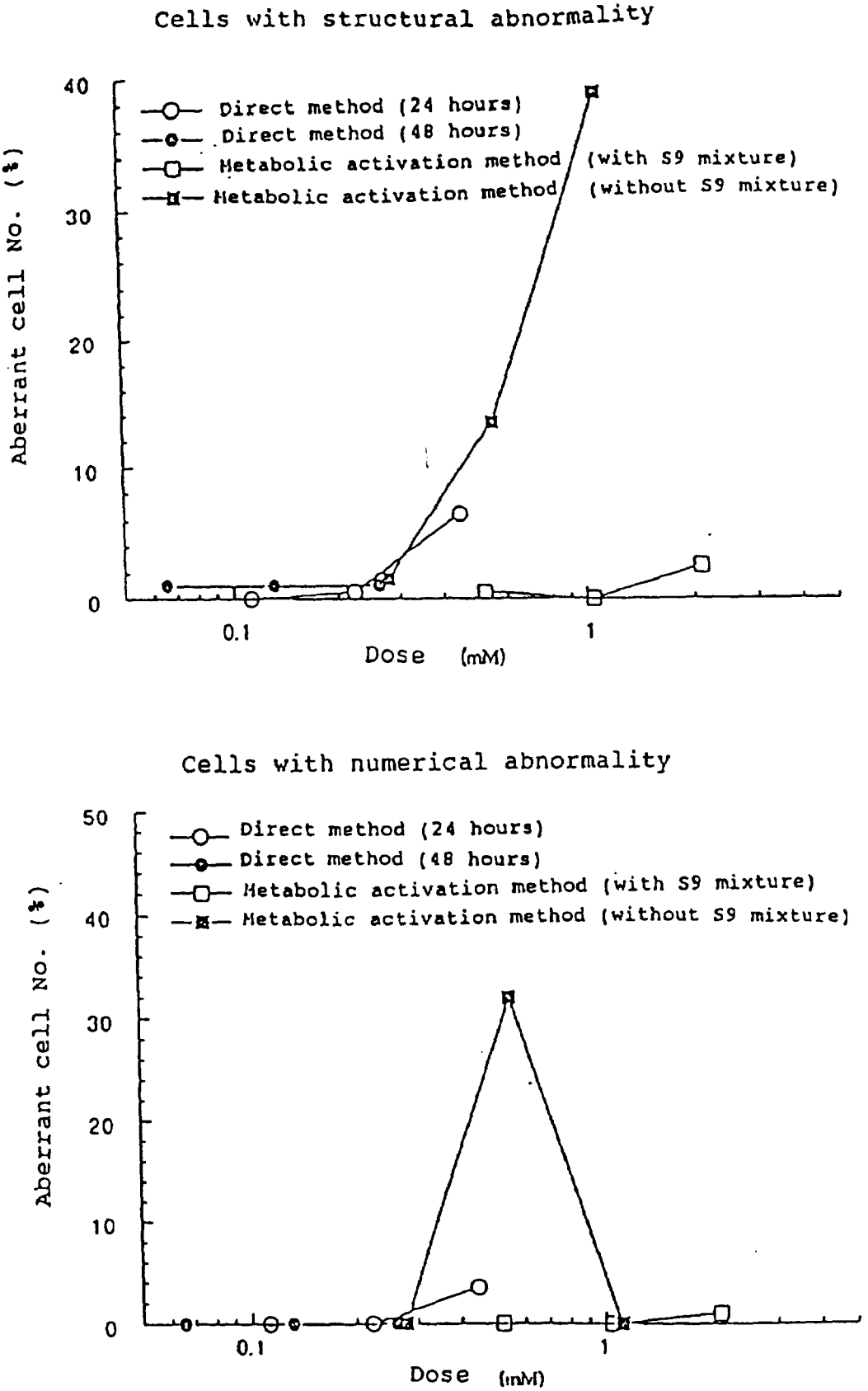

Fig. 3.4.5.1.: Incidence of chromosomal aberration with olmesartan medoxomil treatment

3.4.6. Chromosome Aberration Test of Olmesartan Medoxomil in CHL Cells. Second study (Report #RPD143-027, Study #3222 (129-058). Vol 17.

This GLP study is similar to the previous study (#TR140-049) except that it was conducted by a contract lab [redacted] and the test compound was synthesized by a different method. The study was initiated on October 4, 1996 and completed on February 21, 1997.

