TABLE 3.4.8.1

RESULTS OF CHROMOSOME ABERRATION TEST OF RNH-8097 IN CMC SUSPENSION (CHL CELLS). DIRECT ACTIVATION METHOD

Treatment[1] (hr)	S9 mix	Agent	Dose (mM)	No. of cells analyzed	Polyploidy	Judgement	Structural aberrations[2] gap	ctb	cte	csb	csc	others	TA[3]	TAG[4]	Final Judgement
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		1	0	1	0	0	0	1	2	
				100	0		1	0	1	0	0	0	1	2	
		RNH-8097	1.25	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	
			2.50	50	0	-	0	0	0	0	0	0	0	0	
				50	0		0	0	0	2	0	0	2	2	
				100	0		0	0	0	2	0	0	2	2	
			5.00	50	0	-	0	3	1	0	0	0	4	4	
				50	0		0	3	2	1	0	0	4	4	
				100	0		0	6	3	1	0	0	8	8	±
		Positive	50[6]	50	1	-	1	8	14	0	1	1	20	21	
				50	1		0	5	16	1	1	0	21	21	
				100	2		1	13	30	1	2	1	41	42	+ +
48-0	-	Untreated		50	0	-	0	0	0	1	0	0	1	1	
				50	0		0	1	1	0	0	0	2	2	
				100	0		0	1	1	1	0	0	3	3	
		Vehicle[5]		50	0	-	0	0	0	0	0	0	0	0	
				50	0		0	0	0	1	0	0	1	1	
				100	0		0	0	0	1	0	0	1	1	
		RNH-8097	0.625	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	
			1.25	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	
			2.50	50	0	-	0	0	0	1	0	0	1	1	
				50	1		0	0	0	0	0	0	0	0	
				100	1		0	0	0	1	0	0	1	1	
		Positive	50[6]	50	0	-	0	5	18	1	1	2	20	20	
				50	0		0	6	13	1	2	1	19	19	
				100	0		0	11	31	2	3	3	39	39	+ +

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

3.4.9. Mutagenicity Studies of Compounds Related to Olmesartan Medoxomil. Chromosome Aberration Test of HMPIC, MBT, Diacetyl (Report #TR142-048, Study #94-B052) and Acetoin (Report #TR144-116, Study #94-B070) in CHL Cells. Vol 18.

These non-GLP studies were conducted by Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between June 24, 1994 and June 20, 1995 and September 8, 1994 and May 19, 1998, respectively. The studies investigated the cytogenetic effects in CHL cells of metabolites and other compounds related to olmesartan medoxomil (OM). (See section 3.4.4. for structures of these compounds and their relationship to OM.)

The experimental design and methodology are similar to that described in the previous sections. All test substances were used in suspension form by preparing them in a CMC solution. The lot numbers of HMPIC and MBT are not given. Diacetyl (lot #24H3497) was purchased from [redacted] while acetoin was from (batch #V4H4056) [redacted] Positive control compounds were mitomycin C (direct method) and benzo(a)pyrene (activation method). A fibroblastic cell line derived from the lung of a female Chinese hamster (CHL cells) was used. Cells were exposed for 24 or 48 hours with the direct method, and for 6 hours when used with the metabolic activation method (±S-9 mix), 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 was carried out with all test substances. For **HMPIC**, the mitotic indices were more than 50% of vehicle control even at the maximum concentration of 10 mM with both the direct and metabolic activation methods. Thus, 10 mM was the maximum concentration set for the final study. The MI ratios for the **MBT** groups with the direct method were 12.5%, 34.7% and 108.3% of vehicle control in the 24 hr treatment group and 0%, 38% and 72% of vehicle control in the 48 hr treatment group, respectively, at concentrations of 0.156 mM, 0.0781 mM and 0.0391 mM. With the metabolic activation method, ratios of mitotic indices for the treatment groups relative to vehicle control at 0.625 and 0.313 mM with the S-9 mix and at 0.156 and 0.0781 mM in the absence of S-9 mix were 75.9, 106.5, 33.3 and 109.9%, respectively. On the basis of these results, the maximum doses of MBT for the 24- and 48-hr treatments with the direct method and the metabolic activation method without the S-9 mix were set at 0.156 mM and the maximum dose for the metabolic activation method with the S-9 mix was set at 0.625 mM. The MIs for the **diacetyl** groups at 2.5 mM in the 24 hr treatment group and at 0.313 mM in the 48 hr treatment group with the direct method were 64.3% and 53.1% of vehicle control, respectively. With the metabolic activation method, the MI ratio for 10 mM diacetyl relative to vehicle control in the system with the S-9 mix was 48.8%, and MI ratios at 10, 5 and 2.5 mM in the system without S-9 mix were 0, 112.1 and 53.4%. Hence, the maximum doses for the 24- and 48-hr treatments with the direct method and the metabolic activation method with and without the S-9 mix were set at 2.5, 0.313, 10 and 5 mM, respectively. The MI for **acetoin** with the direct method was 49% of vehicle control at 5 mM in the 24 hr treatment group and 88.2% of vehicle control at 10 mM in the 48 hr treatment group. With the metabolic activation method, with or without S-9 mix, the MI ratio was >82% at 10 mM. Based on these results, the main study was carried out at concentrations of 5, 2.5 and 1.25 mM with the 24-hr direct method and 10, 5, and 2.5 mM with the 48-hr direct method and metabolic activation method (± S-9).

Results

HMPIC, MBT and acetoin produced no structural or numerical aberrations exceeding 2% under any of the study conditions. MBT was severely toxic, whereas HMPIC and acetoin were less toxic. Only diacetyl (derived from RNH-8097) induced chromosomal abnormalities. It may be noted that RNH-8097 also induced chromosomal aberrations (see previous section). Diacetyl produced structural abnormalities at 1.25 mM (14%) and 2.5 mM (17%) with the 24-hour treatment direct method; at 10 mM (15%) with S9; and at 5 mM (36%) without S9. Mitomycin C but not benzo(a)pyrene produced the appropriate response for the positive control. Benzo(a)pyrene is not an appropriate positive control for testing in the absence of metabolic activation.

Based on the results of this study, the sponsor concludes that diacetyl might be the causal agent producing chromosome aberrations seen with olmesartan medoxomil.

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TABLE 3.4.9.1

RESULTS OF CHROMOSOME ABERRATION TEST OF DIACETYL IN CMC SUSPENSION (CHL CELLS). DIRECT METHOD

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			
24-0	–	Untreated		50	0	–	0	0	0	1	0	0	1	1	–
				50	0		0	0	0	0	0	0	0	0	
				100	0		0	0	0	1	0	0	1	1	
		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	
		Diacetyl	0.625	50	1	–	0	0	2	0	1	0	3	3	±
				50	1		1	0	1	0	1	0	3	2	
				100	2		1	0	3	0	2	0	6	5	
			1.25	50	0	–	0	4	3	0	0	0	7	7	+
				50	0		0	4	3	0	0	0	7	7	
				100	0		0	8	6	0	0	0	14	14	
			2.50	50	2	–	0	4	2	0	0	0	6	6	+
				50	0		0	9	2	0	1	0	11	11	
				100	2		0	13	4	0	1	0	17	17	
		Positive	50 [6]	50	1	–	0	3	16	1	0	0	18	18	+ +
				50	0		0	3	16	0	0	0	18	18	
				100	1		0	6	32	1	0	0	36	36	
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	0	0	0	0	0	0	–
				50	0		0	0	0	0	0	0	0	0	
				100	1		0	0	0	0	0	0	0	0	
		Diacetyl	0.0781	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	
			0.156	50	2	–	0	1	0	0	0	0	1	1	–
				50	0		0	0	0	0	0	0	0	0	
				100	2		0	1	0	0	0	0	1	1	
			0.313	50	0	–	0	2	2	0	0	0	4	4	±
				50	2		0	0	1	0	0	0	1	1	
				100	2		0	2	3	0	0	0	5	5	
		Positive	50 [6]	50	0	–	0	7	15	0	0	5	22	22	+ +
				50	0		0	10	22	0	0	0	24	24	
				100	0		0	17	37	0	0	5	46	46	

1) Treatment time – Recovery time 2) ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange, others : maltiple
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 3.4.9.2

RESULTS OF CHROMOSOME ABERRATION TEST OF DIACETYL IN CMC SUSPENSION (CHL CELLS). METABOLIC ACTIVATION METHOD

Treatment[1] (hr)	S9 mix	Agent	Dose (mM)	No. of cells analyzed	Polyploidy	Judgement	Structural aberrations[2] gap	ctb	cte	csb	cse	others	TA[3]	TAG[4]	Final Judgement
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	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	
		Diacetyl	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	1		0	0	0	0	0	0	0	0	
				50	0	−	0	0	0	0	0	0	0	0	−
				100	1		0	0	0	0	0	0	0	0	
			10.0	50	4		0	3	5	0	1	0	8	8	
				50	0	−	0	1	6	0	0	0	7	7	+
				100	4		0	4	11	0	1	0	15	15	
		Positive	10.0[6]	50	1		0	2	7	1	0	0	8	8	
				50	0	−	0	1	6	0	0	0	8	8	+
				100	1		0	3	13	1	0	0	16	16	
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	0	0	0	0	0	0	0	
				50	0	−	0	0	0	0	0	0	0	0	−
				100	1		0	0	0	0	0	0	0	0	
		Diacetyl	1.25	50	2		0	0	0	0	0	0	0	0	
				50	0	−	0	0	0	0	0	0	0	0	−
				100	2		0	0	0	0	0	0	0	0	
			2.50	50	3		0	1	1	0	1	0	3	3	
				50	2	±	0	1	2	0	0	0	3	3	±
				100	5		0	2	3	0	1	0	6	6	
			5.00	50	2		0	14	7	0	0	0	17	17	
				50	0	−	0	16	6	0	0	1	19	19	+ +
				100	2		0	30	13	0	0	1	36	36	
		Positive	10.0[6]	50	0		0	0	0	0	0	0	0	0	
				50	0	−	0	1	0	0	0	0	1	1	−
				100	0		0	1	0	0	0	0	1	1	

1) Treatment time - Recovery time 2) ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange, others : maltiple
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

3.4.10. Gene Mutation at the Thymidine Kinase Locus of Mouse Lymphoma Cells with Olmesartan Medoxomil (Report #TRC142-028, Study #730/3) Vol. 18

This GLP study was conducted by a contract laboratory, [redacted] The study was initiated on February 8 and experimental work was completed on March 2, 1995. Olmesartan medoxomil was investigated for its ability to induce mutation at the tk locus (5-trifluorothymidine resistance) in L5178Y cells.

A preliminary cytotoxicity dose range-finder followed by two independent main experiments were conducted in the presence and absence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9 mix). Olmesartan medoxomil (OM) (batch #NH006C1) was dissolved in DMSO. The cytotoxicity range-finder experiment tested concentrations ranging from 62.5 to 2000 µg/ml, separated by 2-fold intervals. Complete cytotoxicity was observed at the two highest concentrations (1000 and 2000 µg/ml). The highest concentration resulting in some survival was 500 µg/ml (3.8% and 28.4% relative to control survival in the absence and presence of S-9 mix, respectively). Accordingly, the following concentrations were chosen for the 2 main experiments. Negative and positive control treatments were included in each experiment in the absence and presence of S-9 mix. Additionally, for the negative and positive controls, and for each dose of OM showing a significant increase in mutant frequency, the number of wells containing small colonies and the number containing large colonies were scored.

Test System		Concentrations (µg/ml)	Positive control
Experiment 1	-S9 mix	0, 31.25, 62.5, 125, 250, 500, 750	4-nitroquinoline 1-oxide
	+S9 mix	0, 62.5, 125, 250, 500, 750	Benzopyrene
Experiment 2	-S9 mix	0, 50, 100, 200, 300, 400, 500	4-nitroquinoline 1-oxide
	+S9 mix	0, 50, 100, 200, 300, 400, 500, 600, 700	Benzopyrene

Results

In experiment 1, the top two doses, 500 and 750 µg/ml (±S9) were rejected from statistical analyses due to excessive toxicity (see Table 3.4.10.1). The top dose analyzed was 250 µg/ml which yielded 87.1% and 89.3% relative survival in the absence and presence of S-9 mix, respectively. In both cases, no statistically significant increases in mutant frequency were observed (Table 3.4.10.1). In experiment 2, a narrower dose range was tested. The top doses selected were 500 and 600 µg/ml, which yielded 18.1% and 9.7% relative survival in the absence and presence of S-9 mix. In this study, statistically significant increases in mutant frequency were observed at 400 and 500 µg/ml in the absence of S-9 and 500 and 600 µg/ml in the presence of S-9. A linear trend was observed in both cases (Table 3.4.10.1). The probable reason for the absence of increases in mutant frequency in experiment 1 is the wider dose level interval (2-fold) and the steep toxicity curve observed. Thus, the data strongly suggest that OM is mutagenic in this test system. Mutant frequencies in negative control cultures were within the normal ranges. The positive control chemicals induced significant increases in mutant frequencies.

