

TABLE 3.4.3.2
MUTAGENIC ACTIVITY OF ALISKIREN IN V79 CELLS WITHOUT 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	77.75	3.75	4.82
Positive control EMS 0.3 μ l/ml	48.75	940.13	1928.46
CGP 60536 B:			
400.0000 μ g/ml	78.00	4.75	6.09
100.0000 μ g/ml	78.33	2.63	3.35
25.0000 μ g/ml	79.58	3.50	4.40
6.2500 μ g/ml	78.42	3.63	4.62

Treatment	Mean mutant frequency ($\times 10^6$)	Mean mutant factor	Significance (P)
Negative control	2.41		
Positive control EMS 0.3 μ l/ml	964.23	399.83	P<0.001
CGP 60536 B:			
400.0000 μ g/ml	3.04	1.26	Ns
100.0000 μ g/ml	1.68	0.69	Ns
25.0000 μ g/ml	2.20	0.91	Ns
6.2500 μ g/ml	2.31	0.96	Ns

Linear relation: Ns

TABLE 3.4.3.3
MUTAGENIC ACTIVITY OF ALISKIREN IN V79 CELLS WITH METABOLIC ACTIVATION
1ST CONFIRMATORY EXPERIMENT

Treatment	Mean of via- bility clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	73.00	12.38	16.95
Positive control DMN 1 μ l/ml	54.67	113.00	206.71
<u>CGP 60536 B:</u>			
1200.0000 μ g/ml	*	*	*
400.0000 μ g/ml	75.08	11.00	14.65
133.3333 μ g/ml	70.00	6.13	8.75
44.4444 μ g/ml	76.42	11.13	14.56

Treatment	Mean mutant frequency ($\times 10^6$)	Mean mutant factor	Significance (P)
Negative control	8.48		
Positive control DMN 1 μ l/ml	103.35	12.19	P<0.001
<u>CGP 60536 B:</u>			
1200.0000 μ g/ml	*	*	*
400.0000 μ g/ml	7.33	0.86	Ns
133.3333 μ g/ml	4.38	0.52	Ns
44.4444 μ g/ml	7.28	0.86	Ns
Linear relation:	P<0.001		

*: No data due to high toxicity

TABLE 3.4.3.4
MUTAGENIC ACTIVITY OF ALISKIREN IN V79 CELLS WITHOUT METABOLIC ACTIVATION
1ST CONFIRMATORY EXPERIMENT

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	89.42	6.38	7.13
Positive control EMS 0.3 µl/ml	70.75	919.25	1299.29
<u>CGP 60536 B:</u>			
500.0000 µg/ml	53.25	5.00	9.39
166.6667 µg/ml	91.50	6.25	6.83
55.5556 µg/ml	96.42	6.75	7.00
18.5185 µg/ml	90.33	8.00	8.86

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.56		
Positive control EMS 0.3 µl/ml	649.65	182.24	P<0.001
<u>CGP 60536 B:</u>			
500.0000 µg/ml	4.69	1.32	0.02<P<0.05
166.6667 µg/ml	3.42	0.96	Ns
55.5556 µg/ml	3.50	0.98	Ns
18.5185 µg/ml	4.43	1.24	0.02<P<0.05

Linear relation:	Ns
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TABLE 3.4.3.5
MUTAGENIC ACTIVITY OF ALISKIREN IN V79 CELLS WITH METABOLIC ACTIVATION
2ND CONFIRMATORY EXPERIMENT

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	93.42	6.00	6.42
Positive control DMN 1 µl/ml	78.33	138.25	176.49
<u>CGP 60536 B:</u>			
1000.0000 µg/ml	72.42	5.38	7.42
500.0000 µg/ml	88.42	6.63	7.49
250.0000 µg/ml	92.58	3.88	4.19
125.0000 µg/ml	92.00	4.88	5.30

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.21		
Positive control DMN 1 µl/ml	88.24	27.48	P<0.001
<u>CGP 60536 B:</u>			
1000.0000 µg/ml	3.71	1.16	Ns
500.0000 µg/ml	3.75	1.17	Ns
250.0000 µg/ml	2.09	0.65	Ns
125.0000 µg/ml	2.65	0.83	Ns

Linear relation:	Ns
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3.4.4. In vivo Micronucleus Assay in Rats with Aliskiren Hemifumarate

Key Findings: Aliskiren tested negative for inducing micronuclei in rat bone marrow.

Study No: 966161

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 01 and terminated on May 28, 1997.

GLP Compliance: Yes

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

Methods

Prior to the main study, the maximum tolerated dose (MTD) of the test substance was determined according to the procedure described by Mackay and Elliot (*Mutation Research* 271: 97-99, 1992). For this MTD study, 6 to 7 week old male and female rats (Tif: RAI[fSPF]) weighing between 231 and 300 gm, and 167 and 197 gm, respectively, were used. The highest dose tested was 2000 mg aliskiren hemifumarate/kg given orally by gavage to a male and a female rat. Both rats showed hunched posture, ataxia, and reduced locomotor activity within an hour of treatment. The male rat developed convulsions and tonic spasms an hour after treatment and had to be sacrificed moribund after 2 hours. The female rat survived. In a second step, 1250 mg/kg was administered to a pair of rats. Both male and female rats showed hunched posture, ataxia, dyspnea, and reduced locomotor activity, 15 min after treatment. They were sacrificed moribund the day after treatment. A third dose (oral), 800 mg/kg, also resulted in dyspnea, hunched posture, ataxia, and reduced locomotor activity. The male was found dead 3 hr after administration, while the female was sacrificed moribund after 2 days of observation. A lower dose, 500 mg/kg, produced similar symptoms, leaving the male dead a day after administration (the female survived). A male receiving 320 mg/kg (female not dosed at this level) survived, exhibiting reduced locomotor activity and ataxia 15 min after treatment. Acceptability of the doses of 500 mg/kg (female) and 320 mg/kg (male) was confirmed by treating a second pair of rats. Both survived but demonstrated reduced locomotor activity and ataxia 15 min after treatment, which continued for the next 2 days. Based on these results, maximum doses of 500 and 320 mg/kg were chosen for female and male rats, respectively, in the micronucleus test.

The main study animals were male and female rats (Tif: RAI[fSPF]) that were approximately 6 to 7 weeks old and weighed 189 to 231 gm and 153 to 191 gm, respectively. Aliskiren hemifumarate (batch #817196) was dissolved in double distilled water and administered orally by stomach tube (10 ml/kg body weight) to groups of male (doses: 80, 160 or 320 mg/kg) and female (doses: 125, 250 or 500 mg/kg) rats. A negative control group was treated with the vehicle, and a positive control group was treated (p.o.) with cyclophosphamide (40 mg/kg). Animals from the high dose and the negative control groups were sacrificed 24 and 48 hr after administration (5/sex/harvest time). There was only one harvest (24 hr) for the other groups (5/sex). Bone marrow was harvested from the shafts of both femurs with fetal calf serum. The ratio of PCE to NCE was determined and 2000 PCEs were scored for micronuclei. The results were evaluated

with respect to the mean number of PCEs with micronuclei. A test substance is considered to be active if the mean number of micronucleated PCEs exceeds 0.2% and the incidence is statistically significantly greater than the negative control.

Results

Most of the animals in the high dose groups displayed reduced locomotor activity and ataxia. In the mid dose groups, only one female showed ataxia at time of sacrifice. No signs of toxicity were observed with any animal in the low dose groups.

The test substance did not induce a statistically significant increase in the frequency of micronucleated PCEs over the levels observed in the vehicle controls at any of the harvest times. The positive control, CP, induced statistically significant increases in micronucleated PCEs as compared to the vehicle controls (1.0 and 1.1% for the female and male treated groups vs. 0.09 and 0.14% for the female and male vehicle control groups, Table 3.4.4.1). Aliskiren hemifumarate did not significantly decrease the PCE:NCE ratio, demonstrating the absence of cytotoxicity to the bone marrow. Aliskiren hemifumarate is considered to have tested negative in this rat bone marrow micronucleus assay.

TABLE 3.4.4.1
MICRONUCLEUS TEST ON RAT BONE MARROW CELLS. SUMMARY DATA

Test Article:	Dose (mg/kg)	No. of animals	Ratio PCE/NCE	Mean % MN-PCEs
Vehicle: distilled water	0 (24 h)	5M	0.42	0.14
	0 (24 h)	5F	0.38	0.09
	0 (48 h)	5M	0.34	0.06
	0 (48 h)	5F	0.53	0.10
Aliskiren	80 (24 h)	5M	0.49	0.18
	125 (24 h)	5F	0.31	0.16
	160 (24 h)	5M	0.47	0.12
	250 (24 h)	5F	0.47	0.12
	320 (24 h)	5M	0.48	0.13
	500 (24 h)	5F	0.40	0.15
	320 (48 h)	5M	0.39	0.12
	500 (48 h)	5F	0.43	0.12
Cyclophosphamide	40	5M (24 h)	0.15	1.10 *
		5F (24 h)	0.13	1.00 *

Number of cells analyzed per animal: 2000

PCE = polychromatic erythrocytes, NCE = normochromatic erythrocytes, MN = micronucleated

Chi-Squared-Contingency Test: *, $p < 0.05$

3.4.5. In vivo Oral Comet Assay in Rats

Key Findings: Aliskiren tested negative for inducing DNA migration in liver, colon and cecum cells of male rats.

Study No: 0512502

Location of Report: EDR

Conducting Laboratory and Location: Safety Profiling and Assessment, Genetic Toxicology and Safety Pharmacology, Novartis Pharmz, Toxicology/Genetic Toxicology, CH-4002 Basel, Switzerland

Dates of Study: Dosed on November 28 and terminated on December 20, 2005.

GLP Compliance: Yes

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

Drug, batch #: Aliskiren hemifumarate, 0444029, drug content —

Vehicle: Tap water

Positive Control: Ethyl methanesulfonate (EMS) dissolved in tap water.

Methods

Animals: Male — WI (Han) rats (from —) were 7-8 weeks old and weighed 246-279 gm at the beginning of the dose-finding and main experiments. After dosing, animals were housed individually. Food and water were provided *ad libitum*.

Dose-finding Study: Prior to the main study, the maximum tolerated dose (MTD) of the test substance was determined. Two male rats were initially given single oral doses of 668 mg aliskiren hemifumarate/kg by gavage (10 ml/kg). Clinical signs (salivation, dyspnea and piloerection), noted in one animal, were attributed to aspiration of the dosing solution into the airway region. An additional 4 rats were given 2000 mg aliskiren hemifumarate/kg by gavage. No clinical signs were noted in 3 animals. The fourth animal showed clinical symptoms (salivation, dyspnea) which were attributed to aspiration of the dosing solution into the airway region.

Main Study: Male rats were dosed twice by gavage with 1105 or 2210 mg aliskiren hemifumarate/kg (1000 or 2000 mg in terms of the free base, respectively) or with vehicle (10 ml/kg) (n=5/dose), with an interval of 21 hr between administrations. Four positive control animals were dosed, once by gavage, with 300 mg EMS/kg. The experimental design is shown in Table 3.4.5.1. Animals were sacrificed 3 hr after the second administration. The latter sampling time was chosen based on the T_{max} as determined in previous studies (see ADME section).

For tissue sampling, the animals were dissected and liver, colon and cecum mucosa cells were isolated. Two slides were prepared for each tissue and each animal (40-50 cells/slide). After electrophoresis, the cells were stained and analyzed for the induction of DNA damage. This was measured as increased DNA migration in the comet assay. The sensitivity of the test system was shown by the increased DNA migration (determined as tail moment) in EMS-treated animals. The tail moment is defined as the product of the amount of DNA and the mean distance of migration in the tail of comet. The tail moment is the parameter commonly used to analyze the extent of DNA damage.

TABLE 3.4.5.1
GROUPING OF ANIMALS AND SAMPLING TIMES

	Group 1 Negative control	Group 2 Positive control EMS	Group 3 SPP100	Group 4 SPP100	Reserve animals SPP100	Reserve animals SPP100
Dosage (mg/kg)	0	300	1000	2000	1000	2000
Concentration (mg/ml)	0	60	100	200	100	200
Application volume (ml/kg)	10	5	10	10	10	10
Number of animals	5 males	4 males	5 males	5 males	1 male	1 male
Animal no.	21-25	26-29	30-34	35-39	40	41
Animal dissection	3 h after second admin.	3 h after first admin.	3 h after second admin.	3 h after second admin.	3 h after second admin.	3 h after second admin.