The experimental design and methodology are essentially similar to that described in the previous section (#3.4.5). OM (lot #NH203C) was dissolved in DMSO. Positive control compounds, mitomycin C (direct method) and cyclophosphamide (activation method) were dissolved in sterile water for injection. A fibroblastic cell line derived from the Chinese hamster lung (CHL) was employed as a tester cell strain. Cells were exposed for 24 or 48 hours with the direct method, and for 6 hours when used (± S-9) with the metabolic activation method, followed by continued culture for an additional 18 hours. An S-9 mix (prepared from livers of male Sprague-Dawley rats pretreated with phenobarbital and 5,6-benzoflavone) was obtained from a commercial source.

A dose-finding growth-inhibition study demonstrated 50% growth-inhibition at 352 and 139 μg/ml, respectively, with the 24- and 48-hour direct method, 566 and 1864 μg/ml with the activation method without and with the S9 mixture, respectively. Based on the growth-inhibition test and the results of the previous study, the main study was carried out using the following doses.

Test System	Concentrations (μg/ml)
Direct method (24 hour)	43.8, 87.5, 175, 350, 700, 1400
Direct method (48 hour)	7.73, 15.5, 30.9, 61.9, 124, 248
Activation method (-S9)	124, 248, 495, 990, 1980
Activation method (+S9)	248, 495, 990, 1980

Two plates were used at each dose level. One hundred metaphases on each slide (200 metaphases for each dose) were observed microscopically for gaps, chromatid breaks, chromosome breaks, chromatid exchanges and chromosome exchanges, along with structural abnormalities classified as other. Incidence of polyploidies was also scored. Test substance was judged as positive when the incidence rate was more than 10% and the response was reproducible or dose-dependent. The results were considered to be equivocal (±) if the incidence rate was > 5 <10%. No statistical analyses were done.

Results

The incidence rates of cells possessing chromosomal aberrations including gaps and excluding gaps at the lowest concentrations in the direct method tests (both 24 hr and 48 hr treatment) were similar to that of the solvent control group. However, the incidence rates of structural aberration increased with further increases in dose (>87.5 and >61.9 μg/ml with 24 hr and 48 hr treatment, respectively). The rates were significantly different from solvent control at 175 and 124 μg/ml with 24 hr and 48 hr treatment, respectively (Table 3.4.6.1). Growth inhibition at these

concentrations was less than 40% (Fig. 3.4.6.1 and 3.4.6.2). In the highest dose group, "appraisable metaphases were not practically observed" because of the cytotoxicity of olmesartan (>65% growth inhibition). The incidence of polyploidy in the treatment groups was less than 3%. A structural abnormality incidence of ≥39% was observed in the positive control group.

TABLE 3.4.6.1
CHROMOSOME ABERRATION TEST IN CHL CELLS (DIRECT METHOD)

Compound	Dose µg/ml	No. of cells with structural aberrations						total %		polyploid cells %	Final judgement
		gap	ctb	cte	csb	cse	other	+gap	-gap		
A. Direct method 24-hour treatment group											
DMSO	0	1	0	0	0	0	0	0.5 -	0.0 -	0.5 -	-
OM	43.8	0	1	0	0	0	0	0.5 -	0.5 -	0.0 -	-
	87.5	5	2	0	0	0	0	3.0 -	1.0 -	1.0 -	-
	175.0	19	40	17	0	0	0	29.5 +	24.0 +	0.5 -	+
	350[a]										
MMC[c]	0.05	20	33	62	0	1	0	42.5 +	39.0 +	2.0 -	+
B. Direct method 48-hour treatment group											
DMSO	0	1	0	0	0	0	0	0.5 -	0.0 -	0.0 -	-
OM	30.9	2	2	2	0	0	0	3.0 -	2.0 -	0.0 -	-
	61.9	5	2	4	0	0	0	4.5 -	3.0 -	0.0 -	-
	124.0[b]	28	43	42	0	3	0	40.0 +	37.0 +	3.0 -	+
	248.0										
MMC[c]	0.025	20	37	67	1	1	0	48.5 +	42.5 +	1.5 -	+

ctb: chromatid break; cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange
a: The 50% growth inhibition concentration is reported to be 352 µg/ml (see Fig. 3.4.5.1) and the sponsor labels 350 µg/ml as 'toxic'; b: The 50% growth inhibition concentration in 48 hr method is 139 µg/ml (see Fig. 3.4.5.2); c: mitomycin, positive control.

In the activation method without S-9 mix, the incidence rates of aberration increased with dose (124 thorough 495 µg/ml) and the test was judged positive at all concentrations. A qualitatively similar result with a lower incidence of aberrations was obtained in cells treated with S-9 mix and judged to be equivocal even at high concentrations (Table 3.4.6.2, Fig. 3.4.6.3). The incidence of polyploidy in the treatment groups was 0.5 to 1%. A structural abnormality incidence ≥53% was observed in the positive control group with S-9. However, no abnormality was observed in the positive control group without S-9 since cyclophosphamide is not an appropriate positive control for this method.