Both large and small colony mutant frequencies were estimated for experiment 2 only. The test substance induced both large and small colonies (Table 3.4.10.2). The large colonies most likely represent intragenic mutations caused by missense events and microdeletions or additions of a few DNA base pairs, whereas the small colonies are probably due to drug-induced chromosome aberrations, extragenic "suppressors", and gene amplification events (Applegate, M.L. *et al. Proc Natl Acad Sci.*, 87: 51-55, 1990). In conclusion, olmesartan is mutagenic in the absence and presence of S-9 under the conditions employed in the study.

TABLE 3.4.10.1
GENE MUTATION TEST WITH OLMESARTAN MEDOXOMIL IN MOUSE LYMPHOMA CELLS

Experiment 1

Treatment (µg/mL)	-S-9 %RS	-S-9 Mutant frequency#	Treatment (µg/mL)	+S-9 %RS	+S-9 Mutant frequency#
0	100.0	136.36	0	100.0	105.89
31.25	101.4	94.31 NS	62.5	104.3	105.76 NS
62.5	110.5	104.56 NS	125	85.6	95.99 NS
125	97.2	103.87 NS	250	89.3	87.92 NS
250	87.1	147.47 NS	500 X,$$	26.9	
500 X	5.4		750 X	2.0	
750 $	0.0				
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	82.6	766.68	2	67.3	927.72
0.1	62.2	990.62	3	50.6	1276.74

Experiment 2

Treatment (µg/mL)	-S-9 %RS	-S-9 Mutant frequency#	Treatment (µg/mL)	+S-9 %RS	+S-9 Mutant frequency#
0	100.0	134.00	0	100.0	157.12
50	86.1	123.82 NS	200	106.6	157.25 NS
100	104.4	115.51 NS	300	95.8	136.06 NS
200	82.0	143.07 NS	400	46.7	159.54 NS
300	84.3	160.58 NS	500	19.7	366.20 *
400	44.6	249.94 *	600	9.7	325.12 *
500	18.1	489.15 *	700 $	1.2	
Linear trend		***	Linear trend		***
NQO			BP		
0.05	57.0	1011.96	2	83.5	1226.21
0.1	35.0	1665.48	3	37.7	1575.00

\# per 10^6 viable cells
$ not plated for viability / 5-TFT resistance
$$ treatment excluded due to excessive heterogeneity
x treatment excluded from test statistics
NS not significant, NQO 4-nitroquinoline 1-oxide, BP benzo(a)pyrene
*, **, *** significant at 5%, 1% and 0.1% level, respectively

TABLE 3.4.10.2

SMALL AND LARGE COLONY MUTANT FREQUENCIES FOR NEGATIVE AND POSITIVE CONTROLS AND DOSES OF OLMESARTAN MEDOXOMIL SHOWING A SIGNIFICANT INCREASE IN MUTANT FREQUENCY IN MOUSE LYMPHOMA CELLS

Experiment	Concentration (μg/mL)	S-9	Mutant frequency*		Proportion small colony mutants
			Small colony	Large colony	
1	0	-	78.5	51.7	0.60
	NQO 0.05		495.4	158.6	0.76
	NQO 0.1		621.0	226.1	0.73
	0	+	40.3	62.7	0.39
	BP 2		395.0	294.4	0.57
	BP 3		619.5	314.7	0.66
2	0	-	64.1	65.8	0.49
	400		149.5	97.0	0.61
	500		279.5	217.0	0.56
	NQO 0.05		556.4	238.0	0.70
	NQO 0.1		805.8	428.7	0.65
	0	+	97.6	52.7	0.65
	500		214.8	139.0	0.61
	600		200.2	115.2	0.63
	BP 2		575.1	377.2	0.60
	BP 3		853.5	447.6	0.66

* per 10^6 viable cells
BP benzo(a)pyrene
NQO 4-nitroquinoline 1-oxide

In a second study (report #TR 143-058, a non-GLP study), the sponsor tested OM along with candesartan cilexetil (CC) and losartan in mouse lymphoma cells. In this study, the criteria for a positive assay were mutant frequency induction rates more than twice as great as vehicle control and evidence of a dose response. Methylmethane sulfonate served as the positive control. DMSO was used as the vehicle control for OM and CC, and growth medium (RPMI medium 1640) for losartan.

OM increased gene mutation frequency dose dependently (200 to 600 µg/ml). At 600 µg/ml, mutant frequency was 24-fold higher than vehicle control (plating efficiency, 12.3% of vehicle control). In addition, both large and small colonies for OM-treated cells produced an increasing tendency in size and morphology. Losartan also increased gene mutation frequency dose-dependently, but to a lesser extent, with a value of 2.6-fold higher at the maximum dose, 1000 µg/ml, than vehicle control. Plating efficiency at the high dose was 74.6% of vehicle control. Losartan induced an increasing tendency in size and morphology of small colonies (indicative of chromosomal aberrations) only. Large colonies had no important changes (Table 3.4.10.3). For CC, the maximum test concentration was 100 µg/ml. Higher doses (200 and 300 µg/ml) resulted in severe cytotoxicity. The highest concentration, 100 µg/ml, proved "too toxic to allow cells to be inoculated in the plate". There were no gene mutation at any of the doses tested (20 to 80 µg/ml). (Table 3.4.10.3).

In conclusion, results from both studies are consistent to suggest that OM is a strong mutagenic in this test system.

TABLE 3.4.10.3
GENE MUTATION TEST WITH OLMESARTAN MEDOXOMIL (CS-866), CANDESARTAN CILEXETIL (TCV-116) AND LOSARTAN (DUP753) IN MOUSE LYMPHOMA CELLS

Treatment	Dose (µg / ml)	PE0[4] (%)	PE2[5] (%)	RS0[6] (%)	RTG[7] (%)	Mutation Frequency($x10^{-6}$)	Inductivity	Ratio of SC(%)
CS-866	600	3.3	10.6	2.9	0.4	5646.5	24.28	83.1
	500	6.9	33.7	6.0	2.2	2658.4	11.43	63.1
	400	27.3	0.0	24.0	18.0	1767.1	7.60	63.0
	300	72.7	77.0	63.8	34.7	835.8	3.59	61.5
	200	74.8	98.0	65.6	68.3	345.7	1.49	71.1
TCV-116	80	0.0	0.0	0.0	0.0	0.0	0.00	0.0
	60	23.4	66.7	20.5	13.1	253.0	1.09	69.2
	40	89.3	84.1	78.4	80.9	274.5	1.18	65.3
	20	147.9	72.7	129.8	83.9	237.2	1.02	67.7
Vehicle[1]		114.0	84.1	100	100	232.6	1	64.1
Positive[2]	10	116.0	89.3	101.8	79.4	1164.3	5.01	60.1
DuP753	1000	26.3	84.1	29.0	6.7	457.1	2.64	88.8
	800	42.0	130.0	46.4	17.4	254.8	1.47	73.5
	600	92.1	116.0	101.5	54.8	213.5	1.23	60.9
	400	108.2	101.2	119.3	73.9	208.1	1.20	57.0
	200	98.0	141.4	108.1	125.9	116.8	0.68	66.8
Vehicle[3]		90.7	102.9	100	100	173.0	1	60.1
Positive[2]	10	120.3	86.6	132.7	59.1	1277.7	7.39	45.6

1) Dimethylsulfoxide (DMSO) 2) Methyl methanesulfonate 3) RPMI 0
4) PE0 : Plating efficiency in the cytotoxicity test
5) PE2 : Plating efficiency in the gene mutation test at 2 days after the initial treatment
6) RS0 : Relative surviving ratio (PE0 in treatment group / PE0 in vehicle control)
7) RTG : Total growth in the gene mutation test

3.4.11. *In Vitro* Transformation of Syrian Golden Hamster Embryo Cells with Olmesartan Medoxomil (Study #18548-0-485R, Report #TRC 145-002) Vol. 18

This GLP study was conducted by a contract laboratory [redacted] between May 20 and September 15, 1997. Olmesartan medoxomil (OM) was investigated for its potential to induce morphological transformation in the Syrian hamster embryo (SHE) cell transformation assay.

In the initial cytotoxicity assay, approximately 60-100 target SHE cells were added to petri dishes containing about 4 x 10^4 X-irradiated feeder SHE cells in medium seeded about 24 hr earlier. The cultures were incubated at 37°C for approximately 24 hr. OM (lot #NH204C) dissolved in DMSO was added at concentrations ranging from 10-1500 µg/ml (15 dishes/concentration). The dishes were incubated for approximately 7 days. Cytotoxicity was evaluated on the basis of relative plating efficiency (RPE) and relative colony density (RCD). A reduction in RPE was observed with increasing concentration. No toxicity was seen at 10 µg/ml, 5% toxicity was seen at 25 µg/ml, 13% at 50 µg/ml, 49% at 100 µg/ml and there was 100% toxicity at each of the remaining concentrations. When cytotoxicity was evaluated on the basis of RCD, three dose levels (ranging from 10 to 50 µg/ml) showed a greater than control colony density while the 100 µg/ml dose level showed a relative toxicity of 37%. All of the remaining dose levels were completely toxic. Using a stereomicroscope, the stained dishes were screened and individual colonies evaluated for transformed morphology. Criteria for morphological transformation were: piled up cells, extensive random-oriented three dimensional growth, crisscrossing cells with increased cytoplasmic basophilia at the perimeter of the colony and cells with decreased cytoplasm/nucleus ratios relative to normal cells. Benzo(a)pyrene was used as the positive control.

Based on the results of the initial cytotoxicity assay, the dose levels initially selected for use in the definitive transformation assay (#1) were 20, 40, 60, 80 and 100 µg/ml (25 dishes per concentration). However, the results of the definitive assay showed the relative toxicity to be much greater than that predicted by the preliminary (cytotoxicity) assay; 21, 38, 71, 100 and 100% toxicity at concentrations of 20, 40, 60, 80 and 100 µg/ml, respectively. Based on these results, the following dose levels were selected for a second definitive transformation assay: 10, 20, 30, 40 and 50 µg/ml. Two additional dose groups, at concentrations of 40 and 50 µg/ml, were cell adjusted, i.e., the number of target cells seeded was adjusted to yield approximately the same number of colonies per dish as that of the solvent vehicle. Two trials were conducted. The total colony number and the number of colonies with transformed morphology for each test group were recorded. Based on this data, plating efficiencies and transformation frequencies were calculated.

The criterion for a positive result was a statistically significant increase in morphological transformation frequency with at least two dose levels compared to concurrent control. In addition, a test was considered positive if one dose level showed a statistically significant treatment-related increase in transformation frequency and there was an indication of a statistically significant ($p \leq 0.05$) positive dose-trend. A test was considered negative if there was no dose level associated with a statistically significant treatment-related increase in

transformation frequency and the uppermost dose of the test substance demonstrated a sufficient level of toxicity (a 50% reduction in plating efficiency or colony density).

Results

Statistically significant increases in transformation frequency (compared to concurrent control) were observed at 30 and 50 µg/ml when pooled data from both trials were analyzed using the Fishers Exact test (Table 3.4.11.1). However, the 50 µg/ml cell-adjusted culture (50CA) did not show a significant response (p =0.0735). The sponsor argues that a reduction in colony number/dish (681 colonies at 50 µg/ml) due to cytotoxicity can produce significant responses, and the results from the cell-adjusted cultures (1714 colonies at 50CA µg/ml) negate results obtained from their non-adjusted counterparts. There was no significant dose-response trend since 40 µg/ml and 40CA µg/ml did not produce statistically significant increases in transformation frequency compared to concurrent control (p >0.05) (Fig. 3.4.11.1). Therefore, the only concentration of olmesartan considered to produce a statistically significant response was 30 µg/ml. All controls showed an appropriate response and all acceptance criteria for a valid assay were met. Therefore, according to the assay evaluation criteria, OM tested negative for potential to induce morphological transformation.