The results of the comet assay were evaluated according to the following criteria: a test substance is classified as positive if the mean tail moment of the treated group is statistically significantly higher than the mean tail moment of the vehicle control group. The 1-sided t-test was used for the assessment of a significant difference between negative and positive control groups, as well as the assessment of significant difference(s) between negative control group and treatment groups. In addition to evaluating DNA migration, the liver, cecum and colon tissues of all animals were sampled at necropsy, processed and examined microscopically.

Results

No statistically significantly increased DNA damage values (determined as mean tail moment) were recorded in liver cells, colon cells or cecum mucosa cells of male rats in any of the aliskiren-treated groups relative to the concurrent control and all values were within the range of historical control values. No dose-response relationship was observed (Table 3.4.5.2). However, tail moment values above the historical vehicle control range were measured in cecum mucosa cells of three animals in the control group. The tail moment values observed in the same animals in the liver and the colon were all within the corresponding historical vehicle control ranges. The high tail moment values were attributed to mechanical damage of the cecum during dissection. The sensitivity of the test system was shown by the increased DNA migration after treatment with the positive control, ethyl methanesulfonate. Thus, the study concludes that aliskiren, up to a dose of 2000 mg/kg p.o., does not induce DNA damage in the liver, colon or cecum of rats.

Histopathological examination revealed the presence of minimal to slight mucosal basophilia in the cecums of 4/5 rats receiving 1000 or 2000 mg aliskiren/kg. Minimal mucosal basophilia was also seen in the colons of 2/5 rats at 2000 mg/kg. There was no evidence of increased cell death/apoptosis.

TABLE 3.4.5.2
DNA MIGRATION (MEASURED AS TAIL MOMENT) IN THE LIVER, COLON AND CECUM OF RATS

test item	dose (mg/kg)	no. of animals	mean tail moment \pm standard deviation		
			Liver	Colon	Caecum mucosa
vehicle	0	5 males	0.12 \pm 0.08	0.25 \pm 0.17	1.64 \pm 0.99
SPP100	1000	5 males	0.11 \pm 0.05	0.34 \pm 0.13	0.87 \pm 0.14
SPP100	2000	5 males	0.09 \pm 0.004	0.27 \pm 0.13	0.59 \pm 0.22
EMS	300	4 males	3.06 \pm 0.98*	2.51 \pm 1.23*	7.06 \pm 2.29*

T-test, * p<0.05

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ON ORIGINAL**

3.5. Reproductive and Developmental Toxicology

3.5.1. Fertility and Early Embryonic Development Study in Rats

Key Study Findings: Aliskiren hemifumarate up to a maximum oral dose of 250 mg aliskiren/kg/day did not affect male or female fertility or induce early embryonic developmental toxicity.

Study No.: — 1940/04-D6154

Location of Report: EDR

Conducting Laboratory and Location: —

Dates of Study: Dosing was initiated on September 25, 2001 for both males and females. Males were necropsied on November 6, while cesarean females were necropsied on October 27, 2001.

GLP Compliance: Yes

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

Drug, Lot #: Aliskiren hemifumarate, batch #S100B-2001002, — lot #5.

Formulation: Aliskiren hemifumarate was dissolved in sterile water at weekly intervals. The formulations were sampled in duplicate at the beginning and at the end of the dosing period. Mean concentrations for all formulations were within — of nominal.

Animals

Species/Strain: — CD(SD)IGSBR rats from —

#/Sex/Group: 24/sex/group

Age: 12 to 14 weeks at the time of dosing

Weight: males: 359.8 to 530.7 gm, females: 239.5 to 302.6 gm

Husbandry: Animals were housed individually in stainless steel cages. Food and water were given *ad libitum* throughout the study period.

Dosing

Aqueous solutions of aliskiren hemifumarate were administered orally by gavage (5 ml/kg) once daily, to groups of 24 males and 24 females each at doses of 50, 150 or 250 mg aliskiren/kg. The control animals received the vehicle (5 ml/kg body weight). Males were dosed daily for 2 weeks before mating with females of the same dosage group and throughout the mating period until the day before necropsy in week 8. The females were also treated for 2 weeks prior to mating and continued until day 6 of gestation. The doses were selected on the basis of an embryo-fetal development study (see section 3.4.1, study #994006, original IND review dated November 11, 2001) in the same rat strain in which aliskiren produced mild clinical symptoms (decreased and soft stool, salivation, diarrhea) at 300 or more mg/kg/day and decreased body weight gain during gestation at 600 mg/kg/day.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for general condition, mortality and moribundity.

Body Weights: Males were weighed a week before treatment, on the first day of dosing and at weekly intervals prior to necropsy. Females were weighed weekly until confirmation of mating and on days 0, 3, 6, 10 and 13 of pregnancy. Food intake was

measured weekly during the pairing periods and then for mated females for days 0 to 3, 3 to 6, 6 to 10 and 10 to 13 of gestation.

Laparotomy: All surviving females with evidence of mating were euthanized on gestation day 13. The ovaries and uteri were removed and examined. The numbers of corpora lutea, implantations, living fetuses and dead embryos/fetuses (post-implantation losses) were recorded. The male animals were killed (in week 8 of study) after the review of the female data. Seminology investigations were not performed in the absence of an effect on fertility. The following organs and tissues were collected from all animals. Reproductive organs from only the control and high dose animals were examined microscopically.

Cervix
Coagulating gland
Ovaries
Pituitary gland
Prostate gland
Seminal vesicles
Testes
Epididymides
Uterus
Vagina
Lesions

Results

Mortality: All mated animals survived to the scheduled necropsy.

Clinical Signs: All animals in the high dose group exhibited paddling of the forelimbs, salivation and rubbing of the mouth/face against the cage immediately after dosing and continued throughout the dosing period.

Body Weights: No adverse effects of treatment on male or female body weight or body weight gain.

Food Consumption: No treatment-related findings.

Reproductive Performance: There was no effect of test substance on mating behavior, estrous cycle, mean numbers of corpora lutea, implantations or pre-and post-implantation losses (Table 3.5.1.1). There were no macroscopic or microscopic findings in the reproductive organs of either males or females indicative of an adverse effect of treatment. In conclusion, administration of aliskiren at doses of up to 250 mg/kg/day elicited no parental toxicity and had no adverse effects on fertility or early embryonic development.

TABLE 3.5.1.1
SUMMARY OF GROUP MEAN UTERINE/IMPLANTATION DATA

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of females with live embryos on Day 13 gestation	24	23	24	24	
Mean number of corpora lutea per female	17.3	16.8	16.8	16.2	J
Mean number of implantations per female	15.8	15.3	14.7	14.5	J
Pre-implantation loss:					
mean%	8.1	8.5	11.7	10.7	
number of dams affected	15	15	14	15	F+
Early intrauterine deaths:					
mean number	0.8	0.5	0.9	0.7	
number of dams affected	10	10	14	10	F+
Late intrauterine deaths:					
mean number	0.0	0.0	0.0	0.0	
number of dams affected	0	0	0	0	F+
Post-implantation loss:					
mean%	4.7	3.4	6.4	4.2	
number of dams affected	10	10	14	10	F+
Mean number of embryos per female	15.0	14.7	13.8	13.8	J

J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon
 F+ = Cochran-Armitage and Fisher's Exact (upper tail)

Group 1: Control group; Groups 2, 3 and 4: 50, 150 and 250 mg aliskiren/kg/day groups, respectively.

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3.5.2. Embryo-Fetal Development Study in Rats

Key Study Findings: Aliskiren hemifumarate administered to pregnant rats on gestation days 6 through 17, at oral doses of up to 600 mg aliskiren/kg/day, did not adversely affect embryo-fetal development.

Study No.: 974006

Location of Report: EDR

Conducting Laboratory and Location: Safety Evaluation Facility, Preclinical Safety, Novartis Pharmaceuticals Corporation, Summit, NJ

Dates of Study: Dosing was initiated on February 4, and ended February 21, 1997

GLP Compliance: Yes

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

Drug, Lot #: Aliskiren hemifumarate, lot #817196.

Formulation: Aliskiren hemifumarate was dissolved in sterile water for injection.

Animals

Species/Strain: ✓ COBS CD[SD]BR rats from —

#/Sex/Group: 25 mated females/group

Age: 10 to 12 weeks at the time of dosing

Weight: 222-331 gm at the time of dosing (day 0 of gestation)

Husbandry: Animals were housed individually in stainless steel cages. Food and water were given *ad libitum* throughout the study period.

Dosing

Aqueous solutions of aliskiren hemifumarate were administered orally by gavage (10 ml/kg) once daily to groups of presumed pregnant females at doses of 60, 300 or 600 mg aliskiren/kg on gestational days 6 through 17. A control group of 25 mated females received distilled water in similar fashion. The doses were selected based on a 2-week oral toxicity study in non-pregnant rats in which 1000 mg/kg produced mortality and 300 mg/kg produced mild toxicity.

Observations and Measurements

Clinical Signs: All animals were observed daily for general condition, mortality and moribundity.

Body Weights: Individual body weights were recorded on gestation days 0, 6, 9, 12, 15, 18 and 20.

Food Consumption: Recorded on days of body weight measurements.

Laparotomy: On day 20, each dam was sacrificed and major visceral organs, including the placenta, were macroscopically examined and discarded. The combined weight of the uterus and its contents and the ovaries and oviducts was determined. The numbers of corpora lutea, implantations, live fetuses and dead fetuses were recorded. All live fetuses were sexed, weighed and examined externally for abnormalities. Fetuses were "sacrificed by immersion" in an appropriate fixative and then processed for visceral and skeletal examinations.

Results

Analysis of Formulations: The achieved concentration of test substance in the formulation was — % of the targeted concentration.

Mortality: One female receiving 300 mg/kg/day died on gestation day 12. This dam had salivation after dosing on gestation day 9 but displayed no other clinical signs before death. Necropsy revealed distended intestine and autolysis of internal organs. The cause of death could not be ascertained.

Clinical Signs: Treatment-related clinical signs were observed at all dose levels. Decreased and soft stool at doses ≥ 60 mg/kg/day, salivation at doses ≥ 300 mg/kg/day and diarrhea at 600 mg/kg/day were observed. At necropsy, one mid dose dam (different from the one that died) showed coagulated blood surrounding two uterine implants and had greenish fluid (amnion) surrounding fetuses in the left uterine horn. In the absence of other findings, the sponsor does not consider this related to treatment with the test substance.

Body Weights: There were no effects on body weights but a statistically significant decrease in body weight gain was noted for high dose group animals on gestation days 6-9 (9.6 gm *versus* 13.8 gm for the control). This decrease correlated with decreased food consumption at the same time (25.4 gm *versus* 28.4 gm for the control).

Laparo-hysterectomy: Pregnancy rate was not affected by test substance. Dead fetuses were not observed in the study. There were no effects of treatment on reproductive parameters, fetal numbers, weights or sex (Table 3.5.2.1). One grossly malformed fetus with anencephaly was observed in the high dose group of 22 litters. The malformation was considered by the sponsor to be spontaneous in nature. Skeletal examination of the fetuses revealed a statistically significant increase in the incidence of non-ossified caudal vertebrae (a skeletal variation rather than malformation) in the 600 mg/kg group (8 fetuses from 8 litters *versus* 7 fetuses from 4 litters in the control group). It can be concluded that the no adverse effect dose for aliskiren in parent animals on this study was < 60 mg/kg/day, and the NOAEL for embryo-fetal toxicity or teratogenicity was > 600 mg/kg/day.