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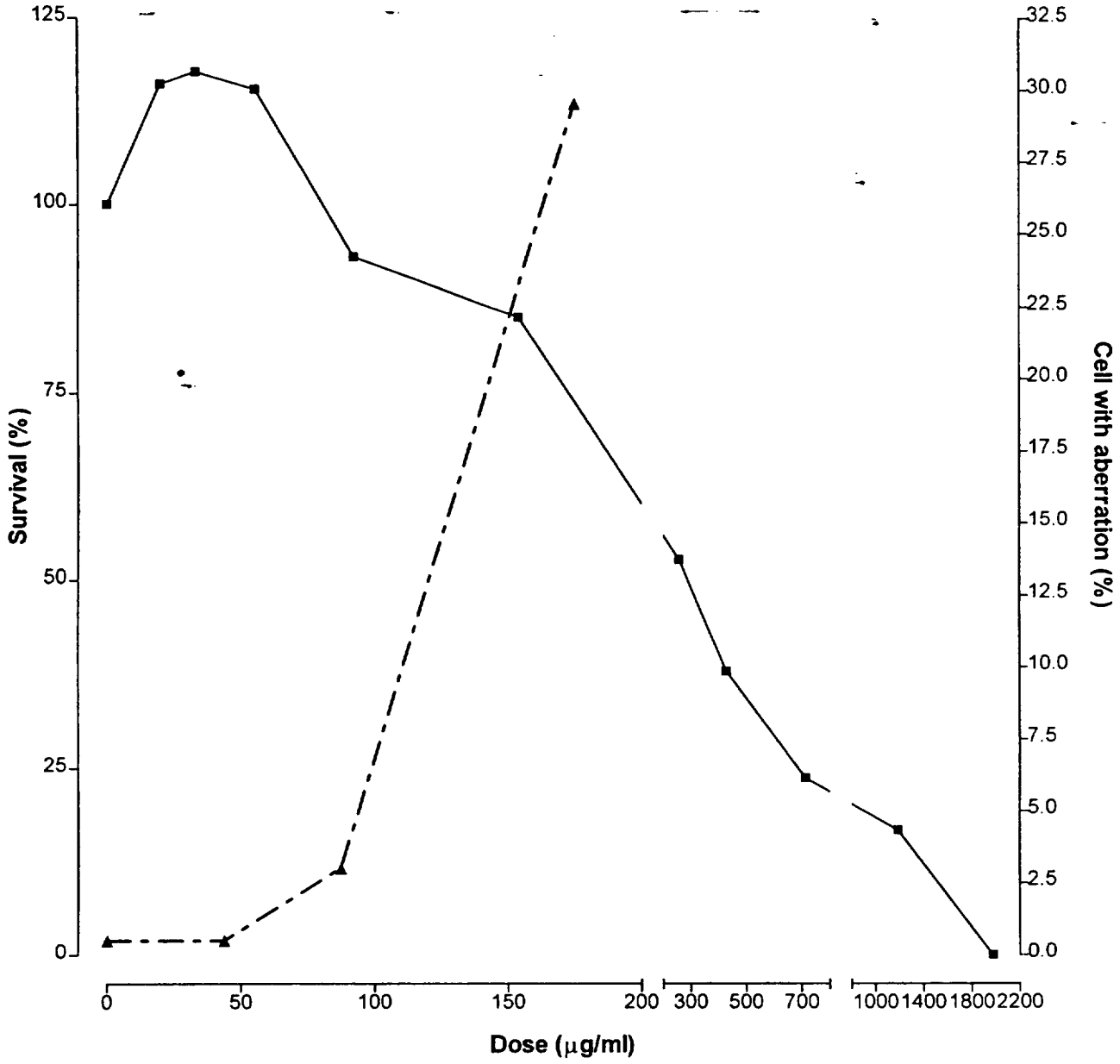

Fig. 3.4.6.1.: *In vitro* chromosome aberration test of OM in CHL cells, direct method, 24 hr treatment group. Dose-survival (%) is plotted on left Y-axis (solid squares with solid line), while the incidence rates of cells (%) showing chromosomal aberrations is plotted on right Y-axis (solid triangle with broken lines). Note the 50% growth inhibition concentration was estimated to be 352 μg/ml.

