

TABLE 3.2.5.1
EXPERIMENTAL DESIGN

Gp #	Test substance	Dose ^a mg/kg/day	Dose vol ml/kg	# of animals: M, F	
				Toxicology	Toxicokinetic ^b
1	Vehicle	10 ml/kg	10	20 M, 20F	None
2	Aliskiren hemifumarate	50	10	20 M, 20F	12 M, 12F
3		150	10	20 M, 20F	12 M, 12F
4		250	10	20 M, 20F	12 M, 12F

^a : Doses are expressed as free base equivalents.

^b : satellite animals for toxicokinetic investigations only; no other experimental observation data from these animals are reported in the submission.

Observations and Measurements (only in Toxicology Groups)

Clinical Signs: All animals were observed at least twice a day for mortality, moribundity and clinical signs.

Body Weights: Recorded before treatment on the first day of dosing and then at weekly intervals and on the day of scheduled necropsy.

Food Consumption: Recorded weekly as amount of food consumed by each cage of animals. Consumption was calculated as gm/animal/week.

Ophthalmoscopy: Conducted prior to the initiation of dosing on all animals and in week 25 in control and high dose animals only.

Hematology and Clinical Chemistry: Blood samples were collected from the lateral caudal vein of the first 10 animals/group/sex (fasted overnight) in week 13 and from the orbital sinus (under halothane anesthesia) in week 26 for males and from the abdominal aorta at necropsy in week 27 for females. In addition, blood samples were collected from the next 10 animals/group/sex (fasted overnight) from the tail vein in week 13 for immunological evaluation.

Urinalysis: Urine samples were collected overnight from the first 10 animals/group/sex in weeks 12 and 25. Food and water were removed during collection.

Gross Pathology: A complete necropsy was conducted on all toxicology group animals (fasted overnight) including those found dead or euthanized *in extremis*.

Organs Weighed: All animals at the scheduled necropsy.

Histopathology: Microscopic examination was performed on all tissues listed in Table 3.2.5.2 from all animals in the control and high dose groups at the scheduled necropsy and for all animals euthanized *in extremis* or found dead during the study. The liver, spleen, pancreas, kidneys, mesenteric lymph nodes, trachea and lungs were preserved from all animals in the mid and low dose groups. However, only trachea, lungs and kidney were scheduled for evaluation.

Toxicokinetics: Blood samples for test substance determination were collected from 3 rats/sex/group/time point at 0.5, 1, 2, 4, 8 and 24 hours after dosing on study day 1 (first day of dosing) and in week 26. Blood was collected from the lateral caudal vein or retro-orbital sinus from halothane anesthetized animals. The animals were euthanized and discarded following the final blood collection.

TABLE 3.2.5.2
26 WEEK TOXICITY STUDY IN RATS: TISSUES/ORGANS SAMPLED
FOR HISTOPATHOLOGICAL EXAMINATION

Adrenals*	Lacrimal glands ¹	Sciatic nerve
Aorta	Liver*	Seminal vesicle
Bone with marrow, femur,	Lungs	Skin
sternum	Lymph nodes, mesenteric*,	Spinal cord, cervical, thoracic,
Bone marrow smear ¹	mandibular*	lumbar
Brain*	Mammary gland (females)	Spleen*
Cecum	Muscle, quadriceps	Stomach
Colon	Nasal turbinates	Testes + Epididymides*
Duodenum	Nasopharynx ¹	Thymus*
Esophagus	Ovaries*	Thyroid with parathyroids*
Eyes	Optic nerves	Tongue
Gross lesions	Pancreas	Trachea
Harderian glands ¹	Peyer's patches	Trachea bifurcation
Head	Pituitary*	Urinary bladder
Heart*	Prostate*	Uterus
Ileum	Rectum	Vagina
Jejunum	Salivary glands	Zymbal glands
Kidney*		

¹: Preserved with the head *in situ*

*: Organ weight obtained

Results

Mortality: A total of 6 animals died or were euthanized *in extremis* during the study (Table 3.2.5.3). None of these deaths was attributed by the sponsor to an effect of the test substance. Three males, one each in the control, low and mid dose groups; and a control female were sacrificed moribund because of hemorrhage. A female receiving 150 mg aliskiren/kg/day was found dead and the cause of death could not be established. A high dose female was sacrificed in a moribund condition due to diaphragmatic hernia.

TABLE 3.2.5.3
FOUND DEAD OR EUTHANIZED *IN EXTREMIS*

Dose, mg/kg/day	# of deaths	Study day and cause of death
Control	1 M	#20 euthanized <i>in extremis</i> on day 179 ⁿ
	1 F	#91 euthanized <i>in extremis</i> on day 182 ⁿ
50	1 M	#36 euthanized <i>in extremis</i> on day 182 ⁿ
150	1 M	#57 euthanized <i>in extremis</i> on day 179 ⁿ
	1 F	#139 found dead on day 145*
250	1 F	#147 euthanized <i>in extremis</i> on day 80 due to diaphragmatic hernia.

ⁿ: Sacrificed in moribund condition because of hemorrhage, which is considered to be a consequence of blood sampling.

*: Cause of death could not be established

Clinical Signs: Noisy breathing was noted in three high dose males, #70, #71 and #60 in weeks 3, 4, 11; 3 and 5, respectively. It was also noted at a later stage in week 18 in two other high dose males (#73 and #74). Due to concern over their condition, animal #70 was taken off dose for one day in week 3 and animal #74 was taken off dose for two consecutive days in week 18 and monitored until recovery. A high dose female (#147) had protruded eye, hunched posture and labored breathing in weeks 11/12. This animal was taken off dose for 5 consecutive days in weeks 11/12 and killed without resuming dosing. Hunched posture was noted in one high dose male (#67, weeks 22 through 24) and two high dose females (#147, weeks 5 through 12; #14, weeks 22 through 26). Salivation and paddling were noted occasionally in a few animals in the low dose group and in most of the animals in the remaining dose groups that persisted throughout the study. These observations were absent in the control animals.

Body Weights: There was no effect of treatment on mean body weight or mean body weight gain relative to control in the first 12 weeks of study. However, a significant ($p < 0.05$) reduction in body weight gain between weeks 12 and 26 was recorded at 250 mg/kg/day. At this dose, weight gain for males and females were 85% and 84% of control, respectively. A reduced body weight gain for the same period, 86% of control, was also noted for mid dose females (Table 3.2.5.4).

Food Consumption: No treatment-related effects

Ophthalmoscopy: No significant changes

Hematology and Clinical Chemistry: There were no significant differences between drug treated and control groups at either the week 13 or 26/27 analyses.

Urinalysis: The only change of significant note was reduction in specific gravity relative to control. A dose-dependant lower than control mean specific gravity was noted ($p < 0.05$ to 0.001) for males at all doses (0.7% to 1.2% lower) and for females at the high dose (1.7% lower) at week 12 measurements. At week 25, the differences from control reached significance ($p < 0.01$) only for females given 150 or more mg/kg/day (2% lower).

Organ Weights: There were no changes relative to control group.

Gross Pathology: No treatment-related findings.

Histopathology: Test substance-related effects were confined to the minor inflammatory and degenerative changes of the respiratory epithelium at the tracheal bifurcation and lungs in a male (#49) and a female (#131) in the 150 mg/kg/day group, and in 6 males (#67, 70, 71, 73, 74, 77) and a female (#147) in the 250 mg/kg/day group. These findings correlated with the noisy breathing noted earlier in clinical findings for these groups.

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TABLE 3.2.5.4
26 WEEK TOXICITY STUDY IN RATS: GROUP MEAN BODY WEIGHT GAINS

Week of study		Mean body weight gains (g) for Group:				Statistics
		1M	2M	3M	4M	
Start to 4	Mean	102.2	104.2	101.2	108.0	A
	SD	16.17	14.13	13.98	17.63	
Start to 12	Mean	191.1	190.6	185.7	202.0	A
	SD	25.34	24.44	23.19	29.71	
Start to 26	Mean	251.9	248.7	244.0	254.5	A
	SD	34.82	31.74	31.28	37.87	
4 to 12	Mean	88.9	86.4	84.6	94.0	A
	SD	15.30	13.96	12.61	15.13	
12 to 26	Mean	61.7	56.1	56.8	52.5	DR* A
	SD	13.33	12.33	11.68	11.59	
		1F	2F	3F	4F	Statistics
Start to 4	Mean	49.9	53.7	51.1	50.4	A
	SD	5.80	6.56	7.22	8.34	
Start to 12	Mean	89.5	91.6	91.7	88.2	A
	SD	10.25	10.06	13.60	12.42	
Start to 26	Mean	114.1	117.2	114.1	109.5	A
	SD	16.15	15.36	17.66	15.11	
4 to 12	Mean	39.6	37.9	40.6	37.3	A
	SD	5.08	7.36	8.13	7.58	
12 to 26	Mean	25.4	25.6	21.9	21.3	DR* A
	SD	9.43	8.71	7.13	5.59	

DR: Significant ($p < 0.05$) dose response test. A = ANOVA, regression and Dunnett's. Groups 1, 2, 3 and 4 are, respectively, Control, 50, 150 and 250 mg aliskiren/kg/day.

Toxicokinetics: Small concentrations of test substance were detected in control animals on day 1 (peaked at 4 hr). C_{max} values for control males and females were 2% and 0.5%, while AUC_{0-24h} values were 14% and 5% of the corresponding low dose group values, respectively. A relatively high concentration of test substance was detected in control animals at week 26 (5 to 10-fold more than on day 1). C_{max} values for control males (at

24 hr) and females (at 2 hr) at week 26 were 6% and 11%, while AUC_{0-24h} values were 15% and 17%, of low dose group values, respectively. After single or repetitive measurement, systemic exposure (AUC_{0-24h}) to aliskiren in aliskiren hemifumarate-treated males and females increased with increasing dose but not proportionately. Plasma levels of aliskiren were higher at week 26 than on day 1. Maximum plasma concentration occurred 1 to 4 hr post dose (Table 3.2.5.5). Though males exhibited a slightly higher exposure to aliskiren than females (for all dose groups), individual variation and relatively small sample size (n=3/time point) precluded statistical interpretation.

TABLE 3.2.5.5
26 WEEK TOXICITY STUDY IN RATS: TOXICOKINETICS OF ALISKIREN

Day 1					
Dose Level (mg/kg/day)	Group	Sex	C_{max} (ng/mL)	$AUC_{(0-24)}$ (h*ng/mL)	t_{max} (h)
0	1	Male	2.2640	39.0269	4
0	1	Female	0.4826	11.5200	4
50	2	Male	101.3405	281.2104	2
50	2	Female	96.6643	212.7092	2
150	3	Male	334.9832	1453.4036	2
150	3	Female	193.0961	998.0966	2
250	4	Male	627.5786	3174.8521	4
250	4	Female	442.2792	2791.2643	2
Week 26					
Dose Level (mg/kg/day)	Group	Sex	C_{max} (ng/mL)	$AUC_{(0-24)}$ (h*ng/mL)	t_{max} (h)
0	1	Male	16.5917	146.1210	24
0	1	Female	30.8849	111.3766	2
50	2	Male	262.9824	1006.1686	2
50	2	Female	283.4997	649.6779	1
150	3	Male	738.2480	2403.2009	1
150	3	Female	420.8870	1545.1999	1
250	4	Male	465.3043	3335.3168	1
250	4	Female	563.8126	2963.3352	1

3.2.6. Two-Week Oral Toxicity Study in Marmosets

Key Study Findings: Oral administration of aliskiren hemifumarate to marmosets for 14 days was associated with a reduction in body weight relative to control for females receiving 100 mg/kg/day. Marked increases in BUN from pretest values were noted at all dosage levels. Arteriolar hypertrophy in the kidneys and single cell necrosis of the liver were noted in females at 100 mg/kg/day.

Study No: 956141, Report #001/96/SL; Toxicokinetics: Study #95-9007, Report #BPK(F) 1996/008)

Location of Report: EDR

Conducting Laboratory and Location: _____

Dates of Study: Dosing was initiated on September 29 and necropsies were begun on October 13, 1995.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, lot #800195, purity ✓

Formulation: Aliskiren hemifumarate was dissolved in purified water. Dosages were prepared daily. All dosages and concentrations were expressed in terms of the salt.

Animals

Species/Strain: Marmoset (*Callithrix jacchus*)

#/Sex/Group: Except for the control group, each group consisted of 2 males and 2 females. The control group (by error) included 1 male and 3 females.

Age: At study start: 19 to 31 months for males and 15 to 34 months for females

Weight: At study start: Males: 325-446 gm, Females: 290-490 gm

Husbandry: Animals were housed in pairs of the same sex in steel cages. Food, twice a day and purified water, *ad libitum*, were given throughout the study period except during the period of fasting (prior to sampling of urine for urinalysis) when food and water were withheld.

Dosing

Doses: 0, 10, 50 or 100 mg aliskiren hemifumarate/kg/day (doses expressed as salt). The doses were selected based on an oral rising dose study in which 100 mg/kg/day for 7 days caused reductions in body weight and food consumption, increased plasma levels of urea nitrogen, creatinine, and magnesium. Additionally, this high dose was associated with elevated alanine aminotransferase and alkaline phosphatase activities, and increased cholesterol and bilirubin concentrations.

Route, Mode and Duration of Administration: Orally by gavage (10 ml/kg), once daily, for 14 days. The control group received the vehicle.

Observations and Measurements

Clinical Signs: All animals were observed daily for clinical signs and mortality.

Body Weights and Food Consumption: Determined weekly and daily, respectively.

Hematology¹ and Clinical Chemistry²: Blood samples were collected pretest and before dosing on day 14 from the femoral vein (non-fasted).

¹ erythrocytes, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, reticulocytes, hematocrit, fibrinogen, white blood cell count (total and differential), thrombocytes, prothrombin time, activated partial thromboplastin time, inclusion bodies.

Urinalysis³: Urine was collected pretest and study day 11 from each animal overnight, under food and water deprivation.

Toxicokinetics: Details are given under Results on page 112.

Gross Pathology: All animals were necropsied one day after last dose administration. The following organs/tissues were taken from all animals for histopathological evaluation. Selected organs were weighed (Table 3.2.6.1).

Histopathology: Microscopic examination was performed on all tissues listed in Table 3.2.6.1 from all animals.

TABLE 3.2.6.1
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenal glands*	jejunum	Skin
Aorta	Kidneys*	Spinal cord (cervical, thoracic, lumbar)
Bone with bone marrow (femur, sternum)	Knee joint (articular surface)	Spleen*
Brain*	Liver*	Stomach (cardia, fundus, pylorus)
Cecum	Lungs*	testes*
Cervix	Lymph nodes -axillary - mesenteric	Thigh muscle
Colon	Mammary area	Thymus*
Duodenum	Ovaries*	Thyroid*/parathyroids
Epididymides	Pancreas	Tongue
Esophagus	Pituitary gland*	Trachea
Eyes with optic nerves	Prostate*	Urinary bladder
Gall bladder	Rectum	Uterus
Gross lesions	Salivary glands	Vagina
Heart*	Sciatic nerve	
Ileum	Seminal vesicles	

* Organ weighed

Results

Mortality: No animals died during the study.

Clinical Signs: Both high dose males and one high dose female vomited (with or without test substance) on two occasions during study week 2. The sponsor considered the vomiting to be stress-related. Diarrhea was noted on occasions in males and a female receiving 100 mg/kg/day.

Body Weights: Changes in body weight were not consistent across study weeks and were not dose-dependent. An 8-10% reduction in body weight relative to initial pretreatment

² alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, calcium, glucose, sodium chloride, magnesium, triglycerides, cholesterol, phosphate, urea, bilirubin, creatinine, potassium, proteins (total and differential).

³ volume, specific gravity, pH, bilirubin, ketones, protein, blood, leucocytes, sodium casts, oxalates, epithelial cells, urates, erythrocytes, phosphates, urobilinogen, glucose, potassium.

weight and control animals was observed in high dose females in both study weeks 2 and 3 (day 15) (Table 3.2.6.2).

TABLE 3.2.6.2
EFFECT OF ALISKIREN ON BODY WEIGHT AND BODY WEIGHT GAIN IN 2-WEEK MARMOSET STUDY

Study days		Dose mg/kg/day							
		Control		10		50		100	
		M	F	M	F	M	F	M	F
-1 to -7	B.wt., g	352	341	389	410	380	403	387	351
1-7	B.wt., g	359	348	397	410	385	416	400	352
	% diff ^s	+2	+2	+2	0	+1.3	+3.2	+3.3	0
8-14	B.wt., g	352	340	399	387	350	401	389	324
	% diff ^s	0	0	+2.6	-5.6	-7.9	0	0	-7.7
15	B.wt., g	356	348	403	384	348	392	388	316
	% diff ^s	+1	+2	+3.6	-6.3	-8.4	-2.7	0	-10.0

^s: % difference from week -1 (initial) body weight

Food Consumption: Food consumption was reduced for all treated animals during study week 2. However, the data was not consistent and did not follow a dose pattern.

Hematology: In all dose groups, hematological parameter values did not differ from control values.

Clinical Chemistry: Increases in BUN from pretest levels were noted for all treated groups. The (mean) increases were dose-dependent. For males, the values for low, mid and high dose groups were, respectively, 5, 105 and 166% of concurrent control on day 14. For females, the values for low, mid and high dose groups were, respectively, 69, 274 and 401% of concurrent control on day 14. Creatinine and magnesium increased moderately (130 to 156%) in animals receiving 50 and 100 mg/kg/day. Alkaline phosphatase levels were increased (2-fold over initial values) at the end of the study in both high dose females. (One of these females showed single cell necrosis (moderate) in one liver lobe.)

Urinalysis: There were no significant changes.

Organ Weights: There were no differences from concurrent control organ weights associated with treatment with aliskiren.

Gross Pathology: There were no macroscopic findings that could be attributed to the test substance.

Histopathology: Microscopic examination revealed arteriolar hypertrophy in the kidneys of one high dose female and single cell necrosis of the liver (moderate, grade 3) in the other high dose female. Since the number of animals per dose group was so small, the statistical significance of these findings could not be evaluated.

Toxicokinetics: Toxicokinetics information was determined in marmosets that were not dosed in parallel with the main study animals. They received a single dose (day 1) on October 3 (males) or October 5, 1995 (females) and were rested for 14 days. Treatment resumed on October 18 (males) or 20, 1995 (females) and continued for 14 days. Test substance was administered orally (as described earlier) at doses of 10, 50 or 100 mg/kg/day. The control group received the vehicle. Each group except the control consisted of 8 male and 8 female marmosets. The control group contained 4 males and 4

females. Blood samples were collected from all animals on day 1 in "single dose regimen" and after 14 consecutive days of treatment in "14-day treatment regimen". It should be noted that the sponsor did not determine the plasma concentrations of aliskiren on day 1 of the "14-day treatment schedule". Animals from each treated group were divided into two equal subgroups, A and B. Animals in group A were bled predose and at 2 and 8 hr after administration of test substance. Animals in group B were bled at 1, 4 and 24 hr post treatment. In the case of control animals, blood samples were collected 2 hr after dosing on days 1 and 14 of the "single dose regimen" and "14-day treatment regimen", respectively.