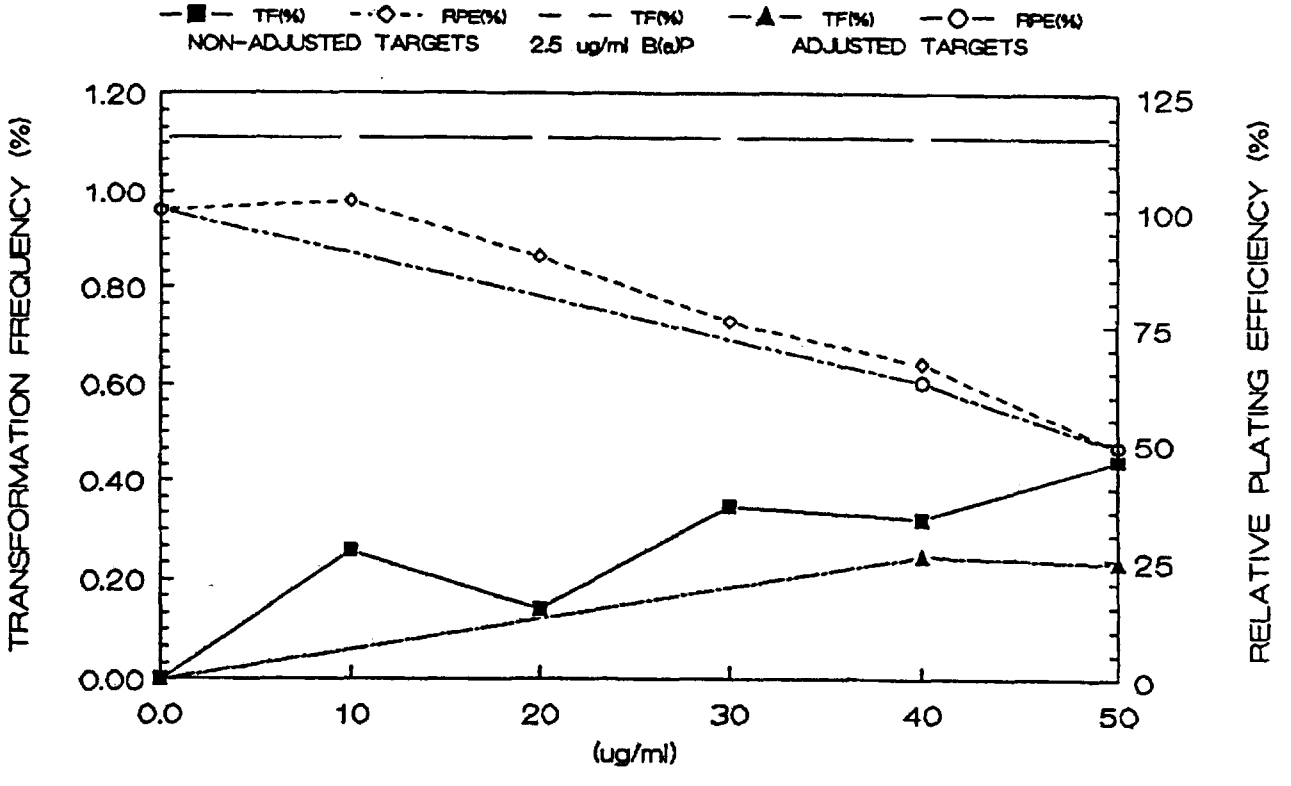

Fig. 3.4.11.1: Syrian hamster embryo cell transformation assay of varying concentrations of OM. Data shows relative plating efficiency and transformation frequency of olmesartan and positive control, benzo(a)pyrene, after 7 days continuous exposure

TABLE 3.4.11.1

Syrian Hamster Embryo Cell Transformation Assay of Varying Concentrations of OM and Positive Control, Benzopyrene, after 7 days Continuous Exposure.

	Dose	Total Colonies Scored	MT[1]/ MT Freq.[2]	P.E.[3] ± S.E.[4]	Relative P.E.[5] (%)	MT P value 1 Tail Fisher's[6]
	DMSO (0.2%)	1577	0/ 0.000	49 ±1.0	100	TR=0.0946[8]
	MEDIUM	1381	3 /0.217	44 ±1.1	90	
B(a)P	1.25 µg/ml	1382	13 /0.941	43 ±0.8	88	0.0000[7]
	2.5 µg/ml	1439	16 /1.112	45 ±1.0	92	0.0000[7]
Dose:	10 µg/ml	1554	4 /0.257	50 ±1.2	102	0.0606
	20 µg/ml	1418	2 /0.141	44 ±0.9	90	0.2241
	30 µg/ml	1153	4 /0.347	37 ±1.2	76	0.0317[7]
	40 µg/ml	935	3 /0.321	33 ±1.2	67	0.0515
	40CA µg/ml	1619	4 /0.247	31 ±0.9	63	0.0657
	50 µg/ml	681	3 /0.441	24 ±1.0	49	0.0273[7]
	50CA µg/ml	1714	4 /0.233	24 ±0.6	49	0.0735

1: MT = Combined total no. of morphologically transformed colonies
2: MT Frequency = MT / Total colonies scored X 100
3: Average PE = Average PE of combined trials
4: Standard Error (SE) = $\frac{\text{Standard deviation of combined average PE (plating efficiency)}}{\sqrt{\text{Combined total no. of dishes counted}}}$
5: Relative PE = Average PE / Average PE of solvent control X 100
6: MT P value = Probability of statistically significant treatment related effects using Fishers Exact Test compared to control groups.
7: Statistically significant treatment related difference between the morphological transformation frequency (MTF) of the solvent control group compared to the MTF of the treatment group at $p \leq 0.05$ using a 1-tailed Fishers Exact Test
8: TR = Data set tested for statistically significant treatment related exact trend increase in MTF of the solvent control group compared to the MTF of the treatment group at $p \leq 0.05$ using a 1-tailed exact test for trend.
9: CA = Cell Adjustment of dose equalized to controls:

No. Target cells needed = $\frac{\text{(No. Target Cells seeded for Controls) X (100)}}{\text{RPE of dose level (from estimation / interpolation of cytotoxicity)}}$

For the 40 µg/ml dose: 130 = $\frac{\text{(80 cells seeded for controls) X (100)}}{\text{62 (estimation of RPE for 40 µg/ml dose group)}}$

For the 50 µg/ml dose: 178 = $\frac{\text{(80 Cells seeded for Controls) X (100)}}{\text{45 (estimation of RPE for 50 µg/ml dose group)}}$

3.4.12. *In Vitro* Transformation of Syrian Golden Hamster Embryo Cells with Olmesartan (Study #18550-0-485R, Report #TRC 145-004) Vol. 18

This GLP study was conducted by a contract laboratory, [redacted] between May 20 and September 20, 1997. Olmesartan, the active metabolite of olmesartan medoxomil (OM), was investigated for its potential to induce morphological transformation in the Syrian hamster embryo cell transformation assay.

The assay was performed as previously described for OM (section 3.4.11). In the initial cytotoxicity assay, olmesartan (lot #F-352) dissolved in DMSO was tested at concentrations ranging from 0.1-100 µg/ml (15 dishes/concentration). Relative cytotoxicity was determined by comparing test substance treated groups with vehicle treated controls. When cytotoxicity was evaluated by the % reduction in relative plating efficiency (% RPE), olmesartan showed less than 10% toxicity for each of the 10 dose levels between 0.10 µg/ml and 25 µg/ml, followed by 40% toxicity at 50 µg/ml, 70% at 75 µg/ml and 84% toxicity at 100 µg/ml. When cytotoxicity was evaluated by % reduction in relative colony density (%RCD), three dose levels (ranging from 1 to 5 µg/ml) showed either no toxicity or a slight enhancement in relative colony size. The relative toxicity increased to 20% at 7.5 µg/ml, then down to 12% at both 10 and 25 µg/ml before increasing to 42% at 50 µg/ml. The toxicity was 59% and 67% at concentrations of 75 and 100 µg/ml, respectively. A second cytotoxicity assay was performed with the test substance at concentrations ranging from 25 to 60 µg/ml. The results of this 2nd cytotoxicity test, evaluated by RPE and RCD, were as follows:

Conc. µg/ml	Cytotoxicity by Relative Plating Efficiency		Cytotoxicity by Relative Colony density	
	Average PE	RPE, %	Colony density, x 10^4	RCD, %
DMSO (0.2%)	52	100	2.86	100
25	41	79	2.22	77
30	37	71	2.10	73
35	33	63	2.51	88
40	35	67	2.80	98
45	29	56	1.88	66
50	27	52	2.04	71
55	19	37	2.33	81
60	15	29	2.54	89

Based on the results of the 2nd cytotoxicity assay, the dose levels selected for use in the definitive transformation assay (2 independent experiments) were 10, 20, 30, 40 and 50 µg/ml (25 dishes per concentration). Two additional dose groups, at concentrations of 40 and 50 µg/ml, were cell adjusted, i.e., the number of target cells seeded was adjusted to yield approximately the same number of colonies per dish as that of the solvent vehicle. Benzo(a)pyrene and DMSO were used as the positive and negative controls, respectively.

Results

A statistically significant increase in the frequency of morphological transformation, compared to concurrent control, was observed at 50 µg/ml (non-target cell adjusted). However, the 50 µg/ml cell-adjusted dose did not result in a similar significant transformation frequency (Table

3.4.12.1). In addition, no positive dose-response trend was observed. The authors of the study report argue that the pattern of differential transformation response as seen at the 50 µg/ml dose level is attributable (at least in part) to the effect of the test agent on the relative colony number per plate (non-adjusted dose resulted in fewer colonies, 778 compared to cell adjusted counterpart, 1399, see Table 3.4.12.1, Fig. 3.4.12.1). Test substance-induced cytotoxicity that results in a 50% reduction in colony number/dish (relative to control) can increase MTF by virtue of that reduction. Further, the authors conclude that the results from the cell-adjusted cultures should negate results obtained from their non-adjusted counterparts when one group has significantly fewer than 1000 colonies and the other group exceeds that value. All controls showed an appropriate response and all acceptance criteria for a valid assay were met.

Based on the above argument advanced by the sponsor, olmesartan is considered to have tested negative for its potential to induce morphological transformation under the conditions of the assay.

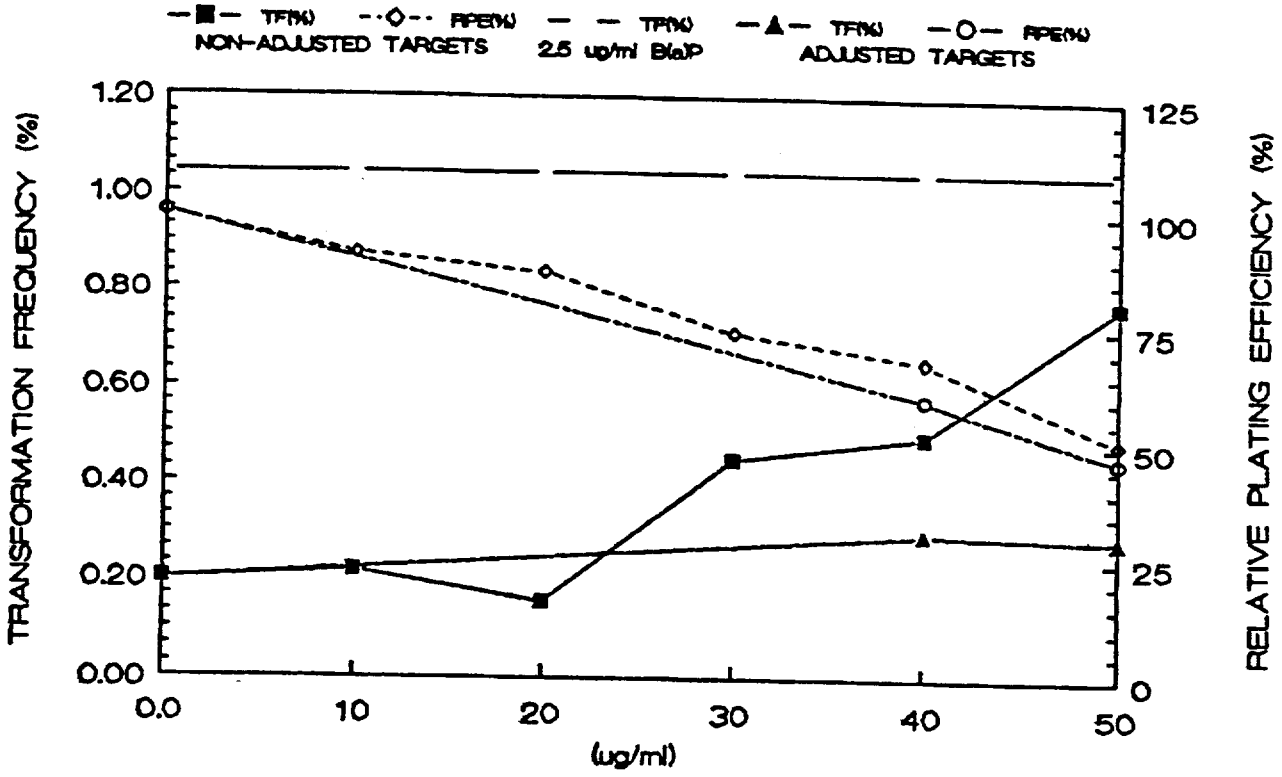

Fig. 3.4.12.1: Syrian hamster embryo cell transformation assay with varying concentrations of olmesartan. Data shows relative plating efficiency and transformation frequency of RNH-6270 and positive control, benzo(a)pyrene, after 7 day continuous exposure.

TABLE 3.4.12.1

SYRIAN HAMSTER EMBRYO CELL TRANSFORMATION ASSAY OF VARYING CONCENTRATIONS OF OLMESARTAN AND POSITIVE CONTROL, BENZO(A)PYRENE, AFTER 7 DAYS CONTINUOUS EXPOSURE.