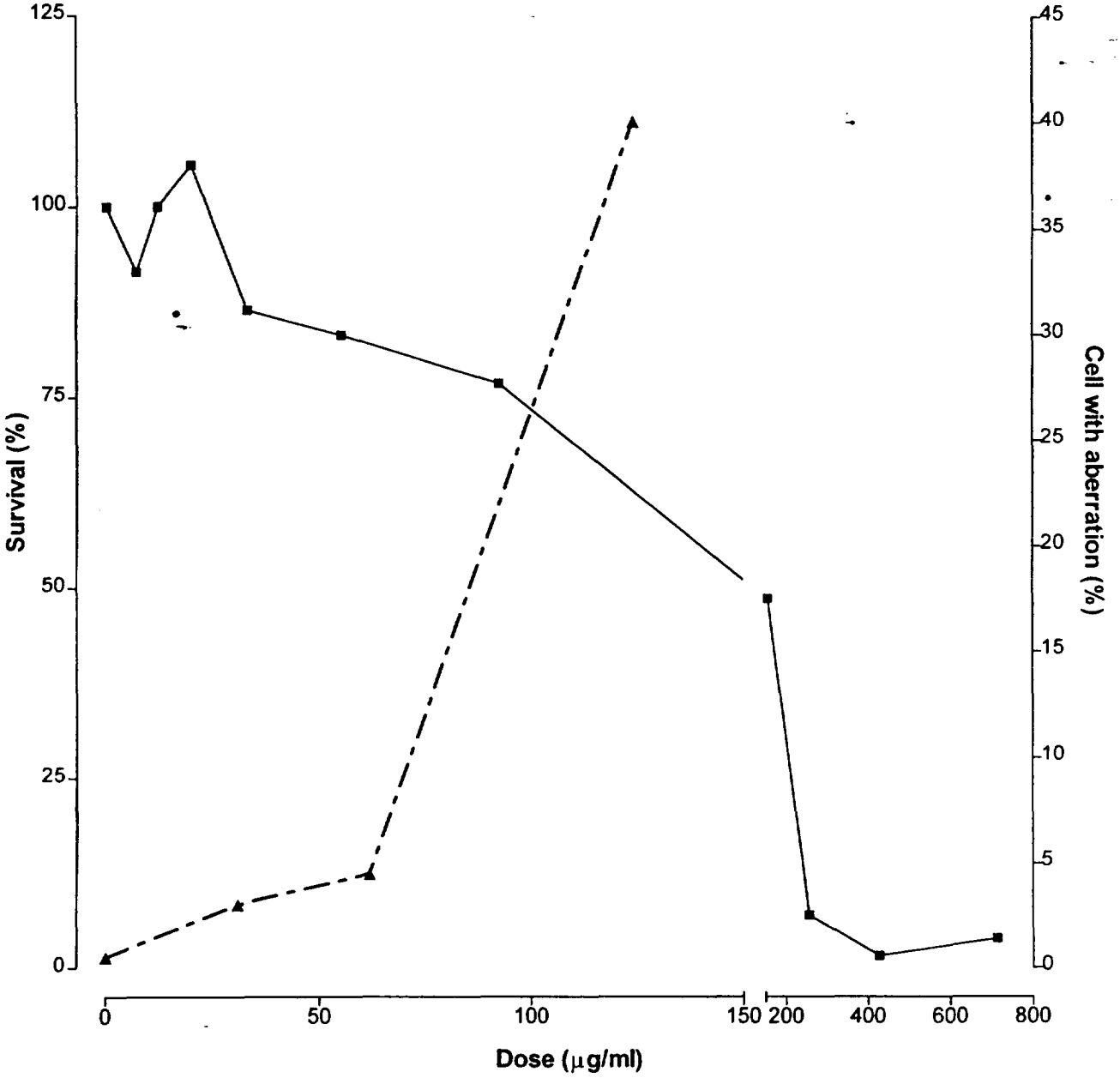

Fig. 3.4.6.2.: *In vitro* chromosome aberration test of OM in CHL cells, direct method, 48 hr treatment group. Dose-survival (%) is plotted on left Y-axis (solid squares with solid line), while the incidence rates of cells (%) showing chromosomal aberrations is plotted on right Y-axis (solid triangle with broken lines). Note the 50% growth inhibition concentration was estimated to be 139 μg/ml.