Plasma levels of aliskiren increased similarly in both sexes after administration of the drug, reaching maximum concentrations at 1 and 2 hr on day 1 and after 14 days of repeated administration, respectively. An increase in the dose from 50 mg/kg to 100 mg/kg did not further increase the plasma levels of test substance. The inter-animal variability of the plasma levels was similar in both sexes and the overall CV was around 40%. AUC values were similar for male and female animals both on day 1 and after 14 days of treatment (Table 3.2.6.3). The plasma concentrations of aliskiren measured after 14 days of treatment were moderately higher than those measured after single dose treatment. Thus, in male marmosets, the plasma levels after 14 days of treatment were, on average, 1.47, 1.73 and 1.36 times higher than those of day 1 for the dose levels of 10, 50 and 100 mg/kg, respectively. Similarly, in females these values were, respectively, 1.40, 1.83 and 1.69 times higher than those measured on day 1 (Table 3.2.6.4). This suggests a moderate accumulation of test substance during prolonged treatment.

Elimination of aliskiren from plasma was rapid in a first phase (4 to 8 hr post administration) and then slower in a second phase with mean terminal elimination half-lives ranging from 5.63 to 12.8 hr (depending on time and sex, Table 3.2.6.3). Mean $t_{1/2}$ for high dose males was about 50% longer on day 1 than on day 14, and appeared to be dose-dependent. (Insufficient sampling for similar assessment in females.)

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TABLE 3.2.6.3
MEAN PHARMACOKINETIC PARAMETERS OF ALISKIREN IN MARMOSETS DETERMINED ON DAY 1
OF TREATMENT AND AFTER 14 DAYS OF TREATMENT WITH ALISKIREN HEMIFUMARATE

Dose (mg/kg)	PK parameter	Male		Female	
		Day 1	Day 14	Day 1	Day 14
10	C _{max}	3.960	5.770	3.960	6.730
	C _{maxspec}	0.396	0.577	0.396	0.673
	t _{max}	1	2.000	1.000	1.000
	t _{1/2}	na	5.630(3)	na	na
	AUC _(0-24h)	22.00	33.900	21.300	29.00
	AUC _{spec}	2.200	3.390	2.130	2.900
	AUC	na		na	
50	C _{max}	6.290	12.300	8.830	10.100
	C _{maxspec}	0.126	0.246	0.177	0.202
	t _{max}	1.000	2.000	1.000	1.000
	t _{1/2}	7.37(3)	na	na	8.190(3)
	AUC _(0-24h)	58.100	107.000	56.200	109.000
	AUC _{spec}	1.160	2.140	1.120	2.180
	AUC	64.000		na	
100	C _{max}	7.200	9.670	7.940	10.600
	C _{maxspec}	0.072	0.097	0.079	0.106
	t _{max}	1.000	2.000	1.000	2.000
	t _{1/2}	12.800(3)	7.800(4)	na	na
	AUC _(0-24h)	76.400	109.000	84.000	142.000
	AUC _{spec}	0.764	1.090	0.840	1.420
	AUC	103.000		na	

C_{max}: maximum concentration (µg/ml); C_{maxspec}: maximum concentration corrected to a dose of 1 mg/kg (µg/ml per mg/kg); t_{max}: sampling time (h) corresponding to C_{max}; t_{1/2}: terminal elimination half-life (h), the number of time points taken for the calculation is indicated in parentheses; AUC_(0-24h): area under the concentration curve calculated over the time interval 0-24 hour (µg.h/ml); AUC_{spec}: AUC_(0-24h) corrected to a dose of 1 mg/kg (µg.h/ml per mg/kg); AUC: area under the concentration curve extrapolated to time infinity (µg.h/ml); na = not available (the number of concentration time-points was not sufficient for the calculation of t_{1/2} and the corresponding AUC could not be calculated).

TABLE 3.2.6.4
ACCUMULATION FACTOR FOR ALISKIREN

Dose (mg/kg)	Male			Female		
	Day 1	Day 14	Ratio ^a	Day 1	Day 14	Ratio ^a
10	22.0	339	1.54	21.3	29	1.36
50	58.1	107	1.84	56.2	109	1.94
100	76.4	109	1.43	84.0	142	1.69

AUC_(0-24h) values (µg.h/ml) calculated using mean aliskiren plasma concentrations measured on day 1 of treatment and after 14 days of treatment.

^a: ratio AUC_(0-24h) day 14/ AUC_(0-24h) day 1 (accumulation factor).

3.2.7. Thirteen-Week Oral Toxicity Study in Marmosets With a 4 Week Recovery

Key Study Findings: Oral administration of aliskiren hemifumarate to marmosets for 13 weeks was associated with the moribund sacrifice of 1 of 3 females receiving 50 mg/kg/day and 1 of 3 females receiving 20 mg/kg/day. Both of these animals as well as scheduled sacrifice (in all treated groups) showed cortical tubular degeneration/regeneration of the kidney. Arteriolar hypertrophy in the kidney cortices was observed in all treatment groups (4 of 6 each at 20 and 50 mg/kg/day, and 1 of 6 at 5 mg/kg/day) and was still evident following the 4-week recovery period. Loss of body weight was noted for males receiving 20 or more mg/kg/day and females receiving 5 or more mg/kg/day. A no observed adverse effect level was not established in this study.

Study No: Report #NVR019/974195; Toxicokinetics: Study #NVR/019, Report #DBPK(F) 1998/013

Location of Report: EDR

Conducting Laboratory and Location: _____

Dates of Study: Dosing was initiated on July 7 and necropsied on November 5, 1997.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, lot #817196, purity _____

Formulation: Aliskiren hemifumarate was dissolved in deionized water. Dosages were prepared weekly. All dosages and concentrations were expressed in terms of the salt.

Animals

Species/Strain: Marmoset (*Callithrix jacchus*)

#/Sex/Group: See Table 3.2.7.1

Age: At study start: 15 to 42 months

Weight: At study start: Males: 333-462 gm, Females: 322-510 gm

Husbandry: Animals were housed in pairs of the same sex in steel cages. Food, twice a day and water, *ad libitum*, were given throughout the study period except during the period of fasting (prior to blood and urine sampling) when food and water were withheld.

Dosing

Doses: 0, 5, 20 or 50 mg aliskiren hemifumarate/kg/day (doses are expressed as salt). It should be noted that the animals selected for the toxicokinetic study had been used previously for a preclinical safety study (mainly for the purpose of collection of blood samples for toxicokinetic analysis) at least 8 months before the start of dosing. The doses were selected based on the previous 2-week study (section 3.2.6) in which a moderate to marked increase in BUN was observed in both sexes receiving 50 or more mg/kg/day, a reduction in body weight was noted for females receiving 100 mg/kg/day and a female from this 100 mg/kg/day group showed arteriolar hypertrophy in the kidneys.

Route, Mode and Duration of Administration: Orally by gavage (10 ml/kg), once daily, for 91 days. The control group received the vehicle. All surviving main study animals were sacrificed on study day 92. Recovery phase animals were treated for 91 days and were then allowed at least 4 weeks of recovery following the cessation of treatment; these animals were killed on recovery day 29.

TABLE 3.2.7.1
STUDY DESIGN

Group	Treatment	Dosage* mg/kg/day	Main Study		Recovery Phase		Toxicokinetic Study	
			Male	Female	Male	Female	Male	Female
1	Control		3	3	2	2	-	-
2	Aliskiren	5	3	3	-	-	3	3
3	Hemi-	20	3	3	-	-	3	3
4	fumarate	50	3	3	2	2	3	3

*all dosages are expressed as salt

Observations and Measurements

Clinical Signs: All animals were observed daily for clinical signs and mortality.

Body Weights: Recorded for all animals before treatment on the first day of dosing and then at weekly intervals and on the day of scheduled necropsy. Recovery animals were weighed weekly.

Food Consumption: Recorded for all main study and recovery animals on a daily basis during the pretest, treatment and recovery periods.

Water Consumption: Recorded for all main study and recovery animals on a daily basis pretest and during study weeks 3 and 4.

Ophthalmoscopy: Conducted for the main study and the recovery phase animals pretest, during week 12 of treatment and during the last week of the recovery phase.

EKG: Recorded from all main and recovery animals in the conscious state, pretest, and 4 hr after dosing during treatment week 12 and recovery week 3. Indirect blood pressures were taken for all animals pretest, during treatment week 12, and recovery weeks 3 and 4 (females and males, respectively).

Hematology¹ and Clinical Chemistry²: Blood samples were collected (from the femoral vein) from all main study and recovery animals (food and water were withheld) pretest and during weeks 6 and 13 of treatment. Additional samples were collected from the recovery animals during the last week of the recovery phase.

Urinalysis³: Urine was collected overnight, under food and water deprivation, from all main study and recovery animals pretest and during weeks 5 and 12 of treatment. Additional samples were collected from the recovery animals during the third week of the recovery phase.

Toxicokinetics: Blood samples were collected (from the femoral vein) from satellite animals on days 1 and 91 at 1, 2, 4, 8, and 24 hours after dosing from the femoral vein.

Gross Pathology: Only animals from the main study and recovery phase were necropsied at the end of the study. The following organs/tissues were taken from all main study animals for histopathological evaluation. Selected organs were weighed (Table 3.2.7.2).

¹ erythrocytes, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, reticulocytes, hematocrit, fibrinogen, white blood cell count (total and differential), thrombocytes, prothrombin time, activated partial thromboplastin time.

² alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, calcium, glucose, sodium, chloride, magnesium, triglycerides, cholesterol, phosphate, urea, bilirubin, creatinine, potassium, proteins (total and differential).

³ volume, specific gravity, pH, bilirubin, ketones, protein, blood pigments, leucocytes, sodium casts, epithelial cells, erythrocytes, phosphates, urobilinogen, glucose, potassium.

Histopathology: Microscopic examination was performed on the tissue sections listed in Table 3.2.7.2 from all main study animals killed on completion of the treatment and recovery periods, and on any tissue/organ noted as abnormal during macroscopic examination.

TABLE 3.2.7.2
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenal glands*	Jejunum	Sciatic nerve
Aorta	Kidneys*	Seminal vesicles
Brain*	Lachrymal glands	Skeletal muscle- thigh
Bronchi	Liver*	Skin
Cecum	Lungs	Spinal cord
Cervix	Lymph nodes -bronchial	Spleen*
Colon	-mandibular	Sternum
Duodenum	-mesenteric	Stomach
Epididymides	Mammary glands	Testes*
Esophagus	Ovaries*	Thymus
Eyes with optic nerves	Pancreas	Thyroid*/parathyroids
Femur	Pituitary gland*	Tongue
Gall bladder	Prostate*	Trachea
Gross lesions	Rectum	Urinary bladder
Heart*	Salivary glands	Uterus*
Ileum	-submandibular	Vagina
Ileo-cecal junction	-parotid	

* organ weighed

Results

Mortality: There were two treatment-related deaths. A female receiving 50 mg/kg/day was sacrificed moribund on day 57 due to the severity of signs observed. Marked diarrhea with red staining and mucus, weight loss, labored respiration and hunched posture were recorded. Week 6 hematology findings included slightly decreased RBC counts with increased reticulocytes. Histopathology findings included acute inflammation of the ileum and cortical tubular degeneration/regeneration of the kidney, which was attributed to hypotension and poor renal perfusion resulting from treatment with aliskiren. A female receiving 20 mg/kg/day was killed on day 73 following sudden body weight loss (-13 gm compared to the previous week and -34 gm relative to pretest weight), abnormal behavior and ataxia. The kidney changes were similar to those of the deceased high dose female.

Clinical Signs: An increased incidence of vomiting and postdose salivation and frequent incidences of diarrhea (minimal to marked) were observed in the high dose animals.

Body Weights: Both mid and high dose males and females showed a reduction in body weight during the study. It was pronounced and statistically significant in females relative to control values (mean body weights of control females did not decrease, Table 3.2.7.3). No significant differences in body weight were observed between the controls and the high dose animals during the recovery period.

Food Consumption: There were no changes in food consumption between controls and treated animals.

Ophthalmoscopy: No abnormalities

TABLE 3.2.7.3
MEAN BODY WEIGHTS (GM) IN 13-WEEK MARMOSSET STUDY

Study week		Dose mg/kg/day							
		Control		5		20		50	
		M	F	M	F	M	F	M	F
-1	B.wt., g	376	377	425	403	383	423	380	379
1	B.wt., g	375	371	427	371	381	408	366	376
3	B.wt., g	371	373	427	414	370	421	361	372
6	B.wt., g	382	380	416	419	378	429	366	373
9	B.wt., g	380	385	433	441	385	417	376	368 ^d
12	B.wt., g	375	389	429	427	372	439 ^c	362	358
13	B.wt., g	368	379	422	403 ^a	369	431	362	352
0-13	diff rel to pretest, g [§]	-8	3	-2	18 ^b	-14	-30 [†]	-19	-21 [†]
	n	5	5	3	2	3	2	5	4

^a: weight missing for the 3rd animal, n=2

^b: calculated using week 12 body weight since week 13 body wt. was missing for a female, n=2

^c: one humane kill in week 11. This animal weighed far less than the other 2 animals in week -1, n=2.

^d: one humane kill in week 9, n=4.

[†]: p < 0.05

[§]: differences in weight between week -1 and 13 were calculated for each animal and then averaged

ECG: No significant changes. Though reduction in blood pressure could not be demonstrated directly with test substance, according to the sponsor, a drop in blood pressure was reflected in the vascular changes observed in the kidneys.

Hematology: Individual animals receiving 20 or more mg/kg/day showed minimal to moderate reductions in red cell parameters and minimal increases in reticulocyte counts. Red blood cell counts, hemoglobin and hematocrit concentrations were statistically significantly different (p < 0.05) for mid and high dose male and high dose female groups relative to control group at study weeks 6 and 13.

Clinical Chemistry: Biochemical findings included a slight or moderate increase in blood urea concentrations in animals receiving 20 or more mg/kg/day at both weeks 6 and 13. However, the group mean data was significant (p < 0.05) for high dose females only (about 50% increase relative to control values). The high dose female group also showed a small but statistically significant increase in chloride concentrations relative to control group at both week 6 and week 13.

Urinalysis: There were no significant findings.

Gross Pathology: There were no drug-related organ weight or gross pathology findings.

Histopathology: Test substance-related histopathology was confined to the kidneys.

Degeneration/regeneration of cortical tubules was observed in all treated groups (including 1 of 3 male controls). Arteriolar hypertrophy in the kidney cortices was observed in 4 of 6 animals each in the mid and high dose groups and in one female in the low dose group. No information was available as to relative severity of these lesions in the different groups. Arteriolar hypertrophy was still evident following the 4-week recovery period. (Table 3.2.7.4). According to the sponsor, reduced b.p. over a period of

time has effects on kidney function, particularly glomerular filtration rate and tubule perfusion and function.

TABLE 3.2.7.4
RENAL PATHOLOGY IN 13-WEEK MARMOSSET STUDY

Kidney Findings	Dose mg/kg/day											
	Main Study								4-Week Recovery			
	0		5		20		50		0		50	
	M	F	M	F	M	F	M	F	M	F	M	F
No. examined	3	3	3	3	3	3	3	3	2	2	2	2
Papillary mineralization	1	0	1	0	0	1	2	2	0	2	0	1
Chronic interstitial nephritis	1	1	1	1	1	2	2	2	2	2	2	2
Degeneration/regeneration of cortical tubules	1	0	1	2	1	1	3	1	0	0	0	0
Arteriolar hypertrophy	0	0	0	1	2	2	2	2	0	0	2	2
Dilatation of cortical tubules	0	2	3	1	1	1	0	2	1	1	2	1
Cortical cyst(s)	0	0	0	0	0	1	0	0	0	0	0	0
Medullary cyst(s)	0	0	0	0	0	1	0	0	0	0	0	0

Toxicokinetics: Plasma levels of aliskiren on day 1 increased with increasing dose in females but not males, which developed the highest plasma concentration of test substance following the 20 mg/kg/day dose. On the other hand, the concentrations of aliskiren measured after 91 days of treatment increased with increasing dose in both males and females. Except for day 1 in males, systemic exposure increased over proportionally in both males and females with increasing dose. Exposures tended to be higher in males than in females (Table 3.2.7.5). However, due to high inter-animal variability of plasma levels, it is difficult to conclude a sex difference. Plasma levels of aliskiren decreased similarly in both sexes over 24 hr after administration of the drug. After a single dose, C_{max} values were achieved between 1 and 2 hr post administration. There was evidence of a dose-dependent accumulation in females but not in males when day 1 and day 91 C_{max}s and AUCs were compared. Although exposures were higher on day 91 than on day 1 in males at the 5 and 50 mg/kg doses, there was no increase with repeated administration at the 20 mg/kg dose (Table 3.2.7.6).

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TABLE 3.2.7.5
MEAN PHARMACOKINETIC PARAMETERS OF ALISKIREN IN MARMOSETS DURING 13-WEEK TOXICITY STUDY (N=3)

Dose mg/kg/day	Day	C _{max} , ng/mL		T _{max} , h		AUC [1-24h] ng.h/ml		Normalized AUC (ng.h/ml)/(mg/kg)	
		M	F	M	F	M	F	M	F
5	1	384.64	552.40	1.67	1.00*	1411.75	1913.58	282	383
	91	543.10	183.31	2.00	1.67	4550.82	834.53	910	167
20	1	8323.56	4248.63	1.00	1.33	47709.80	16441.70	2385	822
	91	6534.66	4631.08	1.33	1.67	45987.75	23894.64	2299	1195
50	1	6595.71	7720.98	1.00	1.00	41537.96	38729.85	831	775
	91	11405.62	9462.92	1.00	1.33	119054.46	76362.55	2381	1527

*Only one T_{max} available

TABLE 3.2.7.6
ACCUMULATION OF ALISKIREN IN MARMOSETS DURING 13-WEEK TOXICITY STUDY. RATIOS OF C_{MAX} AND AUC BETWEEN DAY 91 AND DAY 1

Dose mg/kg/day	C _{max} Day 91/ C _{max} Day 1		AUC Day 91/AUC Day 1	
	M	F	M	F
5	1.41	0.33	3.22	0.44
20	0.79	1.09	0.96	1.45
50	1.73	1.23	2.87	1.97

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3.2.8. Thirty-Nine Week Oral Toxicity Study in Marmosets

Key Study Findings: Three males and 4 females (including 2 control females) died or were euthanized *in extremis* during the study. None of these deaths was attributed by the sponsor to the drug substance. The principal drug-related finding was minimal hyperplasia of the juxtaglomerular apparatus of the kidneys at 20 mg/kg/day in 2 males and a female. The hyperplasia was not evident in 8 week recovery group animals. The NOAEL for this study was 5 mg/kg/day.

Study No: 1940-007 Report #1715-1940-007)

Location of Report: EDR

Conducting Laboratory and Location:

Dates of Study: Dosing was initiated on July 3, 2001. Terminal necropsies were begun on April 2 for main study animals and on May 28, 2002 for recovery study animals.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, batch #S100B-2001002

Formulation: Aliskiren hemifumarate was dissolved in distilled water at weekly intervals.

Analyses showed that the formulation of test substance was stable for 7 days at room temperature. Analysis of the formulations for achieved concentration and homogeneity was performed in weeks 1, 13, 27 and 39 of the study.

Animals

Species/Strain: Marmoset (*Callithrix jacchus*)

#/Sex/Group: 5/sex/group for toxicology; 3/sex/group for toxicokinetics

Age: 1.5 to 5 years old at start of study

Weight: males: 131.8 to 175 gm, females: 106 to 140 gm

Husbandry: Animals were housed individually in stainless steel cages. Food, twice a day and mineral water, *ad libitum*, were given throughout the study period except during the period of fasting (prior to sampling of blood for clinical pathology studies) when food but not water was withheld.