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TABLE 3.5.2.1
PREGNANCY STATUS IN RATS TREATED ORALLY WITH ALISKIREN AND C-SECTIONED ON DAY 20
OF GESTATION. DATA GIVEN AS MEAN ± STANDARD DEVIATION

Parameters	Dose (mg/kg/day)			
	Control (0)	60	300	600
N	25	25	25	25
Pregnant and survived to terminal sacrifice	23	21	24	22
Corpora lutea	17.43 ± 2.71 (23)	17.57 ± 2.50 (21)	17.75 ± 2.52 (24)	17.82 ± 1.79 (22)
Implantation sites	15.35 ± 3.23 (23)	15.95 ± 3.88 (21)	15.33 ± 3.55 (24)	15.86 ± 2.96 (22)
Early resorptions	0.96 ± 1.07 (23)	1.29 ± 1.38 (21)	0.96 ± 1.23 (24)	1.18 ± 1.33 (22)
Late resorptions	0.00 ± 0.00 (23)	0.00 ± 0.00 (21)	0.04 ± 0.20 (24)	0.05 ± 0.21 (22)
Total resorptions	0.96 ± 1.07 (23)	1.29 ± 1.38 (21)	1.00 ± 1.25 (24)	1.23 ± 1.34 (22)
Live fetuses	14.39 ± 2.89 (23)	14.67 ± 3.72 (21)	14.33 ± 3.61 (24)	14.64 ± 3.54 (22)
Dead fetuses	0.00 ± 0.00 (23)	0.00 ± 0.00 (21)	0.00 ± 0.00 (24)	0.00 ± 0.00 (22)
Postimplantation loss	0.96 ± 1.07 (23)	1.29 ± 1.38 (21)	1.00 ± 1.25 (24)	1.23 ± 1.34 (22)
% Postimplantation loss	5.71 ± 6.07 (23)	7.63 ± 8.48 (21)	6.48 ± 8.12 (24)	9.45 ± 12.81 (22)
Fetal sex ratio, % males	52	48	51	52
Male fetal weight, gm	3.65 ± 0.06 (23)	3.60 ± 0.07 (20) ^a	3.71 ± 0.06 (24)	3.70 ± 0.07 (21) ^b
Female fetal weight, gm	3.49 ± 0.07 (23)	3.39 ± 0.08 (21)	3.50 ± 0.07 (24)	3.57 ± 0.08 (22)

^a: dam #40, no male fetuses

^b: dam #97, no male fetuses

number of animals are given in parentheses

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3.5.3. Embryo-Fetal Development Study in Rabbits

Key Study Findings: Aliskiren hemifumarate at an oral dose of 200 mg aliskiren/kg/day resulted in 3 deaths and 9 morbid sacrifices. The remaining members of this group were terminated as a result of inappetence and loss of body weight. Furthermore, three and 4 females receiving 50 and 100 mg aliskiren/kg/day, respectively, died or were killed due to signs of abortion or actual abortion in late gestation. Animals in the low and mid dose groups showed small but significant decreases in mean body weight gain, food and water intake during the study. There were no adverse effects of treatment on the reproductive performance of does. However, a dose-related significant decrease in mean litter weight was noted. The NOAEL for maternal and embryo-fetal toxicity was less than 50 mg/kg/day.

Study No.: — 1940/06-D6154

Location of Report: EDR

Conducting Laboratory and Location: —

Dates of Study: Dosing initiated on August 6, 2001 (day 7 of gestation); necropsy completed on September 7, 2001

GLP Compliance: Yes

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

Drug, Lot #: Aliskiren hemifumarate, batch #S 100B-2001002, — lot #5.

Formulation: Aliskiren hemifumarate was dissolved in sterile water. Solutions were prepared at weekly intervals. The formulations were sampled in duplicate at the beginning and end of the dosing period. Mean concentrations for all formulations were within — of nominal.

Animals

Species/Strain: — NZW/Kbl BR rabbits from —

#/Sex/Group: 24 females/group

Age: 4 to 5 months at the time mating

Weight: 2.51 to 4.26 kg at the time of dosing (day 7 of gestation)

Husbandry: Animals were housed individually in stainless steel cages. Food and water were given *ad libitum* throughout the study period.

Dosing

Aqueous solutions of aliskiren hemifumarate were administered orally by gavage (5 ml/kg), once daily, to groups of 24 presumed pregnant females at doses of 50, 100 or 200 mg aliskiren/kg. The control animals received the vehicle (5 ml/kg body weight). All animals were treated on gestation days 7 through 28. The doses were selected on the basis of a dose range-finding study in pregnant rabbits of the same strain in which the test substance, administered from gestation day 7 to gestation day 19, produced deaths at doses ≥ 400 mg/kg/day; the no adverse effect level was 200 mg/kg/day.

Observations and Measurements

Clinical Signs: All animals were observed at least once daily for general condition and twice daily to detect any mortality and moribundity. Animals were observed at intervals of up to 4 hr after dosing for signs of reaction to treatment.

Body Weights: Individual body weights were recorded on gestation days 4, 7, 8, 9, 12, 15, 19, 24, 28 and 29.

Food and Water Consumption: Recorded for each animal daily from day 4 to day 29 of gestation.

Toxicokinetics: Blood samples were collected from the marginal ear vein from 12 animals in the high dose group (#85 to #96) at 30 min post dose and just prior to necropsy.

Laparotomy: All surviving maternal rabbits, except in the high dose group, were euthanized on gestation day 29 and examined macroscopically. The ovaries and uteri were removed, examined, and the following data recorded: pregnancy status, gravid uterus weight, number of corpora lutea, number and intrauterine position of implantations that included live and dead fetuses. The high dose group was terminated early. Samples of the following tissues were retained.

Adrenals	Ileum	Rectum
Brain	Jejunum	Spleen
Cecum	Kidneys	Stomach
Colon	Liver	Thymus
Duodenum	Lungs	Thyroids
Esophagus	Lymph nodes, mesenteric,	Trachea
Gall bladder	Ovaries	Urinary bladder
Gross lesions	Pancreas	Uterus
Heart	Pituitary	Vagina

Fetuses were sexed, weighed and examined externally for abnormalities. Live fetuses were killed, viscera examined and heads of approximately one half of the fetuses in each litter were preserved. The hearts of approximately one half of the fetuses in each litter were fixed; several coronal slices of each heart were sectioned and examined (rationale for studying the morphology of heart is not provided). Each carcass was processed for skeletal examination. Fetal abnormalities were classified as malformations and variations.

Results

Mortality: Three high dose females died (#78, 81 and 84 on gestation days 13, 15 and 15, respectively). Nine animals in this group were killed in moribund condition between gestation days 15 and 21. Necropsy examination of these animals showed macroscopic indications of severe inappetence and body weight loss. Due to these findings and signs of lack of appetite in the remaining 12 high dose animals, the group was terminated early (between gestation days 11 and 21) after sampling blood for plasma analysis of test substance. No further observations were reported for the high dose group in the study report. In the low dose group, one animal (#48) was killed in a moribund condition on GD 48, while two (#38, 44) aborted in late gestation, on days 23 and 21, respectively. In the mid dose group, a doe (#72) was found dead on GD 25, two (#61, 68) were euthanized *in extremis* on GD 26 and 23, and one (#60) aborted in late gestation, day 25. All of these animals had shown severe inappetence and consequent body weight loss. According to the study report, this resulted in abortion or total embryo-fetal loss.

Clinical Signs: Signs of thinness were noted in 3 mid dose females in late gestation.

Body Weights: A dose-related decrease in body weight was noted for does in the low and mid dose groups ($p < 0.05$) (Table 3.5.3.1). The mean body weight gain over the dosing period (days 7 to 29 of gestation) was 11.6, 6.7 and 5.5% for the control, low and mid dose groups, respectively. After allowing for difference in gravid uterine weight, all groups lost weight during this period (about 8% loss for each of the two treated groups versus 4% loss for the control group).

TABLE 3.5.3.1
EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS
GROUP MEAN BODY WEIGHTS DURING GESTATION

	Group 1	Group 2	Group 3	Statistics
Mean body weight (kg) on Day 7	3.48	3.65	3.56	
Mean body weight (kg) on Day 29	3.88	3.90	3.74	
% body weight change Days 7 - 29	11.6	6.7	5.5	
Mean gravid uterus weight (kg)	0.54	0.54	0.46	C
Mean corrected body weight (kg) on Day 29	3.34	3.36	3.28	A
% body weight change (corrected) Days 7 - 29	-4.0	-7.9	-7.7	

C = ANCOVA and Dunnett's

A = ANOVA, regression and Dunnett's

Group 1: Control group; Groups 2 and 3: 50 and 150 mg aliskiren/kg/day groups, respectively.

Food Consumption: Mean food consumption for low and mid dose groups was lower than that of the control group but the differences were only marginally dose-related. The difference from control was significant ($p < 0.05$) for the low dose group between GDs 19 and 24 and for the mid dose group between GDs 24 and 28 (Table 3.5.3.2).

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TABLE 3.5.3.3
EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS
SUMMARY OF FETAL DATA

Number of animals:	Group 1	Group 2	Group 3	Group 4
In group	24	24	24	24
Not pregnant	1	2	2	0
Pregnant (%)	23 (95.8)	22 (91.7)	22 (91.7)	24 (100.0)
Died/killed	0	1	3	24+
Aborted and killed	0	2	1	0
With total embryo/foetal loss	2	0	0	0
With live foetuses on Day 29	21	19	18	0
Number of females with live foetuses on Day 29 gestation	21	19	18	Statistics
Mean number of corpora lutea per female	12.0	11.9	11.1	J
Mean number of implantations per female	10.6	10.8	9.3	J
Pre-implantation loss:				
mean number of dams affected	12.2	9.8	17.0	
number of dams affected	12	12	12	F+
Early intrauterine deaths:				
mean number	0.3	0.4	0.2	
number of dams affected	6	6	3	F+
Late intrauterine deaths:				
mean number	1.0	0.5	0.7	
number of dams affected	9	6	7	F+
Dead foetuses:				
mean number	0.0	0.1	0.0	
number of dams affected	1	1	0	X
Post-implantation loss:				
mean number of dams affected	12.1	8.8	10.1	
number of dams affected	12	10	8	F+
Mean number of foetuses per female	9.2	9.8	8.3	J
Number of male foetuses	98	85	79	
Number of female foetuses	96	102	71	
Mean ± male foetuses	52.0	44.6	53.4	J
Mean litter weight (g)	366.4	358.1	301.5	DR* A
Mean placental weight (g)	5.11	4.94	5.35	J
Mean foetal weight (g)	40.1	36.5	38.0	J
Mean foetal weight (g) - males only	40.7	37.8	38.8	J
Mean foetal weight (g) - females only	39.5	35.6**	37.0	J

J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon
A = ANOVA, regression and Dunnett's

* P<0.05
** P<0.01
*** P<0.001

DR = significant dose response test

F+ = Cochran-Armitage and Fisher's Exact (upper tail); X = not analyzed

Group 1: Control group; Groups 2, 3 and 4: 50, 150 and 250 mg aliskiren/kg/day groups, respectively.

No external, skeletal or visceral fetal variations were noted in the drug treated groups. Fetal malformations were noted in 6, 8 and 5 fetuses from 6, 7 and 2 litters in the control, low and mid dose groups, respectively (Table 3.5.3.4). The study laboratory contends that these defects are commonly noted in this strain of rabbit and thus do not represent an adverse effect of treatment.