TABLE 3.4.6.2
CHROMOSOME ABERRATION TEST IN CHL CELLS (ACTIVATION METHOD)

Compound	Dose µg/ml	No. of cells with structural aberrations						total %		polyploid cells %	Final judgement
		gap	ctb	cte	csb	cse	other	+gap	-gap		
A. – S9 MIX											
DMSO	0	1	0	1	0	0	0	1.0 -	0.5 -	0.0 -	-
OM	124	5	8	0	0	0	0	6.5 ±	4.0 -	0.0 -	±
	248	18	19	10	0	0	0	19.0 +	12.0 +	0.5 -	+
	495	47	83	49	2	0	0	59.5 +	54.0 +	1.0 -	+
	990										
CP[a]	12.5	2	2	0	1	0	0	2.5 -	1.5 -	0.5 -	-
B. + S9 MIX											
DMSO	0	1	0	1	0	0	0	1.0 -	0.5 -	0.0 -	-
OM	495	1	0	2	0	0	0	1.0 -	1.0 -	1.0 -	-
	990	3	3	10	0	0	0	8.0 ±	6.5 ±	0.5 -	±
	1980[b]	5	5	9	0	0	0	9.5 ±	7.0 ±	0.5 -	±
CP[a]	12.5	20	34	89	0	1	0	55.0 +	52.5 +	0.5 -	+

ctb: chromatid break; cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange
a: cyclophosphamide, positive control; b: visible precipitation observed at the end of exposure period

D_{20} (defined as the concentration that causes abnormalities in 20% of metaphases) and TR [numerical expression for the incidence of the exchange type abnormalities; incidence rate of chromatid exchanges (cte) per unit of concentration (mg/ml) of test agent] values for OM were calculated from the above results and are given below.

Test system	D_{20} (mg/ml)	TR
Direct method (24 hour)	0.144	48.6
Direct method (48 hour)	0.069	169
Activation method (+S-9)	0.177	49.5

In summary, with both the 24-hr and the 48-hr direct methods as well as the activation method without the S9 mixture, an apparent induction of chromosome aberration was observed in a dose-dependent manner, and the response was considered to be positive. According to the sponsor, the D_{20} and TR obtained with the 48 hr direct method, each of which represents a comparative strength of mutagenicity, suggest that OM should be classified as a middle grade mutagen. Thus, the study confirms the previous study findings that OM induces chromosomal aberrations in the absence (but not in the presence) of metabolic activation. Furthermore, the positive response is independent of the new synthetic source.