Dosing

Doses: See Table 3.2.8.1. Dose levels were based on the results of a previous 13 week oral toxicity study in the same strain in which gavage administration of aliskiren hemifumarate resulted in the moribund sacrifices of one female at 20 mg aliskiren/kg/day and another female at 50 mg aliskiren/kg/day. Both had renal cortical tubular degeneration/regeneration attributed to hypotension and poor renal perfusion. Arteriolar hypertrophy in the kidney cortices was observed at 5 or more mg aliskiren/kg/day. Furthermore, loss of body weight was noted for males receiving 20 or more mg/kg/day and females receiving 5 or more mg/kg/day.

Route, Mode and Duration of Administration: Orally by gavage, 10 ml/kg for all groups for 39 weeks. Recovery phase animals were also treated for 39 weeks (273 days) but killed 57 days later (study day 329).

TABLE 3.2.8.1
EXPERIMENTAL DESIGN

Gp #	Test substance	Dose* mg/kg/day	Dose vol ml/kg	# of animals/group		Necropsy after	
				Toxicology	Toxicokinetics	39 weeks	47 weeks
1	Vehicle	10 ml/kg	10	5 M, 5F	3 M, 3F	3 M, 3F	2 M, 2F
2	Aliskiren hemifumarate	2	10	5 M, 5F	3 M, 3F	3 M, 3F	2 M, 2F
3		5	10	5 M, 5F	3 M, 3F	3 M, 3F	2 M, 2F
4		20	10	5 M, 5F	3 M, 3F	3 M, 3F	2 M, 2F

*: Doses are expressed as free base equivalent.

Observations and Measurements

Clinical Signs: All animals were observed at least twice a day for mortality, moribundity and clinical signs.

Body Weights: Recorded for all animals before treatment on the first day of dosing and then at weekly intervals and on the day of scheduled necropsy.

Food Consumption: Estimated daily for main study animals but no calculations were performed.

Water Consumption: Recorded daily for all animals.

Ophthalmoscopy: Conducted on all main study animals prior to the initiation of dosing and in weeks 13, 26, at the end of the dosing period during study week 38, and on all surviving animals at the end of the recovery period.

ECG: Recorded from all main study animals pre-dose, in study weeks 13, 26 and 39, and from all surviving animals at the end of the recovery period.

Hematology and Clinical Chemistry: Blood samples were collected (from femoral or brachial vein) from all overnight fasted main study animals pre-dose and during weeks 13, 26 and 38. Additional samples were collected from the recovery animals at the end of the recovery period (week 47). Blood was collected at additional time points for analyses of urea, creatinine and serum electrolytes.

Urinalysis: Urine samples were collected overnight from all main study animals pre-dose and during weeks 13, 26 and 38 of treatment and in week 47 for recovery animals. Water was removed during collection except for week 47.

Gross Pathology: A complete necropsy was conducted on all toxicology group animals (fasted overnight) including those found dead or euthanized *in extremis*. Bone marrow smears were prepared at necropsy.

Organs Weighed: All main study animals at the scheduled necropsy. See Table 3.2.8.2

Histopathology: Microscopic examination was performed on all tissues listed in Table 3.2.8.2 from all main study animals at the scheduled necropsy and for all animals euthanized *in extremis* or found dead during the study.

Toxicokinetics: Blood samples for test substance determination were collected from all toxicokinetics study animals (3/sex/group/time point) at 1, 2, 4, 8 and 24 hours after dosing on study day 1 (first day of dosing) and in week 39. Blood was collected from the brachial vein.

TABLE 3.2.8.2
39 WEEK TOXICITY STUDY IN MARMOSETS: TISSUES/ORGANS SAMPLED
FOR HISTOPATHOLOGICAL EXAMINATION

Adrenals*	Lacrimal glands	Seminal vesicles
Aorta	Liver*	Skin
Bone with marrow, femur ¹ , sternum	Lungs	Spinal cord, cervical, thoracic,
Bone marrow smear	Lymph nodes, mesenteric,	lumbar
Brain*	mandibular	Spleen*
Cecum	Mammary gland	Stomach
Colon	Muscle, skeletal	Testes*
Duodenum	Nasal cavity ¹	Thymus
Epididymides*	Ovaries*	Thyroid with parathyroids*
Esophagus	Pancreas	Tongue
Eyes with optic nerves	Parotids	Trachea
Gross lesions	Pituitary*	Urinary bladder
Heart*	Prostate*	Uterus*
Ileum with Peyer's patches	Rectum	Vagina
Jejunum	Salivary glands, mandibular	
Kidney*	Sciatic nerve	

¹: Preserved, but not examined
 *: Organ weight obtained

Results

Mortality: Three main study and 4 toxicokinetic study animals died or were euthanized *in extremis* during the study (Table 3.2.8.3). Cause of death for these animals included unspecific cachexia, intestinal inflammation, pulmonary inflammation and hepatic necrosis. None of these deaths, according to the sponsor, was attributable to an effect of the test substance.

TABLE 3.2.8.3
FOUND DEAD OR EUTHANIZED IN EXTREMIS

Dose, mg/kg/day	# of deaths	Animal #, Mortality on study day and cause of death
Control	2 F	#952 (TK) Found dead on day 228 ^a #953 (TK) Found dead on day 237 ^a
5	1 M	#305 Euthanized <i>in extremis</i> on day 140 ^b
	2 F	#352 Euthanized <i>in extremis</i> on day 121 ^c #973 (TK) Found dead on day 51 ^d
20	2 M	#402 Found dead on day 232 ^e #932 (TK) Found dead on day 140 ^f

^a: succumbed to marmoset wasting syndrome
^b: poor condition was due to marked acute peritonitis with severe pleuritis of the diaphragm and lung and a severe acute pneumonitis. Elevated BUN, creatinine; enlarged spleen, adrenals, kidneys and liver
^c: displayed peritonitis and ulceration of the cecum, marked abscessation of the diaphragm and acute necrotizing inflammation of the lung.
^d: marked acute hepatitis with severe necrosis accompanied by subacute inflammation of the heart.
^e: marked acute peritonitis of the stomach and severe ulceration of the rectum
^f: acute inflammation of the lung accompanied by acute pleuritis.

Clinical Signs: There were no clinical signs throughout the study period that could be ascribed to treatment with test substance. Clinical signs were restricted to individual animals and noted at a low incidence. Clinical observations in those animals that died or were sacrificed in a moribund state included emaciation, sluggishness, rough hair coat, labored breathing, rapid respiration, prostration, dehydration, diarrhea and hunched posture. There was a continuous loss of body weight in these animals preceding death. The following animals were temporarily withdrawn from treatment due to transient poor physical condition:

Control: #953F, Study days 231 to 236

5 mg/kg/day: #302M, Study days 58 to 62; #353F, Study days 92 to 97

20 mg/kg/day: #405M, Study days 57 to 61; #451F, Study days 143 to 148

Body Weights: There were sporadic and non-statistically significant changes in mean body weight or mean body weight gain relative to control.

Water Consumption: No significant changes

Ophthalmoscopy: There were no treatment-related ocular changes across groups.

ECG: No significant changes.

Hematology and Clinical Chemistry: There were no drug-related hematological changes during the study period. Mean BUN was higher than concurrent control for high dose males in weeks 4 (118% higher, $p < 0.05$) and 39 (100% higher, $p > 0.05$) and slightly elevated above concurrent control for high dose females in study weeks 21 (87% higher, $p > 0.05$) and 39 (42% higher, $p > 0.05$) and for mid dose females in week 39 (32% higher, $p < 0.05$). The differences were primarily contributed by a marked increase in single animals in each of these groups. BUNs of recovery group animals were comparable to the concurrent control.

Urinalysis: There were no significant changes.

Organ Weights: Significant ($p < 0.05$) increases in mean absolute and relative (to final body weight) kidney (58%) and liver (46%) weights were noted for the high dose males relative to control males. Kidney and liver weights of recovery group animals were comparable to the concurrent control.

Gross Pathology: No treatment-related findings at terminal and recovery sacrifices.

Histopathology: Principal histopathological findings in the decedents included acute inflammation of the lung/pleuritis (#305M, #352F, #932M), subacute inflammation of intestine and unspecific cachexia (#402M, #952F, #953F) and one case of acute hepatitis with severe necrosis (#973F). Terminal necropsy revealed test substance-related renal changes consisting of minor grades of hyperplasia of the juxtaglomerular apparatus in 2 males (#401, #403) and a female (#453) of the high dose group. Additionally, a male (#401) and a female (#453) in the high dose group displayed a slight to moderate interstitial inflammation of the kidneys. These findings were not seen in recovery group animals, suggesting regression during the treatment-free period. Also, these findings were regarded as expected pharmacological effects rather than adverse toxic events. There were no histopathological correlates for the increased liver weight in high dose males.

Toxicokinetics: After single or repetitive measurements, systemic exposure (AUC_{0-24h}) to aliskiren in males and females increased with increasing dose but not proportionately. Males exhibited a slightly higher exposure to aliskiren than females (all dose groups) at the week 39 measurement, whereas the opposite appeared to be the case for

determinations made on day 1. Maximum plasma concentration (T_{max}) occurred 1 to 4 hr post dose (Table 3.2.8.4).

TABLE 3.2.8.4
TOXICOKINETICS OF ALISKIREN AFTER 39 WEEK ORAL DOSING IN MARMOSETS

Day 1						
Dose Level (mg/kg/day)	Group	Sex	C_{max} (ng/mL)	AUC ₀₋₂₀ (h*ng/mL)	t_{max} (h)	
0	1	Male	NA	NA	NA	
0	1	Female	NA	NA	NA	
2	2	Male	21.5388	228.8073	1	
2	2	Female	48.1795	564.9119	2	
5	3	Male	87.5419	940.7776	2	
5	3	Female	78.2352	926.1238	1	
20	4	Male	1439.5333	9820.4784	1	
20	4	Female	5377.4334	35285.6597	1	
Week 39						
Dose Level (mg/kg/day)	Group	Sex	C_{max} (ng/mL)	AUC ₀₋₂₀ (h*ng/mL)	t_{max} (h)	
0	1	Male	NA	NA	NA	
0	1	Female	NA	NA	NA	
2	2	Male	76.4371	433.7770	4	
2	2	Female	50.7779	317.7857	2	
5	3	Male	270.3620	2182.1004	1	
5	3	Female	350.9064	2160.9845	2	
20	4	Male	6555.8417	27523.8546	1	
20	4	Female	1120.9704	7742.9477	1	

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3.3. Carcinogenicity

3.3.1. 104-Week Oral (feed admixture) Carcinogenicity Study in Rats

Key Study Findings: No test substance-related mortality/morbidity was noted and there were no statistically significant differences in survival between control and aliskiren-treated animals. Statistically significant, dose-dependent reductions from concurrent control in mean body weight (6 to 28.5%) and mean body weight gain (10 to 47%) were observed at all doses. Microscopic gastrointestinal changes (mucosal epithelial hyperplasia, erosion/ulceration of the cecum and colon) and mesenteric lymph node sinusoid dilation were observed in both sexes at doses ≥ 750 mg/kg/day. One colonic adenoma and one cecal adenocarcinoma were observed in males receiving 1500 mg/kg/day.

Study No.: 0370063

Conducting Laboratory and Location: Safety Profiling and Assessment, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey

Dates of Study: Dosing was initiated on June 23, 2003 and terminal necropsies were from June 21-30, 2005

GLP Compliance: Yes

QA Report: yes (X) no ()

Drug, Batch #, and % Purity: Aliskiren hemifumarate; batch #0323008, 0323009, 0323010, 0444016, 0424025, 0424026, 0524036 and 0444029; drug content: —

Methods

Doses: 250, 750 and 1500 mg/kg/day of base (equivalent to 276.3, 828.8 and 1657.5 mg/kg/day hemifumarate salt, respectively)

Basis of Dose Selection: Toxicity based endpoint (MTD). The doses administered in this study were approved by the Executive CAC (see attachment #1 for eCAC meeting minutes)

Species/Strain: IGS Wistar Hannover rats — WI(Glx/BRL/Han)IGS BR

Number/Sex/Group: 60 for main study, 10 additional animals for gastrointestinal toxicity assessment (see Interim Sacrifice, below), 6 of which were used for toxicokinetics assessment

Route, Formulation: Orally as a feed admixture

Frequency of Dosing: Continuous with feed

Animal Age: 8 weeks at initiation of dosing

Animal Weight: males: 182.7 to 268.2 g, females: 131.6 to 200.5 g

Animal Housing and Feeding: Animals were housed in pairs in stainless steel cages.

Restriction paradigm for dietary restriction studies: Food and water were given *ad libitum*

Drug Stability/Homogeneity: Feed admixture was stable at room temperature for 21 days and formulation homogeneity was confirmed.

Interim Sacrifice: 10/sex/group at 52 weeks for gastrointestinal toxicity assessment.

Observations / Measurements

Mortality: Twice daily on week days and once daily on all other days

Clinical Signs: Once daily and recorded once weekly. Palpable mass examinations were performed on all animals every 4 weeks for the first 52 weeks and every two weeks thereafter until the start of necropsy.

Body Weights: Once weekly during weeks 1 to 14, every four weeks from weeks 18 to 78, and every two weeks thereafter until the start of necropsy.

Food Consumption: As per body weight schedule

Gross Pathology: A complete necropsy was conducted on all animals (fasted overnight) with a recording of macroscopic observations for all protocol tissues.

Histopathology: Peer review: yes (X), no ()

All tissues specified in the table below were processed and examined for all animals.

TABLE 3.3.1.1

104 WEEK CARCINOGENICITY STUDY IN RATS: TISSUES/ORGANS PROCESSED (P) FOR HISTOPATHOLOGICAL EXAMINATION

P adrenal	P jejunum	P sciatic nerve
P aorta	P kidney	P seminal vesicle
blood and bone marrow smears	P lacrimal gland	P skeletal muscle
P bone marrow (in bone)	P liver	P skin
P brain	P lung	P spinal cord
P cecum	P lymph node – bronchial	P spleen
P cervix	P lymph node – mandibular	P sternum
P clitoral gland	P lymph node – mesenteric	P stomach
P colon	P mammary gland	P testis
P duodenum	nasal pasage	P thymus
P epididymis	P ovary	P thyroid
P esophagus	P pancreas	P tongue
P eye	P parathyroid	P trachea
P femur/tibia	P pituitary	P urinary bladder
P Harderian gland	P preputial gland	P uterus
P heart	P prostate	P vagina
P ileum	P rectum	P macroscopic lesions
	P salivary gland	animal identification

Toxicokinetics: Blood samples for test substance determination were collected from the first 6 animals/sex/group designated for interim sacrifice at 52 weeks at 0, 8 and 16 hours after the lights came on (5 AM) during study weeks 4 and 26 (2/sex/group/time point). Blood was collected from the retro-orbital venous plexus of anesthetized animals.

Results

Analysis of Feed Admixture: Samples of the admixtures were analyzed at frequent intervals. At all intervals, the concentrations were — of target. The mean actual cumulative drug consumption (ACD) in each treatment group was — of target, but ACD was estimated on the basis of predicted food consumption rather than measured food consumption (Table 3.3.1.2).

TABLE 3.3.1.2
ACTUAL CUMULATIVE DRUG CONSUMPTION OF ALISKIREN HEMIFUMARATE VIA DIETARY EXPOSURE

Target dose (mg/kg/day) (hemifumarate salt)	Group 2 276.3 mg/kg/day		Group 3 828.8 mg/kg/day		Group 4 1657.5 mg/kg/day	
	Males	Females	Males	Females	Males	Females
ACD ^a , mean ± SD	271.7 ± 19	272.0 ± 19	813.3 ± 51	818.1 ± 51	1650.5 ± 191	1659.0 ± 171
% of target ^b	98.3	98.5	98.1	98.7	99.6	100.1

^a Actual cumulative dose, mg/kg = concentration of aliskiren hemifumarate in feed X mean predicted food consumption ÷ mean mid-period body weight. Represents mean values based on body weight and food consumption through day 722.

^b Calculation of actual cumulative dose for group 2 males did not include week 100-101 values.

Mortality: No test substance-related deaths occurred in the study. There were no statistically significant differences in survival between the control and aliskiren-treated groups. At the end of the 104-week dosing period, survival was 63% for controls (both sexes) and ranged from 77% (250 mg/kg/day) to 83% (1500 mg/kg/day) for males and from 65% (250 mg/kg/day) to 81% (1500 mg/kg/day) for females receiving drug (Table 3.3.1.3). For both sexes, mortality decreased with an increase in dose (a negative trend).

TABLE 3.3.1.3
SURVIVAL SUMMARY ^a

Study Week	Males				Females			
	Dose (mg/kg/day) – Base				Dose (mg/kg/day) – Base			
	0	250	750	1500	0	250	750	1500
1	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)
14	70 (100%)	70 (100%)	70 (100%)	70 (100%)	69 (99%)	70 (100%)	70 (100%)	70 (100%)
26	70 (100%)	70 (100%)	70 (100%)	70 (100%)	69 (99%)	70 (100%)	70 (100%)	69 (99%)
52	68 (97%)	67 (96%)	70 (100%)	68 (97%)	68 (97%)	70 (100%)	69 (99%)	68 (97%)
78	56 (93%)	56 (93%)	57 (95%)	56 (93%)	50 (83%)	54 (90%)	54 (90%)	54 (90%)
105	37 (62%)	46 (77%)	48 (80%)	50 (83%)	38 (63%)	39 (65%)	40 (67%)	48 (80%)
105 ^b	37 (63%)	46 (77%)	48 (80%)	50 (83%)	38 (63%)	39 (65%)	40 (67%)	48 (81%)
IS ^c	9	8	10	10	10	10	9	10
Died ^d	24	16	12	10	22	21	21	12

Note: n=70 (60 + 10) for each dose group through week 52. Following interim sacrifice in week 53, n=60/group for the remainder of the term.

a: % survival calculated based on the number of animals alive at the beginning of the week.

b: % survival calculated based on the number of animals alive at the beginning of the week *versus* an adjusted n number excluding early sacrifices (female #4547 on day 250 and male #1032 on day 598) due to injury.

c: Interim sacrifice in week 53. A number less than 10 in each group indicates early sacrifices

d: Died/sacrificed moribund, week 1 through week 105

Clinical Signs: No apparent increases in the incidence of palpable masses were noted either during the course of the study or prior to scheduled necropsy. At the end of the

104-week dosing period, the percent of males with palpable masses was 24% in control and ranged from 8% (1500 mg/kg/day) to 17% (750 mg/kg/day) in test substance-treated animals; the percent of palpable masses in females was 39% in control and ranged from 15% (1500 mg/kg/day) to 26% (250 mg/kg/day) in test substance-treated animals. Aliskiren-related clinical signs such as fecal changes (diarrhea, soft and mucoid feces, at doses ≥ 750 mg/kg/day) and perineal staining (at doses ≥ 250 mg/kg/day in females; at doses ≥ 750 mg/kg/day in males) were present with dose-related increases in incidence, duration and/or severity. Bloody feces were noted for both sexes at 1500 mg/kg/day. Additional clinical signs attributed to aliskiren included thin appearance (males at doses ≥ 750 mg/kg/day; females at 1500 mg/kg/day), hunched posture (males at doses ≥ 750 mg/kg/day), pale appearance and unkempt coat (both sexes at 1500 mg/kg/day). Body Weights: Significantly lower than concurrent control body weights and body weight gains were noted for both sexes in all treatment groups (Table 3.3.1.4).