TABLE 3.5.3.4
EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS
SUMMARY OF FETAL MALFORMATIONS

Group	Dam/Foetus	Malformation
1	12/R8	Skull, nasal fused
	16/R3	Heart, ventricles – no recognisable structures present
	19/R1	Blood vessels, subclavian artery arising from descending aorta; blood vessels, subclavian artery retro-oesophageal
	21/R4	Blood vessels, subclavian artery arising from descending aorta; blood vessels, subclavian artery retro-oesophageal
	22/R2	Head, frontal/parietal region swelling – soft/skin covered; head, orbital region reduced in size; head, Buccal cavity, palate misshapen; head, nasal region absent; head, nasal region not patent; head, craniofacial region shortened; eye(s) reduced in size; eye(s) displaced – medial; eye(s) abnormally positioned within orbit; skull, frontal misshapen; skull, maxilla misshapen; skull, nasal misshapen; skull, premaxilla misshapen; skull, palatine misshapen
	24/R9	Diaphragm thinned and displaced; sternum shortened; sternum abnormal cleft; trunk, abdomen opening in body wall involving umbilicus and viscera within sac outside body cavity; forelimb absent, humerus, radius and ulna absent, digits and metacarpal absent; sternbrae cleft and absent; pectoral girdle, scapula reduced in size
2	31/R7	Thoracic arch, additional arch between; rib branched and displaced – cranial
	33/L2	Thoracic vertebra absent; rib absent
	33/L3	Thoracic arch reduced in size; thoracic centrum hemicentric, right side present
	35/L4	Blood vessels, interrupted aortic arch
	37/R4	Neck – subcutaneous oedema; diaphragm herniation, stomach protruding; diaphragm, adhered liver; heart, interventricular septum incomplete; skull, fontanelle enlarged; zygomatic arch fused; sternbrae fused and cleft; hindlimbs, tibia and fibula bent
	41/L1	Ureter distended – fluid contents; increased kidney cavitation
	43/R3	Sternebrae fused
	46/R10	Head, fronto/parietal region opening in skull, nervous tissue exposed; skull, frontal and parietal misshapen, interparietal and supraoccipital reduced in size
3	49/R3	Heart, interventricular septum incomplete
	55/L4	Forelimb wrist joint flexed
	55/L5	Forelimb wrist joint flexed; Forelimb elbow joint malrotated
	55/R3	Forelimbs wrist joint flexed; brain, lateral ventricles dilated
	55/R4	Forelimbs wrist joint flexed

3.5.4 Pre- and Postnatal Development Study in Rats

Key Study Findings: Aliskiren hemifumarate administered to pregnant rats from gestation day 6 through lactation day 21 at oral doses of up to 250 mg aliskiren/kg/day did not induce maternal toxicity or adverse effects on the development or reproductive performance of the F₁ generation.

Study No.: — 1940/05-D6154

Location of Report: EDR

Conducting Laboratory and Location: —

Dates of Study: Dosing was initiated on August 6, 2001 (day 6 of gestation); F₀ necropsy: September 16, 2001 (maternal lactation day 21); F₁ necropsy: January 16, 2002.

GLP Compliance: Yes

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

Drug, Lot #: Aliskiren hemifumarate, batch #S100B-2001002. — lot #5.

Formulation: Aliskiren hemifumarate was dissolved in sterile water at weekly intervals. The formulations were sampled in duplicate at the beginning of the dosing period. Mean concentrations for all formulations were within — of nominal.

Animals

Species/Strain: — CD(SD)IGSBR rats from —

#/Sex/Group: 24 females/group

Age: 9 to 11 weeks at the time of dosing

Weight: 190.5 to 297.7 gm at the time of dosing (day 6 of gestation)

Husbandry: Animals were housed individually in stainless steel cages. Food and water were given *ad libitum* throughout the study period.

Dosing

Aqueous solutions of aliskiren hemifumarate were administered orally by gavage (5 ml/kg) once daily to groups of presumed pregnant females at doses of 50, 150 or 250 mg aliskiren/kg. The control animals received the vehicle (5 ml/kg body weight). All animals were treated from gestation day 6 through lactation day 21. The doses were selected on the basis of an embryo-fetal development study (study #994006) in the same rat strain in which aliskiren produced mild clinical symptoms (decreased and soft stool, salivation, diarrhea) at 300 or more mg/kg/day and decreased body weight gain during gestation at 600 mg/kg/day.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for general condition, mortality and moribundity.

Body Weights: Individual body weights were recorded on gestation days 4, 6, 7, 8, 9, 12, 15, 17 and 20 and on lactation days 1, 4, 7, 14 and 21. F₁ male body weights were recorded weekly, while F₁ female body weights were recorded weekly prior to mating, through mating and on gestation days 0, 3, 6, 10 and 13.

Food Consumption: Recorded for F₀ females on days of body weight measurement during gestation and for lactation days 1 to 4, 4 to 7, 7 to 14 and 14 to 21. For the F₁ animals, food consumption values were recorded weekly prior to pairing and, for F₁ females, it was also recorded on gestation days 0 to 3, 3 to 6, 6 to 10 and 10 to 13.

Parturition: All dams were allowed to deliver naturally and rear their offspring to weaning (post-natal day 21). The duration of gestation and litter size were recorded. Immediately after parturition (PND 1) to day 21 postpartum, the following parameters were recorded:

- The number of pups born (live and dead)
- Daily live litter size and sex (reported on lactation days 1, 4, 7, 14 and 21)
- Daily clinical observations
- Individual pup weights on days 1, 4, 7, 14 and 21 postpartum
- Necropsy findings for dead and culled pups where condition permitted

Litters were culled to 4 males and 4 females on day 4 of lactation. All surviving main study group F₀ animals with viable pups on lactation day 21, or with total litter loss (within 24 hr of litter loss) and those that did not deliver were euthanized. A gross necropsy was performed on each of these females. The uterus was stained and the number of implantations recorded. Although no histopathological examination was performed, at necropsy the following tissues were retained from all F₀ and F₁ animals. Additionally, the kidneys from one male and one female F₁ pup per litter (randomly selected from surplus pups) were weighed and retained.

Cervix	Uterus
Coagulating gland	Vagina
Ovaries	Lesions
Pituitary gland	Lungs*
Prostate gland	Larynx*
Seminal vesicles	Trachea*
Testes	Head*
Epididymides	

*Tissues were retained from all F₀ females only.

Postnatal Evaluation: Twenty offspring of each sex were randomly selected from each group to form the F₁ generation. All other pups were euthanized and necropsied. The following development parameters were recorded for each pup: day of pinna unfolding, incisor eruption, eye opening, vaginal opening and balano-preputial separation. The following function tests were performed on all pups in each litter: surface righting reflex on day 1 postpartum, air righting reflex on day 17 postpartum, and grip strength reflex, papillary reflex, auditory response and visual placing response on day 21 postpartum. Neurobehavioral tests for effects on learning ability and motor activity were conducted in weeks 5 and 4 for all F₁ animals in the control and high dose groups, respectively. At 12 weeks post-weaning, F₁ animals were cohabited (1 male and 1 female per litter but avoiding sibling mating) within the same treatment group for up to 15 days. The day on which evidence of mating was identified was termed gestation day 0. The males and females were separated and the females were housed in individual mesh cages until gestation day 13, when laparohysterectomies were performed. The ovaries and uteri were removed and the following data were recorded: pregnancy status, number of corpora lutea, number and intrauterine position of implantations subdivided into- live embryos, early intrauterine deaths and late intrauterine deaths.

Results

Mortality: One F₀ female was killed *in extremis* on day 8 of lactation following clinical observation of noisy respiration and gasping. The death was attributed to dosing error as necropsy revealed pale and inflated lungs with dark areas.

Clinical Signs: No significant clinical signs that could be attributed to test substance.

Body Weights: A dose-related significant ($p < 0.05$) increase in body weight gain relative to control was noted for mid and high dose groups between gestation days 17 and 20. Group mean body weights and body weight gains were comparable in all groups for the remainder of the study.

Food Consumption: No significant changes for any of the dose groups relative to control.

Reproductive Performance: F₀ generation: No effect of treatment on the mean duration of gestation (22.1 days). Pregnancy rates in treated and control groups were comparable. Two females in each of the control and low dose groups, and one in each of the mid and high dose groups, showed total litter loss between birth and lactation day 6. The deaths were not attributed to the test substance administration. The mean number of implantations and mean litter sizes were comparable among groups. The survival rates of pups from birth to PND 4 and from PND 4 to lactation day 21 were comparable in all groups. There was no adverse effect of treatment on mean pup body weight gain over the lactation period (Table 3.5.4.1). Necropsy examination of F₀ females sacrificed on lactation day 21 did not reveal any adverse effect of test substance. Also, the necropsy of the surplus F₁ pups from all groups revealed no macroscopic findings that could be attributed to maternal treatment with aliskiren hemifumarate.

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TABLE 3.5.4.1
REPRODUCTIVE PERFORMANCE OF F₀ FEMALES AND SUMMARY OF
F₁ GENERATION PRIOR TO WEANING

	Group 1	Group 2	Group 3	Group 4	Statistics	
Number in group	24	24	24	24		
Number not pregnant	0	0	1	0		
Number pregnant (%)	24 (100.0)	24 (100.0)	23 (95.8)	24 (100.0)		
Number of females with live pups at Day 21 post-partum	22	22	22	22		
Mean duration of gestation (days)	22.1	22.3	22.0	22.1	X	
Mean number of implantation sites	13.7	14.0	13.9	14.4	J	
Mean number of pups born	12.2	13.3	13.1	13.3	J	
Mean number of pups alive Day 1	11.9	12.7	12.7	13.0	X	
Mean % male pups Day 1	50.8	50.4	54.0	54.0	J	
Mean number of pups alive Day 4 before culling	11.4	12.3	12.4	12.9	X	
Mean number of pups culled Day 4	3.7	4.3	4.4	4.9	X	
Mean number of pups alive Day 4 after culling	7.7	8.0	8.0	8.0	X	
Mean number of pups alive Day 7	7.6	8.0	8.0	8.0	X	
Mean number of pups alive Day 14	7.2	7.5	7.2	7.3	X	
Mean number of pups alive Day 21	7.1	7.5	7.2	7.3	X	
Post-implantation survival index †	89.0	95.4	94.0	92.3	F-	
Live birth index †	97.4	96.1	97.2	98.2	F-	
Viability index 1 †	95.7	96.8	97.4	99.4	F-	
Viability index 2 †	98.9	99.4	100.0	100.0	F-	
Viability index 3 †	94.2	93.8	90.3	90.9	F-	
Viability index 4 †	98.5	100.0	99.4	100.0	F-	
Mean weight (g) Day 1:						
Male	6.4	6.2	6.4	6.4	J	
Female	6.0	5.8	6.0	6.1	J	
Combined	6.2	6.0	6.3	6.2	J	
Mean weight (g) Day 4:						
Male	8.8	8.5	8.7	8.7	J	
Female	8.4	8.1	8.4	8.4	J	
Combined	8.6	8.3	8.6	8.6	J	
Mean weight (g) Day 7/8:						
Male	14.8	14.0	14.8	14.3	J	
Female	13.9	13.4	14.3	14.0	J	
Combined	14.4	13.7	14.6	14.2	J	
Mean weight (g) Day 14:						
Male	32.1	31.0	32.5	31.8	J	
Female	30.3	29.7	30.7	30.9	J	
Combined	31.0	30.5	31.6	31.4	J	
Mean weight (g) Day 21:						
Male	52.0	51.0	53.5	53.6	J	
Female	50.0	49.7	51.1	51.5	J	
Combined	51.1	50.2	52.2	52.7	J	
† weight change Days 1 - 21	Combined	733.6	741.8	736.9	752.3	J

Group 1: Control group; Groups 2, 3 and 4: 50, 150 and 250 mg aliskiren/kg/day groups, respectively.

X : not analyzed

J : Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon

F-: Cochran-Armitage and Fisher's Exact (lower tail)

$$\begin{aligned} \text{Live birth index \%} &= \frac{\text{Number of pups alive Day 1}}{\text{Number of pups born}} \times 100 \\ \text{Viability index 1 \%} &= \frac{\text{Number of pups alive Day 4 before culling}}{\text{Number of pups alive Day 1}} \times 100 \\ \text{Viability index 2 \%} &= \frac{\text{Number of pups alive Day 7}}{\text{Number of pups alive Day 4 after culling}} \times 100 \\ \text{Viability index 3 \%} &= \frac{\text{Number of pups alive Day 14}}{\text{Number of pups alive Day 7}} \times 100 \\ \text{Viability index 4 \%} &= \frac{\text{Number of pups alive Day 21}}{\text{Number of pups alive Day 14}} \times 100 \end{aligned}$$

F₁ Generation. Postnatal Development: All developmental landmarks and functional test results were similar among the control and F₁ animals in the treated groups. One male in the high dose group was killed in week 13 following clinical observation of body weight loss, paleness, hunched posture, reduced activity, cold, piloerection and staining around the mouth. Necropsy examination revealed gaseous distension of the jejunum and cecum and markedly enlarged liver and spleen. All F₁ animals survived to the scheduled necropsy. No clinical findings that could be attributed to F₀ test article administration were noted at any dose level. Mean male and female body weights and body weight gains were similar (p >0.05) for treated and control groups during the post-weaning period and from sexual maturity to necropsy. Group mean food intake was similar in all groups. There were no effects on F₁ mating indices, fertility indices or estrous cycles as a result of F₀ test article administration. Intrauterine growth and survival of the F₂ fetuses were unaffected by F₀ maternal treatment at all dose levels. There were no significant differences in mean pre- and post-implantation losses, numbers of corpora lutea, implantation sites and live embryos between control and treated groups (Table 3.5.4.2).