	Dose	Total Colonies Scored	MT,[1] MT Freq.[2]	Average P.E.[3] ± S.E.[4]	Relative P.E.[5] (%)	MT P Value 1 Tail Fisher's[6]
	DMSO (0.2%)	1504	3 /0.199	47 ±1.2	100	[8] TR=0.2281
	MEDIUM	1348	2 /0.148	42 ±1.2	89	
B(a)P	1.25 µg/ml	1442	16 /1.110	45 ±1.1	96	0.0016[7]
	2.5 µg/ml	1437	15 /1.044	45 ±0.9	96	0.0028[7]
Dose:	10 µg/ml	1376	3 /0.218	43 ±1.2	91	0.6138
	20 µg/ml	1296	2 /0.154	41 ±1.3	87	0.5694
	30 µg/ml	1112	5 /0.450	35 ±1.4	74	0.2143
	40 µg/ml	1006	5 /0.497	32 ±1.2	68	0.1745
	40CA[9] µg/ml	1350	4 /0.296	28 ±1.0	60	0.4411
	50 µg/ml	778	6 /0.771	24 ±1.2	51	0.0469[7]
	50CA µg/ml	1399	4 /0.286	22 ±0.8	47	0.4605

For descriptions of the superscript numbers 1 to 8, see Table 3.4.11.1.

9: CA = Cell Adjustment of dose equalized to controls:

$$\text{No. Target cells needed} = \frac{\text{(No. Target Cells seeded for Controls) X (100)}}{\text{RPE of dose level (from estimation / interpolation of cytotoxicity)}}$$

$$\text{For the 40 µg/ml dose: } 120 = \frac{\text{(80 cells seeded for controls) X (100)}}{\text{67 (estimation of RPE for 40 µg/ml dose group)}}$$

$$\text{For the 50 µg/ml dose: } 160 = \frac{\text{(80 Cells seeded for Controls) X (100)}}{\text{50 (estimation of RPE for 50 µg/ml dose group)}}$$

3.4.13. Unscheduled DNA Synthesis in Rat Liver, *In Vivo* (Study #SNY 340/951225, Report #TRC 142-023) Vol. 18

This GLP study was conducted by a contract laboratory, [redacted] between May 9 and July 2, 1995. Olmesartan medoxomil was investigated for potential DNA-damaging activity in the *in vivo* rat hepatocyte DNA repair system. The method detects unscheduled DNA synthesis due to a lesion induced by a chemical, by measuring the incorporation of tritiated thymidine in the DNA of non-replicating rat hepatocytes in primary culture.

In the initial toxicity test, 4 male Sprague-Dawley rats were dosed orally (by gastric intubation) once with OM (batch #NH 006 C1) at 2000 mg/kg (10 ml/kg), the maximum dose recommended by the OECD guidelines. Following dosing, the animals were observed regularly during the working day for a period of 4 days. No mortalities and no clinical signs of toxicity were observed. Based on these results, in the main study, groups of 4 male Sprague-Dawley rats (6 weeks old and weighing 174-204 gm) were treated once with olmesartan (suspension in 0.5% w/v SCMC) at 600 and 2000 mg/kg orally by gastric intubation (10 ml/kg). A negative control group was treated with the vehicle, aqueous 0.5% SCMC, and a positive control group was treated (p.o.) with dimethylnitrosamine at 4 mg/kg (for the 2 hr expression) or 2-acetylaminofluorene at 50 mg/kg (for the 14 hour expression).

Animals were killed 2 or 14 hour after treatment with test substance, vehicle or positive control. Four animals were assessed at each experimental point, with the exception that only 2 animals from the positive control group were assessed at each expression time. Hepatocytes were isolated from each rat by enzymatic dissociation of the liver using a perfusion procedure. The cells were suspended in tissue culture medium and incubated with 10 μCi/ml tritiated thymidine at 37°C to radiolabel replicating DNA. After 4 hours, cells were washed, fixed and autoradiograms were prepared. The incorporated radioactive thymidine results in deposition of silver grains in the overlying autoradiographic emulsion. Increases in the nuclear grain density of cells from treated animals are indicative of DNA repair and hence DNA damage. Each slide was examined microscopically for cytoplasmic and gross nuclear grain count. The cytoplasmic grain count was subtracted from the gross nuclear grain count to give the net nuclear grain count. The number of cells with a net grain count greater than or equal to 5 was recorded as an indication of cells undergoing repair. A positive response is indicated by a substantial dose-associated statistically significant increase in the net nuclear grain count, which is accompanied, by a substantial increase in the gross nuclear grain count over concurrent control values.

Results

There were no mortalities or clinical signs of toxicity. Olmesartan medoxomil, at doses up to 2000 mg/kg did not show any significant increase ($p > 0.05$) in the gross or net nuclear grain count at either the 2 or 14 hour expression time. Grain counts (net nuclear, -2.5 at 2000 mg/kg) were similar to vehicle control values (-2.3) and were within the range of historical control values. Animals treated with positive control chemicals showed a highly significant increase ($p < 0.001$) in the net nuclear grain count which was accompanied by a substantial increase in the gross nuclear grain count. Thus, it is concluded that OM did not elicit any evidence of DNA-damage in the rat liver in this *in vivo* test system.

3.4.14. Micronucleus Test of Olmesartan Medoxomil in Mice (Study #93-0031, Report #TR 140-033). Vol. 17

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between February 23, 1993 and April 7, 1993. The aim of the study was to investigate the potential of olmesartan medoxomil to produce clastogenic or aneugenic effects in mice.

An *in vivo* micronucleus test was carried out in 8-week-old male CD-1 mice. An initial dose range-finding study was conducted using groups of 3 male mice (weight: 34.8 to 37.8 gm). Olmesartan medoxomil (OM) (lot NH001C3) was suspended in 0.5% SCMC made up with physiologic saline in appropriate concentrations to allow intraperitoneal dosing at a constant volume, 20 ml/kg. Doses of 2000, 400, 80, 16, and 3.2 mg/kg were used to determine lethality at 24 hours. No mice died. For the main study, groups of 6 male mice (weight: 36.7 to 42.2 gm) were given OM intraperitoneally at dose levels of 2000, 1000, and 500 mg/kg. Negative (vehicle) and positive control (mitomycin C, 0.5 mg/kg) groups were also included, six male mice being used for each treatment group. Bone marrow cells were collected from femurs 24 hours after drug administration, stained and examined microscopically. The number of micronucleated cells were recorded in 1000 polychromatic erythrocytes and 1000 normochromatic erythrocytes per mouse. At the same time, the ratio between PCE and NCE per 1000 erythrocytes was calculated.

Results

OM was well tolerated and there were no deaths. Mice treated with OM exhibited polychromatic to normochromatic erythrocyte ratios of 80.2, 79.3 and 68.1% for the 500, 1000 and 2000 mg/kg groups, respectively (vehicle control, 100%), suggesting adequate exposure of the bone marrow. As the dose levels increased, PE/NE ratios decreased but not significantly. (The lack of statistical significance for the rather large mean difference between the high dose and vehicle control groups appears to be due to high intragroup variability.) The incidences of micronucleated polychromatic erythrocytes were 0.12%, 0.10% and 0.07% for the 2000, 1000, and 500 mg/kg doses, respectively. The incidence for the vehicle control was 0.18%, and for mitomycin C, 1.23% ($p < 0.01$). The corresponding incidences of micronucleated normochromic erythrocytes were 0.12%, 0.08%, and 0.10%, not significantly different from the incidence in the vehicle control (0.12%).

It is concluded that olmesartan medoxomil has no clastogenic activity in this *in vivo* test system.

3.4.15. Micronucleus Test of Olmesartan Medoxomil in Mice. Second study (Study #B-3528, Report #ZRC144-001) Vol. 18

This GLP study was conducted by a contract lab, [redacted] Dosing was initiated on December 9, 1996 and bone marrow smear observation was completed on December 13, 1996. This study examined the micronucleus-inducing potential in bone marrow erythrocytes of olmesartan medoxomil (ΘM) produced by a different manufacturing method from that used for production of the lot tested in the previous study.

The experimental design and methodology are essentially similar to that described in the previous section (#3.4.9). OM (lot #NH204C) suspended in 0.5% CMC-Na was administered intraperitoneally to male mice (Crj:CD-1 SPF, body weight: 33.5 to 39.1 gm) at three dose levels: 500, 1000 and 2000 mg/kg (n=6/dose). The positive control group animals received single i.p. doses of 2 mg mitomycin C/kg. All animals were observed for toxic signs and behavioral abnormalities before, immediately after, and 24 hours after dosing.

Results

Test substance did not affect the general condition of the animals. The mean body weight for the vehicle and positive control groups was 0.6 to 1.0 gm greater on the day following administration than before administration. In contrast, the drug treated groups showed mean decreases of 1.8 to 2.5 gm (5 to 6.9%) from their pretreatment weights,

The incidences of micronucleated polychromatic erythrocytes (MNPCE) per 1000 polychromatic erythrocytes counted were 0.07, 0.15 and 0.22% for the 500, 1000 and 2000 mg/kg doses, respectively. The incidence of MNPCEs in the negative and positive control groups were 0.08 and 6.22%, respectively. The Fisher's exact probability test suggested no significant difference between the negative control and the drug treated groups. But the incidence of MNPCEs showed a dose-response when assessed by Cochran Armitage's trend test. However, the authors of the study report consider this finding not biologically significant because it arose from an incidental increase in one mid dose animal.

The present study confirms the previous findings that olmesartan medoxomil has no micronucleus inductivity.

3.4.16. Gene Mutation Asssay in Transgenic Mice with Olmesartan Medoxomil (Study #3926, Report #TRC146-013) Vol. 18

This GLP study was conducted by a contract lab. [redacted] between May 1 and June 17, 1988. Olmesartan medoxomil (OM) is intended for oral dosing and is metabolized in the intestine, so the intestine was examined as a potential target organ for genetic toxicity.

Animals used for the study were CD2-LacZ80/HazfBR (BALB/CxDBA/2) male mice (Muta™ mice). Groups of 5 mice (21 to 33.5 gm, 7 weeks of age) were given OM (lot #NH206C) orally by gavage once daily on 5 consecutive days at doses of 100, 1000 or 2000 mg/kg. Two additional groups of mice were dosed with vehicle (10 ml/kg, 0.5% SCMC) or N, N-dimethylhydrazine (DMH) (5 mg/kg, dissolved in water). Dose selection was based on a previous gene mutation test in the Muta™ mouse in which 5 daily doses of 2000 mg/kg/day day resulted in a statistically significant increase in mutant frequency in intestinal mucosal cells (Report #TRC 145-008).

Body weights and clinical signs of toxicity were recorded on the first day of treatment and 1 and 14 days after treatment. Animals were sacrificed 1 or 14 days after the 5th dose. DNA from mucosal epithelial samples from small and large intestine was isolated, solubilized, and packaged in bacteriophages. *E coli* strain C (gal E$^-$) was incubated with the bacteriophage to infect the *E coli*. The *E coli* were then plated onto P-gal plates and incubated for 16 hours to allow expression of *lacZ* mutant plaques. The same procedure was followed without addition of P-gal to obtain the total number of plaques. Mutation frequency was calculated by dividing the number of *lacZ* mutant plaques by the total number of plaques.

The genetic makeup of the lactose operon (*lac*) of a normal bacterium can be written $Z^+Y^+A^+$, which indicates that active forms of all three proteins are produced. A mutation in one of the genes (here it is the Z gene that codes for ß-galactosidase) can result in a bacterium that produces an altered or completely inactive form of the corresponding protein. The $Z^-Y^+A^+$ mutant would respond to the presence of lactose by producing permease (Y+) and transacetylase (A+) but no effective ß-galactosidase. The donor (phage) does not produce ß-galactosidase (because of mutation) in the absence of inducer (lactose) but does it in its presence. The recipient (E.coli) produces no active ß-galactosidase in either condition because their Z gene is defective.

Results

There were no treatment-related changes in the general condition of animals on day 1 of treatment. On day 14, one high dose animal showed dermal irritation around the anus. Furthermore, a slight suppression of body weight gain (not significant) was noted in mid and high dose groups on day 14.

The mutant frequencies were statistically significantly increased in intestinal mucosa in all the OM-treated groups at 1 day post-dosing relative to vehicle control (Fig 3.4.16.1). For the 14 day expression period group, the mutation frequency was statistically significantly increased relative to vehicle control in the middle and the highest dose groups (Fig 3.4.16.2). A dose-dependent

increasing trend in the frequency was demonstrated at both 1 and 14 days post-dosing. The average mutation frequencies in the positive control groups were significantly increased compared to the vehicle control groups.

Based on these results, it was concluded that olmesartan medoxomil caused a statistically significant increase in mutation frequency in intestinal mucosal cells under the conditions of this assay. However, the sponsor in an amendment to the study report (May 25, 2000) retracts this conclusion, citing a lack of international agreement on evaluation criteria for this system.