TABLE 3.3.1.4
SUMMARY OF DECREASES IN MEAN BODY WEIGHT AND BODY WEIGHT GAIN FOR
ALISKIREN HEMIFUMARATE-TREATED RATS

Body weight parameter	Interval (week)	Sex	Dose level (mg/kg/day)		
			250	750	1500
% Decrease in mean body weight ^a	26	M	2.9	8.9*	19.7*
		F	3.6*	8.6*	14.2*
	50	M	3.3	11.0*	22.6*
		F	4.2*	11.5*	20.0*
	104	M	7.8*	14.8*	28.5*
		F	6.3	18.1*	27.4*
% Decrease in mean body weight gain ^a	26	M	5.8*	17.8*	39.1*
		F	8.5*	20.8*	37.1*
	50	M	6.1*	19.8*	39.8*
		F	8.4*	23.7*	42.9*
	104	M	13.1*	23.6*	45.3*
		F	10.1	29.6*	47.0*

^aValues are presented as the mean percent decrease relative to concurrent controls.

*Mean value was significantly different from control at $p \leq 0.05$.

Food Consumption: Significant decreases (ranging from 3 to 12%) in food consumption relative to concurrent control were seen in all treatment groups (beginning on day 22 at 250 mg/kg/day and on day 8 at 750 mg/kg/day). The decreases in food consumption at these dose levels likely contributed to the observed decreases in body weight. Evaluation of food consumption at 1500 mg/kg/day was not possible due a high incidence of food

spillage which was dose-related (observed for both sexes at 750 mg/kg/day with a marked increase at 1500 mg/kg/day). The sponsor suggests that this was due to a palatability problem. However, a rising dose palatability study (up to 2500 mg aliskiren/kg/day) showed no significant difference between control and treated groups at doses of up to 2000 mg/kg/day administered for 2 weeks following 2 weeks, each, at doses of 1000 and 1500 mg/kg/day (see section 3.2.3). In the 104 week carcinogenicity study, animals in mid and high dose groups probably ate less food as evidenced by their thin and pale appearance and reduced weight gain. Since there is no toxicokinetics data beyond 26 weeks, it is difficult to know whether the animals in the mid and high dose groups received the targeted doses for the full duration of the study.

Gross Pathology: An increased incidence of cystic mesenteric lymph nodes was noted for males at doses ≥ 750 mg/kg/day (0, 0, 11 and 16 animals at doses of 0, 250, 750 and 1500 mg/kg/day).

Histopathology:

Non-neoplastic: Histopathological changes considered to be related to treatment were noted in the gastrointestinal tract in a dose-dependent manner at both the 52-week and 104-week sacrifices. At the 52 weeks sacrifice (n=10/sex/dose), mucosal epithelial hyperplasia of the cecum and colon were observed at 750 and 1500 mg/kg/day. Cecal erosion and ulceration were noted, respectively, in one male at 750 mg/kg/day and one female at 1500 mg/kg/day (Table 3.3.1.5). In animals sacrificed at 104 weeks, an increased incidence of epithelial hyperplasia of the duodenum of males and the cecum, colon and rectum of both sexes were noted at doses of 750 or more mg/kg/day. Mean severity of the hyperplasia tended to increase with dose. This finding was associated with the presence of inflammation, inflammatory exudates in the lumen and/or erosions/ulcerations in some of these animals in both the mid and high dose groups (Table 3.3.1.6). The incidence and severity of dilation of sinusoids in mesenteric lymph nodes increased slightly in high dose females and substantially in males receiving 750 or more mg/kg/day. Mesenteric lymph nodes also contained aggregates of large, eosinophilic-staining macrophages, which were present at a high incidence in all groups/sexes/segments, and were of increased severity in both sexes at 750 or more mg/kg/day (Table 3.3.1.7). The proliferative changes observed in the intestinal epithelium were considered secondary to the irritant properties of aliskiren in view of the increased incidence and/or severity of aliskiren-related inflammation in the intestines (with or without erosions or ulcers) and sinusoidal dilatation and macrophage aggregates in mesenteric lymph nodes.

TABLE 3.3.1.5
INCIDENCE OF GASTROINTESTINAL TRACT FINDINGS IN ANIMALS SACRIFICED AFTER 52 WEEKS OF TREATMENT

Microscopic Findings	Dose (mg/kg/day)							
	Male				Female			
	0	250	750	1500	0	250	750	1500
<i>Number examined</i>	10	10	10	10	10	10	10	10
Stomach: squamous hyperplasia, grade 1	1	1	2	2	3	1	1	2
grade 2		1		1				
Total	1	2	2	3	3	1	1	2
<i>Mean severity</i>	1.0	1.5	1.0	1.3	1.0	1.0	1.0	1.0
Stomach: epithelial vacuolation, grade 1	2	2		1	1	2	1	2
grade 2				2				1
Total	2	2		3	1	2	1	3
<i>Mean severity</i>	1.0	1.0		1.7	1.0	1.0	1.0	1.3
Cecum: mucosal hyperplasia, grade 1			5	9			2	9
erosion, grade 1			1					
ulceration, grade 2								1
Colon: mucosal hyperplasia, grade 1			2	3			2	6

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TABLE 3.3.1.6
SELECTED INTESTINAL FINDINGS IN ANIMALS SACRIFICED AFTER 104 WEEKS OF TREATMENT

Microscopic Findings	Dose (mg/kg/day)							
	Males				Females			
	0	250	750	1500	0	250	750	1500
1. Cecum (No. examined)	60	60	60	60	60	60	60	60
Mucosal hyperplasia	12	24	47	49	5	9	48	55
<i>Mean severity</i>	1.3	1.1	1.6	1.7	1.0	1.1	1.3	1.6
Inflammation	2	0	17	9	0	1	1	3
Inflammatory exudate in lumen	2	0	10	2	0	1	1	1
Erosion/ulceration	0	0	8	3	0	0	1	1
Crypt dilatation	1	0	8	1	0	0	1	4
2. Colon (No. examined)	60	60	59	60	60	60	60	60
Mucosal hyperplasia	7	9	33	34	2	1	28	39
<i>Mean severity</i>	1.0	1.3	1.2	1.5	1.0	2.0	1.3	1.4
Inflammation	0	2	3	7	0	1	1	0
Inflammatory exudate in lumen	0	0	1	1	0	1	0	0
Erosion	0	0	0	2	0	0	0	0
Crypt dilatation	0	1	1	0	0	0	0	0
3. Rectum (No. examined)	60	60	60	60	60	60	60	60
Mucosal hyperplasia	4	1	18	28	0	1	7	31
<i>Mean severity</i>	1.0	1.0	1.2	1.3	-	2.0	1.1	1.3
Inflammation	1	2	2	2	0	1	0	0
Inflammatory exudate in lumen	1	2	3	0	0	1	0	0
Crypt dilatation	0	0	0	1	0	0	0	0
4. Duodenum (No. examined)	60	60	60	60	60	60	60	60
Mucosal hyperplasia	8	11	15	16	5	7	6	6
<i>Mean severity</i>	1.0	1.0	1.3	1.5	1.0	1.0	1.0	1.0
Inflammation	1	0	1	0	0	0	0	0
Erosion/ulceration	0	0	0	0	0	1	0	0
5. Jejunum (No. examined)	60	60	60	60	60	60	60	60
Mucosal hyperplasia	3	2	0	2	2	0	0	0
Inflammation	2	0	0	0	0	1	0	0
Erosion/ulceration	2	0	0	0	0	0	0	0
Ileum	60	59	60	60	60	60	60	60
Mucosal hyperplasia	1	1	4	4	1	1	0	1
Inflammation	0	0	0	1	0	1	0	0

TABLE 3.3.1.7
SELECTED MESENTERIC LYMPH NODE FINDINGS IN ANIMALS SACRIFICED AFTER 104 WEEKS OF TREATMENT

Microscopic findings	Dose (mg/kg/day)							
	Males				Females			
	0	250	750	1500	0	250	750	1500
Mesenteric lymph node (No. examined)	60	59	60	60	60	60	60	60
Macrophage aggregates: total	51	56	53	47	51	57	52	54
<i>Mean severity</i>	1.1	1.1	1.4	1.7	1.0	1.1	1.1	1.4
Dilation sinusoids	2	3	20	26	0	0	1	7
<i>Mean severity</i>	1.5	1.0	2.6	2.7	-	-	2.0	2.1

Neoplastic: At week 91, there were at least 48 males and 46 females alive in each group, demonstrating that sufficient number of animals lived long enough to provide adequate exposure to the test substance. A large decrease (18 to 47% relative to concurrent control) in body weight at 750 or more mg/kg/day suggests the attainment of MTD. No aliskiren-induced effects on the 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 for either sex of rats that were killed or died during the treatment period, or killed at term. A detailed tumor incidence summary is given in Table 3.3.1.8. The sponsor performed Peto trend tests and incidences of appropriate combinations of tumors were analyzed based on the work of McConnell¹. Although not statistically significant, a colonic adenoma was found in one male (#4002) and a cecal adenocarcinoma was found in another male (#4028) (both historically rare tumors), both at 1500 mg/kg/day (P = 0.075, FDA combined analysis). These were the only large intestine tumor observed in any of the study groups. The FDA analysis also showed no statistically significant positive trend for any tumor type (alone or combined) for either male or female rats.

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¹ McConnell, E.E., Solleveld, H.A., Swenburg, J.A., et al.: Guidelines for combining neoplasms for evaluation of rodent carcinogenicity studies. *J Natl Cancer Inst*: 6:283-289, 1986.

TABLE 3.3.1.8
24 MONTH CARCINOGENICITY STUDY IN RATS. INCIDENCE OF PRIMARY NEOPLASMS

SEX : MALE					SEX : FEMALE				
DOSE GROUP:	1	2	3	4	DOSE GROUP:	1	2	3	4
NO. ANIMALS:	60	60	60	60	NO. ANIMALS:	60	60	60	60
ABDOMEN	1	-	-	-	ABDOMINAL CAVITY	1	-	-	-
ABDOMINAL CAVITY	-	-	-	1	- Metastasis, Carcinoma:	1	-	-	-
- Sarcoma, NOS	-	-	-	1	ADIPOSE TISSUE	8	7	4	3
ADIPOSE TISSUE	1	-	1	5	- Metastasis, Carcinoma:	1	-	-	1
ADRENAL GLANDS	60	60	60	60	ADRENAL GLANDS	60	60	60	60
- Adenoma Cortical	1	-	-	1	- Adenoma Cortical	-	-	1	1
- Pheochromocytoma (B):	-	1	-	-	- Metastasis, Carcinoma:	-	-	-	1
- Pheochromocytoma (M):	1	-	-	-	- Metastasis, Sarcoma	-	-	-	1
AORTA	60	60	60	60	- Pheochromocytoma (B):	1	-	1	-
BILE DUCT EXTRAHEP.	2	2	3	2	- Pheochromocytoma (M):	-	-	-	1
BONE	-	1	1	-	AORTA	60	60	60	60
- Metastasis, Sarcoma	-	-	1	-	- Hemangiosarcoma	-	-	1	-
BONE MARROW	60	60	60	60	BILE DUCT EXTRAHEP.	1	-	-	1
BRAIN	60	60	60	60	BONE MARROW	60	60	60	60
- Meningioma, Benign	1	-	-	-	BRAIN	59	60	60	60
- Schwannoma	-	-	1	-	- Glioma, Anaplastic	1	-	-	-
- Tumor Granular C. (B):	1	-	-	-	- Metastasis, Carcinoma:	2	-	-	-
- Tumor Granular C. (M):	-	1	-	1	- Sarcoma, Meningeal	1	-	-	-
BROWN ADIPOSE TISSUE	-	-	1	-	- Tumor Granular C. (B):	1	-	-	-
- HIBERNOMA	-	-	1	-	BROWN ADIPOSE TISSUE	-	-	1	-
CECUM	60	60	60	60	CECUM	60	60	60	60
- Adenocarcinoma	-	-	-	1	- Metastasis, Carcinoma:	-	-	-	1
COLON	60	60	60	60	CERVIX	60	60	60	60
- Adenoma	-	-	-	1	- Adenocarcinoma	1	-	-	-
DEFERENT DUCTS	-	1	-	-	- Leiomyosarcoma	-	-	-	1
- Metastasis, Carcinoma:	-	1	-	-	- Metastasis, Carcinoma:	-	-	-	2
DIAPHRAGM	1	-	-	-	- Metastasis, Sarcoma	-	-	2	-
- Metastasis, Sarcoma	1	-	-	-	CLITORAL GLANDS	58	55	59	60
DUODENUM	60	60	60	60	- Hemangioma	1	-	-	-
- Metastatic carcinoma:	-	1	-	-	COLON	60	60	60	60
EARS	3	-	-	-	DIAPHRAGM	1	-	-	3
EPIDIDYMIDES	60	60	60	60	- Metastasis, Carcinoma:	1	-	-	1
- Mesothelioma	-	1	1	1	- Metastasis, Sarcoma	-	-	-	1
- Metastasis, Carcinoma:	-	1	-	-	DUODENUM	60	60	60	60
- Sarcoma, NOS	-	-	1	-	- Metastasis, Sarcoma	-	-	-	1
ESOPHAGUS	59	60	60	60	EARS	-	-	1	-
EYES	59	60	60	60	ESOPHAGUS	60	60	60	60
- Schwannoma Malignant:	1	-	-	-	- Metastasis, Sarcoma	-	1	-	1
					EYES	60	60	60	60

TABLE 3.3.1.8 (Continued)
24 MONTH CARCINOGENICITY STUDY IN RATS. INCIDENCE OF PRIMARY NEOPLASMS

	SEX : MALE					SEX : FEMALE					
	DOSE GROUP:	1	2	3		4	DOSE GROUP:	1	2	3	4
	NO. ANIMALS:	60	60	60	60		NO. ANIMALS:	60	60	60	60
FEMUR	:	60	58	60	60	FEMUR	:	59	57	58	59
- Osteosarcoma	:	1	-	-	-	HARDERIAN GLANDS	:	60	60	60	60
HARDERIAN GLANDS	:	60	60	60	60	HEART	:	60	60	60	60
- Metastasis, Sarcoma	:	1	-	-	-	- Metastasis, Carcinoma:	:	-	-	-	1
HEART	:	60	60	60	60	HINDLEGS	:	-	2	-	-
- Schwannoma, Endoc. (M)	:	1	1	1	-	ILEUM	:	60	60	60	60
HINDLEGS	:	9	8	7	1	JEJUNUM	:	60	60	60	60
ILEUM	:	60	59	60	60	- Leiomyoma	:	-	2	-	-
- Metastasis, Sarcoma	:	-	-	1	-	- Metastasis, Sarcoma	:	-	-	1	-
JEJUNUM	:	60	60	60	60	KIDNEYS	:	60	60	60	59
KIDNEYS	:	60	60	60	60	- Lipoma	:	-	-	1	-
- Adenoma, Tubule	:	-	-	-	1	- Metastasis, Carcinoma:	:	1	-	-	-
LACRIMAL GLANDS	:	60	60	60	60	LACRIMAL GLANDS	:	60	60	60	60
LIVER	:	60	60	60	60	LIVER	:	60	60	60	60
- Adenoma, Hepatocell.	:	2	5	-	-	- Adenoma, Hepatocell.	:	2	-	4	-
- Carcinoma, Hepatocell.	:	2	-	-	-	- Metastasis, Carcinoma:	:	1	-	-	1
- Cholangiocarcinoma	:	-	1	1	-	- Metastasis, Sarcoma	:	-	1	1	1
- Metastasis, Sarcoma	:	1	-	-	-	LUNGS	:	60	60	60	60
LUNGS	:	60	60	60	60	- Adenoma, Bronchioalv.	:	-	-	1	-
- Adenoma, Bronchioalv.	:	-	-	1	-	- Carcinoma, Bron.-Alv.	:	-	1	-	-
- Carcinoma, Bron.-Alv.	:	1	-	-	-	- Metastasis Carcinoma:	:	-	-	-	1
- Metastasis, Sarcoma	:	2	-	-	-	- Metastasis, Sarcoma	:	-	-	1	-
LYMPH NODES	:	3	5	3	1	LYMPH NODES	:	1	-	-	3
MAMMARY GLAND AREA	:	54	54	54	52	- Hemangioma	:	1	-	-	-
- Adenolipoma	:	1	-	-	-	- Metastasis, Sarcoma	:	-	-	-	1
MANDIB. LYMPH NODES	:	59	60	60	60	MAMMARY GLAND AREA	:	60	60	60	60
MEDIAST. LYMPH NODES	:	7	9	8	7	- Adenocarcinoma	:	6	1	5	2
- Hemangioma	:	-	-	-	1	- Adenoma	:	3	1	3	1
MEDIASTINUM	:	1	-	-	-	- Fibroadenoma	:	10	17	7	5
- Metastasis, Sarcoma	:	1	-	-	-	MANDIB. LYMPH NODES	:	59	60	60	60
MESENT. LYMPH NODES	:	60	59	60	60	MEDIAST. LYMPH NODES	:	2	1	3	-
- Hemangioma	:	1	-	1	-	MEDIASTINUM	:	-	-	-	1
- Hemangiosarcoma	:	5	-	3	1	- Metastasis, Carcinoma:	:	-	-	-	1
- Metastasis, Carcinoma:	:	-	-	-	1	MESENT. LYMPH NODES	:	60	60	60	60
- Metastasis, Sarcoma	:	2	-	-	-	- Hemangiosarcoma	:	1	-	1	-
MESENTERY	:	2	-	1	1	- Metastasis, Sarcoma	:	-	1	1	1
- Hemangioma	:	-	-	1	-	MESENTERY	:	2	1	1	2
- Metastasis, Sarcoma	:	1	-	-	-	- Hemangiosarcoma	:	-	1	-	-
NASAL CAVITY	:	1	-	-	-	- Metastasis, Carcinoma:	:	1	-	-	1
PALATE	:	1	-	-	-	- Metastasis, Sarcoma	:	-	-	1	1
- Metastasis, Sarcoma	:	1	-	-	-	ORAL CAVITY	:	-	1	1	-
OVARIES	:	60	60	60	60	- Carcinoma Squam. Cell:	:	-	1	-	-
- Carcinoma Yolksac	:	1	-	-	-						
- Cystadenocarcinoma	:	1	1	1	-						
- Metastasis, Sarcoma	:	-	-	-	1						