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TABLE 3.5.4.2
REPRODUCTIVE PERFORMANCE OF F₁ GENERATION

	Group 1	Group 2	Group 3	Group 4
Males				
Number in group	20	20	20	20
Number died/killed	0	0	0	1
Number inducing pregnancy	19	20	18	18
Females				
Number in group	20	20	20	20
Number not pregnant	1	0	2	0
Number pregnant (%)	19 (95.0)	20 (100.0)	18 (90.0)	20 (100.0)
Number of females with live embryos on Day 13 gestation	19	20	18	20
Mean number of corpora lutea per female	17.0	16.7	17.0	17.2
Mean number of implantations per female	15.6	15.5	15.3	15.6
Pre-implantation loss:				
mean	8.6	6.6	10.9	8.6
number of dams affected	12	11	14	12
Early intrauterine deaths:				
mean number	0.7	1.1	0.5	1.0
number of dams affected	11	12	9	11
Late intrauterine deaths:				
mean number	0.1	0.1	0.2	0.2
number of dams affected	1	1	3	3
Post-implantation loss:				
mean	4.6	8.3	4.2	7.4
number of dams affected	12	12	10	11
Mean number of embryos per female	15.0	14.4	14.7	14.5

Group 1: Control group; Groups 2, 3, and 4 are 50, 150 and 250 mg aliskiren/kg/day groups, respectively.

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4.0. OVERALL SUMMARY AND EVALUATION

Aliskiren represents a new class of non-peptide, low molecular weight renin inhibitor for the treatment of hypertension. Blockade of the enzyme, renin, at a higher level in the cascade than the currently available ACE inhibitors, blocks the generation of angiotensin I and, consequently, leads to reduced levels of angiotensin II. Aliskiren was evaluated for its potential antihypertensive efficacy in *in vitro* and in various animal models of hypertension. Safety pharmacology studies were conducted to assess its safety in critical organ systems. The disposition of aliskiren across species was conducted using radiolabeled compound. A number of *in vitro* and *in vivo* studies were performed to characterize its kinetics, protein binding, metabolite profile and the enzymes involved in its metabolism. Aliskiren has been tested in a complete range of animal toxicity studies, including carcinogenicity, genotoxicity and reproductive toxicity studies. The present review describes these studies and evaluates their adequacy as support for the administration of aliskiren hemifumarate to hypertensive patients in accordance with the proposed product labeling.

Pharmacodynamics

Selectivity, specificity and potency of aliskiren for human renin was tested *in vitro*. Aliskiren was shown to inhibit human plasma renin with an IC_{50} of 0.6 nM. It was less potent in inhibiting plasma renin from marmoset, dog, rabbit, and rat (IC_{50} s of 2.0, 7.0, 11.0, and 80 nM, respectively). Since renin inhibition with aliskiren was selective for primates, blood pressure lowering effects were evaluated in marmoset monkeys. Though hypertensive rat models are commonly used for researching drugs acting on the RAS, the species specificity exhibited by renin calls for alternate models, especially for drugs such as aliskiren, a selective human renin inhibitor. Thus, aliskiren was tested in transgenic mice and rats. Since aliskiren has poor oral bioavailability, it was administered subcutaneously in some of the studies.

Studies with aliskiren hemifumarate in severely or mildly sodium depleted marmosets demonstrate that aliskiren is effective in lowering mean arterial blood pressure (MAP) and blocking the renin angiotensin system (RAS). The duration of action but not the magnitude of the response increased from 6 to 16 hr after increasing the dose from 3 to 10 mg aliskiren/kg. Both diastolic and systolic blood pressures were lowered. Evidence for RAS blockade is the inhibition of plasma renin activity (PRA) and the rise in plasma renin concentration (PRC), the latter indicating blockade of the RAS at the level of the juxtaglomerular cell¹. Despite large increases in PRC, there was no observed tolerance to the hypotensive effects of aliskiren, and after the cessation of eight days of treatment, no rebound increase in MAP was observed. The smaller MAP-lowering response in the mildly sodium depleted marmosets reflects the observation that

¹ Azizi, M. *et al.*: Pharmacologic demonstration of the synergistic effects of a combination of the renin inhibitor aliskiren and the AT1 receptor antagonist valsartan on the angiotensin II-renin feedback interruption. *J Am Soc Nephrol*; 15:3126-3133, 2004.

Inhibition of PRA by aliskiren decreases the availability of ang II at the receptor sites. This results in rise in PRC as a result of positive feedback.

the magnitude of the hypotensive response to blockers of the RAS in normotensive animals, DOCA-salt treated rats or normal human volunteers is dependent on the degree of activation of the RAS. Test substance (50 mg aliskiren/kg/day for 4 weeks *via* subcutaneous osmotic minipump) significantly ($p < 0.001$) reduced MAP (37 to 47 mm Hg drop relative to sham vehicle group) in both renin-dependent (Goldblatt or 2K1C model²) and -independent (uninephrectomy and clipping of both renal arteries or 1K1C model) hypertensive ApoE^{-/-} mice³. Additionally, aliskiren produced a 94% inhibition of modestly elevated PRA in 2K1C (but not 1K1C) model mice relative to vehicle control. These studies suggest that aliskiren has potential to affect experimental hypertension that may not dependent on the RAS.

Effects of single oral doses (0.3 to 100 mg/kg) and repeat oral doses (1 to 10 mg/kg, once a day for 10 days) of aliskiren were studied in conscious, unrestrained male double transgenic rats⁴ (dTGR [(h-REN)L10J x (h-AOGEN)L1623]). Aliskiren rapidly and dose-dependently reduced mean arterial blood pressure following single doses (13 to 74 mm Hg drop relative to baseline) or repeat doses (30 to 50 mm Hg drop relative to baseline). The highest single oral dose of aliskiren (100 mg/kg) resulted in a prolonged antihypertensive effect that lasted for more than 24 hr, and recovery of MAP to baseline required up to 48 hours. The PK/PD relationship was relatively log-linear over a 10-fold range of doses and over the entire duration of action from peak to recovery. Aliskiren log-linearly and concentration-dependently lowered MAP over its therapeutic range in the dTGRs. The long PK half-life translated into a prolonged duration of antihypertensive effect. The oral hypotensive potency of aliskiren in dTGR is similar to that observed in the sodium-depleted marmoset.

In *safety pharmacology studies*, a single intravenous administration of aliskiren hemifumarate (0.3 or 1 mg aliskiren/kg) produced no adverse effects on the CNS. Intravenous administration of aliskiren hemifumarate (0.3, 1 and 3 mg aliskiren/kg) to anesthetized normotensive rats induced dose-dependent transient reductions in both systolic and diastolic b.p. and a concurrent slight fall in heart rate. However, there were no significant effects on the ECG, respiratory or renal function. Aliskiren hemifumarate was also evaluated *in vitro* for its affinity toward 16 different neurotransmitter receptors. It showed no affinity (at a concentration of 10 μ M aliskiren) for adrenergic, serotonergic, muscarinic, NMDA, AMPA and kainate receptors. Very weak binding was demonstrated at histamine-1 and opiate μ -type receptors. Electrophysiological investigations in the isolated rabbit heart did not reveal any effect of aliskiren (up to 100 μ M) on action potential duration, inter-ventricular conduction or pacemaker activity. Aliskiren was evaluated for effects on potassium current in cloned hERG channels. In this preparation, aliskiren produced a very shallow dose-response over a 100-fold concentration range (10 to 1000 μ M) and failed to

² 2K1C model: a renin-dependent acute renal hypertension, left renal artery is clamped. In 1K1C, a renin-independent chronic hypertension, right kidney is removed and both the renal arteries are clamped at the aortic level.

³ C57/BL/6 background, one renin gene animals, and are hypercholesterolemic.

⁴ Human renin does not effectively cleave rat angiotensinogen, and *vice versa*. The single transgenic rats (i.e., transgenic for either human angiotensinogen or renin) are normotensive. However, when cross-bred, the dTGR offspring develop (at 3 weeks of age) severe and sustained hypertension (due to life-long over-expression of human renin and angiotensinogen. i.e., over-stimulated RAS) with severe organ damage and do not live beyond the seventh or eighth week of age (Pilz, B., *et al.* Aliskiren, a human renin inhibitor, ameliorates cardiac and renal damage in double-transgenic rats. *Hypertension* 46: 569-576, 2005).

achieve a 50% inhibition (IC₂₅ at 670.9 µM). The lowest concentration, 10 µM, had no significant effect.

Drug Disposition (ADME)

The ADME studies for aliskiren were conducted following single and multiple dose (oral or intravenous) administrations across species. Some of these studies involved administration of ¹⁴C-labeled compound. *In vitro* studies were performed to characterize the blood/plasma distribution, plasma protein binding, metabolism and to characterize the enzymes involved in the metabolism of aliskiren in animals and human.

Absorption

Aliskiren hemifumarate, following single or multiple oral administration, was rapidly absorbed in mice, rats and marmosets with T_{max} values of 0.25 hr to 2 hr. Oral absorption was highly variable in all species and ranged from 2 to 25% of the dose (≥ 3.4%, ≥ 4.2%, 25%, ≥ 3% of dose in the rat, mouse, marmoset and human, respectively). Oral bioavailability of aliskiren calculated from AUC values, corrected for dose, was 3%, 2.4%, 1.5% and 1.9 to 2.6% in marmosets, rats, mice and humans, respectively (Table 4.1).

TABLE 4.1
PHARMACOKINETIC PARAMETERS OF ALISKIREN IN PLASMA AFTER A SINGLE ORAL DOSE IN VARIOUS SPECIES

PK parameters	Mouse	Rat	Marmoset	Rabbit	Human
Dose (mg/kg)	500	100	3	200	4.3 ^a (300 mg)
T _{max} (h)	1	0.25	2	0.5	3
C _{max} (µmol/L)	1.45	0.27	0.395	6.59	0.46
C _{max} / dose (µmol/L)/(mg/kg)	0.0029 ^b	0.0027 ^b	0.132 b	0.033 ^b	0.107 ^b
AUC (µmol·h/L)	7.04	3.01	4.55	14.3	2.008
AUC / dose (µmol·h/L)/(mg/kg)	0.014 ^b	0.030	1.52 ^b	0.072 ^b	0.469 ^b
Apparent terminal t _{1/2} (h)	n.a.	n.a.	n.a.	n.i.	48.7 ^c
Absorption (% of dose)	≥ 4.2	≥ 3.4	25	≥ 1.5	≥ 3
Bioavailability (%)	1.5	2.4	3	n.a.	1.9 / 2.6 ^d
Study #	R0301336	R0300781	R0400300	R0300778-01	2223, 0029
Review section #	2.1.1	2.1.2	2.1.4	2.1.5	Not reviewed

n.a.: not applicable. n.i.: not investigated.

a: dose in mg/kg calculated by assuming a body weight of 70 kg

b: calculated value

c: calculated between 48 and 144 hr post administration [study 2223]

d: BAV from study 0029 after oral dosing of 75 mg (1.1 mg/kg) as solution or capsule, respectively

Following oral dosing, the concentration-time profiles of aliskiren (and total radioactivity in case of radiolabeled compound) were highly variable and peaked between 0.25 hr (rat) and 3 hr (human) in all species investigated. In humans and rats, the specific AUC values (after normalization) were lower than in the marmoset and the rabbit. After intravenous administration, aliskiren declined rapidly and multi-exponentially in all species investigated. Terminal half-lives of about 23 and 36 hours were calculated in rats and marmosets, respectively. In humans, similar

terminal half-lives of about 24 to 49 hours were observed. The half-lives for aliskiren and total radioactivity were similar, consistent with aliskiren being the major circulating component.