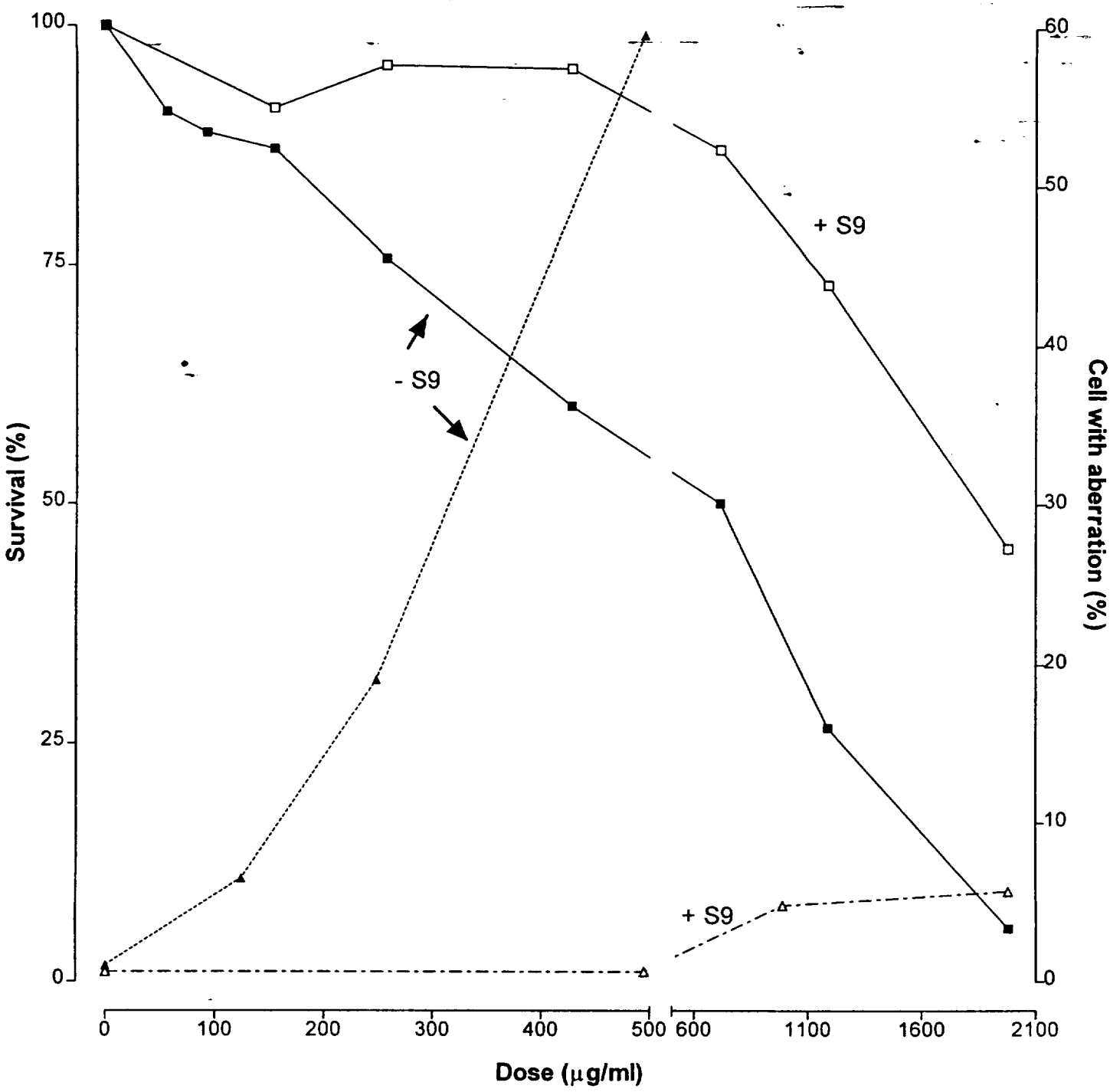

Fig. 3.4.6.3.: *In vitro* chromosome aberration test of OM in CHL cells, activation method, ± S9 mix as indicated against each line on the plot. Dose-survival (%) is plotted on left Y-axis (solid and open squares with solid line), while the incidence rates of cells (%) showing chromosomal aberrations is plotted on right Y-axis (solid and open triangles with broken lines). See Table 3.4.6.2. for actual numbers.

3.4.7. Chromosome Aberration Test of Olmesartan in CHL Cells (Report #TR-141-027, Study #94-B026). Vol. 17.

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between March 10 and May 19, 1994. Discussion includes the amendments issued to the report on August 18, 1997 and June 22, 1999. Since olmesartan medoxomil (OM) had been earlier shown to produce structural and numerical chromosome aberrations, the current test was conducted to confirm whether olmesartan, the active metabolite of OM, had a clastogenic action.

Tests employing a Chinese hamster lung (CHL) fibroblast cell line were done for olmesartan with and without metabolic activation. The methods used were essentially similar to that described in section 3.4.5. In addition to DMSO, a common solvent for aberration studies, suspension in 1% CMC solution "intending a compulsory incorporation of olmesartan into cells" was also studied. Olmesartan (Lot #5) dissolved in DMSO at a maximal concentration of 1.25 mM, produced no reduction in mitotic indices in a dose-finding test and no relevant chromosome aberrations at this and lower concentrations. However, when given as a suspension in 1% CMC in a dose-finding test, mitotic indices were reduced both at the high concentrations used in the 24-hour direct test (1.25 mM to 5 mM) and at the relatively low concentrations in the 48-hour direct test (0.0391 mM to 0.156 mM). Mitotic indices were not reduced in the metabolic activation test, in spite of the use of high concentrations (2.5 mM to 10 mM). Based on these findings, the same concentrations were used in the main part of the study.

Results

No numerical or structural aberrations were detected in any treatment group where DMSO was used as solvent. In contrast, in groups where 1% CMC was used, 11% of cells were identified as having structural aberrations at 0.0781 mM (not the highest concentration), which was the median dose for the 48-hr continuous treatment in a direct method test. Further, with the 48-hr direct method, 6% and 7% of total cells were identified as having a numerical abnormality (polyploidy) at 0.156 and 0.0391 mM (Table 3.4.7.1). The mitotic index (MI) at 0.0781 mM was 27.3% of the vehicle control index suggesting strong cytotoxicity of the agent. Absence of a clear dose response relationship indicates, according to the sponsor, the aberration could have resulted from a non-specific reaction attributable to turbulence of homeostasis of the cells rather than genotoxicity of olmesartan. The responses to the test agent were negative with the 24-hr direct method.

With the metabolic activation method (6-hr exposure) without the S9 mixture, olmesartan did not induce chromosomal aberrations. On the other hand, structural aberrations (22%) were found in the metabolic activation test group given the S-9 mix at the highest concentration (10 mM) but none were found at lower concentrations. The predominant type of aberration was a chromatid-exchange type replacement aberration (16%). No numeric aberrations were noted. The MI at the aberration-inducing concentration was relatively high (79.2% that of the vehicle control) suggesting a weak cytotoxicity. The findings indicate that olmesartan has a sporadic clastogenic effect when given as a suspension in CMC in this test system, at concentrations that are associated with reduced mitotic indices (i.e., toxic concentrations) under similar experimental

conditions. No clastogenic effects of olmesartan were found when the metabolite was dissolved in DMSO. It is likely that the differences in results for olmesartan between those obtained when it is given in CMC and those obtained when it is given in DMSO are related to an effect CMC may have on incorporation of the compound into the cells. This is supported by the fact that a positive finding for OM dissolved in DMSO was not identified with the S-9 mix treatment (see section 3.4.6). It is concluded that olmesartan liberated in cells was involved in the clastogenic effects identified for olmesartan medoxomil. Positive and negative control results except for benzo(a)pyrene produced appropriate responses. It may be noted that benzo(a)pyrene is not an appropriate positive control for testing in the absence of metabolic activation.