TABLE 3.4.16.1
MUTANT FREQUENCIES FROM ANALYSIS OF SMALL INTESTINE MUCOSA OF MALE MICE AFTER OLMESARTAN MEDOXOMIL TREATMENT (EXPRESSION TIME: 1 DAY)

Compound	Dose (mg/kg)	Number of animals	Number of mutations	Number of plaques	Mutant frequency ($\times 10^{-6}$) [Mean ± S.D.]
0.5% CMC-Na a)	0×5	5	143	2720100	51.7 ± 11.5
CS-866	100×5	5	264	3068700	82.7 ± 18.2 *
	1000×5	5	244	2871900	87.1 ± 19.2 **
	2000×5	5	287	2844900	101.4 ± 14.4 ***
DMH b)	5×5	5	264	2546500	100.8 ± 16.4 ***

a) : Vehicle control
b) : Positive control (*N, N*-Dimethylhydrazine)
Significant difference from control *:p<0.05, **:p<0.01, ***:p<0.001

TABLE 3.4.16.2
MUTANT FREQUENCIES FROM ANALYSIS OF SMALL INTESTINE MUCOSA OF MALE MICE AFTER OLMESARTAN MEDOXOMIL TREATMENT (EXPRESSION TIME: 14 DAYS)

Compound	Dose (mg/kg)	Number of animals	Number of mutations	Number of plaques	Mutant frequency ($\times 10^{-6}$) [Mean ± S.D.]
0.5% CMC-Na a)	0×5	5	128	2832300	51.0 ± 10.7
CS-866	100×5	5	249	3120300	76.9 ± 24.4
	1000×5	5	348	3328200	107.9 ± 17.8 ***
	2000×5	5	236	2398400	88.6 ± 29.1 **
DMH b)	5×5	5	264	3060000	120.8 ± 24.5 ***

a) : Vehicle control
b) : Positive control (*N, N*-Dimethylhydrazine)
Significant difference from control **:p<0.01, ***:p<0.001

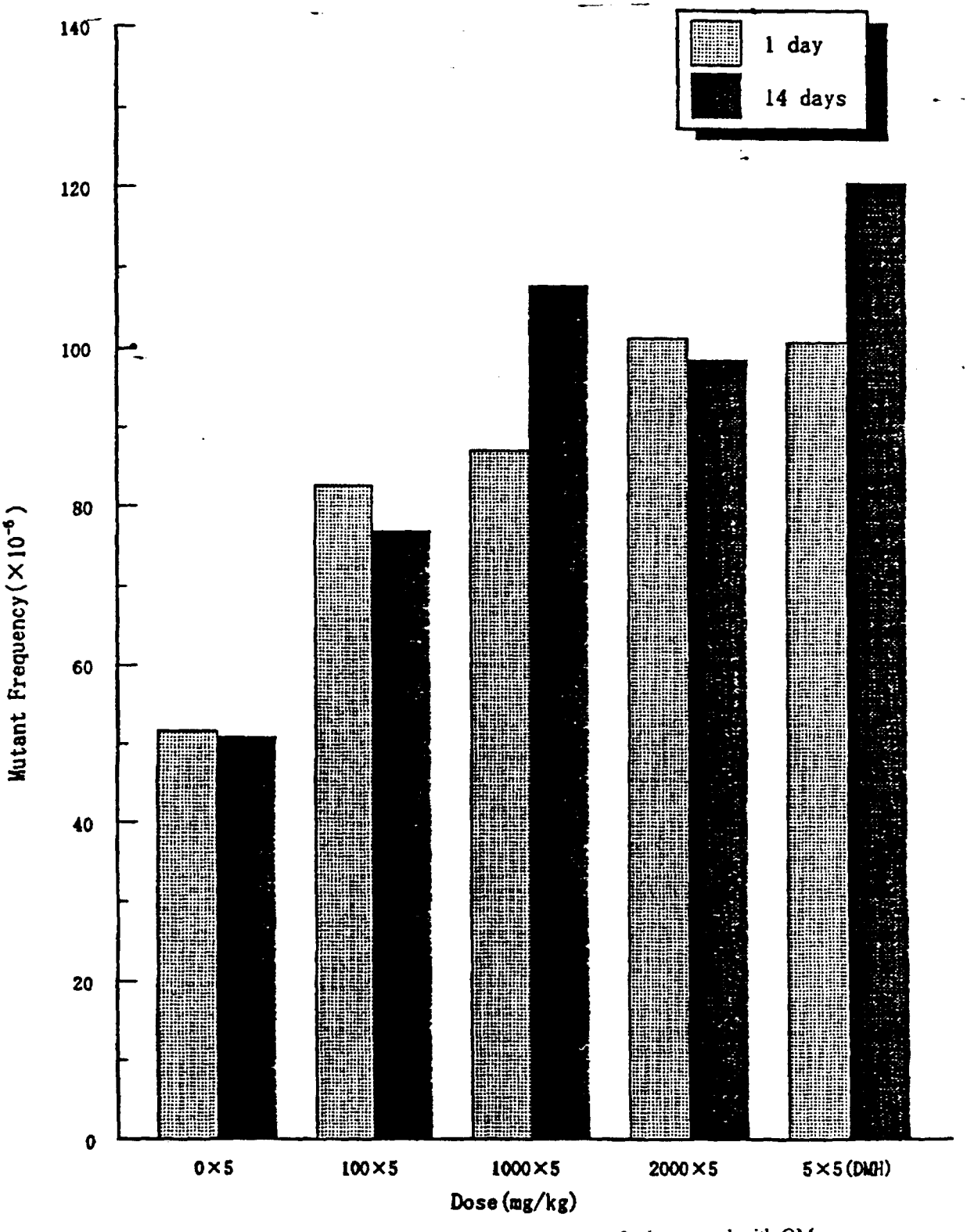

Fig. 3.4.16.1.: Mutant frequencies obtained from small intestine mucosa of mice treated with OM

3.4.17. Gene Mutation Assay in Transgenic Mice (Muta™ Mouse Intestine) with Olmesartan Medoxomil –Repeat Study (Study #00-B072, Report #APR147-155) Amendment of August 2, 2001

This non-GLP study was conducted by a contract lab, [redacted] between November 28, 2000 and July 16, 2001.

In the previous two gene mutation assays of olmesartan medoxomil (OM) in Muta™ mice (section 3.4.16), small but statistically significant increases in *LacZ* mutation frequency were observed in the intestinal epithelium 1 and 14 days after 5 days of OM administration at all dose levels. But the sponsor contends that the increases in mutation frequency were neither obvious (less than 2-fold) nor dose-dependent. Additionally, the sponsor argues that "the validity of these studies could be suspicious because of insufficient response to the positive control substance N, N-dimethylhydrazine (DMH)". In this third study, mutation frequency at the *cII* locus as well as the *LacZ* locus was analyzed and ethyl nitrosourea (ENU) was used as a positive control instead of DMH. The sensitivity of the *cII* system is comparable to that of the *LacZ* system (Jakubczak, J.L. *et al.*, Proc. Natl. Acad. Sci., **93**: 9073-9078, 1996). Both the *cII* and the *LacZ* genes were inserted into the bacteriophage λ transgene, which was stably integrated into the genome of the Muta™ mouse.

Animals used in this study were similar to those used in the previous studies [CD2-LacZ80/HazfBR (BALB/CxDBA/2) male mice (Muta™ mice)]. Groups of 10 mice (18.9 to 24.1 gm, age not given) were given OM (lot #OS-001C1, 2000 mg/kg/day), vehicle (0.5% CMC) or ENU (100 mg/kg/day) orally by gavage once daily on 5 consecutive days. Animals were housed individually with *ad libitum* access to a commercial diet and tap water.

Animals were sacrificed 1 or 14 days after the 5th dose. DNA from mucosal epithelial samples from the upper part of the small intestine (from the area next to the pylorus of the stomach) was isolated, solubilized, and packaged in bacteriophages. *E coli* strain *gal E*$^-$ for *lacZ* selection and *E coli* strain *G1225* for *cII* selection were incubated with the bacteriophage to infect the *E coli*. The mixtures containing *E coli* were poured onto agar plates and incubated at 37°C overnight to allow expression of *lacZ* or *cII* mutant plaques. Mutation frequency was calculated by dividing the number of *lacZ* or *cII* mutant plaques by the total population (titer of the adsorbed phage). Mutation frequency data was statistically analyzed using Student's t-test with Bonferroni adjustment.

Results

Clinical signs of toxicity in the vehicle control group were limited to polypnea in 4 of 10 animals, which was noted between days 1 and 4. All animals appeared normal after day 5. In the OM group, polypnea was observed in 5 of 10 animals. Three days after the last dose, one of the OM-treated mice had polypnea, labored respiration, hypoactivity, ataxia and limited use of hind limbs and died the next day. In the positive control group, 3 of the 10 animals had polypnea and one exhibited labored respiration.

No statistically significantly increases in *lacZ* mutant frequencies (MFs) were observed in the intestinal epithelium 1 and 14 days after 5 days of OM administration. In contrast, OM tested positive for *cII* gene mutations 14 days (not 1 day) after 5 days of OM administration ($p = 0.04$). For both loci, MFs were less than twice vehicle control (Table 3.4.17.1). MFs in the ENU administered group were increased significantly at both sampling times. MFs for ENU were 25.5 and 35.4 times vehicle control on day 1 and day 14, respectively, for *lacZ* selection and 24 and 63 times vehicle control on day 1 and day 14, respectively, for *cII* selection (Table 3.4.17.1). The results thus suggest equivocal effect of OM in the intestinal epithelium.

TABLE 3.4.17.1
MUTATION FREQUENCY ANALYSIS IN MUTA™ MOUSE USING *lacZ* AND *cII* SELECTION SYSTEMS

Exp day	Chemical	*CII* gene			*LacZ* gene		
		Mutation Frequency ($\times 10^{-6}$)		p-value	Mutation Frequency ($\times 10^{-6}$)		p-value
		Average ± S.D	Fold induction		Average ± S.D.	Fold induction	
1	Vehicle[a]	35.64 ± 10.36	-	-	60.49 ± 22.03	-	-
	ENU[b]	858.38 ± 468.75	24.08	0.0022	1542.99 ± 1181.83	25.51	0.0124
	OM	35.22 ± 11.57	0.99	0.4766	67.21 ± 37.99	1.11	0.3707
14	Vehicle[a]	31.59 ± 11.91	-	-	80.27 ± 26.07	-	-
	ENU[b]	1992.17 ± 982.24	63.06	0.0011	2838.82 ± 1040.65	35.36	0.0002
	OM	51.33 ± 17.16	1.62	0.0401	90.43 ± 21.80	1.13	0.2767

a) 0.5% CMC.
b) Ethyl nitrosourea.

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3.4.18. Gene Mutation Assay in Transgenic Mice (Muta™ Mouse Kidney) with Olmesartan Medoxomil (Study #APE-148-016B, Report #APR148-071) Amendment of August 30, 2001

This non-GLP study was conducted by Medicinal Safety Research Laboratories, Sankyo Co. Ltd., Japan, between June 21 and September 7, 2001. Using similar techniques and methods as described for the small intestine (section 3.4.17), the sponsor conducted another Muta™ mouse study to determine the effects of olmesartan medoxomil (OM) on the kidney. Unlike in the previous study, mutation frequency at the *LacZ* locus only was analyzed and streptozotocin (STZ) was used as the positive control

Animals used in this study were similar to those used in the previous studies [CD2-LacZ80/HazfBR (Muta™ mouse)]. Groups of 10 male mice (21 to 29 gm, age not given) were given OM (lot #100K1677, 600 or 2000 mg/kg/day), or vehicle (0.5% CMC). The positive control, STZ, was administered intraperitoneally once daily for 1 or 5 consecutive days at 150 mg/kg/day (n=5/treatment). Animals were housed individually and both tap water and a commercial diet were available *ad libitum*.

Animals given vehicle or test drug were sacrificed 1 or 14 days after the 5th dose (n=5/sampling time). STZ treated animals were all sacrificed 14 days after the last dose administration. The kidney was excised, longitudinally cut open and rinsed with buffer. The *lacZ* selection was performed as described in the previous section. The samples collected from animals receiving 600 mg OM/kg/day were stored but not used for mutant frequency (MF) analysis. Mutation frequency data was statistically analyzed using Student's t-test with Bonferroni adjustment.

Results

No clinical signs of toxicity were observed in the vehicle control or drug treated groups. All 5-day STZ-treated animals were found dead (day of death is not given). However, no clinical signs of toxicity were observed in animals receiving 1-day STZ.

No statistically significant increases in *lacZ* MF observed in the kidney 1 and 14 days after 5 days of OM administration. MFs were 0.62 ($p = 0.052$) and 1.06 ($p = 0.42$) times vehicle control on day 1 and day 14, respectively, after 5 days of OM administration. On the other hand, MF in the single STZ administered group was increased significantly at day 15 (no sampling done on day 6 for STZ treated group). MF for STZ was 2.43 times vehicle control (Table 3.4.18.1).