TABLE 4.2
PHARMACOKINETIC PARAMETERS OF ALISKIREN IN PLASMA AFTER A SINGLE INTRAVENOUS DOSE IN VARIOUS SPECIES

PK parameters	Mouse	Rat	Marmoset	Human
Dose (mg/kg)	10	10	1	0.29 ^a (20 mg)
AUC ($\mu\text{mol}\cdot\text{h/L}$)	9.0	12.6	49	4.20
AUC / dose ($\mu\text{mol}\cdot\text{h/L}$)/(mg/kg)	0.090 ^b	1.26 ^b	49 ^b	14.7 ^b
CL (L/h/kg)	2.01	1.2 ^{b,c}	0.036	0.13
V _{ss} (L/kg)	1.05	7.8 ^{b,c}	0.58	4.1 ^e
Apparent terminal t _{1/2} (h)	n.i.	23.1 ^{b,c}	36 ^d	23.7
Study #	R0301336	R0300781	R0400300	2223, 0029
Review section #	2.1.1	2.1.2	2.1.4	Not reviewed

n.a.: not applicable; n.i.: not investigated.

a: dose in mg/kg calculated by assuming a body weight of 70 kg.

b: calculated value

c: calculated between 8 and 96 hr. The time range 1-24 hr used in the study report was considered too short.

d: calculated between 48 and 168 hr post administration.

e: defined in [study 0029] as volume of distribution during the terminal elimination phase (V_{dβ}).

Distribution

Aliskiren distributed well into the blood cells although, after both i.v. and oral doses, the radioactivity in plasma was much higher than in blood in all species studied. Volume of distribution at steady-state (V_{ss}) and plasma clearance (CL) showed high variability within studies and between the species investigated (Table 4.2). *In vitro* plasma protein binding was highest in marmoset (92%) followed by mouse (71%), rat (62%), rabbit (58%) and human (49-52%). Measurements of the concentrations of the radioactivity in the tissues by whole-body autoradioluminograms showed that the overall exposure to test substance was marginal after either single or multiple (daily) oral doses (for 3, 7 or 10 days) which is in line with the poor to moderate oral absorption. The highest radioactivity concentration was observed in the lumen of the gastrointestinal tract, especially the colon and cecum, reflecting mostly non-absorbed material. Radioactivity was also detected in liver, brown fat and kidney medulla. Radioactivity was eliminated within 24 hr from all tissues, except the intestinal wall and brown fat. On the other hand, intravenous administration of radioactive aliskiren hemifumarate demonstrated rapid and even distribution of aliskiren in all organs and tissues of rats. Highest levels were found in the liver and kidney, followed by moderate levels in the gastrointestinal tract. Embryo-fetal transfer of radioactivity was noted in pregnant rabbits (day 17 of gestation) following single oral doses of radiolabeled aliskiren hemifumarate. Significant concentrations of radioactivity were found in placenta, amniotic fluid and fetuses, suggesting a free passage of parent compound and its metabolites.

Metabolism

In all species investigated, biotransformation occurred from a low (humans and marmosets) to a moderate (mice, rats and rabbits) extent since unchanged parent compound accounted for most of the radioactivity (>90%) in plasma and excreta after both i.v. and oral dosing with [¹⁴C]aliskiren hemifumarate. Only a small part of the absorbed aliskiren was metabolized. The primary biotransformation reactions of aliskiren in rats, mice, marmosets and rabbits were oxidation at the phenolic moiety and the attached side chain by *O*-demethylation, *O*-dealkylation and/or alcohol oxidation to M1, M2, M3 and M4 (Figure 2.1.1.1 in section 2.1.1). CYP3A4/5 enzymes catalyzed these oxidations predominantly. Additionally, amide hydrolysis was observed as well as some glucuronidation of oxidated metabolites to the minor metabolites M5 and M6. All metabolites observed in plasma were also found in the excreta either in free or conjugated form. The same metabolites were found in a human ADME study with ¹⁴C-labeled compound. In human, marmoset and rat feces, additional minor metabolites M12, M13 and M14 were detected. See Table 4.3 for species comparison of metabolites detected in plasma, urine, feces and bile. *In vitro* studies with rat, marmoset and human liver microsomes suggested the following rank order of disappearance of parent compound: marmoset > human > rat. Inhibition of major human cytochrome P450 isoforms by aliskiren was demonstrated *in vitro* in a human lymphoblastoid cell line and human liver microsomes. Aliskiren at 200 μM showed significant and distinct inhibition of CYP2C9 (32%), CYP2C19 (43%) and CYP2D6 (33%). At a lower, more *in vivo* relevant, concentration (25 μM), none of the investigated cytochromes were substantially inhibited by aliskiren. This suggests that metabolic drug-drug interactions of aliskiren with co-medications are unlikely.

TABLE 4.3
OCCURRENCE OF METABOLITES ACROSS SPECIES

Metabolite	Human	Mouse	Rat	Rabbit	Marmoset
M1	X	X	X	X	-
M2	X	X	X	X	X
M3	X	X	X	X	X
M4	X	X	X	X	X
M5	-	X	X	X	-
M6	X	X	X	X	-
M7 ^a	-	-	-	X	-
M8	-	-	X	X	-
M9	X	-	X	X	X
M10 / M11	X	X	X	X	X
M12 ^e	X	-	X ^d	-	-
M13 / M14 ^{b,e}	X	-	X ^d	-	X
Study #	2223	R0301336	R0300781, R0300779,	R0300778-02	R0400300
Review section #	Not reviewed	2.1.1	R0301337, 2.1.2, 2.1.3, 2.3.4	Not reviewed	2.1.4

a: for M7, the two biotransformation steps leading to M2 and M1 were combined.

b: M13 and M14 are isomeric metabolites which contained an additional of C3H4O2 group - they were probably formed in the intestine.

d: M12 and M13 were detected in the rat after multiple oral dosing [study #R0300779].

e: M12, M13/M14 are fecal metabolites

Excretion

Excretion of aliskiren and its metabolites occurred mainly *via* the hepato-biliary route (about 70% of the dose in bile) into feces. Most of the administered radioactivity (>70%) was excreted during the first 24 hr. The main route of excretion was feces, with 91% of an i.v. dose in mice, 82-90% of an i.v. dose in rats and 72-78% of an i.v. dose in marmosets indicating a high biliary excretion. Renal excretion was minor in all species (including human) regardless of route of administration. Urinary recovery of radioactivity after oral or intravenous dosing was approximately 2% of the dose in mice (0-72 hr), 0.3%-10% in rats (0-168 hr), 2%-15% in marmosets (0-168 hr) and 0.6% in human (0-168 hr). Unchanged aliskiren was the main excretory product in urine and feces in all of the species investigated. Only minor proportions of metabolites were detected in excreta. About 0.08% of the administered dose was estimated to be excreted in the milk of lactating rats between 0 and 72 hours. About 27% of the radioactivity in milk was from unchanged aliskiren. Metabolites M2, M5/M6 were detected in trace amounts. Assuming a systemic availability of 6.7% after oral administration for total radioactivity, approximately 1.1% of the systemically available radioactivity was eliminated *via* milk in rats (the sponsor did not provide the basis for this number).

Toxicology

Acute Toxicity

A single dose oral gavage toxicity study was conducted with aliskiren hemifumarate in female rats. No adverse effects were noted at the highest dose administered (2000 mg aliskiren/kg).

Repeat Dose Toxicity

Aliskiren hemifumarate was administered orally to mice for up to 13 weeks, rats for up to 26 weeks and marmosets for up to 39 weeks.

Mice

Dietary administration of aliskiren hemifumarate to mice at doses of 2500 and 5000 mg aliskiren/kg/day was stopped after a week of dosing in a 13 week study due to adverse effects on food consumption and body weight. For the next 2 weeks, the animals in these two groups were fed a diet containing no test substance until they had recovered, after which dosing for these two groups was resumed at 1500 and 2000 mg/kg/day, respectively, and continued for at least 13 weeks. Decrements in body weight gain ($p < 0.05$) were noted for all treated groups (1000 or more mg/kg/day) relative to control in both 4 and 13 week studies. Males were more affected than females. No other treatment-related effects were noted.

Rats

The chronic toxicity of aliskiren was studied at gavage doses of up to 750 mg/kg/day for 13 weeks and 250 mg/kg/day for 26 weeks; and at dietary doses of up to 2500 mg/kg/day for 2 weeks and 1000 mg/kg/day for 13 weeks. Oral gavage administration of aliskiren hemifumarate to rats resulted in deaths or moribund sacrifices of 6 males and 2 females at 500 mg aliskiren/kg/day and 7 males and 8 females at 750 mg aliskiren/kg/day during study days 10 to 91. Histopathology findings in these animals included ulceration and inflammatory exudates in the nasal cavities and nasopharynx, which were, according to the sponsor, due to aspiration of small amounts of the test article either during or after administration. Additionally, moderate erosion in the cecum and ulceration of the colon were noted in the deceased animals. Clinical

signs such as labored respiration, rales, tachypnea, hunched posture and emaciation were noted in animals receiving 500 or more mg/kg/day. A significant ($P < 0.05$) reduction in group mean body weight gain relative to control was noted for males and females at 150 (8 and 14%) and 250 (15 and 16%) mg aliskiren/kg/day between study weeks 12 and 26. There were no changes in food consumption. Oral gavage administration of test substance resulted in histopathological changes that were confined to the respiratory tract and large intestine. Alterations in nasal cavities, nasopharynx and larynx which included minimal to marked inflammatory exudate, minimal to marked ulceration, minimal to moderate squamous metaplasia and slight mucoserous exudate were noted in rats receiving 500 or more mg/kg/day. Minor inflammatory and degenerative changes of the respiratory epithelium at the tracheal bifurcation and lungs were evident in a few animals receiving 150 or 250 mg/kg/day at the 26 week sacrifice. Test substance-related changes in the large intestine, a moderate erosion in the cecum and ulceration in the colon, were observed in 3 females (2 decedents and one terminal sacrifice) receiving 750 mg/kg/day by gavage.

Dietary administration of aliskiren hemifumarate (up to 1000 mg aliskiren/kg/day) for 13 weeks did not result in premature deaths. Body weights were less affected with the dietary administration compared to gavage administration at equivalent doses. At 1000 mg/kg/day, males and females had mean body weight gains at study day 15 that were 19% and 34% lower than control, respectively ($p < 0.05$), but by the end of the study (13 week) the differences from control had decreased to 6% and 8%, respectively ($p > 0.05$). More severe reductions in body weight gain were observed when doses of 1500 or more mg/kg/day were administered for 2 weeks (23 to 67% for males and 12 to 20% for females). In these studies, females exhibited a slightly higher exposure to test substance than did males. There were no test substance-related macroscopic or microscopic findings observed at the scheduled necropsy in animals treated by dietary administration. Gavage administration resulted in a higher AUC and a greater accumulation potential than dietary administration. A significantly greater exposure without deaths or irritant effects (to respiratory tract) was obtained with dietary administration.

Marmosets

The repeat dose toxicity of aliskiren hemifumarate was evaluated in marmosets at oral (gavage) doses of up to 100 mg aliskiren/kg/day for 2 weeks, 50 mg aliskiren/kg/day for 13 weeks (followed by a 4 week drug-free recovery) and 20 mg aliskiren/kg/day for 39 weeks (followed by an 8 week drug-free recovery). Two females, one dosed at 20 and the other dosed at 50 mg/kg/day, were sacrificed moribund on treatment days 73 and 57, respectively. Both had renal cortical tubular degeneration/regeneration attributed to hypotension and poor renal perfusion resulting from treatment with the drug. Treatment-related clinical findings in these studies included increased incidences of vomiting, salivation and diarrhea. Reduction in body weight was evident in the 13 week study. Both males and females at doses of 20 or more mg/kg/day showed mean reductions (4-7%) in body weight. However, the reductions were statistically significant only for females ($P < 0.05$) and were not dose dependent. There were no changes in food consumption. Red blood cell indices (RBC, hemoglobin, and hematocrit) decreased significantly but non-dose dependently in males at 20 or more mg/kg/day and in females at 50 mg/kg/day at study weeks 6 and 13. Significant and dose-dependent increases above concurrent control mean BUN (50 to 266%) were observed at doses as low as 20 mg/kg/day at all weeks (4 to 39) of measurement during the treatment period. Such increases were no longer evident

following a 4- or 8-week recovery period. At termination of 39 weeks of treatment, significant ($p < 0.05$) increases above concurrent control in mean absolute and relative kidney and liver weights were noted for males receiving 20 mg/kg/day. The weights of these organs in the recovery group animals were similar to concurrent control weights. Histopathology considered to be related to treatment was seen in the kidneys. Degeneration/regeneration of cortical tubules and arteriolar hypertrophy were observed in both sexes at doses as low as 5 mg/kg/day administered for 13 weeks and was also evident in recovery group animals. On the other hand, minor grades of hyperplasia of the juxtaglomerular apparatus (JGA) of the kidney and renal interstitial inflammation noted in both sexes at 20 mg/kg/day coupled with significant ($p < 0.05$) increases in creatinine, blood urea nitrogen and absolute and relative kidney weights relative to control were not observed in the recovery group animals. The hyperplasia of the JGA was, according to the sponsor, an expected pharmacological effect rather than an adverse toxic event. According to the sponsor, prolonged periods of reduced blood pressure adversely affect kidney function, particularly glomerular filtration rate and tubule perfusion and function.