TABLE 3.3.1.8 (Continued)
24 MONTH CARCINOGENICITY STUDY IN RATS. INCIDENCE OF PRIMARY NEOPLASMS

	SEX : MALE					SEX : FEMALE			
	DOSE GROUP:					DOSE GROUP:			
	1	2	3	4		1	2	3	4
	NO. ANIMALS:					NO. ANIMALS:			
	60	60	60	60		60	60	60	60
PANCREAS	60	60	60	60	PANCREAS	60	60	60	60
- Adenoma, Islet Cell	3	1	3	3	- Adenoma, Islet Cell	2	-	-	-
- Carcinoma, Islet Cell	1	-	-	-	- Carcinoma, Islet Cell	-	-	-	1
- Metastasis, Carcinoma	-	1	-	-	- Metastasis, Carcinoma	1	-	-	1
- Metastasis, Sarcoma	1	-	-	-	- Metastasis, Sarcoma	-	-	1	1
PARATHYROID GLANDS	57	57	58	59	PARATHYROID GLANDS	58	55	59	58
- Adenoma	2	2	1	-	- Adenoma	-	1	-	-
PERIPHER. NERVE	32	37	33	40	PERIPHER. NERVE	33	32	33	32
PINEAL BODY	-	1	-	-					
PITUITARY GLAND	60	59	60	60	PITUITARY GLAND	60	60	60	60
- Adenoma P. Distalis	18	18	16	12	- Adenocarcin., P. Dist.	2	-	-	-
- Adenoma, P. Intermed.	1	1	-	-	- Adenoma P. Distalis	36	30	27	26
PREPUTIAL GLANDS	60	59	59	59	- Adenoma, P. Intermed.	1	1	1	2
PROSTATE	60	60	60	59	- Metastasis, Sarcoma	1	-	-	-
- Adenocarcinoma	-	1	-	-	RECTUM	60	60	60	60
- Adenoma	1	-	1	-	- Hemangiosarcoma	-	1	-	-
- Metastasis, Carcinoma	-	1	-	-	SALIVARY GLANDS	60	60	60	60
RECTUM	60	60	60	60	SCIATIC NERVE	58	58	59	59
SALIVARY GLANDS	59	60	60	60	SKELETAL MUSCLE	59	58	59	59
- Adenocarcinoma	-	-	1	-	SKIN	60	60	60	60
- Schwannoma	-	-	1	-	- Fibroma	1	-	-	-
SCIATIC NERVE	59	59	60	57	- Keratoacanthoma	1	-	1	1
SEMINAL VESICLES	60	60	60	59	- Metastasis, Carcinoma	-	-	-	1
- Metastasis, Carcinoma	-	2	-	-	- Papilloma, Squam. Cell	2	-	-	-
- Metastasis, Sarcoma	1	-	-	-	SPINAL CORD	60	60	60	60
SKELETAL MUSCLE	60	59	60	58	SPLEEN	60	60	60	60
- Hemangiosarcoma	1	-	-	-	- Metastasis, Carcinoma	1	-	-	1
- Metastasis, Sarcoma	-	-	-	1	STERNUM	60	60	60	60
SKIN	60	60	60	60	STOMACH	60	60	60	58
- Adenoma Sebaceous	1	-	-	-	- Leiomyosarcoma	-	1	1	-
- Fibroma	-	2	1	1	- Metastasis, Carcinoma	1	1	-	1
- Fibrosarcoma	3	-	-	-	- Metastasis, Sarcoma	-	-	-	1
- Hemangiosarcoma	1	-	-	-	- Stromal Sarcoma	1	-	-	-
- Keratoacanthoma	1	2	5	-					
- Lipoma	-	1	1	1					
- Papilloma, Squam. Cell	3	-	1	-					
- Schwannoma, Malignant	-	1	-	-					
- Tumor, Basal Cell (B)	-	-	1	-					
SPINAL CORD	60	60	60	60					
- Meningioma	-	1	-	-					
SPLEEN	60	60	60	60					
- Metastasis, Carcinoma	-	1	-	-					
- Metastasis, Sarcoma	1	-	-	-					
STERNUM	60	60	60	60					
STOMACH	60	60	60	60					
- Metastasis, Carcinoma	-	1	-	-					
- Stromal Sarcoma	1	-	-	-					

TABLE 3.3.1.8 (Continued)
24 MONTH CARCINOGENICITY STUDY IN RATS. INCIDENCE OF PRIMARY NEOPLASMS

	SEX : MALE					SEX : FEMALE					
	DOSE GROUP:	1	2	3		4	DOSE GROUP:	1	2	3	4
	NO. ANIMALS:	60	60	60	60		NO. ANIMALS:	60	60	60	60
SYSTEMIC NEOPLASMS	:	60	60	60	60	SYSTEMIC NEOPLASMS	:	60	60	58	60
- Histiocytic Sarcoma	:	1	-	1	-	- Lymphoma: malignant	:	3	1	2	1
- Leukemia, Myeloid	:	1	-	-	-	THORACIC CAVITY	:	-	1	-	-
- Lymphoma: malignant	:	-	1	-	-	THYMUS	:	59	59	60	58
TESTES	:	60	60	60	60	- Thymoma, Benign	:	4	4	2	2
- Adenoma Interst. Cell	:	-	2	1	1	THYROID GLAND	:	60	60	60	60
- Mesothelioma	:	-	1	-	-	- Adenoma C-cell	:	9	9	7	14
- Metastasis Sarcoma	:	-	-	1	1	- Adenoma Follic. Cell	:	2	1	4	2
THORACIC-CAVITY	:	-	-	-	1	TONGUE	:	60	60	60	60
THYMUS	:	59	59	56	59	TRACHEA	:	60	60	60	60
- Thymoma, Benign	:	1	1	-	1	TRACHEOBRONCHIAL LNN	:	55	51	55	56
THYROID GLAND	:	59	60	60	59	URINARY BLADDER	:	60	60	60	60
- Adenocarcinoma, Foll.:	:	-	1	-	-	- Metastasis, Carcinoma:	:	-	-	-	1
- Adenoma C-cell	:	8	11	10	4	- Papilloma, Transit. C.:	:	2	-	-	-
- Adenoma Follic. Cell	:	3	6	6	4	UTERUS	:	60	60	60	60
TONGUE	:	60	60	60	60	- Adenocarcinoma	:	-	3	1	4
TRACHEA	:	60	60	60	60	- Leiomyoma	:	1	-	-	-
TRACHEOBRONCHIAL LNN	:	57	55	54	56	- Polyp Endom. Stromal:	:	6	9	5	8
- Metastasis, Carcinoma:	:	-	1	-	-	- Sarcoma, Endom. Strom.:	:	-	1	2	2
URETERS	:	-	1	-	-	VAGINA	:	60	60	60	60
URINARY BLADDER	:	60	60	60	59	- Granul. Cell. Tum. Ben.:	:	-	3	4	-
- Metastasis, Carcinoma:	:	-	1	-	-	- Metastasis, Sarcoma	:	-	-	2	1
- Metastasis, Sarcoma	:	-	-	-	1	- Polyp	:	-	1	-	-
ZYMBALIS GLANDS	:	-	-	1	1	ZYMBALIS GLANDS	:	1	-	1	1
- Carcinoma Sebaceous	:	-	-	1	1	- Carcinoma Sebaceous	:	1	-	-	1
						- Carcinoma Squam. Cell:	:	-	-	1	-

Group 1: Control

Group 2: Aliskiren 250 mg/kg/day

Group 3: Aliskiren 750 mg/kg/day

Group 4: Aliskiren 1500 mg/kg/day

Toxicokinetics: No significant differences in aliskiren plasma concentrations were noted between males and females. The plasma concentrations of aliskiren increased in a dose-dependent (although not dose-proportional) manner, and for all dose groups the concentration in week 26 was slightly higher than in week 4 (Table 3.3.1.9). Mean plasma concentrations of aliskiren at any given time were highly variable for the high dose group, somewhat less variable for the mid dose group and least variable for the low dose group. Variability was much higher in week 26 than in week 4 (Fig. 3.3.1.1). The AUC_{0-24hr} /dose ratios for mid to low and high to low dose male groups in week 4 were similar (3.25 and 3.38, respectively). In contrast, the ratios at week 26 were 2.57 and 1.43 for mid to low and high to low dose male groups, respectively. This suggests an inadequate exposure of high dose group males to the test substance, a suggestion supported by reduced food consumption at doses ≥ 750 mg/kg/day (study notes an increase in the occurrence of spilled feed for both sexes at 750 mg/kg/day and a marked increase in spill at 1500 mg/kg/day.) Note that plasma concentrations of aliskiren were not determined beyond 26 weeks.

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TABLE 3.3.1.9
TOXICOKINETIC PARAMETERS OF ALISKIREN IN RAT PLASMA

Sampling period	Dose (mg/kg/day)					
	250		750		1500	
	Male	Female	Male	Female	Male	Female
Week 4						
Tmax	16	0	8	8	16	0
Cmax	17.5	21.8	198	155	314	383
Cmax/Dose	0.07	0.0872	0.264	0.207	0.209	0.255
AUC(0-24h) ± SE	320 ± 62	438 ± 88	3120 ± 471	3060 ± 543	6490 ± 354	8260 ± 1028
AUC(0-24h)/Dose ± SE	1.28 ± 0.248	1.75 ± 0.352	4.16 ± 0.628	4.09 ± 0.724	4.33 ± 0.236	5.50 ± 0.685
Week 26						
tmax	8	0	0	16	16	0
Cmax	40.9	64.1	485	174	408	590
Cmax/Dose	0.164	0.256	0.647	0.232	0.272	0.393
AUC(0-24h) ± SE	874 ± 77	890 ± 252	6740 ± 2390	3390 ± 313	7530 ± 1361	10200 ± 1108
AUC(0-24h)/Dose ± SE	3.50 ± 0.308	3.56 ± 1.01	8.99 ± 3.18	4.52 ± 0.417	5.02 ± 0.907	6.83 ± 0.739

Tmax in hours; Cmax in ng/mL; Cmax/dose in (ng/mL)/(mg/kg/day); AUC(0-24h) in (ng/ml) x hours and AUC(0-24)/dose in ((ng/mL)x hours)/(mg/kg/day)
 For AUC(0-24h) calculation, concentration at time 24 h was considered equal to concentration at 0 h (assuming steady state was reached).

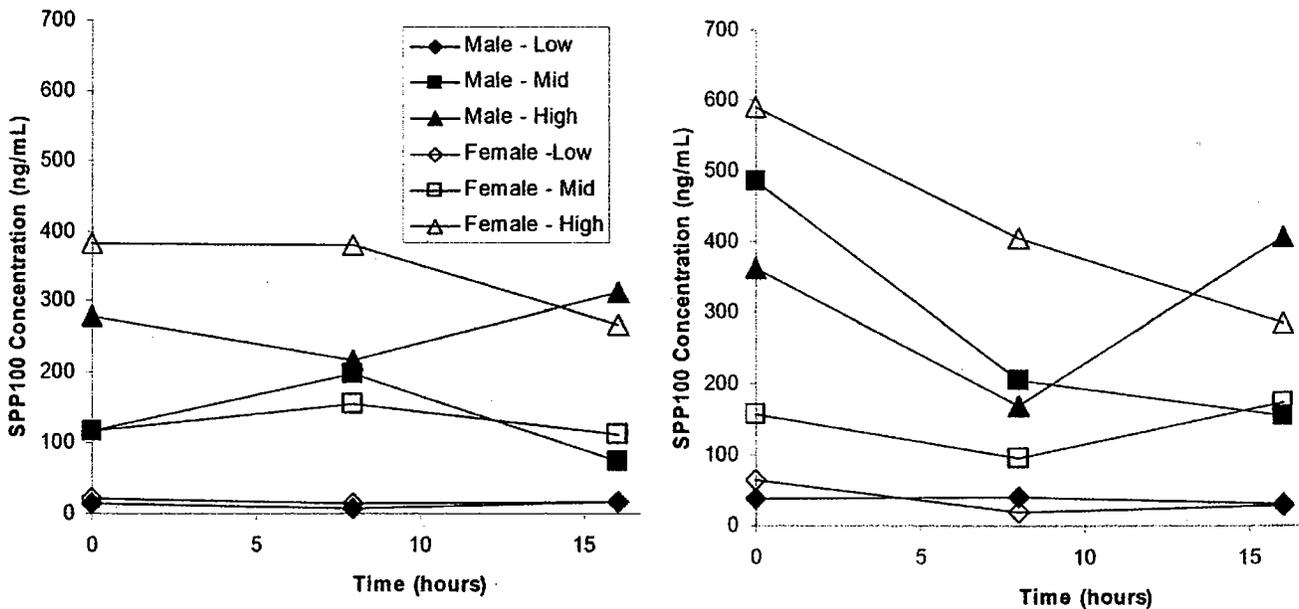


Fig. 3.3.1.1: Mean plasma concentration of aliskiren in rats at week 4 (left side) and at week 26 (right side)

3.3.2. 26-Week Oral (dietary) Carcinogenicity Study in CB6F1- rasH2 Mice

Key Study Findings: Statistically significant reductions in survival rates were noted for males receiving 1500 mg aliskiren/kg/day relative to concurrent control males. Statistically significant reductions in body weight gain relative to control were noted for males in all treatment groups (250 or more mg/kg/day) and for females in the high dose group. The main target organs for non-neoplastic lesions (noted at 750 or more mg/kg/day) were nasal cavity, small and large intestine, mesenteric lymph node and gall bladder in both sexes, and reproductive organs and bone marrow in females. Focal atypical hyperplasia of the colon (a pre-neoplastic lesion) was noted in high dose animals (1 male and 3 females). Other findings included an increased incidence of hypertrophy/hyperplasia in the small intestine and cecum, and atrophic changes in the female reproductive organs. None of these findings were observed in concurrent control mice. The types of neoplastic findings seen with the aliskiren-treated groups were similar to those reported for untreated Tg-rasH2 mice and there were no incidences above concurrent control levels. The NOAEL for the present study was less than 250 mg/kg/day.

Study no.: 0410091

Conducting laboratory and location: Exploratory Development, Safety Profiling and Assessment, Toxicology, Novartis Pharma AG, Basel, Switzerland

Dates of Study: Dosing was initiated on November 15, 2004 and terminal necropsies were from May 17-23, 2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, batch #, and % purity: Aliskiren hemifumarate; batch #0444017 and 0444029;
drug content: —

CAC concurrence: Yes

Methods

Doses: 250, 750 and 1500 mg/kg/day of base (equivalent to 276.3, 828.8 and 1657.5 mg/kg/day hemifumarate salt, respectively).

Basis of Dose Selection (MTD, MFD, AUC etc.): Toxicity based endpoint (MTD). The doses administered in this study were approved by the Executive CAC (see attachment #2 for eCAC meeting minutes)

Species/strain: CB6F1/Jic-TgrasH2 hemizygous mice (from —)

Number/Sex/Group: 25 for main study, 6 for toxicokinetics

Route, Formulation: Test substance was administered in the diet as a feed admixture.

Frequency of Dosing: Continuous with feed

Positive Control: N-methyl-N-nitrosourea (MNU) was administered as the positive control to 25 males and 25 females at a single intraperitoneal dose of 75 mg/kg, on day 1 of the study. MNU was dissolved in citrate-buffered saline.

Animal Age: About 10 weeks at initiation of dosing

Animal Weight: males: 20.9 to 26.6 g, females: 16.1 to 22.3 g

Animal Housing and Feeding: Males individually, females in groups (number/group not specified). Food and water were given *ad libitum*.

Drug Stability/Homogeneity: Samples of each diet formulation were taken for analysis in study weeks 1, 13 and 26. No decrease in test substance concentration was noted in feed admixtures stored under experimental conditions for 42 days.

Interim Sacrifices: None

Deviations from Original Study Protocol: Ophthalmic examinations were not conducted since earlier studies did not indicate test substance-related changes in this parameter. Blood sampling for toxicokinetic measurements started with the afternoon time point (8 hr after the onset of lights) rather than at 6 am (indicated in the original study protocol) on the assumption that steady state conditions were achieved and, further, variability in plasma concentrations at this time is considered negligible.

Observations and Measurements

Mortality: Checked whenever other activities were performed

Clinical Signs: Once daily and recorded once weekly.

Body Weights: Once weekly during weeks 1 to 14, every four weeks from weeks 18 to 78, and every two weeks thereafter until the start of necropsy.

Food Consumption: Mean consumption per animal was calculated from the total cage value.

Gross Pathology: Satellite animals were excluded from this procedure. A complete necropsy was conducted on all main study animals. All tissues/organs specified in the table below were taken from all animals. In addition, all organs/tissues showing macroscopic abnormalities (including palpable masses) during necropsy were sampled.

Histopathology: Peer review: yes (X), no ()

Histopathological examination of all tissues listed in Table 3.3.2.1 were conducted for animals in the control, the high dose and the MNU groups. Additionally, lungs, stomach, small and large intestine, spleen, thymus, mesenteric lymph node, liver, gall bladder, clitoral glands, ovaries, uterus, vagina, knee joint (with femur and bone marrow, females only) and nasal cavity of all animals from low and mid dose groups were examined microscopically.

Toxicokinetics: Blood samples for test substance determination were collected from all satellite study animals (2/sex/group/time point) at 2 pm, 10 pm and 6 am (immediately after lights came on in animal rooms) in study weeks 4 and 22. Blood was collected from the retro-orbital venous plexus of anesthetized animals.

TABLE 3.3.2.1
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE: TISSUES/ORGANS PROCESSED (P) FOR HISTOPATHOLOGICAL EXAMINATION

P	W	adrenals	P	W	ovaries (with oviduct)
P		aorta	P		pancreas
P	W	brain	P		peripheral nerves
P		cecum	P		pituitary ^b
P		clitoral gland	P		preputial gland
P		colon	P	W	prostate
P		duodenum	P		rectum
P		epididymides	P		salivary glands
P		esophagus	P		seminal vesicles
P		eyes with optic nerves ^b	P		skeletal muscle
P		gall bladder (together with liver)	P		skin
P		Harderian glands ^b	P		spinal cord
P	W	heart	P	W	spleen
P		ileum	P		sternum (with bone marrow)
P		jejunum	P		stomach
P	W	kidneys	P	W	testes
P		knee joint (with femur and bone marrow)	P	W	thymus
P		lacrimal glands	P		thyroid (with parathyroid) ^a
P		larynx	P		tongue
P	W	liver	P		trachea
P		lung	P		ureters
P		lymph node – mandibular	P		urinary bladder
P		lymph node – mesenteric	P	W	uterus (with cervix)
P		mammary gland	P		vagina
P		nasal cavity	P		Zymbal glands ^b

^a: taken with trachea

^b: was not separated but fixed with the skull

P : processed, W: organ weighed

Results

Analysis of Feed Admixture: The concentrations of aliskiren measured in the feed samples ranged from _____ of target. The mean actual drug consumption levels over the 26 week exposure period were _____ of target.

Mortality: Survival rates after 26 weeks of treatment were 100, 96, 100 and 84% (21/25) for the males and 96, 96, 96 and 100% for the females in the control, 250, 750 and 1500 mg/kg/day groups. Though the sponsor's analysis revealed no statistical differences in survival between the control group and aliskiren-treated male groups, the FDA analysis showed a statistically significant increase in mortality (log-rank test, P=0.0253,

permutation trend test, $P=0.0064$). Although cause of death for 3 of the 4 high dose males that failed to survive the study was not determined, the study report notes that none of these four deaths were related to drug treatment (Table 3.3.2.2).

TABLE 3.3.2.2
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE: CAUSE OF DEATH

Group	Sex / animal #	Day of death	Cause of death
Control	F / 37	148	malignant tumor of the mammary gland
250 mg/kg	M / 61	148	malignant tumor of the lung
	F / 87	132	cause of death not detectable
750 mg/kg	F / 148	162	malignant tumor of the nasal cavity
1500 mg/kg	M / 162	150	intussusception of colon/cecum
	M / 153	103	cause of death not detectable
	M / 171	27	cause of death not detectable
	M / 175	128	cause of death not detectable

In the MNU treated group, 20 males and 19 females were killed moribund or were found dead and one female died accidentally. The cause of death was related to neoplasms or proliferative changes, most frequently malignant lymphomas, squamous cell carcinoma or papilloma in the forestomach or skin and adenoma in the lung in both sexes. This group was terminated on day 172 (males) and on day 154 (females) when only 5 surviving animals remained.

Clinical Signs: No palpable masses were observed in any of the aliskiren hemifumarate-treated or untreated control animals during the study. Nor were test substance-related clinical signs noted, except for nonspecific signs of toxicity noted in decedents, such as reduced activity, hunched posture, abnormal gait and piloerection. A female in the positive control group showed a single palpable mass at the inguinal region. Many animals in this group showed proliferations of the skin and exhibited swellings on the tail.