TABLE 3.4.18.1

lacZ MUTANT FREQUENCIES IN THE KIDNEY AFTER 1 OR 5 DAYS OF ADMINISTRATION OF VEHICLE, OLMESARTAN MEDOXOMIL OR STZ IN MALE MUTA™ MICE

Exp. day	Chemical	Mutation Frequency (x 10^{-6})		Statistical analysis[c]	
		Average ± S.D	Fold induction	p-value	Evaluation[d]
6	Vehicle[a]	72.00 ± 20.62	-	-	
6	OM 2000 mg/kg/day x 5 days	44.75 ± 10.85	0.62	0.0523	
19	Vehicle[a]	53.80 ± 19.73	-	-	
15	STZ[b] 150 mg/kg/day x 1 day	130.75 ± 25.17	2.43	0.0048	§
19	OM 2000 mg/kg/day x 5 days	57.11 ± 18.63	1.06	0.4182	

a) 0.5% CMC.
b) streptozotocin
c) Student's t-test with Bonferroni adjustment.
d) § $p<0.01$

o3.4.19. A Comet Assay With Olmesartan Medoxomil in Rats (Study #APE-148-012B, Report #APR148-062) Amendment of August 30, 2001

This non-GLP study was conducted by Medicinal Safety Research Laboratories, Sankyo Co. Ltd., Japan, between May 25 and August 27, 2001. The comet assay detects DNA damage that includes strand breaks and/or alkali-labile sites in DNA, but does not detect chromosome aberrations.

Male F344/DuCrj rats (9 weeks of age, body weight not given) were given single oral doses of 600 or 2000 mg/kg olmesartan medoxomil (OM) (lot #OS-00Cl) or vehicle (10 ml/kg, 0.5% SCMC) by gastric tube. The positive control, methyl methanesulfonate, was administered intraperitoneally at a rate of 1 ml/100 gm (80 mg/kg). Animals were sacrificed 3 or 24 hours (n=3/time/treatment) after the administration. The left kidney was removed, minced and homogenized. The suspension was centrifuged and the supernatant containing the nuclei was electrophoresed and 50 nuclei in one specimen per animal were observed to calculate DNA migration. Nuclei with increased DNA damage display increased migration of DNA from the nucleus toward the anode.

Measuring the length of the whole comet (length) and the diameter of the head (diameter) using an image analysis system quantitates the migrating DNA. The migration is calculated by subtracting the diameter from the length. DNA migration for each animal was defined as the average value of the 50 migrations calculated per animal. Group values for DNA migration were compared using one-way analysis of variance. Differences between the treatment groups and vehicle control groups were compared using the Dunnett's test. A p-value less than 0.05 was considered statistically significant. The Center's Genetic Toxicology Committee, which reviewed the results of the assay, does not agree with the way the sponsor has analyzed the data. According to the committee, although it is appropriate to compare the means of the treated and control groups, the proportion of damaged cells should also be analyzed (see Appendix #1).

Results

Based on the sponsor's analysis, DNA migration 3 or 24 hours after administration in the groups treated with OM (600 or 2000 mg/kg) was not significantly different than the DNA migration in the corresponding vehicle control group (Table 3.4.19.1). In the positive control group, DNA migration was significantly increased at both sampling times. Thus, the sponsor concludes that OM does not induce DNA damage in the kidneys of rats. In contrast, the Committee's analysis of the data suggests a "positive" finding at a significance level of <0.01 (see Appendix 1).

TABLE 3.4.19.1
DNA MIGRATION IN KIDNEYS OF RATS

Treatment period	Substances	Dose	Animal No.	DNA migration (μm)[1] Individual value[2]	Group value[2]
3hr	Vehicle		9	7.49 ± 10.97	7.57 ± 0.74
			8	8.35 ± 13.87	
			11	6.87 ± 8.35	
	CS-866	600 mg/kg	16	6.82 ± 6.58	7.59 ± 0.94
			1	7.31 ± 7.00	
			18	8.64 ± 8.39	
		2000 mg/kg	24	10.25 ± 13.98	8.88 ± 1.47
			12	7.32 ± 8.20	
			22	9.07 ± 10.89	
	MMS	80 mg/kg	15	25.85 ± 13.93	29.45 ± 3.28**
			17	32.27 ± 11.08	
			19	30.24 ± 11.33	
24hr	Vehicle		4	7.60 ± 11.08	8.04 ± 0.83
			7	8.99 ± 10.17	
			23	7.52 ± 10.14	
	CS-866	600 mg/kg	20	7.02 ± 6.53	8.32 ± 1.16
			2	8.73 ± 11.19	
			6	9.22 ± 7.96	
		2000 mg/kg	10	9.37 ± 9.42	9.30 ± 1.44
			21	7.83 ± 8.63	
			14	10.70 ± 13.94	
	MMS	80 mg/kg	13	24.14 ± 13.71	21.22 ± 2.80**
			5	20.94 ± 14.01	
			3	18.57 ± 9.36	

Vehicle, 0.5% carboxymethylcellulose sodium; MMS, methyl methanesulfonate.

**$p<0.01$ when compared with the corresponding vehicle control groups by Dunnett's test (n=3).

[1] DNA migration is the difference between the whole comet length and the nuclear diameter.

[2] Values are mean ± S.D. of DNA migration.

3.4.20. A Comet Assay With Olmesartan Medoxomil in Aged Rats (Study #APE-148-037B, Report #APR148-081) Amendment of August 30, 2001

This non-GLP study was conducted by Medicinal Safety Research Laboratories, Sankyo Co. Ltd., Japan, between July 30 and September 10, 2001. Techniques and methods were as described previously (section 3.4.19), except that only the 2000 mg/kg dose of olmesartan medoxomil (OM) was tested and aged rats were used.

Male F344/DuCrj rats, 9 months age, were given single oral doses of 2000 mg/kg OM (lot #OS-00Cl) or vehicle (10 ml/kg, 0.5% SCMC) by gastric tube. An additional group of rats, 6 weeks of age, received the vehicle. The positive control, methyl methanesulfonate (MMS), was administered intraperitoneally at a rate of 1 ml/100 gm (80 mg/kg) to rats at 9 months of age. Animals were sacrificed 3 or 24 hours (n=4/time/treatment) after administration with all treatments except for rats receiving MMS and rats receiving vehicle at 6 weeks of age, which were sacrificed only 3 hr after treatment. The rest of the methodology, including data analysis, is similar to that described in the previous section.

Results

DNA migration 3 or 24 hours after administration in the groups treated with OM (2000 mg/kg) was not significantly different than DNA migration in the corresponding vehicle control group (Table 3.4.20.1). DNA damage in 9 month old (vehicle treated) rats was greater than in 6 week old rats ($p < 0.01$). DNA migration could not be measured in the positive control group due to "severe damage". The authors of the report claim that MMS induced DNA damage and, therefore, the results are valid. The results thus suggest that OM does not induce DNA damage in the kidneys of aged rat (in which a higher rate of spontaneous DNA damage is observed than in 6 week old rats).

The genetic toxicology committee concurs with the sponsor's interpretation of the data (see Appendix 2).

TABLE 3.4.20.1
DNA MIGRATION IN KIDNEYS OF RATS

Substance	Age	Treatment Period	Dose	Animal No.	DNA migration (μm)[1] Individual value[2]	DNA migration (μm)[1] Group value[2]
Vehicle	6 weeks	3 hr	-	21	10.22 ± 8.49	9.68 ± 0.90
				22	10.65 ± 7.88	
				23	8.82 ± 7.24	
				24	9.01 ± 7.51	
	9 months	3 hr	-	19	17.38 ± 10.50	16.73 ± 1.74**
				6	18.12 ± 7.78	
				2	17.22 ± 7.50	
				8	14.19 ± 4.80	
		24 hr	-	13	18.72 ± 7.02	17.37 ± 1.80**
				3	15.08 ± 7.29	
				17	16.78 ± 8.36	
				18	18.89 ± 10.13	
CS-866		3 hr	2000 mg/kg	16	19.23 ± 7.41	15.89 ± 2.37
				20	14.65 ± 5.04	
				10	13.87 ± 6.14	
				7	15.81 ± 7.44	
		24 hr	2000 mg/kg	11	14.03 ± 7.65	16.54 ± 1.98
				1	18.67 ± 4.51	
				4	17.38 ± 7.01	
				14	16.06 ± 9.13	
MMS	9 months	3 hr	80 mg/kg	12	-[3]	-
				15	-[3]	
				9	-[3]	
				5	-[3]	

Vehicle, 0.5% carboxymethylcellulose sodium; MMS, methyl methanesulfonate.

**p<0.01 when compared with the vehicle control group at 6 weeks by Dunnett's test (n=4).

[1] DNA migration is the difference between the whole comet length and the nuclear diameter.

[2] Values are mean ± S.D. of DNA migration.

[3] Measurement could not be carried out due to severe damage.

Reproductive Toxicity Studies

3.5.1. Fertility and Early Embryonic Development (Segment I) Study in Rats (Study #94-0044, Report #TR 141-121). Vol. 25

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between April 19, 1994 and August 16, 1995. Dosing was initiated on May 9, 1994 and completed on July 27, 1994. The study investigated the effect of olmesartan medoxomil (OM) on gonadal function, estrous cycle, conception rate, fertility and early embryonic development through implantation.

Animals

Crj:CD rats [redacted] Males were approximately 6 weeks of age and weighed 177-214 gm, while females were approximately 8 weeks of age and weighed 195-235 gm, at initiation of dosing. Males and females were housed individually and fed *ad libitum*.

Mode of Administration/Dosage Levels

Suspensions of OM (lot #NH005C1) were prepared in 0.5% CMC in distilled water and administered orally by stomach tube (5 ml/kg) once daily to groups of 25 males and 25 females each at doses of 40, 200 or 1000 mg/kg. Control animals (25/sex) received the vehicle in a similar manner. Males were dosed for 9 weeks before mating and throughout the 2 week mating period. The females were treated from 2 weeks prior to mating until day 7 of gestation. The doses were selected on the basis of a teratogenicity study (see report #TR140-064) in which doses of 200 and 1000 mg OM/kg/day, administered on gestation days 7 to 17, were associated with decreases in mean body weight gain and food intake.

Observations/Measurements

All animals were observed daily for general condition. Body weights of males were measured once a week until the day of autopsy and those of females on 14, 11, 7 and 4 days before mating, the day of start of mating, and days 0, 3, 7, 10, 14, 17 and 20 of pregnancy. Food intake was measured from the day treatment started to the day of mating for males, and on days of body weight measurements (except day 0 of pregnancy) for females. Females were killed on gestation day 20 (under anesthesia) and thoracic and intraperitoneal organs were macroscopically observed. The numbers of corpora lutea, implantations, living fetuses and post-implantation losses (dead embryos/fetuses) were recorded. The living fetuses were examined for abnormality in external appearance and for determination of sex. Fetuses showing external anomalies were fixed in Bouin' fluid and preserved in ethanol. Skeletal examinations were not conducted on these or dead fetuses; skeletal examinations were conducted in the remaining living fetuses. Visceral observations were not made on either living or dead fetuses. Those females in which mating was not confirmed were autopsied 20 days after the end of the mating test. The male animals were killed after the mating period, and thoracic and intraperitoneal organs were macroscopically observed, testes were weighed and epididymides removed. The tail of the

epididymis was minced in a petri dish and an aliquot of the sample was examined under a microscope for the presence or absence of sperm and for sperm locomotor activity.

Results

There were no deaths and no clinical signs observed during the study. Statistically significantly lower body weight and food intake were recorded for males and females of all treated groups relative to control. Mean body weight gain was statistically but not dose-dependently decreased for all OM-treated male groups during the entire treatment period (Table 3.5.1.1, Fig 3.5.1.1). For females, a statistically significant reduction in body weight gain was recorded for the high dose group 7 days before mating and nondose-dependently for all dose groups between days 0 and 20 of pregnancy (Table 3.5.1.2, Fig 3.5.1.2).