Carcinogenicity

The carcinogenic potential of aliskiren hemifumarate was evaluated at dietary doses of up to 1500 mg aliskiren/kg/day in Wistar Hannover rats for 104 weeks and in Tg-rasH2 transgenic mice for 26 weeks.

Rat

The doses selected for the carcinogen bioassay were based on toxicity end-points from the 90 day oral (gavage and dietary) study and the 8 week rising dietary dose palatability study in Wistar rats. A statistically significant decrease in body weight gain (84 to 38% of control) was observed for males (dose-dependent) and females (non-dose-dependent) as the dose was raised from 1000 to 1500 to 2000 to 2500 mg/kg/day (2 weeks at each dose level). A dose of 1000 mg/kg/day was associated with only a small decrease in mean body weight gain for males and females (6% and 8% of control, respectively, $p > 0.05$) after 90 days. There was no other evidence of dose-limiting toxicity. Comparison of AUC values for aliskiren in humans and in rats indicated that the maximum dose chosen for the 2 year rat study (1500 mg/kg/day) provided systemic exposure approximately 4 times that achieved in humans at a clinical dose of 300 mg/day. The Executive CAC concurred with doses proposed by the sponsor (1500, 750 and 250 mg/kg/day) based on body weight data from both the 90 day study and the 8 week rising dose study.

In the 24 month carcinogenicity study, dietary administration of aliskiren at dose levels of up to 1500 mg/kg/day was associated with a dose-dependent decrease in mortality (a negative trend). A dose-related increase in incidence, duration and/or severity of clinical signs consisting of fecal changes, perineal staining, pale appearance and hunched posture was noted for both sexes at 750 or more mg/kg/day. Bloody feces were noted for both sexes at 1500 mg/kg/day suggesting irritant effects of the test substance on the colon and cecum. Statistically significant, dose-dependent reductions in mean body weight (6 to 28.5% that of concurrent control) and mean body weight gain (10 to 47% that of concurrent control) were observed at all doses, suggesting attainment of an MTD. There was a high incidence of food spillage at doses of 750 or more mg/kg/day and less eating might have contributed to the thin and pale appearance of animals in the high dosage group. Microscopic gastrointestinal changes (mucosal epithelial hyperplasia of

duodenum, colon, cecum and rectum; erosion/ulceration of the cecum and colon) and mesenteric lymph node changes (sinusoid dilation and aggregates of large macrophages) were observed in both sexes at doses ≥ 750 mg/kg/day at the 52 week and 104 week sacrifices, with increased incidence and severity at the 102 week sacrifice. The proliferative changes in the intestinal epithelium were considered a consequence of the irritant properties of aliskiren. One colonic adenoma and one cecal adenocarcinoma were observed in different males receiving 1500 mg/kg/day. The incidences of these relatively rare (historical incidence $<0.1\%$) neoplasms (separate or combined) were not statistically significant.

Mouse

Dose selection for the mouse carcinogenicity was based on a 4 week dose range-finding dietary administration study in CB6F1 wild-type mice in which 4 dose levels (500, 1000, 1500 and 2000 mg/kg/day) of aliskiren were evaluated. The sponsor considered 1500 mg aliskiren/kg/day to be the maximum practical dose level for both males and females for the 26-week study in CB6F1-rasH2 mice. This dose was associated with marked decreases in body weight gain (78% of control gain for males and 27% of control gain for females) over the 28 day dosing period, although, by the end of the study (day 29), the difference from control in absolute body weight had decreased to 8% for males and 5% for females from a peak difference of 13% and 8%, respectively (day 8), largely as a consequence of mean body weight gains being much greater than control during the last week of treatment (550 and 300 % of control gain for males and females, respectively). Doses larger than 1500 mg/kg/day were not likely to be tolerated as evidenced by a mean weight loss (7%) for males and a marked (82%) decrease in mean weight gain for females at 2000 mg/kg/day. Comparison of AUC values for aliskiren in humans and wild-type mice indicated that the maximum dose chosen for the 26 week transgenic mouse study provided systemic exposure levels approximately 2.0 to 2.5 times that achieved in humans at a clinical dose of 300 mg/day. The Executive CAC concurred with doses proposed by the sponsor (1500, 750 and 250 mg/kg/day) based on body weight data from the 4 week study.

In the 26 week CB6F1-TgrasH2 mouse study, dietary administration of aliskiren at dose levels up to 1500 mg/kg/day did not elicit clinical signs of toxicity. However, the FDA analysis showed a statistically significant increase in mortality for males (log-rank test, $P=0.0253$) (cause of high dose deaths not determined). Statistically significant decreases in mean body weight or reduced body weight gain relative to control were noted for males at all doses and for females in the high dose group. The main target organs for non-neoplastic lesions noted at 1500 mg/kg/day were: nasal cavity, small and large intestine (hypertrophy of cecum at 750 mg/kg/day), mesenteric lymph node (germinal center development) and gall bladder (dilatation, biliary calculus and increased bile concentration) in both sexes, and reproductive organs and bone marrow (hypocellularity) in females. Delayed or disturbed estrous cycle coupled with ovarian findings (absence or decrease in size/number of corpora lutea with increased presence of old corpora lutea) and endometrial atrophy and decreased organ weights were noted in females receiving 1500 mg/kg/day. The local irritating properties of aliskiren were reflected in a minimal increase in incidence and/or severity of microphage accumulation and/or inflammation in the lung and cytoplasmic inclusion in the respiratory epithelia of the nasal cavity in males at all dose levels (nondose-dependent) and in females at 1500 mg/kg/day, and diffuse hyperplasia in the small intestine and cecum (both sexes) at 1500 mg/kg/day. Focal atypical hyperplasia (pre-neoplastic finding) was observed in the colons of one high dose male and three high dose females. It was

not observed in concurrent controls and is not considered a common spontaneous lesion. The types of neoplasms noted in aliskiren-treated groups were similar to those observed in untreated Tg-rasH2 transgenic mice and the occurrence of these tumors was not considered by the sponsor to be treatment-related. The FDA/CDER analysis also showed no evidence of aliskiren-related tumorigenicity for male or female mice. Positive control mice treated with methyl nitrosourea were characterized by a high incidence of tumors (malignant lymphoma, squamous cell carcinoma or papilloma in the forestomach or skin and adenoma in the lung in both sexes), suggesting a sensitive animal model to identify potential carcinogens.

Toxicokinetics

Comparative systemic exposure ratios at no observed adverse effect dose levels (NOAELs) in the toxicity species are given in Table 4.4. Exposure to aliskiren at the NOAELs was generally less than or similar to that in humans at 300 mg, the maximum recommended dose. The NOAEL (50 mg/kg/day) in the rat study was based primarily on findings associated with the local irritating properties of aliskiren in the respiratory tract following gavage administration, effects not seen with dietary administration at doses up to 1000 mg/kg/day. In marmosets, altered kidney function and early deaths because of marked hypotension were the main dose-limiting effects. A significantly greater exposure without deaths or irritant effects could be obtained with dietary administration.

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TABLE 4.4
ALISKIREN HUMAN EXPOSURE MULTIPLES IN TOXICITY STUDIES

Species	Study number/Rev section	NOAEL ^a (mg/kg)	Sex	AUC _(0-24h) ^b (ng·h/ml)	C _{max} ^b (ng/ml)	Exposure Multiples ^c	
						Based on AUC _(0-24h)	Based on C _{max}
13-wk dietary mice	1940/19	<1000	male	3119	196	1.50	0.60
			female	4605	314	2.20	1.00
26-wk dietary carcinogenicity mice ^e	0410091	<250 ^d	male	77	5	0.04	0.02
			female	69	5	0.03	0.02
26-wk gavage Rat	1940/18 3.2.5	50	male	1006	263	0.50	0.80
			female	650	283	0.30	0.90
104-wk dietary carcinogenicity rats ^f	0370063	<250 ^d	male	874	41	0.40	0.13
			female	890	64	0.40	0.20
13-wk gavage marmoset	NVR019/ 974195	<5 ^d	male	4551	543	2.10	1.70
			female	835	183	0.40	0.60
39-wk gavage marmoset	1940/007	5	male	2182	270	0.20	0.20
			female	2161	351	0.10	0.20
Human ^c	2202	-	male	2135	321	-	-

a: No observed adverse effect level

b: Values at the end of the stated treatment-period except for the mouse carcinogenicity study, see foot note, e

c: based on 300 mg dose in healthy male volunteers, values obtained on day 10, Study #2202

d: A NOAEL was not established in this study

e: Systemic exposures were determined on study days 25 and 151. For males, parameters for day 151 could not be calculated due to an outlier value. Thus, day 25 values, which were not significantly different from day 151 values for both sexes, are used in the Table.

f: Systemic exposures were determined in study weeks 4 and 26. Values determined in week 26 are given in the Table.

Genetic Toxicology

A standard battery of (four) genetic toxicity tests was performed (Ames assay, *in vitro* CHO chromosomal aberration assay, *in vitro* gene mutation test with Chinese hamster V79 cells, and *in vivo* rat micronucleus assay). No positive results for mutagenicity or clastogenicity were obtained. Additionally, a comet assay, which detects DNA damage, was carried out in rats of the strain employed in the 2 year carcinogenicity study, and the effects on liver, colon and cecum mucosa cell DNA evaluated. Aliskiren showed no potential to induce DNA damage in these tissues.

Reproductive Toxicity

Aliskiren hemifumarate was evaluated at doses of up to 250 mg aliskiren/kg/day for its effects on fertility and early embryonic development and pre- and postnatal development in rats. Aliskiren elicited no parental toxicity at the highest tested dose level of 250 mg/kg/day. Furthermore, this

dose did not result in any adverse effects on fertility, early embryonic development, or on the development or reproductive performance of the F₁ generation. No evidence of embryo-fetal toxicity or teratogenicity was noted at doses as high as 600 mg aliskiren/kg/day in a developmental toxicity study conducted in pregnant rats. However, maternal toxicity (decreased and soft stool, diarrhea, salivation) was observed at doses as low as 60 mg/kg/day, the lowest dose evaluated. A developmental toxicity study in pregnant rabbits demonstrated maternal toxicity (deaths and abortions) at doses as low as 50 mg aliskiren/kg/day. A group treated with 200 mg aliskiren/kg/day was terminated early (after 7 doses) as a result of a large number of deaths. All of those rabbits showed inappetence and loss of body weight. Rabbits receiving 50 or more mg/kg/day showed a small but significant dose-dependent reduction in mean body weight gain, food and water intake relative to control. There were no adverse effects of treatment on reproductive performance, although a statistically significant and dose-dependent decrease (2 to 18%) in mean litter weight was noted at 50 or more mg/kg/day. The study failed to identify a NOAEL for maternal or fetal toxicity. Toxicokinetics of aliskiren in plasma were not determined in any of the reproductive toxicity studies. The maximum dose evaluated for teratogenic potential in rats (600 mg/kg/day) is, on a mg/m² basis, about 22 times the maximum recommended human dose of aliskiren (300 mg/day), and the maximum dose that could be evaluated for teratogenic potential in rabbits (100 mg/kg/day) is, on a mg/m² basis, only about 7 times the MRHD of aliskiren. On the other hand, fetal birth weight was adversely affected in rabbits (not in rats) at doses as low as 50 mg/kg/day, the lowest dose used in the study (about 3.6 times the MRHD of aliskiren).