TABLE 3.4.7.1
CHROMOSOME ABERRATION STUDY OF OLMESARTAN IN CMC SUSPENSION. DIRECT METHOD

Treatment[1] (hr)	Agent	Dose (mM)	No. of cells analyzed	Polyploidy	Judgement	Structural Aberrations[2]						TA[3]	TAG[4]	Final Judgement
						gap	ctb	cte	csb	Cse	others			
24-0	Untreated		50	0	-	0	0	0	0	0	0	0	0	-
			50	0		0	0	0	0	0	0	0	0	
			100	0		0	0	0	0	0	0	0	0	
	Vehicle[5]		50	0	-	0	0	0	0	0	0	0	0	-
			50	0		0	0	0	0	0	0	0	0	
			100	0		0	0	0	0	0	0	0	0	
	OLM	1.25	50	0	-	0	0	1	0	0	0	1	1	-
			50	0		0	0	0	1	0	0	1	1	
			100	0		0	0	1	1	0	0	2	2	
		2.50	50	0	-	0	1	0	0	0	0	1	1	-
			50	0		0	0	0	0	1	0	1	1	
			100	0		0	1	0	0	1	0	2	2	
		5.00	50	0	-	0	2	0	0	0	0	2	2	-
			50	0		0	1	0	0	0	0	1	1	
			100	0		0	3	0	0	0	0	3	3	
	Positive	50[6]	50	0	-	0	7	12	1	0	0	18	18	++
			50	0		0	2	11	1	1	0	14	14	
			100	0		0	9	23	2	1	0	32	32	
48-0	Untreated		50	0	-	0	0	0	0	0	0	0	0	-
			50	0		0	0	0	0	0	0	0	0	
			100	0		0	0	0	0	0	0	0	0	
	Vehicle[5]		50	1	-	0	0	1	0	0	0	1	1	-
			50	1		0	0	0	0	1	0	1	1	
			100	2		0	0	1	0	1	0	2	2	
	OLM	0.0391	50	1	±	0	0	1	0	0	0	1	1	-
			50	6		0	1	2	0	0	0	3	3	
			100	7		0	1	3	0	0	0	4	4	
		0.0781	50	2	-	0	2	5	0	0	0	6	6	+
			50	2		0	3	2	0	1	0	5	5	
			100	4		0	5	7	0	1	0	11	11	
		0.156	50	3	±	0	2	0	0	0	0	2	2	-
			50	3		0	0	0	0	0	0	0	0	
			100	6		0	2	0	0	0	0	2	2	
	Positive	50.0[6]	50	0	-	0	5	14	0	0	0	16	16	++
			50	1		0	5	17	1	3	1	23	23	
			100	1		0	10	31	1	3	1	39	39	

1) Treatment time - Recovery time; 2) ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, csc: chromosome exchange, others: multiple; 3) TA: total aberrant cells; excluding the gap; 4) TAG: total aberrant cells including the gap; 5) 1% Sodium carboxymethyl cellulose in physiological saline (1% CMC suspension); 6) ng/ml Mitomycin C