TABLE 3.5.1.1
BODY WEIGHT CHANGES IN MALE RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of rats	Body weight (g) at each day of treatment											
		0	7	14	21	28	35	42	49	56	63	70	77
0	25	198.0 ±1.68	259.0 ±2.24	318.8 ±3.13	365.0 ±4.01	406.6 ±4.62	441.2 ±4.98	473.3 ±5.57	495.8 ±6.34	518.0 ± 6.84	538.5 ± 7.41	547.9 ± 7.70	562.7 ± 8.06
40	25	197.8 ±1.22	249.1* ±1.96	301.3* ±3.15	342.0* ±4.25	377.2* ±5.78	408.0* ±6.89	436.1* ±7.03	457.5* ±7.50	479.8* ± 8.16	498.4* ± 8.66	501.5* ± 6.05	515.2* ± 8.96
200	25	198.4 ±1.43	248.3* ±2.75	293.6* ±4.53	331.9* ±5.97	362.5* ±7.46	393.2* ±8.59	420.9* ±9.25	444.4* ±9.54	465.6* ±10.17	482.5* ±11.17	489.3* ±11.07	503.5* ±11.75
1000	23	199.0 ±1.58	248.0* ±2.53	294.0* ±3.84	331.2* ±5.38	364.7* ±6.53	398.4* ±7.41	428.4* ±8.11	453.1* ±8.81	475.5* ± 9.92	494.1* ±10.78	497.5* ±10.36	513.8* ±11.19

Data are expressed as mean ± SEM. * Significant at 1 % level compared with control

TABLE 3.5.1.2
BODY WEIGHT CHANGES IN FEMALE RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of rats	Body weight (g) at each day before mating					Number of pregnant females	Body weight (g) at each gestational day					
		14	11	7	4	0		0	3	7	10	14	17	20
0	25	212.6 ±1.91	224.0 ±2.29	238.0 ±2.52	247.2 ±2.75	258.2 ±3.07	23	268.8 ±4.44	292.4 ±4.29	313.1 ±4.85	328.0 ±4.83	355.0 ±4.82	388.8 ±5.11	442.7 ±6.48
40	25	207.7 ±1.82	217.4 ±2.03	229.0* ±2.27	236.5 ±2.29	246.0 ±2.74	22	251.3* ±4.07	271.3* ±4.41	288.8* ±5.25	308.3* ±4.78	331.8* ±4.93	365.2* ±5.19	417.0 ±6.17
200	25	212.4 ±1.89	222.0 ±2.50	231.8 ±2.69	238.0 ±2.71	247.6 ±3.27	25	256.4 ±3.35	275.4* ±3.81	290.0* ±4.14	306.1* ±4.53	333.2* ±4.79	367.6* ±5.02	418.1* ±5.49
1000	23	208.4 ±1.27	218.6 ±1.74	227.0* ±1.76	232.8* ±2.00	241.6* ±2.43	23	247.8* ±2.80	264.8* ±3.44	278.4* ±3.11	288.4* ±3.78	323.1* ±3.12	355.5* ±3.01	407.5* ±4.00

Data are expressed as mean ± SEM. * Significant at 1 % level compared with control

At the end of the treatment period, mean body weights of males and females of all treated groups were approximately 90% of control. OM-treated males consumed significantly less food ($p < 0.05$) on days 14 to 63 of treatment (as early as day 7 for high dose males, Table 3.5.1.2). Low

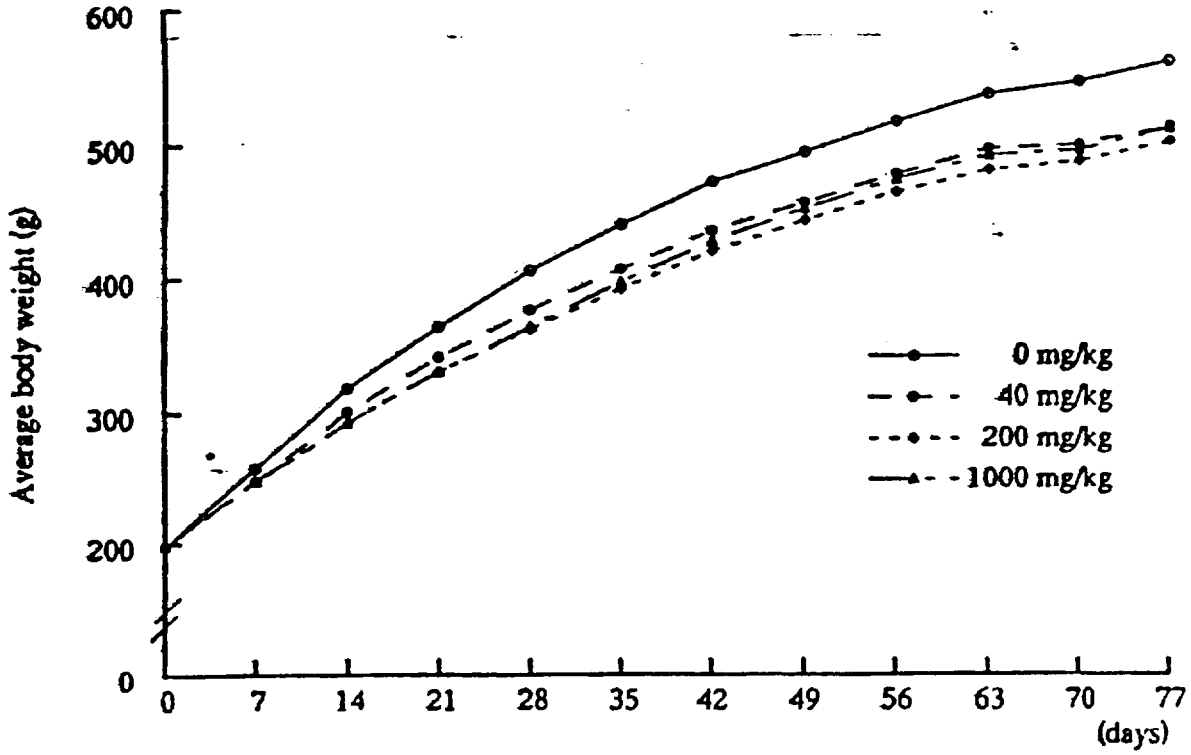

Fig. 3.5.1.1.: Body weight changes in male rats treated orally with olmesartan medoxomil

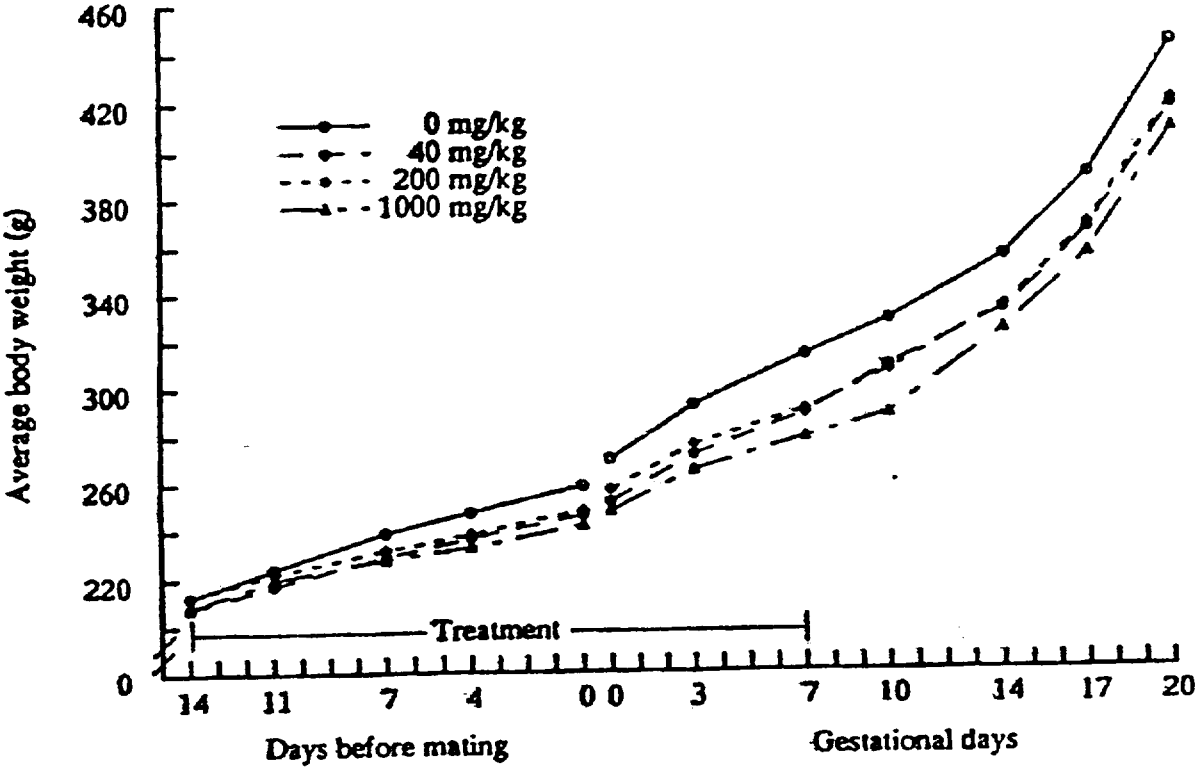

Fig. 3.5.1.2.: Body weight changes in female rats treated orally with olmesartan medoxomil

dose females had a decrease ($p < 0.05$) in food consumption 11 and 7 days before mating and on day 0 of mating; mid dose females had decreased food consumption 7 and 4 days before mating, on day 0 of the mating period and on gestation days 3 and 7; and high dose females had decreased consumption 4 days before mating, day 0 of the mating period and on gestation days 3 and 7 (Table 3.5.1.4).

TABLE 3.5.1.3
FOOD CONSUMPTION IN MALE RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of rats	Food intake (g) at each day of treatment									
		0	7	14	21	28	35	42	49	56	63
0	25	20.8 ±0.48	26.9 ±0.40	29.7 ±0.56	29.7 ±0.56	31.0 ±0.48	30.2 ±0.69	31.9 ±0.62	31.8 ±0.60	35.6 ±0.75	31.8 ±0.61
40	25	21.2 ±0.30	25.4 ±0.38	27.2* ±0.49	26.8* ±0.57	29.2 ±0.48	28.2 ±0.80	30.2 ±0.59	29.0* ±0.61	32.9 ±0.70	28.6* ±0.57
200	25	21.5 ±0.33	25.4 ±0.54	25.5* ±0.66	25.5* ±0.77	25.5* ±0.94	26.3* ±0.80	27.1* ±1.03	28.2* ±0.59	31.9* ±0.77	28.0* ±0.73
1000	23	21.7 ±0.46	24.5* ±0.52	25.0* ±0.69	25.4* ±0.83	27.4* ±0.67	27.6 ±0.69	29.0* ±0.70	29.4 ±0.72	31.3* ±0.95	29.0* ±0.68

Data are expressed as mean ± SEM. * Significant at 1 % level compared with control

TABLE 3.5.1.4
FOOD CONSUMPTION IN FEMALE RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of rats	Food intake (g) at each day before mating					Number of pregnant females	Food intake (g) at each gestational day					
		14	11	7	4	0		3	7	10	14	17	20
0	25	20.6 ±0.41	23.4 ±0.47	23.1 ±0.50	23.5 ±0.48	24.3 ±0.67	23	28.3 ±0.68	29.1 ±0.76	29.3 ±0.87	29.6 ±0.66	31.4 ±0.55	30.2 ±0.59
40	25	19.6 ±0.55	21.0* ±0.40	20.4* ±0.47	21.6 ±0.52	21.4* ±0.69	22	25.6 ±0.66	27.1 ±0.81	29.6 ±0.58	28.5 ±0.50	29.5 ±0.95	29.5 ±0.50
200	25	20.1 ±0.41	21.4 ±0.59	19.4* ±0.55	20.3* ±0.48	21.5* ±0.52	25	26.0* ±0.50	26.1* ±0.72	29.4 ±0.72	28.9 ±0.52	31.0 ±0.50	28.7 ±0.57
1000	23	18.8* ±0.46	20.5* ±0.43	19.7* ±0.34	19.6* ±0.54	20.1* ±0.59	23	23.9* ±0.64	22.9* ±0.62	28.0 ±0.90	27.7 ±0.66	30.2 ±0.60	28.3 ±0.58

Data are expressed as mean ± SEM. * Significant at 1 % level compared with control

There was no effect of test substance on estrous cycle. Also, there were no significant differences in copulation rate or fertility rate between the treated and control groups. At necropsy of the males, a nondose-dependent increase in the relative weight of the testes was recorded in the OM-treated groups, which was probably attributable to the decrease in body weight since there was no increase in the absolute weight of the testes. The difference from concurrent control was statistically significant only at the mid dose group (0.645 ± 0.0151 % *vs.* 0.580 ± 0.0105 % for

concurrent control). One member of this dose group showed atrophy of the testis. No abnormality was observed in the morphology or activity of the epididymal sperm.

At the GD 20 sacrifice and cesarean section, evidence of pregnancy was observed for 23, 22, 25 and 23 of the mated females at 0, 40, 200 and 1000 mg/kg/day, respectively. Statistically significant decreases in the number of corpora lutea relative to the control group were observed in all treated groups. The average number of corpora lutea in the control group was 19.2, while the values in the 40, 200, and 1000 mg/kg/day groups were 17.0, 16.9, and 16.5, respectively (Table 3.5.1.5). The sponsor notes that the statistically significant decrease in corpora lutea observed in the treated groups was considered to be due to an elevated value in the concurrent control group. (Note that both the concurrent control and high dose values are outside the range of the laboratory's historical control values, 16.8 to 17.8.) Examination of the fetuses revealed no treatment-related findings. A trend of increase in the number of ossified caudal vertebrae was observed in all treated groups but differences from control were not statistically significant. The NOEL for parental toxicity was less than 40 mg/kg/day and for fetal toxicity, more than 1000 mg/kg/day.

TABLE 3.5.1.5
PREGNANCY STATUS IN RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of pregnant females	Number of corpora lutea (Mean S.E.M.)	Number of implantations (Mean S.E.M.)	Number of living fetuses (Mean S.E.M.)	Pre-implantation loss[1] (%)	Post-implantation loss[2] (%)	Body weight of fetuses (g) (Mean S.E.M.)	Sex ratio (Male/Female)	Type and number of external malformations (%)
0	23	441 (19.2 0.54)	389 (16.9 0.67)	372 (16.2 0.74)	52(11.8)	17(4.4)	3.59 0.060	0.91 (177/195)	1 (0.3) a):1 (0.3)
40	22	373 (17.0* 0.38)	332 (15.1 0.56)	318 (14.5 0.62)	41(11.0)	14(4.2)	3.75 0.037	1.00 (159/159)	2 (0.6) b):1 (0.3) c):1 (0.3)
200	25	422 (16.9* 0.42)	387 (15.5 0.58)	372 (14.9 0.63)	35(8.3)	15(3.9)	3.74 0.054	1.13 (197/175)	1 (0.3) d):1 (0.3)
1000	23	380 (16.5* 0.29)	358 (15.6 0.46)	338 (14.7 0.65)	22(5.8)	20(5.6)	3.66 0.052	1.10 (177/161)	0

1) (number of corpora lutea – number of implantations) / number of corpora lutea X 100

2) (number of implantations – number of living fetuses) / number of implantations X 100

* Significant at 1% level compared to control

a) omphalocele
b) microphthalmia
c) micro- or anophthalmia
d) microphthalmia and cleft palate

3.5.2. Developmental Toxicity (Segment II) Study in Rats (Study #93-0037, Report #TR 140-064). Vol. 25

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between February 23, 1993 and February 21, 1994. Dosing was completed on April 13, 1993. The study investigated the effects of olmesartan medoxomil (OM) during organogenesis.