Evaluation

Aliskiren is a potent and selective inhibitor of human renin. The efficacy of aliskiren in inhibiting the RAS was demonstrated by a) sustained and prolonged reductions in mean blood pressure in sodium depleted marmosets, double transgenic rats and renal hypertensive hypercholesteromic mice, b) significant increases in plasma concentrations of active and total renin and c) inhibition of plasma renin activity. The disposition of aliskiren was similar in all species, including human; bioavailability was poor (<3% in all species). Biliary/fecal excretion of aliskiren was the major route of elimination. Biotransformation occurred to a low extent and no human specific metabolites were found.

Toxicity studies in rodents identified local irritation in the gastrointestinal tract and respiratory system as adverse effects of aliskiren. In marmosets, local intolerance to aliskiren in the gastrointestinal tract was reflected as salivation, vomiting and diarrhea. In addition, kidney was an organ of toxicity in marmosets.

Test substance-associated pre-neoplastic focal atypical hyperplasia was noted in the colons of transgenic rasH2 mice (1 of 25 males and 3 of 25 females) at 1500 mg/kg/day ($p > 0.05$) in a 26 week carcinogenicity study. This finding was absent in the concurrent control and, according to the sponsor, is not a common spontaneous lesion. Additionally, mucosal hyperplasia/hypertrophy was noted in both sexes in the cecum at 750 and 1500 mg/kg/day and in duodenum, jejunum, ileum and colon at 1500 mg/kg/day and not in any of the control animals. Similarly, in rats, an increased incidence of mucosal epithelial hyperplasia was noted in the small and large intestine of both sexes at 250 or more mg/kg/day. Cecal erosion and ulceration were noted with increased

incidence in both sexes at 750 or more mg/kg/day. One colonic adenoma and one cecal adenocarcinoma were also observed ($p > 0.05$) in males receiving 1500 mg/kg/day for 24 months. In marmosets, diarrhea was the predominant effect after exposure to high oral dietary doses (50 mg aliskiren/kg/day) for 26 weeks. The sponsor attributes the mucosal hyperplasia/hypertrophy in the gastrointestinal tracts of rodents to the combined irritative effect of aliskiren and the increased bile concentration in the colonic environment (bile acids are known carcinogens in humans and rats⁵).

Gene expression changes were noted in the rat jejunum, ileum, cecum and colon at doses of 250 or more mg/kg/day in 1 and 4 week oral dietary mechanistic studies (not reviewed), suggesting the presence of a stress or toxic injury to the mucosal epithelium, potentially leading to a chronic injury/repair/proliferation cycle. Local irritation, triggered by high local concentrations of aliskiren (due to very low absorption) reflects an inflammatory process and subsequent epithelial reaction with proliferation, growth factor induction and modification in signal transduction pathways due to induction of transcription factors, and was seen as early as 1 week into the treatment. However, the sponsor claims that a 13 week mechanistic study (not reviewed) suggest the presence of an adaptation mechanism, leading to a regression of the inflammatory profile identified in the 1 and 4 week studies. In the 13 week mechanistic study, no proliferative or inflammatory changes were noted at 250 mg/kg/day (suggesting an adaptive response); however, a maintained epithelial reaction was noted in the cecum and colon at 750 or more mg/kg/day. This, according to the sponsor, could result in the chronic inflammatory and proliferative changes observed in these dose groups in the carcinogenicity study. In clinical trials, diarrhea was a prominent finding (6-12%) at the 600 mg dose.

An additional toxic potential of aliskiren was reflected in alterations in serum creatinine and blood urea nitrogen together with increased kidney weights and degeneration and regeneration of cortical tubules, arteriolar or juxtaglomerular hypertrophy/hyperplasia and interstitial inflammation in the kidneys of marmosets receiving 20 or more mg/kg/day. Though biochemical changes and hyperplasia of the juxtaglomerular apparatus were reversible following a 4 or 8 week recovery period, the other renal lesions were still evident. The sponsor relates these other effects to prolonged periods of reduced blood pressure at doses 28-fold higher than the minimally effective pharmacological dose in the marmoset (0.7 mg/kg).

Exposure of the fetus, placenta and amniotic fluid to aliskiren was demonstrated in pregnant rabbits. Additionally, excretion into the milk up to 72 hr after dosing accounted for about 0.08% of the administered dose. Concentrations of aliskiren in milk were similar to or higher than concentrations in plasma. The metabolite pattern in the milk was qualitatively similar to the pattern in plasma. Though there were no teratogenic findings in rats and rabbits, the administration of drugs affecting the RAS during the 2nd or 3rd trimester of pregnancy is known to be associated with fetal malformations and neonatal deaths. A recent publication notes a significant increase in the risk of cardiovascular and central nervous system congenital

⁵ 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.

malformations with the use of ACE inhibitors during the first trimester.⁶ Thus, aliskiren is not recommended for use during pregnancy.

In conclusion, aliskiren did not demonstrate carcinogenic, genotoxic or teratogenic activity in animal studies. The results of the preclinical studies suggest that aliskiren can be used safely in humans for the treatment of hypertension, at the intended therapeutic dose and in accordance with the proposed product labeling.

Recommendations: Approvable

Suggested labeling changes: See page 6

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

⁶ Cooper, W.O. *et al.*: Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med* 354: 2443-51, 2006

Friedman, J.M.: ACE Inhibitors and Congenital Anomalies. *N Engl J Med* 354: 2498-00, 2006.

APPENDICES

104-Week Oral Carcinogenicity Study in Rats: Minutes of Executive CAC Protocol Review

Date of Meeting: May 20, 2003

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
David Jacobson-Kram, Ph.D., HFD-024, Member
Abby Jacobs, Ph.D., HFD-540, Member
John Leighton, Ph.D., HFD-150, Alternate Member
Charles Resnick, Ph.D., HFD-110, Team Leader

Presenting Reviewer: Gowra Jagadeesh, Ph.D., HFD-110

Author of Draft: Charles Resnick, Ph.D.

IND # 62976

Drug: Aliskiren hemifumarate

Sponsor: Novartis Pharmaceuticals Corporation

Background: Aliskiren hemifumarate is a renin inhibitor being investigated for safety and efficacy as an antihypertensive agent. The anticipated maximum human dose is 300 mg/day. The carcinogenic potential of aliskiren hemifumarate is to be assessed by daily dietary administration to Wistar rats for 104 weeks. The animals will be housed in pairs, and food and water will be available ad libitum. Dietary drug concentrations are intended to provide doses of 0, 250, 750 or 1500 mg aliskiren/kg/day (50 rats/sex/group). Doses were selected based on the results of a 90-day dietary administration study and an 8 week rising dose dietary administration study (two weeks at each dosage level) in the same strain of rat. For toxicokinetics study, blood will be drawn from the retro-orbital plexus of surviving animals during study weeks 4 and 39. Additional rats (5/sex/group) will be sacrificed after one year for evaluation of GI irritant effects. Complete necropsies will be performed and histopathologic examinations of all protocol listed tissues will be conducted on all animals.

The 90-day dose range-finding study does not address doses beyond 1000 mg/kg/day, a dose at which there was no significant effect on body weight gain (94 and 92% of control weight gain for males and females, respectively) or other evidence of dose-limiting toxicity at the end of the study. The results of the 8 week rising dose study are inconsistent with those of the former study, particularly as far as the body weight effect at 1000 mg/kg/day in females is concerned (female group mean weight gain ranged from 52-68% of control weight gain at doses ranging from 1000 to 2500 mg/kg/day with no relationship to dose level). On the other hand, a clearly dose-related decrease in weight gain (84-38% of control) was observed for males as the dose was raised from 1000 to 1500 to 2000 to 2500 mg/kg/day. On the basis of the 90 day study, the MTD for aliskiren hemifumarate in the Wistar rat is greater than 1000 mg aliskiren/kg/day for both males and females. Results of the 8 week rising dose study, although inconsistent with those of the 90 day study, suggest that dose levels cannot go much higher than 1000 mg/kg/day without

encountering excessive reduction in body weight gain. The division considers the sponsor's choice of 1500 mg/kg/day as the high dose for the 2-year study to be a reasonable choice.

Executive CAC Recommendations and Conclusions

- The Committee concurs with the doses proposed by the sponsor (1500, 750 & 250 mg/kg/day) based on data from both the 90 day dose range-finding study and the 8 week rising dose toxicity study.
- The Committee recommends that satellite animals, rather than main study animals, be used for toxicokinetics evaluation.
- The Committee recommends that the animals in this feeding study be individually housed rather than housed in pairs.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

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/HFD-110
/CResnick, HFD-110
/GJagadeesh, HFD-110
/DAllis, HFD-110
/ASeifried, HFD-024

26-Week Oral Carcinogenicity Study in CB6F1-rasH2 Mice: Minutes of Executive CAC Protocol Review**Executive CAC**

Date of Meeting: October 26, 2004

Committee:

David Jacobson-Kram, Ph.D., HFD-024, Chair
Abby Jacobs, Ph.D., HFD-540, Member
Joseph Contrera, Ph.D., HFD-901, Member
Charles Resnick, Ph.D., HFD-110, Team Leader

Presenting Reviewer: Gowra Jagadeesh, Ph.D., HFD-110

Author of Draft: Gowra Jagadeesh, Ph.D.

IND # 62976

Drug: Aliskiren hemifumarate

Sponsor: Novartis Pharmaceuticals Corporation

Background: Aliskiren hemifumarate is a renin inhibitor being investigated for safety and efficacy as an antihypertensive agent. The anticipated maximum human dose is 300 mg/day.

Carcinogenicity Study Protocol: The carcinogenic potential of aliskiren hemifumarate is to be assessed by daily dietary administration to TgrasH2 mice for 26 weeks. The dietary mode of administration was chosen by the sponsor after doses of 350 or more mg/kg/day administered by oral gavage for 2 weeks resulted in inflammatory lesions in the nasal cavities of CD-1 mice. Similarly administered doses of 1000 mg/kg/day resulted in deaths and/or severe microscopic changes in the respiratory tract that were attributed to aspiration of dosing solution. In the same study, doses as high as 1000 mg/kg/day administered in the diet did not result in similar findings. Animals will be housed individually (males) or in pairs (females), and food and water will be available *ad libitum*. Dietary drug concentrations are intended to provide doses of 0, 250, 750 or 1500 mg aliskiren/kg/day (25 rats/sex/group). Animals will be monitored for clinical signs and effects on body weight and food consumption. For toxicokinetics study, blood will be drawn from satellite animals during study weeks 4 and 22. Complete necropsies will be performed and histopathologic examination of all protocol listed tissues will be conducted on all animals.

Basis for Dose Selection: Effects on body weight and body weight gain in a 4 week dietary administration study in CB6F1 wild-type mice suggest that an MTD was achieved at 1500 mg/kg/day. There is no other basis for selecting a high dose for the 26 week transgenic mouse study. The sponsor considers 1500 mg aliskiren/kg/day to be the maximum practical dose level for both males and females for the 26-week study in CB6F1-rasH2 mice. This dose was associated with marked decreases in body weight gain (78% for males and 27% for females) over the 28 day dosing period, although, by the end of the study (day 29), the difference from control in absolute body weight had decreased to 8% for males and 5% for females from a peak

difference of 13% and 8%, respectively (day 8), largely as a consequence of mean body weight gains being much greater than control during the last week of treatment (550 and 300 % of control for males and females, respectively). Doses larger than 1500 mg/kg/day are not likely to be tolerated as evidenced by a mean weight loss (7%) for males and a marked (82%) decrease in mean weight gain for females at 2000 mg/kg/day. The marked body weight effects observed in this study may have been related to less consumption of food because of poor palatability. However, food consumption was not measured in the dose range-finding study. Comparison of AUC values for aliskiren in humans and wild-type mice indicates that the maximum dose chosen for the 26 week transgenic mouse study provides systemic exposure levels approximately 2.0 to 2.5 times that achieved in humans at a clinical dose of 300 mg/day. The division considers the sponsor's choice of 1500 mg/kg/day as the high dose for the 26 week transgenic mouse study to be a reasonable choice.

Executive CAC Recommendations and Conclusions

The Committee concurs with the doses proposed by the sponsor (1500, 750 and 250 mg/kg/day) based on body weight data from the 4 week study in CB6F1 wild-type mice.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

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/HFD-110
/CResnick, HFD-110
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**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Gowra Jagadeesh
9/27/2006 09:53:56 AM
PHARMACOLOGIST

Charles Resnick
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