Body Weights: Statistically significantly lower than control mean body weight or reduced body weight gain was noted for males at all doses: at 250 mg/kg/day from weeks 16 to 21, at 750 mg/kg/day from week 11 and at 1500 mg/kg/day from week 2 to the end of treatment (Fig. 3.3.2.1). Mean body weight gain for males over the dosing period was 25.5% and 12.5% at 750 or 1500 mg/kg/day, respectively, compared to a 35.8% gain for male controls. For females, a significantly lower ($p < 0.05$) than control mean body weight or body weight gain was noted for high dose group only (13% gain compared to 38.5% gain for control) for week 2 through the end of treatment (Fig. 3.3.2.1). Final mean body weights of animals exposed to aliskiren were 95, 91 and 80% of control for males and 101, 95 and 83% of control for females at 250, 750 or 1500 mg/kg/day, respectively. In the MNU treated group, mean body weights were slightly reduced ($P > 0.05$) for both sexes (85% of control).

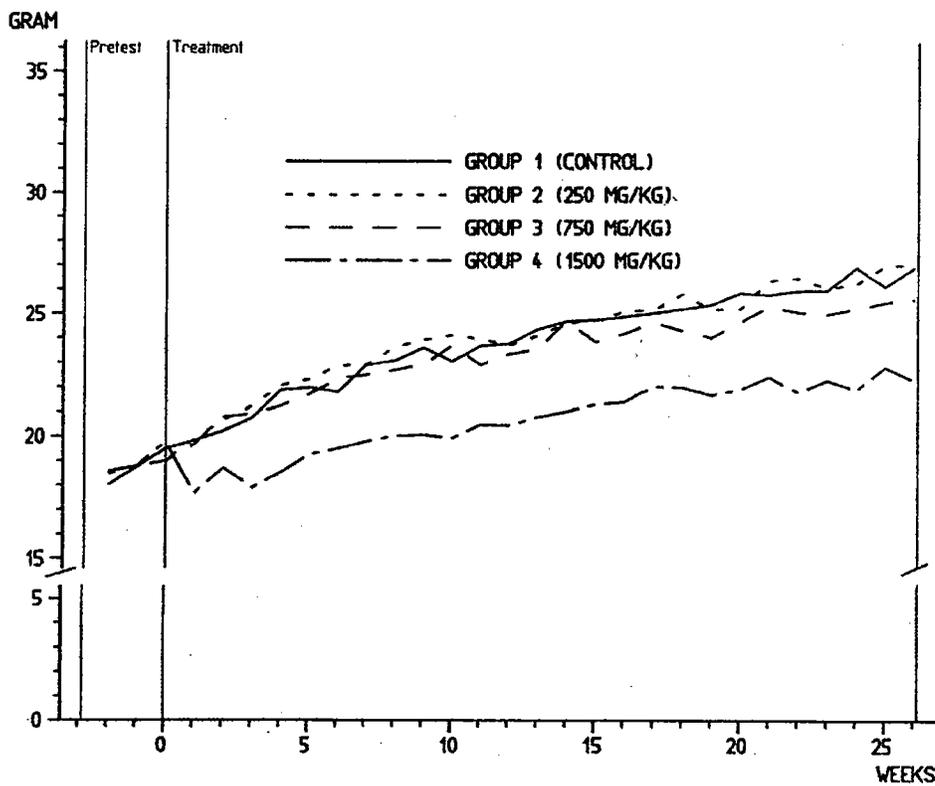
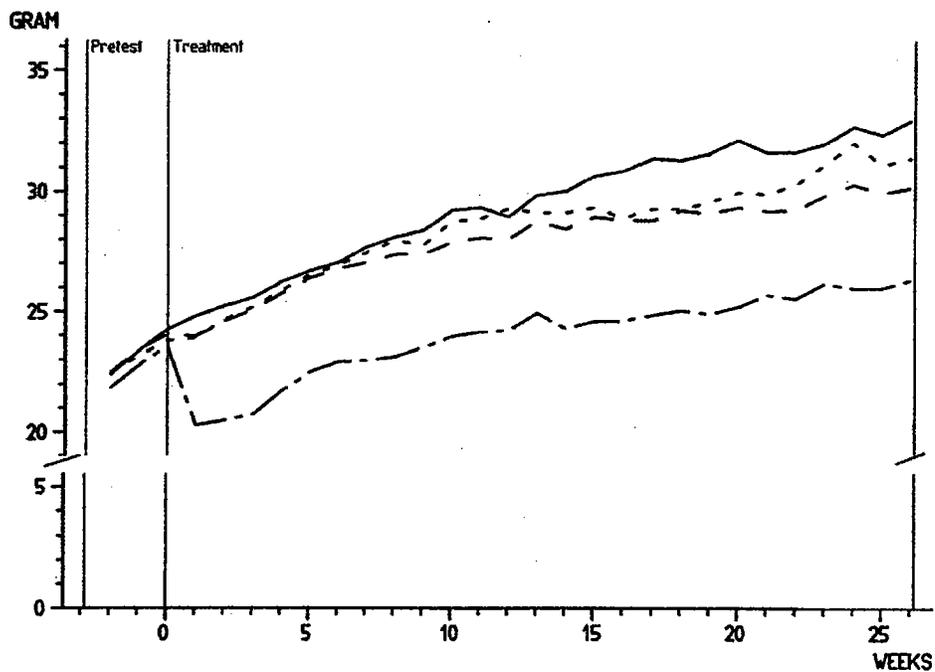


Fig. 3.3.2.1: Mean body weights for mice (male: top panel, female: bottom panel) treated with aliskiren hemifumarate for 26 weeks.

Food consumption: The overall food intake was lower than control ($P < 0.05$) at doses ≥ 750 mg/kg/day (both sexes). For the MNU treated group, overall mean food intake was not affected in males and only slightly reduced ($P > 0.05$) in females.

Organ Weights: Absolute and/or relative (to body and/or brain) weight differences from control were noted for the heart, kidneys, liver, spleen, thymus and brain for both sexes and in ovaries, uterus and adrenal gland for females (Table 3.3.2.3). Except for ovarian and uterine changes and in the absence of pertinent macroscopic and histomorphological changes, the observed weight decreases at 750 or more mg/kg/day were considered to reflect the treatment-related decrease in body weight and thus were not considered a direct effect of the test substance. For the MNU treated group, a marked increase ($P < 0.05$) in both absolute and relative (to body and brain) weights was noted for the spleen (both sexes) and was accompanied by microscopic findings.

Gross pathology: No test substance-related findings. MNU-related macroscopic changes (such as mass, nodule, thickening) were noted in the stomach, liver, kidneys, spleen, thymus, lymph nodes and skin (both sexes).

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TABLE 3.3.2.3
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE: GROUP MEAN ORGAN WEIGHTS

SEX: MALE				SEX: FEMALE			
ORGAN	DOSE GROUP: NO. ANIMALS:						
	01 25	02 25	03 25	04 25	05 25	01 25	02 25
FINAL BODY WEIGHT	24	25	25	21	5	24	24
MEAN WEIGHT (g)	32.10	30.33	29.39	25.75**	26.54**	25.11	24.63
SD	3.32	2.75	1.57	1.27	1.04	1.82	1.44
DEVIAT. FROM CONTR. (%)	-	-5.50	-8.42	-19.76	-17.31	-	-1.92
HEART	25	24	25	21	5	24	24
MEAN WEIGHT (g)	0.21615	0.21288	0.20222	0.15792**	0.23708	0.17188	0.15697
SD	0.037	0.027	0.025	0.016	0.052	0.017	0.016
DEVIAT. FROM CONTR. (%)	-	-2.42	-7.27	-27.61	8.68	-	-8.67
MEAN % BODY	0.68063	0.70881	0.69929	0.61334*	0.89198**	0.68758	0.63944
SD	0.097	0.121	0.085	0.054	0.185	0.067	0.073
DEVIAT. FROM CONTR. (%)	-	4.14	1.27	-9.59	31.05	-	-7.00
MEAN % BRAIN	44.53	43.37	41.43	33.74**	49.75	33.66	31.03*
SD	7.30	5.69	5.20	3.66	9.79	3.39	3.43
DEVIAT. FROM CONTR. (%)	-	-2.62	-6.98	-24.24	11.71	-	-7.83
KIDNEYS	25	24	25	21	5	24	24
MEAN WEIGHT (g)	0.63040	0.60598	0.58022	0.45263**	0.59680	0.45049	0.40819**
SD	0.058	0.055	0.049	0.030	0.046	0.033	0.026
DEVIAT. FROM CONTR. (%)	-	-3.24	-7.96	-28.20	-5.33	-	-9.39
MEAN % BODY	1.97	2.02	1.97	1.76**	2.25**	1.80	1.86*
SD	0.140	0.133	0.140	0.084	0.162	0.141	0.128
DEVIAT. FROM CONTR. (%)	-	2.26	0.21	-10.77	14.14	-	-7.75
MEAN % BRAIN	128.7	124.2	118.7*	96.59**	125.6	98.26	84.53
SD	11.45	12.14	9.07	6.00	8.43	6.82	5.12
DEVIAT. FROM CONTR. (%)	-	-3.48	-7.80	-24.57	-2.47	-	-8.63
LIVER	25	24	25	21	5	24	24
MEAN WEIGHT (g)	1.73	1.55	1.48**	1.22**	1.67	1.50	1.42
SD	0.191	0.182	0.132	0.092	0.224	0.186	0.163
DEVIAT. FROM CONTR. (%)	-	-10.23	-14.40	-29.62	-3.43	-	-5.13
MEAN % BODY	5.38	5.11*	5.03**	4.72**	6.31*	5.96	5.63*
SD	0.291	0.357	0.383	0.245	1.03	0.409	0.436
DEVIAT. FROM CONTR. (%)	-	-4.99	-6.55	-12.35	17.14	-	-0.40
MEAN % BRAIN	352.6	315.8*	302.4**	259.7**	352.3	293.3	278.6
SD	37.71	36.27	24.73	23.94	59.26	37.81	33.72
DEVIAT. FROM CONTR. (%)	-	-10.43	-14.25	-20.35	-9.08	-	-5.02
SPLEEN	25	24	25	20	5	24	24
MEAN WEIGHT (g)	0.08140	0.07636	0.07304**	0.06244**	0.25132**	0.09898	0.10319
SD	0.019	0.007	0.008	0.004	0.153	0.014	0.015
DEVIAT. FROM CONTR. (%)	-	-6.32	-10.28	-23.36	208.73	-	4.25
MEAN % BODY	0.25550	0.25321	0.24874	0.24307	0.95505**	0.39472	0.40956
SD	0.036	0.032	0.024	0.036	0.592	0.046	0.057
DEVIAT. FROM CONTR. (%)	-	-0.90	-2.65	-4.87	274.03	-	0.112
MEAN % BRAIN	16.64	15.52	14.94**	13.29**	53.03**	19.36	20.23
SD	2.09	1.86	1.38	1.68	32.00	2.66	2.83
DEVIAT. FROM CONTR. (%)	-	-6.71	-10.22	-20.14	218.75	-	4.46

Group 1, 2, 3, 4 and 5 are respectively, Control; 250, 750 and 1500 mg aliskiren/kg/day; methyl nitrosourea.
Dunn's test at 5% (#) or 1% (##) level. Dunnett's test are based on pooled variances at 5% (*) or 1% (**) level.

TABLE 3.3.2.3 (continued)
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE: GROUP MEAN ORGAN WEIGHTS

SEX: MALE	ORGAN	DOSE GROUP:									
		01	02	03	04	05	01	02	03	04	05
		NO. ANIMALS:									
	TESTES	n: 25	n: 24	n: 25	n: 21	n: 25	n: 21	n: 25	n: 21	n: 25	n: 5
	MEAN WEIGHT (g)	0.05014	0.04637	0.04323*	0.02938**	0.04922					0.04922
	SD	0.012	0.010	0.010	0.008	0.045					0.045
	DEVIAT. FROM CONTR. (t)	-0.012	-7.63	-13.78	-41.41	-1.83					35.11
	MEAN % BODY	0.15577	0.15222	0.14718	0.11358**	0.18112					0.17459
	SD	0.029	0.030	0.033	0.028	0.158					0.319
	DEVIAT. FROM CONTR. (t)	-	-2.28	-5.32	-27.08	16.27					65.96
	MEAN % BRAIN	10.26	9.46	8.94	6.29**	10.22					16.76
	SD	2.45	2.34	1.90	1.77	9.20					13.62
	DEVIAT. FROM CONTR. (t)	-	-7.79	-13.89	-38.69	-0.40					49.73
	OVARIES	n: 25	n: 24	n: 25	n: 21	n: 25	n: 21	n: 25	n: 21	n: 25	n: 5
	MEAN WEIGHT (g)	0.29063	0.29311	0.29159	0.27361##	0.26020**					0.03454
	SD	0.021	0.017	0.028	0.016	0.026					0.038
	DEVIAT. FROM CONTR. (t)	-	0.65	0.33	-5.66	-10.47					8.97
	MEAN % BODY	0.9168	0.9205	0.99413##	1.06##	0.97962					0.15220
	SD	0.087	0.081	0.102	0.080	0.076					0.147
	DEVIAT. FROM CONTR. (t)	-	6.62	9.04	16.66	7.45					20.07
	MEAN % BRAIN	59.36	59.62	59.75	58.40	54.71*					7.29
	SD	4.12	2.51	6.35	3.51	4.45					7.96
	DEVIAT. FROM CONTR. (t)	-	0.45	0.65	-1.61	-7.84					17.65
	PROSTATE	n: 25	n: 24	n: 25	n: 21	n: 25	n: 21	n: 25	n: 21	n: 25	n: 5
	MEAN WEIGHT (g)	0.05763	0.06890	0.06216	0.04717	0.05514					0.18446
	SD	0.022	0.025	0.024	0.017	0.018					0.117
	DEVIAT. FROM CONTR. (t)	-	19.57	7.87	-18.14	-4.32					-34.55
	MEAN % BODY	0.18224	0.22858	0.21121	0.18302	0.20873					0.85585
	SD	0.075	0.087	0.080	0.067	0.073					0.508
	DEVIAT. FROM CONTR. (t)	-	25.43	15.89	0.42	14.53					10.95
	MEAN % BRAIN	11.72	14.01	12.76	10.05	11.63					38.76
	SD	4.46	5.04	4.98	3.62	3.99					24.96
	DEVIAT. FROM CONTR. (t)	-	19.32	8.84	-14.24	-0.79					3.40
	ADRENAL GLANDS	n: 25	n: 24	n: 25	n: 21	n: 25	n: 21	n: 25	n: 21	n: 25	n: 5
	MEAN WEIGHT (g)	0.00923	0.00873	0.00848	0.00815	0.00726					0.01038
	SD	0.003	0.002	0.002	0.002	0.002					0.002
	DEVIAT. FROM CONTR. (t)	-	-5.81	-8.30	-11.89	-21.53					-5.67
	MEAN % BODY	0.02940	0.02877	0.02884	0.03154	0.02751					0.04936
	SD	0.011	0.006	0.007	0.008	0.007					0.009
	DEVIAT. FROM CONTR. (t)	-	-2.12	-1.89	7.28	-6.41					11.69
	MEAN % BRAIN	1.89	1.78	1.74	1.74	1.54					2.19
	SD	0.616	0.427	0.403	0.481	0.373					0.511
	DEVIAT. FROM CONTR. (t)	-	-5.54	-8.03	-7.77	-18.63					1.21
	BRAIN	n: 25	n: 24	n: 25	n: 21	n: 25	n: 21	n: 25	n: 21	n: 25	n: 5
	MEAN WEIGHT (g)	0.48978	0.49167	0.48912	0.46883**	0.47330					0.47626**
	SD	0.015	0.020	0.024	0.019	0.016					0.024
	DEVIAT. FROM CONTR. (t)	-	0.39	-0.13	-4.28	-2.96					-6.82
	MEAN % BODY	1.54	1.63	1.57#	1.42##	1.79##					2.30*
	SD	0.193	0.160	0.091	0.112	0.043					0.314
	DEVIAT. FROM CONTR. (t)	-	6.03	8.21	18.46	16.31					11.85

Group 1, 2, 3, 4 and 5 are respectively, Control; 250, 750 and 1500 mg aliskiren/kg/day; methyl nitrosourea. Dunn's test at 5% (#) or 1% (##) level. Dunnett's test are based on pooled variances at 5% (*) or 1% (**) level.

Histopathology:

Non-neoplastic: Principal histopathological findings considered related to treatment with aliskiren hemifumarate were noted in the small and large intestine, mesenteric lymph node, gall bladder, femur, lungs and nasal cavity (both sexes) at doses of 750 or more mg/kg/day (Table 3.3.2.4). A minimal to slight diffuse mucosal hypertrophy/hyperplasia was noted with increased incidence in the small intestine (duodenum and jejunum) and large intestine (cecum and colon) in both sexes at 750 or more mg/kg/day. Focal atypical mucosal hyperplasia (considered to be pre-neoplastic) was noted in the colon of 1 male and 3 females receiving 1500 mg/kg/day. The local irritating properties of aliskiren hemifumarate were reflected in a minimal increase in incidence and/or severity of macrophage accumulation and/or inflammation in the lung and cytoplasmic inclusions in the respiratory epithelia of the nasal cavity in males at all dose levels and in females at 1500 mg/kg/day. An increase in incidence and severity of slight to moderate gall bladder dilatation and the presence of biliary calculi were noted in both sexes at 1500 mg/kg/day. A delayed or disturbed estrous cycle was noted in females at 750 or more mg/kg/day. Microscopic changes in the ovaries at these dose levels consisted of an absence or decrease in size/number of corpora lutea with increased presence of old corpora lutea. In addition, an increased incidence and severity of endometrial atrophy was noted in high dose females. Though not dose-dependent, a slightly increased incidence of minimal to slight bone marrow hypocellularity, characterized by an increased number of fat cells, was noted at all doses in females, suggesting reduced hematopoiesis.

MNU-related non-neoplastic changes consisted mainly of atrophic changes in the submandibular salivary glands, preputial and clitoral glands, ovaries, vagina, uterus, retina of eye (outer nuclear layer) and mammary gland (adipose tissue).