Animals

Groups of female Crj:CD rats [redacted] were mated at approximately 10 weeks of age. Those that became pregnant weighed 206-251 gm on day 0 of gestation.

Mode of Administration/Dosage Levels

Suspensions of OM (Lot #NH001C3) were prepared in 0.5% CMC and administered orally by stomach tube (5 ml/kg), once daily, to mated females at doses of 40, 200 or 1000 mg/kg on gestational days 7 through 17. Fifteen pregnant females were used at the 40 mg/kg dose level, 14 at 200 mg/kg and 17 at 1000 mg/kg. A control group of 14 pregnant females was given 0.5% CMC suspension in similar fashion. The doses were selected on the basis of a preliminary study in which doses of 500 or more mg/kg/day were associated with decreases in mean maternal body weight gain and food consumption and a dose-related decrease in mean body weights of live fetuses.

Dams were observed daily, with body weights and food intake measured on alternate days, from day 7 to day 20. On day 20, each dam was sacrificed, organs of the thorax and abdomen macroscopically examined, and the numbers of corpora lutea, implantations, live fetuses and dead fetuses recorded. Live fetuses were sexed and weighed and examined externally for abnormalities. Fetuses without external abnormalities were processed by Dawson's alizarin red method for detection of skeletal malformations. Fetuses with external anomalies were fixed in Bouin' fluid and preserved in ethanol; skeletal examinations were not conducted on these fetuses. Visceral examinations were not conducted on any of the fetuses.

Results

No dams had abnormal clinical signs. Those in the two higher dose groups (200 and 1000 mg/kg) had slightly but significantly ($p < 0.05$) reduced food intake on days 15 and 17 of gestation. This effect was accompanied by a slight reduction ($p > 0.05$) in body weight gain. At necropsy, there were no significant differences between treatment groups and the control group in the numbers of corpora lutea, implantations, live and dead fetuses, or the mean body weights of live fetuses except for a significant increase in fetal body weight at 40 mg/kg/day (Table 3.5.2.1). One dam of the 1000 mg/kg group had live fetuses with markedly lower mean body weights. The sex ratios were similar in all groups. The only external abnormalities observed were edema and a kinky tail in one fetus from the 40 mg/kg group. Macroscopic examination of maternal thoracic and abdominal organs revealed no abnormality in any of the treated groups.

Skeletal examination of the fetuses revealed only one abnormality - a partial sternal cleft in one fetus from the 200 mg/kg group. A number of anatomical variations of a minor nature were encountered in all groups including the control group: cervical rib, split thoracic vertebral body, wavy rib, shortened 13th or 14th rib, 25 presacral vertebrae. The frequency and distribution of these variations were not significantly different from the control group. The number of ossified caudal vertebrae, an indicator of ossification, was decreased in the 1000 mg/kg group, though the difference from control was not statistically significant. It can be concluded that the general toxicological no-effect dose for OM in parent animals on this study was 40 mg/kg/day, and the NOEL for developmental toxicity was 200 mg/kg/day.

TABLE 3.5.2.1
PREGNANCY STATUS IN RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of pregnant females	Number of corpora lutea (Mean±S.E.M.)	Number of implantations (Mean±S.E.M.)	Number of living fetuses (Mean±S.E.M.)	Pre-implantation loss[1] (%)	Post-implantation loss[2] (%)	Body weight of fetuses (g) (Mean±S.E.M.)	Sex ratio (Male/Female)	Type and number of external malformations (%)
Control	14	230 (17.1 ± 0.55)	230 (16.4 ± 0.47)	213 (15.2 ± 0.93)	9(3.6)	17(7.4)	3.53 ± 0.066	1.16 (114 / 99)	0
40	15	251 (16.7 ± 0.43) P=0.6433	242 (16.1 ± 0.31) P=0.5959	222 (14.8 ± 0.48) P=0.6971	9(3.6) P=0.8931	20(8.3) P=0.7243	3.82 ± 0.043 P=0.0211	1.41 (130 / 92) P=0.2899	1 (0.5)[a] P=0.9835
200	14	241 (17.2 ± 0.42) P=0.8450	226 (16.1 ± 0.33) P=0.6204	216 (15.4 ± 0.27) P=0.8284	15(6.2) P=0.2166	10(4.4) P=0.1796	3.69 ± 0.059 P=0.4899	0.80 (96 / 120) P=0.0601	0 ND
1000	17	292 (17.2 ± 0.46) P=0.8797	278 (16.2 ± 0.72) P=0.8306	260 (15.3 ± 0.89) P=0.9446	18(6.5) P=0.3536	16(5.8) P=0.4696	3.58 ± 0.102 P=0.7038	1.00 (130 / 130) P=0.4458	0 ND

1) (number of corpora lutea – number of implantations) X 100
number of corpora lutea

2) (number of implantations – number of living fetuses) X 100
number of implantations

a) edema with kinky tail

3.5.3. Developmental Toxicity (Segment II) Study in Rats. An Extended Study (Study #93-0110, Report #TR 141-086). Vol. 25

This GLP study was conducted by the Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd., Shizuoka, Japan, between October 18, 1993 and March 9, 1995. Dosing initiation and necropsy dates are not given. The study investigated the effects of olmesartan medoxomil (OM) on organogenesis, parturition and post-natal development (until weaning).

Animals

Female Crj:CD rats [redacted] were mated at approximately 10 weeks of age. Those that became pregnant weighed 231-283 gm on day 0 of gestation.

Mode of Administration/Dosage Levels

Suspensions of OM (Lot #NH001C2) were prepared in 0.5% CMC and administered orally by stomach tube (5 ml/kg), once daily, to groups of 40 mated females each at doses of 40, 200 or 1000 mg/kg/day on gestational days 7 through 17. A control group of 40 females was given 0.5% CMC suspension in similar fashion. The numbers of animals showing evidence of pregnancy were 38, 39, 38 and 33 in the control, 40, 200 and 1000 mg/kg/day groups, respectively. The doses were selected on the basis of the previous study (see section 3.5.2).

Observations/Measurements

Dams were observed daily for physical signs. Body weights were recorded on gestation days 0, 7, 9, 11, 13, 15, 17 and 20, and lactation days 1, 4, 8, 15 and 22. Food consumption was determined on days of body weight measurements except on gestation day 0 and lactation day 1. Approximately two-thirds of the dams in each group were sacrificed on day 20 of gestation (25, 26, 25, and 20 in the control, 40, 200, and 1000 mg/kg/day groups, respectively) and half of the fetuses were examined for visceral abnormalities and the other half for skeletal abnormalities. The remaining dams of each group were allowed to deliver naturally for determination of perinatal/postnatal effects. The duration of gestation, litter size, stillbirths and live births were recorded. Each litter was reduced to 4 males and 4 females on post-partum (pp) day 4. Weaning index (live pups on day 22/live pups on day 4 X 100) was determined on pp day 22. Pups were weighed on pp days 1, 4, 8, 15, 22, 29, 36, 43, 50 and 57. The pups were observed/tested for effects on maturational parameters (incisor eruption, appearance of abdominal hair, auricular tract opening, separation of eyelids, testes descendent and vaginal opening), locomotor function, exercise performance (righting reflex, negative geotaxis, free-fall righting reflex) sensory function (pupillary reflex, corneal reflex and Preyer's reflex), emotionality (open field performance test) and learning (conditioned avoidance response). F_0 dams were autopsied during the weaning period.

At 8 weeks of age, 10 pups/sex were selected from each group and raised until 10 weeks of age. Those animals that were not used beyond 8 weeks of age were sacrificed and thoracic and peritoneal viscera were macroscopically examined. Additionally, a male from each litter was selected for examination of morphology and motility of epididymal sperm. At about 10 weeks of

age, avoiding sib mating, 1 male and 1 female from the same treatment group (10/sex/group) were mated to examine reproductive performance. Each dam was allowed to undergo natural parturition and the birth index and the gestation period were determined. The newborn were counted and weighed and their sex determined, and the presence or absence of external abnormalities noted. Necropsies were performed on all F_1 dams.

Results

There were no mortalities and no clinical signs observed in the dams assigned to the teratology portion of the study. However, among dams assigned to natural parturition, four of 13 in the 1000 mg/kg/day group died late in the gestation period (2 on day 22 of gestation, one during the labor on day 21 of pregnancy and one on day 11 postpartum) as did one of 13 dams in the mid dose group (on day 11 postpartum). Clinical signs consisted of decreased locomotor activity and irregular respiration in one of 13 dams in the 200 mg/kg/day group and five of 13 dams in the 1000 mg/kg/day group. There was a significant decrease in body weight gain (92.7% of control) from day 11 to day 20 of gestation in the 200 mg/kg/day group and from day 15 to day 20 of gestation (90.2% of control) in the 1000 mg/kg/day group. Body weight gains were also significantly decreased in these groups on days 1 and 15 of lactation (Table 3.5.3.1). Food consumption was also significantly decreased in these groups during gestation, but not significantly during the lactation period (Table 3.5.3.2).

TABLE 3.5.3.1
BODY WEIGHT CHANGES IN RATS TREATED ORALLY WITH OLMESARTAN MEDOXOMIL

Dose (mg/kg)	Number of pregnant females	Body weight (g) at each gestational day								Number of nursing dams	Body weight (g) at each day after delivery				
		0	7	9	11	13	15	17	20		1	4	8	15	22
Control	38	257.8 ±1.83	304.1 ±2.42	313.2 ±2.43	324.8 ±2.56	334.7 ±2.78	349.3 ±3.06	373.6 ±3.20	426.5 ±3.86	13	314.9 ±6.25	361.0 ±6.23	353.5 ±5.20	356.6 ±4.06	319.2 ±3.29
40	39	257.6 ±1.55 P=0.8915	300.7 ±2.16 P=0.2839	308.3 ±2.27 P=0.1438	318.2 ±2.22 P=0.0554	327.5 ±2.40 P=0.0534	342.4 ±2.61 P=0.0914	366.2 ±2.87 P=0.0968	419.2 ±3.39 P=0.1565	13	303.4 ±6.94 P=0.2951	333.8 ±6.74 P=0.4016	345.2 ±4.34 P=0.2319	349.5 ±3.85 P=0.5732	316.7 ±2.94 P=0.5818
200	38	254.7 ±1.63 P=0.2132	297.6 ±1.66 P=0.0304	304.9 ±1.88 P=0.0157	312.6* ±1.93 P=0.0008	320.9* ±1.95 P=0.0003	330.4* ±2.35 P=0.0000	350.2* ±2.67 P=0.0000	395.6* ±3.38 P=0.0000	13	280.8 ±7.76 P=0.0138	311.3 a) ±9.91 P=0.0168	326.8 ±10.97 P=0.0435	338.0* b) ±2.64 P=0.0037	308.5 ±6.17 P=0.0140
1000	33	256.7 ±2.16 P=0.4632	300.1 ±2.20 P=0.2386	309.2 ±2.10 P=0.2242	317.9 ±2.13 P=0.0445	325.0 ±2.50 P=0.0137	333.7* ±2.64 P=0.0002	351.8* ±2.90 P=0.0000	384.9* ±4.22 P=0.0000	9	270.2* ±9.39 P=0.0057	310.0 c) ±14.60 P=0.0343	331.3 ±8.36 P=0.0291	342.7 ±5.06 P=0.0913	317.9 ±6.80 P=0.8477

See Table 3.5.3.2, below, for description of superscripts

Pregnancy rates were comparable for all groups. No significant group differences in the mean number of corpora lutea, implantation sites, fetuses or pre- and post-implantation losses were noted. A dose-dependent decrease in the mean body weight of surviving fetuses relative to control was significant ($p < 0.05$) for the top two high dose groups (Table 3.5.3.3).

One fetus in the 40 mg/kg/day group had microphthalmia/anophthalmia and one fetus in the 1000 mg/kg/day group had an omphalocele. Regarding visceral abnormalities, ventricular septal defect was observed in all groups including control ($p > 0.05$). Other malformations with incidences that did not differ significantly from control were abnormal origin of subclavian artery and dilatation of the lateral ventricle. A dose-dependent and statistically significant ($p < 0.05$) increase in incidence of dilatation of the renal pelvis (anatomic variation) was observed