TABLE 3.4.7.2
CHROMOSOME ABERRATION STUDY OF OLMESARTAN IN CMC SUSPENSION. METABOLIC ACTIVATION

Treatment[1] (hr)	S9 mix	Agent	Dose (mM)	No. of cells analyzed	Polyploidy	Judgement	Structural Aberrations[2]						TA[3]	TAG[4]	Final Judgement
							gap	ctb	cte	csb	cse	others			
6-18	+	Untreated		50	0	-	0	0	2	0	1	0	2	2	-
				50	0		1	0	0	0	0	0	0	1	
				100	0		1	0	2	0	1	0	2	3	
		Vehicle[5]		50	0	-	0	0	0	0	0	0	0	0	-
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	0	0	0	0	0	0	
		OLM	2.50	50	0	-	0	0	0	0	0	0	0	0	-
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	0	0	0	0	0	0	
			5.00	50	0	-	0	1	1	0	0	0	2	2	-
				50	2		0	0	0	0	0	0	0	0	
				100	2		0	1	1	0	0	0	2	2	
			10.0	50	0	-	0	1	7	0	2	1	10	10	++
				50	1		0	2	9	0	1	2	12	12	
				100	1		0	3	16	0	3	3	22	22	
		Positive	10.0[6]	50	0	-	0	3	11	0	0	0	14	14	++
				50	0		1	2	11	0	0	0	12	12	
				100	0		1	5	22	0	0	0	26	26	
6-18	-	Untreated		50	0	-	0	0	0	0	0	0	0	0	-
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	0	0	0	0	0	0	
		Vehicle[5]		50	1	-	0	1	0	0	0	0	1	1	-
				50	0		0	0	0	0	0	0	0	0	
				100	1		0	1	0	0	0	0	1	1	
		OLM	2.50	50	0	-	0	0	1	0	0	0	1	1	-
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	1	0	0	0	1	1	
			5.00	50	0	-	0	0	0	0	0	0	0	0	-
				50	0		0	0	1	0	0	0	1	1	
				100	0		0	0	1	0	0	0	1	1	
			10.0	50	0	-	0	0	0	0	0	0	0	0	-
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	0	0	0	0	0	0	
		Positive	10.0[6]	50	0	-	0	0	0	0	0	0	0	0	-
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	0	0	0	0	0	0	

1) Treatment time - Recovery time
2) ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, csc: chromosome exchange, others: multiple
3) TA: total aberrant cells excluding the gap
4) TAG: total aberrant cells including the gap
5) 1% Sodium carboxymethyl cellulose in physiological saline (1% CMC suspension)
6) μg/ml Benzo(a)pyrene

3.4.8. Mutagenicity Studies of Compounds Related to Olmesartan Medoxomil Chromosome Aberration Test of RNH-8097 in CHL Cells. (Report #TR141-090, Study #94-B026). Vol 17.

This non-GLP study was conducted by Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between March 10, 1994 and September 9, 1994. The study investigated the cytogenetic effects of RNH-8097, which is the alcoholic moiety in the structure (ester side chain) of olmesartan medoxomil (OM).

The experimental design and methodology were generally similar to that described in the previous sections (#3.4.5 and 3.4.7). The test substance, RNH-8097 (batch #A1548-182), was incorporated in the test system as a DMSO solution or a 1% CMC suspension. Positive control compounds were mitomycin C (direct method) and benzo(a)pyrene (activation method). A fibroblastic cell line derived from the Chinese hamster lung (CHL) was employed as a tester cell strain. Cells were exposed for 24 or 48 hours with the direct method, and for 6 hours with the metabolic activation method, followed by continued culture for an additional 18 hours. An S9 mix (prepared from livers of male Sprague-Dawley rats pretreated with phenobarbital and 5,6-benzoflavone) was obtained from a commercial source.

A dose-finding study using DMSO as vehicle demonstrated a decrease in mitotic index (MI) in the 48 hr direct treatment group only at 10 mM RNH-8097 (MI 67.9% of vehicle control), the maximum dose level set in DMSO treatment groups. In all other treatment groups, the indices were between 91 and 100%. Based on these results, in the main DMSO study, 10 mM was the maximum dose level set with both direct and metabolic activation methods. In the dose-finding experiment using 1% CMC as the vehicle, the MIs at 2.5 and 5 mM in the 24 hr direct treatment groups were, respectively, 89.8 and 39.8%. The indices at 1.25 and 2.5 mM in the 48 hr direct treatment groups were, respectively, 90.3 and 50.0%. With the metabolic activation method, the indices at 10 mM with and without the S-9 mixture were 100 and 50.6%, respectively. Based on these results, in the main CMC study, the maximum dose levels for the 24 hr and 48 hr direct method were set at 5 and 2.5 mM, respectively. The maximum concentration used for the metabolic activation method was 5 and 10 mM, respectively, in the absence and presence of S-9 mix. Test substance was judged as positive when the incidence rate was 10% or more. The results were considered to be equivocal (±) if the incidence rate was more than 5% and less than 10%. No statistical analyses were done.

Results

None of the treatment groups using DMSO as the solvent showed structural or numerical abnormalities exceeding 2%. When 1% CMC-saline suspension was used as the solvent, 8% of the cells were recognized to have structural abnormalities at 5 mM (MI ratio *versus* vehicle, 39.8%) in the 24 hr direct method. According to the criteria set by the sponsor, the result is ambiguous or equivocal. The positive result appeared only at this high dose (Table 3.4.8.1) and low mitotic index. Among other treatments, no numerical abnormality exceeding 1% was recognized. It should be noted that both OM and its active metabolite, olmesartan, induce structural as well as numerical chromosome aberrations. Thus, the results of this study suggest that RNH-8097 is not responsible for the chromosome aberrations induced by OM.