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TABLE 3.3.2.4
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE: SUMMARY INCIDENCE OF GRADING BY ORGAN/GROUP OF RELEVANT NON-NEOPLASTIC LESIONS

SEX : MALE					SEX : FEMALE				
DOSE GROUP:	01	02	03	04	DOSE GROUP:	01	02	03	04
NO. ANIMALS:	25	25	25	25	NO. ANIMALS:	25	25	25	25
CAUSE DEATH/MORIBUND :	25	25	25	25	CAUSE DEATH/MORIBUND :	25	25	25	25
- Intussusception :	-	-	-	1	- Not Detectable :	-	1	-	-
- Not Detectable :	-	-	-	3	- Terminal Sacrifice :	24	24	24	25
- Terminal Sacrifice :	25	24	25	21	- Tumor, Malignant :	1	-	1	-
- Tumor, Malignant :	-	1	-	-					
LUNGS :	25	25	25	25	LUNGS :	25	25	25	25
- Hyperplasia, Br.-Alv.					- Hyperplasia, Br.-Alv.				
TOTAL AFFECTED :	-	3	1	1	TOTAL AFFECTED :	-	1	1	1
MEAN SEVERITY :	-	1.3	1.0	1.0	MEAN SEVERITY :	-	1.0	1.0	1.0
.....								
- Inflammation					- Inflammation				
TOTAL AFFECTED :	-	-	-	3	TOTAL AFFECTED :	-	1	-	2
MEAN SEVERITY :	-	-	-	2.3	MEAN SEVERITY :	-	1.0	-	1.0
.....								
- Macrophages, Alveolar					- Macrophages, Alveolar				
TOTAL AFFECTED :	-	1	-	5	TOTAL AFFECTED :	2	1	1	4
MEAN SEVERITY :	-	1.0	-	1.8	MEAN SEVERITY :	1.5	1.0	1.0	1.0
.....								
DUODENUM :	25	25	25	21	SALIVARY GLANDS :	25	1	1	25
- Hypertrophy, Mucosa					- Atrophy				
TOTAL AFFECTED :	-	-	-	21	TOTAL AFFECTED :	1	1	1	-
MEAN SEVERITY :	-	-	-	1.8	MEAN SEVERITY :	2.0	3.0	4.0	-
.....								
JEJUNUM :	25	25	25	21	DUODENUM :	25	25	25	25
- Hypertrophy, Mucosa					- Hypertrophy, Mucosa				
TOTAL AFFECTED :	-	-	-	20	TOTAL AFFECTED :	-	-	-	24
MEAN SEVERITY :	-	-	-	1.8	MEAN SEVERITY :	-	-	-	1.1
.....								
ILEUM :	25	25	25	20	JEJUNUM :	25	25	25	25
- Hypertrophy, Mucosa					- Hypertrophy, Mucosa				
TOTAL AFFECTED :	-	-	-	7	TOTAL AFFECTED :	-	-	-	23
MEAN SEVERITY :	-	-	-	1.0	MEAN SEVERITY :	-	-	-	1.0
.....								
CECUM :	25	25	25	23	ILEUM :	25	25	25	25
- Hypertrophy, Mucosa					- Hypertrophy, Mucosa				
TOTAL AFFECTED :	-	-	5	10	TOTAL AFFECTED :	-	-	-	4
MEAN SEVERITY :	-	-	1.0	1.0	MEAN SEVERITY :	-	-	-	1.0
.....								
COLON :	25	25	25	23	CECUM :	23	25	25	25
- Hyperplasia, Atypical					- Hypertrophy, Mucosa				
TOTAL AFFECTED :	-	-	-	1	TOTAL AFFECTED :	-	-	5	13
MEAN SEVERITY :	-	-	-	3.0	MEAN SEVERITY :	-	-	1.0	1.0
.....								
- Hyperplasia, Mucosa					COLON :	25	25	25	25
TOTAL AFFECTED :	-	-	-	1	- Hyperplasia, Atypical				
MEAN SEVERITY :	-	-	-	1.0	TOTAL AFFECTED :	-	-	-	3
.....					MEAN SEVERITY :	-	-	-	3.3
- Hypertrophy, Mucosa								
TOTAL AFFECTED :	-	-	-	2	- Hypertrophy, Mucosa				
MEAN SEVERITY :	-	-	-	1.0	TOTAL AFFECTED :	-	-	1	-
.....					MEAN SEVERITY :	-	-	1.0	-
- Inflammation								
TOTAL AFFECTED :	-	-	-	1	- Inflammation				
MEAN SEVERITY :	-	-	-	1.0	TOTAL AFFECTED :	-	-	-	3
.....					MEAN SEVERITY :	-	-	-	1.3

TABLE 3.3.2.4 (continued)
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE: SUMMARY INCIDENCE OF GRADING BY ORGAN/GROUP OF RELEVANT NON-NEOPLASTIC LESIONS

SEX : MALE					SEX : FEMALE				
DOSE GROUP:	01	02	03	04	DOSE GROUP:	01	02	03	04
NO. ANIMALS:	25	25	25	25	NO. ANIMALS:	25	25	25	25
SPLEEN	25	25	25	25	SPLEEN	25	25	25	24
- Deposition, Pigment					- Deposition, Pigment				
TOTAL AFFECTED :	3	4	1	2	TOTAL AFFECTED :	24	24	24	5
MEAN SEVERITY :	1.3	2.3	3.0	1.5	MEAN SEVERITY :	1.0	1.0	1.1	1.8
.....								
- Hemopoiesis, Incr.					- Hemopoiesis, Incr.				
TOTAL AFFECTED :	6	4	4	4	TOTAL AFFECTED :	6	8	7	-
MEAN SEVERITY :	1.0	1.0	1.0	1.0	MEAN SEVERITY :	1.5	1.1	1.0	-
MESENT. LYMPH NODES	25	25	25	25	MESENT. LYMPH NODES	25	24	25	25
- Germ.Center Develop.					- Germ.Center Develop.				
TOTAL AFFECTED :	15	17	21	21	TOTAL AFFECTED :	19	20	21	23
MEAN SEVERITY :	1.1	1.1	1.1	1.4	MEAN SEVERITY :	1.3	1.4	1.3	1.4
LIVER	25	25	25	25	LIVER	25	25	25	25
- Glycogen Decrease					- Glycogen Decrease				
TOTAL AFFECTED :	-	1	-	9	TOTAL AFFECTED :	1	-	6	14
GALLBLADDER	25	25	25	24	GALLBLADDER	24	25	25	25
- Dilatation					- Dilatation				
TOTAL AFFECTED :	-	1	-	11	TOTAL AFFECTED :	1	3	1	21
MEAN SEVERITY :	-	2.0	-	2.6	MEAN SEVERITY :	2.0	1.7	2.0	2.1
.....								
- Hyperpl., Epithelial					- Hyperpl., Epithelial				
TOTAL AFFECTED :	-	-	-	2	TOTAL AFFECTED :	3	-	-	3
MEAN SEVERITY :	-	-	-	2.0	MEAN SEVERITY :	1.3	-	-	1.0
PREPUTIAL GLANDS	25	1	-	25	OVARIES	25	25	25	25
- Atrophy					- C.Lutea, Absent				
TOTAL AFFECTED :	1	-	-	-	TOTAL AFFECTED :	-	-	1	12
MEAN SEVERITY :	2.0	-	-	-	MEAN SEVERITY :	-	-	2.0	3.0
FEMUR/MARROW	25	1	-	25				
- Hypocellularity					- C.Lutea, Decrease				
TOTAL AFFECTED :	-	-	-	1	TOTAL AFFECTED :	-	-	11	10
MEAN SEVERITY :	-	-	-	1.0	MEAN SEVERITY :	-	-	1.8	2.0
NASAL CAVITY	25	25	25	25	UTERUS	25	25	25	25
- Inc., Cyto., Resp.Epi.					- Atrophy, Endometrial				
TOTAL AFFECTED :	-	3	2	6	TOTAL AFFECTED :	-	-	-	7
MEAN SEVERITY :	-	1.7	1.5	1.7	MEAN SEVERITY :	-	-	-	1.7
VAGINA	25	25	25	25	VAGINA	25	25	25	25
- Atrophy, Epithelial					- Atrophy, Epithelial				
TOTAL AFFECTED :	-	-	-	1	TOTAL AFFECTED :	-	-	-	1
MEAN SEVERITY :	-	-	-	3.0	MEAN SEVERITY :	-	-	-	3.0
FEMUR/MARROW	25	25	25	25	FEMUR/MARROW	25	25	25	25
- Hypocellularity					- Hypocellularity				
TOTAL AFFECTED :	2	5	3	14	TOTAL AFFECTED :	2	5	3	14
MEAN SEVERITY :	1.0	1.0	1.0	1.1	MEAN SEVERITY :	1.0	1.0	1.0	1.1
NASAL CAVITY	25	25	25	25	NASAL CAVITY	25	25	25	25
- Hyperplasia, Resp.Ep.					- Hyperplasia, Resp.Ep.				
TOTAL AFFECTED :	-	-	-	1	TOTAL AFFECTED :	-	-	-	1
MEAN SEVERITY :	-	-	-	1.0	MEAN SEVERITY :	-	-	-	1.0
.....								
- Inc., Cyto., Resp.Epi.					- Inc., Cyto., Resp.Epi.				
TOTAL AFFECTED :	6	1	2	8	TOTAL AFFECTED :	6	1	2	8
MEAN SEVERITY :	1.0	1.0	1.0	1.0	MEAN SEVERITY :	1.0	1.0	1.0	1.0

- finding level:
 GRADE 1 = minimal / very few / very small
 GRADE 2 = slight / few / small
 GRADE 3 = moderate / moderate number / moderate size
 GRADE 4 = marked / many / large
 GRADE 5 = massive / extensive number / extensive size

Group 1, 2, 3 and 4 are, respectively, Control, 250, 750 and 1500 mg aliskiren/kg/day

Neoplastic: The incidence and types of neoplastic findings noted with aliskiren hemifumarate-treated groups were similar to the incidence and types of spontaneous tumors reported for Tg-rasH2 transgenic mice (Table 3.3.2.5). In contrast, the focal atypical hyperplasia (a pre-neoplastic finding) noted in the colons of high dose animals (1 male and 3 females, see Table 3.3.2.4) is not a common spontaneous lesion. The sponsor attributes this lesion to the combined irritative effect of aliskiren and the increased bile concentration in the colonic environment (bile acids are known carcinogens in humans and rats¹). Although hemangiomas and/or hemangiosarcomas were more frequent in treated males and females, the distribution showed no dose-dependency and no statistically significant difference from concurrent control. Furthermore, the incidence of these tumors (up to 13 %) is within the range reported for untreated CB6F1-TgrasH2 female mice of this age² and, thus, the occurrence of these tumors was not considered to be treatment-related. The sponsor concludes that there were no differences in neoplastic findings between control and aliskiren hemifumarate-treated male and female groups. The FDA statistical reviewer also arrives at the same conclusion. Major neoplastic findings observed in animals treated with MNU were malignant lymphoma, squamous cell carcinoma or papilloma in the forestomach or skin and adenoma in the lung in both sexes. This suggests that the Tg-rasH2 hemizygous mice strain used in this study is a sensitive animal model to identify potential carcinogens. The NOAEL for this study was less than 250 mg/kg/day.

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¹ Bernstein H, Bernstein C, Payne CM, Dvorakova K and Garewal H (2005). Bile acids as carcinogens in human gastrointestinal cancers Mutation Research 589: 47-65.

² Kanno H, Tanakamaru Z-y, Ishimura Y, Kandori H, Yamasak H, Sasaki S. (2003): Historical background data in CB6F1-Tg-rasH2 mice and CB6F1-nonTG-rasH2 mice over a 26-week experimental period. J Toxicol Pathol 16: 167-274.

Takaoka M, Sehata S, Maejima T, Imai T, Torrii M, Satoh H, Toyosawa K, Tanakamaru ZY, Adachi T, Hisada S, Ueda M, Ogasawara H, Matsumoto M, Koybayashi K, Mutai M, Usui T (2003). Interlaboratory comparison of short-term carcinogenicity studies using CB6F1-rasH2 transgenic mice. Toxicologic Pathology 31: 191-199.

TABLE 3.3.2.5
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE
SUMMARY INCIDENCE OF NEOPLASTIC FINDINGS

SEX : MALE					SEX : FEMALE				
DOSE GROUP:					DOSE GROUP:				
NO. ANIMALS:					NO. ANIMALS:				
	01	02	03	04	01	02	03	04	
LUNGS	25	25	25	25	LUNGS	25	25	25	25
- Adenoma, Bronchioalv.:	3	2	2	-	- Adenoma, Bronchioalv.:	2	2	1	1
- Carcinoma, Bron.-Alv.:	-	1	-	-	- Carcinoma, Bron.-Alv.:	1	-	-	-
SPLEEN	25	25	25	25	SPLEEN	25	25	25	24
- Hemangioma	1	-	-	-	- Hemangioma	-	1	1	-
- Hemangiosarcoma	-	-	-	1	- Hemangiosarcoma	-	1	-	-
HARDERIAN GLANDS	25	1	-	25	KIDNEYS	25	1	1	25
- Adenoma	1	-	-	-	- Hemangioma	-	-	-	1
SKIN	25	1	1	25	UTERUS	25	25	25	25
- Carcinoma, Basal Cell:	-	-	1	-	- Deciduoma	-	1	-	-
NASAL CAVITY	25	25	25	25	MAMMARY AREA	25	1	1	25
- Hemangiosarcoma	1	-	-	-	- Adenocarcinoma	1	-	-	-
					FEMUR/MARROW	25	25	25	25
					- Hemangioma	-	1	-	-
					NASAL CAVITY	25	25	25	25
					- Hemangioma	-	-	-	1
					- Hemangiosarcoma	-	-	1	-

Group 1, 2, 3 and 4 are, respectively, control,
250, 750 and 1500 mg aliskiren/kg/day

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TABLE 3.3.2.6
26 WEEK CARCINOGENICITY STUDY IN TG-rasH2 MICE
SUMMARY INCIDENCE OF NEOPLASTIC FINDINGS IN THE CONTROL (GROUP 1) AND
MNU-TREATED GROUP (GROUP 5)

	DOSE GROUP: 01		05	
	SEX : M	F	M	F
NO. ANIMALS:	25	25	25	25
SYSTEMIC NEOPLASMS :	25	25	25	25
- Leukemia, Granulocyt.:	-	-	1	-
- Lymphoma, Malignant :	-	-	20	17
LUNGS :	25	25	25	25
- Adenoma, Bronchioalv.:	3	2	4	8
- Carcinoma, Bron.-Alv.:	-	1	1	1
SALIVARY GLANDS :	25	25	25	25
- Hemangiosarcoma :	-	-	-	1
STOMACH :	25	25	25	25
- Carcinoma, Squam. Cell:	-	-	6	2
- Papilloma, Squam. Cell:	-	-	15	18
JEJUNUM :	25	25	22	21
- Adenocarcinoma :	-	-	1	1
CECUM :	25	23	21	23
- Adenocarcinoma :	-	-	1	-
SPLEEN :	25	25	25	25
- Hemangioma :	1	-	-	-
- Hemangiosarcoma :	-	-	1	-
MESENT. LYMPH NODES :	25	25	25	23
- Metastasis, Carcinoma:	-	-	-	1
KIDNEYS :	25	25	25	25
- Hemangioma :	-	-	-	1
ADRENAL GLANDS :	25	25	25	25
- Carcinoma, Squam. Cell:	-	-	-	1
- Metastasis, Carcinoma:	-	-	-	1
OVARIES :	-	25	-	25
- Metastasis, Carcinoma:	-	-	-	2
HARDERIAN GLANDS :	25	25	25	25
- Adenoma :	1	-	-	-
SKIN :	25	25	25	25
- Carcinoma, Basal Cell:	-	-	-	2
- Carcinoma, Squam. Cell:	-	-	-	2
- Hemangiosarcoma :	-	-	-	1
- Papilloma, Squam. Cell:	-	-	10	7
MAMMARY AREA :	25	25	25	24
- Adenocarcinoma :	-	1	-	-
NASAL CAVITY :	25	25	25	25
- Adenoma :	-	-	-	1
- Hemangiosarcoma :	1	-	-	-

Toxicokinetics: The systemic exposure to aliskiren was determined on study days 25 and 151. Both C_{max} and AUC values increased with increasing dose (more than dose-proportional) for both sexes. The C_{max} and AUC values were significantly higher towards the end of the dosing period when compared with day 25 for groups receiving 750 and 1500 mg/kg/day. No significant gender difference was noted (Table 3.3.2.7).

TABLE 3.3.2.7
SYSTEMIC EXPOSURE TO ALISKIREN IN 26 WEEK CARCINOGENICITY STUDY IN IN TG-rasH2 MICE

Study day	Dose (mg/kg/day)	Males				Females			
		C _{max}	C _{max} /dose	AUC _(0-24h)	AUC _(0-24h) /dose	C _{max}	C _{max} /dose	AUC _(0-24h)	AUC _(0-24h) /dose
Day 25	250	5	0.0184	77	0.309	5	0.0204	69	0.277
	750	15	0.0195	296	0.394	18	0.0244	380	0.507
	1500	59	0.0391	1080	0.717	65	0.0435	1180	0.789
Day 151	250	*	*	*	*	6	0.0249	83	0.333
	750	32	0.0424	551	0.735	35	0.0469	610	0.813
	1500	145	0.0967	2740	1.830	186	0.124	3540	2.360

* Parameters not calculated due to a high concentration in one animal at 24 hr, which was considered an outlier.

C_{max} in ng/ml; C_{max}/dose in (ng/ml) / mg/kg/day; AUC_(0-24h) in ng·h/ml; AUC_(0-24h)/dose in (ng·h/ml) / mg/kg/day

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3.4. Genetic Toxicology

3.4.1. Ames Assay. In Vitro Bacterial Test of Aliskiren Hemifumarate

Key Findings: Aliskiren was reproducibly negative in all tester strains both with and without metabolic activation.

Study No: 966158

Location of Report: EDR

Conducting Laboratory and Location: Novartis Crop Protection AG, Toxicology/Genetic Toxicology, CH-4002 Basel, Switzerland

Dates of Study: Initiated on March 06 and terminated on May 12, 1997.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Methods

Four *Salmonella typhimurium* strains and one *Escherichia coli* strain were used, with and without metabolic activation. The *S. typhimurium* strains were, TA98, TA100, TA1535, TA1537 and TA102; the *E. coli* strain, WP2uvrA. Test substance was dissolved in double distilled water (concentrations expressed in terms of salt).

A dose range-finding study to determine the highest dose level of aliskiren for the reverse mutation study was carried out with strains *S. typhimurium* TA100 and *E. coli*, WP2uvrA. Aliskiren hemifumarate (batch #817196) was tested at doses of 20.6 to 5000 µg/plate with and without metabolic activation. No toxicity was observed. Thus, the main study was carried out using 5000 µg/plate as the highest dose. One original and one confirmatory experiment were performed using 5 doses of the test substance (312.5, 625, 1259, 2500, and 5000 µg/plate), a negative and a positive control. For each test dose, 3 plates were used. Statistical analyses were not carried out.

Basis of dose selection: Cytotoxicity

Metabolic activation system: S9 homogenate (liver microsomal enzymes) prepared from the livers of a male RAI rat (Tif: RAIf[SPF]) given Aroclor 1254 (500 mg/kg, i.p.) 5 days before sacrifice.

Controls

Negative control: Double distilled water (one group)

Positive controls: Each tester strain was treated with an appropriate positive control substance (Table 3.4.1.1).

Criteria for a valid study: The assay is considered acceptable if the solvent control data for all tester strains are within the laboratory's normal control range for spontaneous mutant frequency and the positive controls induce increases in the mutation frequency which are significant.

Criteria for a positive result: For test substance to be considered positive, it had to produce a dose-dependent effect on revertant colony count, reaching 2 or more times the corresponding negative control count for strains TA98, TA1535, TA1537 or *E. coli* WP2uvrA; 1.5 times the corresponding negative control count for strains TA100 or TA1027.

TABLE 3.4.1.1
BACTERIAL REVERSE MUTATION ASSAY. POSITIVE CONTROLS

Strain	Direct method		Metabolic activation method	
	Substance	Conc. µg/plate	Substance	Conc. µg/plate
TA98	2-Nitrofluorene	5.0	2-aminoanthracene	1.5
TA100	Sodium azide	2.0	2-aminoanthracene	1.5
TA102	Mitomycin-C	0.5	2-aminoanthracene	4.0
TA1535	Sodium azide	2.0	Cyclophosphamide	200.0
TA1537	9-Aminoacridine	80.0	2-aminoanthracene	1.5
WP2uvrA	4-Nitroquinoline	2.0	2-aminoanthracene	20.0

Results

In both the original and the confirmatory experiments performed with and without metabolic activation, treatment of strains TA98, TA100, TA1535, TA1537, TA102, and WP2uvrA with aliskiren hemifumarate did not lead to an increase in the incidence of histidine- or tryptophan-prototrophic mutants in comparison with the corresponding negative controls. In both experiments with the metabolic activation system, reduction in the growth of the background lawn occurred with strains TA100, TA102, and TA1537 at 5000 µg aliskiren hemifumarate/plate. A similar effect was observed in both experiments without metabolic activation with strains TA102 at 2500 and 5000 µg aliskiren hemifumarate/plate (Table 3.4.1.2). The positive control compounds induced reverse mutations in each strain, with revertant colony counts ranging from 4 to 266 times corresponding negative control count. Based on these results it is concluded that aliskiren did not induce gene mutations in bacteria under the conditions of the study.

At a later date (report issue date October 2001), a similar GLP study (#1940/02-D6171) was completed at a contract lab _____) using a new batch of aliskiren hemifumarate (NE-5810-Batch-01-01). In this study, aliskiren was assayed at concentrations up to 5000 µg/plate for mutations in five histidine-requiring strains (TA98, TA100, TA1535, TA1537, TA102), both in the absence and in the presence of a rat liver metabolic activation system (S-9). This study, confirmed the results of the earlier study: aliskiren treatment did not induce any increase in revertant numbers that could be considered as evidence of mutagenic activity. The results are summarized in Table 3.4.1.3.

TABLE 3.4.1.2 (Study #966158)
SUMMARY OF THE BACTERIAL REVERSE MUTATION ASSAY. EXPERIMENTS WITH S9

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	115.67	TA 1535	Negative control	13.00
	312.50 µg/plate	109.33		312.50 µg/plate	15.33
	625.00 µg/plate	105.33		625.00 µg/plate	15.33
	1250.00 µg/plate	92.00		1250.00 µg/plate	15.67
	2500.00 µg/plate	60.00		2500.00 µg/plate	15.33
	5000.00 µg/plate	44.00		5000.00 µg/plate	10.67
	Positive control	1596.33		Positive control	422.67
WP2 uvrA	Negative control	20.33	TA 98	Negative control	25.00
	312.50 µg/plate	20.00		312.50 µg/plate	24.67
	625.00 µg/plate	16.67		625.00 µg/plate	30.33
	1250.00 µg/plate	14.33		1250.00 µg/plate	20.33
	2500.00 µg/plate	14.00		2500.00 µg/plate	21.00
	5000.00 µg/plate	14.67		5000.00 µg/plate	20.00
	Positive control	775.67		Positive control	1063.00
TA 1537	Negative control	8.33	TA 102	Negative control	202.67
	312.50 µg/plate	10.67		312.50 µg/plate	242.33
	625.00 µg/plate	10.00		625.00 µg/plate	202.00
	1250.00 µg/plate	7.00		1250.00 µg/plate	222.00
	2500.00 µg/plate	6.67		2500.00 µg/plate	207.67
	5000.00 µg/plate	0.67		5000.00 µg/plate	0.00
	Positive control	225.33		Positive control	1537.33

SUMMARY OF THE BACTERIAL REVERSE MUTATION ASSAY. EXPERIMENTS WITHOUT S9

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	110.67	TA 1535	Negative control	10.33
	312.50 µg/plate	108.67		312.50 µg/plate	10.67
	625.00 µg/plate	97.67		625.00 µg/plate	14.67
	1250.00 µg/plate	96.33		1250.00 µg/plate	12.67
	2500.00 µg/plate	82.33		2500.00 µg/plate	9.67
	5000.00 µg/plate	65.67		5000.00 µg/plate	10.67
	Positive control	968.33		Positive control	680.67
WP2 uvrA	Negative control	17.00	TA 98	Negative control	14.67
	312.50 µg/plate	18.67		312.50 µg/plate	13.33
	625.00 µg/plate	19.33		625.00 µg/plate	12.00
	1250.00 µg/plate	23.33		1250.00 µg/plate	12.00
	2500.00 µg/plate	17.67		2500.00 µg/plate	16.33
	5000.00 µg/plate	17.67		5000.00 µg/plate	12.33
	Positive control	305.00		Positive control	278.00
TA 1537	Negative control	7.33	TA 102	Negative control	241.67
	312.50 µg/plate	8.33		312.50 µg/plate	273.67
	625.00 µg/plate	8.67		625.00 µg/plate	227.33
	1250.00 µg/plate	8.67		1250.00 µg/plate	179.33
	2500.00 µg/plate	10.33		2500.00 µg/plate	111.33
	5000.00 µg/plate	9.33		5000.00 µg/plate	70.33
	Positive control	2158.00		Positive control	1027.33

TABLE 3.4.1.3 (Study #1940/02-D6171)
SUMMARY OF THE BACTERIAL REVERSE MUTATION ASSAY. EXPERIMENTS WITH S9

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WATER	100 µl	39 ± 2	110 ± 11	17 ± 5	16 ± 6	328 ± 28
SPP100B	1.6	37 ± 9	116 ± 8	12 ± 6	17 ± 1	346 ± 24
	8	33 ± 11	121 ± 4	14 ± 5	20 ± 6	341 ± 5
	40	41 ± 7	109 ± 3	21 ± 4	15 ± 2	353 ± 28
	200	43 ± 7	93 ± 11 (M)	20 ± 5	13 ± 6	300 ± 50
	1000	39 ± 3	97 ± 11	16 ± 8	20 ± 1	264 ± 5
	5000	39 ± 7	74 ± 8 (S)	17 ± 4	11 ± 6	220 ± 17 (S)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	267 ± 9	2153 ± 112	141 ± 12	190 ± 10	1553 ± 172

SUMMARY OF THE BACTERIAL REVERSE MUTATION ASSAY. EXPERIMENTS WITHOUT S9

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WATER	100 µl	42 ± 7	113 ± 14	12 ± 5	15 ± 3	301 ± 37
SPP100B	1.6	39 ± 4	108 ± 13	19 ± 6	10 ± 5	334 ± 32
	8	44 ± 8	109 ± 16	15 ± 10	9 ± 5	310 ± 25
	40	45 ± 11	108 ± 7	25 ± 13	10 ± 3 (M)	311 ± 26
	200	55 ± 5	101 ± 11	12 ± 3	9 ± 5	342 ± 28
	1000	50 ± 3	98 ± 13	10 ± 2 (S)	9 ± 3	352 ± 15
	5000	44 ± 5 (S)	54 ± 13 (S)	18 ± 7 (S)	10 ± 2 (S)	242 ± 15 (S)
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	705 ± 41	707 ± 38	343 ± 58	137 ± 13	572 ± 9

Data are given as mean ± standard deviation. M= plates counted manually, S= slight thinning of lawn

3.4.2. Chromosome Aberration Test of Aliskiren Hemifumarate in Chinese Hamster Ovary Cells *in vitro*

Key Findings: Aliskiren hemifumarate did not show any clastogenic potential in the chromosomal aberration test with Chinese hamster ovary cells.

Study No: 966159

Location of Report: EDR

Conducting Laboratory and Location: Novartis Crop Protection AG, Toxicology/Genetic Toxicology, CH-4002 Basel, Switzerland

Dates of Study: Initiated on March 19 and terminated on July 30, 1997.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Methods

The clastogenic potential of aliskiren was evaluated in Chinese hamster ovary (CHO) cells with and without metabolic activation. The metabolic activation system was S9, prepared from the liver of a male RAI rat (Tif: RAI[SPF]) given Aroclor 1254 (500 mg/kg, i.p.) 5 days before sacrifice. Aliskiren hemifumarate (batch #817196) was dissolved in double distilled water. 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 21 or 45 hours with the direct method, and for 3 hours with the metabolic activation method, followed by continued culture for an additional 18 or 42 hours with a fresh medium excluding the test substance and S9 mixture. Two hours prior to harvesting, the cultures were treated with colcemide (0.4 µg/ml) to arrest cells in metaphase. Whenever possible, 200 well spread metaphases from two vehicle control and two treated cultures (100 metaphases per replicate culture) were scored. At least 50 metaphases were scored in the positive control cultures (25 per replicate culture). Cyclophosphamide (+S9) and mitomycin-C (-S9) were used as positive control cultures. The experimental design was as follows.

Expt. #	Original study		Confirmatory study			
	1	2	1	2	3	4
Metabolic activation	-	+	-	+	-	+
Treatment with aliskiren (h)	21	3	21	3	45	3
Recovery after treatment (h)	-	18	-	18	-	42
No. of drug conc. used	8	8	8	8	8	8
Lowest drug conc. (µg/ml)	39.06	39.06	156.25	312.50	156.25	312.50
Highest drug conc. (µg/ml)	5000.00	5000.00	1875.00	3750.00	1875.00	3750.00
Concentration (µg/ml) selected based on cell growth and mitotic index	312.5	625.0	937.5	1250.0	625.0	1250.0
	625.0	1250.0	1250.0	1875.0	937.5	1875.0
	1250.0	2500.0	1875.0	2500.0	1250.0	2500.0
Conc. (µg/ml) inhibiting cell growth by 100%	2500.0	5000.0	Not tested	3750.0	1875.0*	3750.0

*: 90% suppression

The concentration of test substance that suppresses mitotic activity (cytotoxicity measured as mitotic index) by approximately 50 to 80% relative to the control group was

selected as the highest for the analysis of chromosome aberrations together with two lower concentrations in succession. The slides were examined for the following structural aberrations.

Specific aberrations: chromatic and chromosome deletions (including breaks, deletions and fragments); chromatid exchanges (including triradials, quadriradials, endfusions, acentric rings); chromatid exchanges (including dicentrics, polycentrics, centric and acentric rings)

Multiple aberrations: metaphases containing more than 10 aberrations of different types or more than 5 aberrations of one particular type (excluding gaps)

Unspecific aberrations: gaps (chromatid- and chromosome-)

Incidence of polyploidies was also scored.

The evaluated numbers of specific aberrations were subjected to statistical analysis. The test substance is considered to be positive in the CHO cells if the percentage of metaphases containing specific aberrations in a treatment group is higher than 6 and differs significantly from the respective value of the negative control. Further, there should be a concentration-related increase or a reproducible increase in the number of cells with chromosome aberrations.

Results

The incidence rates of cells possessing chromosomal aberrations, including or excluding gaps at all concentrations in both original and confirmatory studies performed with or without metabolic activation, never exceeded 5% of cells (Table 3.4.2.1). Chromosomal aberrations detected in the negative control ranged from 0.5 to 3.0%. In contrast, the incidence of chromosomal aberration in cultures treated with the positive controls, mitomycin-C and cyclophosphamide, ranged between 48 and 60%.

It is concluded that aliskiren does not have any clastogenic potential under the conditions of this chromosomal aberration test.

TABLE 3.4.2.1

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH ALISKIREN HEMIFUMARATE.
A. CONFIRMATORY STUDY: 21 HR TREATMENT WITHOUT METABOLIC ACTIVATION

Treatment	total no of cells examined	% cells with specific aberrations [#]	Total number of cells with aberrations							pol [%]
			gaps	ct del	ct exc	cs del	cs exc	mab	end	
Solvent control	200	0.5	4	1	0	0	0	0	1	1.5
937.5 µg/ml	200	2.5	3	1	0	2	2	0	1	1.5
1250.0 µg/ml	200	1.0	3	2	0	1	0	0	1	4.0
1875.0 µg/ml	200	1.5	4	0	0	3	0	0	1	3.5
positive control (Mito-C, 0.2 µg/ml)	50	60.0***	11	23	17	5	1	0	0	0.5

B. CONFIRMATORY STUDY: 3 HR TREATMENT WITH METABOLIC ACTIVATION / 18 HR RECOVERY

Treatment	total no of cells examined	% cells with specific aberrations [#]	Total number of cells with aberrations							pol [%]
			gaps	ct del	ct exc	cs del	cs exc	mab	end	
Solvent control	200	3.0	2	4	0	1	1	0	5	3.5
1250.0 µg/ml	200	3.5	3	1	0	5	1	0	4	1.5
1875.0 µg/ml	200	3.5	5	3	0	4	0	0	5	1.0
2500.0 µg/ml	200	2.0	9	2	0	1	1	0	1	1.5
positive control (CPA, 20 µg/ml)	50	48.0 ^{***}	3	11	12	7	2	0	0	0.5

C. CONFIRMATORY STUDY: 45 HR TREATMENT WITHOUT METABOLIC ACTIVATION

Treatment	total no of cells examined	% cells with specific aberrations [#]	Total number of cells with aberrations							pol [%]
			gaps	ct del	ct exc	cs del	cs exc	mab	end	
Solvent control	200	1.0	4	1	0	1	0	0	0	1.5
625.0 µg/ml	200	3.0	2	2	0	2	2	0	0	3.0
937.5 µg/ml	200	2.0	4	1	1	1	1	0	0	3.0
1250.0 µg/ml	200	2.0	2	1	0	3	0	0	0	3.0

D. CONFIRMATORY STUDY: 3 HR TREATMENT WITH METABOLIC ACTIVATION / 42 HR RECOVERY

Treatment	total no of cells examined	% cells with specific aberrations [#]	Total number of cells with aberrations							pol [%]
			gaps	ct del	ct exc	cs del	cs exc	mab	end	
Solvent control	200	1.0	5	1	0	1	0	0	0	1.5
1250.0 µg/ml	200	1.5	3	3	0	0	0	0	0	4.0
1875.0 µg/ml	200	1.5	4	2	0	0	1	0	0	1.0
2500.0 µg/ml	200	2.5	4	2	0	3	1	0	0	2.5

3.4.3. In vitro Gene Mutation Assay in V79 Cells with Aliskiren Hemifumarate

Key Findings: Aliskiren hemifumarate tested negative for inducing gene mutations in Chinese hamster V79 cells both with and without metabolic activation.

Study No: 966160

Location of Report: EDR

Conducting Laboratory and Location: Novartis Crop Protection AG, Toxicology/Genetic Toxicology, CH-4002 Basel, Switzerland

Dates of Study: Initiated on March 06 and terminated on December 10, 1997.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Methods

The Chinese Hamster V79 cell assay system detects mutations (base pair substitutions, frameshifts, deletions and chromosomal rearrangements induced by the test substance) from the parental type to the mutant form, which give rise to a change in an enzymatic protein, HGPRT (hypoxanthine guanine phosphoribosyl transferase). The gene for HGPRT is located on the X chromosome and the role of the HGPRT enzyme is to induce the biosynthesis of purine nucleotides by converting hypoxanthine and guanine to the corresponding nucleoside 5'-monophosphate. Purine analogues such as 6-thioguanine (6-TG) are also converted, but to toxic ribonucleotides, which kill cells with normal enzyme activity. Conversely, mutant cells with a HGPRT-deficient genotype due to a mutation induced by a genotoxic agent can proliferate in a medium containing 6-thioguanine because of their ability to synthesize that required purine via the *de novo* pathway from 5'-ribose phosphate, amino acids and ATP. Experimentally, mutagenic effects are manifested by the appearance of cells resistant to 6-thioguanine (6-TG) and can be quantified by comparison of the numbers of 6-TG resistant colonies in the treated and control cultures.

V79 Chinese hamster cells were originally derived from embryonic lung tissue. The experiment was conducted both in the presence and absence of postmitochondrial fraction S-9, which was prepared from the livers of male RAI rats (Tif: RAI[SPF]) given Aroclor 1254 (500 mg/kg, i.p.) 5 days prior to sacrifice. Aliskiren hemifumarate (batch #817196) was dissolved in DMSO. In addition to a preliminary cytotoxicity test, one original and two confirmatory experiments (one without and another with S-9) were performed under the same experimental conditions with the same batch of test substance. Two replicate cultures were used for each dose group.

V-79 cell cultures were exposed to the test substance for 5 hr in the presence and for 21 hr in the absence of metabolic activation. The treatment was terminated by washing the cultures with phosphate buffered saline. After 7-8 days of growth, the cultures were fixed and stained and the surviving colonies were counted. The highest concentration to be selected for the mutagenicity assay was the one causing about 50-90% reduction of viable cells in comparison with the mean of the two negative controls. Based on the cytotoxicity test, four concentrations of aliskiren in DMSO were used for each mutagenicity test. The

positive control used in the non-activated part of the experiment was ethylmethanesulfonate (0.3 µg/ml). In the presence of rat liver S-9 mix, N-nitrosodimethylamine (DMN) (1.0 µg/ml) was used. A solvent (DMSO) control was included in each mutation assay.

The mutant colonies were counted manually and the mutant frequency was expressed as the number of 6-TG resistant mutants/million viable cells for each concentration. All comparisons were made against the concurrent vehicle control. A test substance is considered to be mutagenic if the mutant frequency at one or more concentrations is significantly greater than that of the negative control and the number of normalized mutant clones in the treated and untreated cultures differs by more than 20. Further, the data should be statistically significant with a dose response relationship (as shown by the linear trend analysis) and be reproducible. The mutant frequency of the solvent controls (spontaneous mutant frequency) should not exceed 35×10^{-6} per 1 million viable cells.

Results

The preliminary cytotoxicity test made in the absence of S-9 mix showed that aliskiren hemifumarate was completely cytotoxic at 500 µg/ml, while the next two lower concentrations, 250 and 125 µg/ml, inhibited growth by 56 and 38%, respectively. In the presence of S-9 mix, aliskiren hemifumarate exerted a complete growth inhibitory effect at 2000 µg/ml, while 1000 and 500 µg/ml inhibited growth by 68 and 27%, respectively. Based on the above data, the sponsor chose four concentrations of aliskiren for each experiment.

No dose-related increase in total mutant clones or mutant frequencies was observed with varying concentrations of aliskiren hemifumarate in the absence or presence of metabolic activation (Tables 3.4.3.1 through 3.4.3.5). The mean mutant frequencies varied between 1 and $7.33/10^6$ viable cells. The slight but statistically significant increase in mean mutant frequencies observed in the original experiment with metabolic activation at the concentration of 25 µg/ml (4.1×10^{-6}) (Table 3.4.3.1) and in the first confirmatory experiment without metabolic activation at the concentrations of 18.52 (4.4×10^{-6}) and 500 (4.7×10^{-6}) µg/ml (Table 3.4.3.4) are within the historical negative control range (1 to 21×10^{-6}) and thus do not meet the criteria for a positive response. These three observed increases are considered by the sponsor to be of spontaneous origin and not related to treatment with the test substance. A second confirmatory experiment in the absence of metabolic activation was not necessary and thus, was not conducted. All the data with test substance were evaluated as negative. In contrast, the positive controls induced a mutation frequency which was clearly in excess of the background. It is thus concluded that aliskiren hemifumarate has no genotoxic activity in this assay under the conditions described.

TABLE 3.4.3.1
MUTAGENIC ACTIVITY OF ALISKIREN IN V79 CELLS WITH METABOLIC ACTIVATION
A. ORIGINAL EXPERIMENT

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	90.00	2.00	2.22
Positive control DMN 1 μ l/ml	72.58	65.88	90.76
<u>CGP 60536 B:</u>			
1600.0000 μ g/ml	*	*	*
400.0000 μ g/ml	89.42	2.13	2.38
100.0000 μ g/ml	87.50	2.75	3.14
25.0000 μ g/ml	89.92	7.38	8.20

Treatment	Mean mutant frequency ($\times 10^6$)	Mean mutant factor	Significance (P)
Negative control	1.11		
Positive control DMN 1 μ l/ml	45.38	40.84	P<0.001
<u>CGP 60536 B:</u>			
1600.0000 μ g/ml	*	*	*
400.0000 μ g/ml	1.19	1.07	Ns
100.0000 μ g/ml	1.57	1.41	Ns
25.0000 μ g/ml	4.10	3.69	0.001<P<0.002

Linear relation:	0.01<P<0.025
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*: No data due to high toxicity