Exposure concentrations and inhaled doses for (R,R)-Formoterol treatment groups.

Group	Minute volume/BW L/min/kg[a]		Exposure Duration (min)	Mean Exposure concentration[b], µg/L		Target exposure concentration, µg/L		Mean Estimated Dose µg/kg/day (% of target)	
	Male	Female		Male	Female	Male	Female	Male	Female
Low Dose	0.510 0.490 0.419	0.708 0.644 0.615	15	0.66	0.65	0.65 0.68 0.80	0.47 0.52 0.54	4.4 (88%)	6.2 (125%)
Mid Dose	0.606 0.537 0.560	0.496 0.390 0.489	15	5.0	5.4	4.4 4.97 4.76	5.38 6.84 5.45	42.6 (106%)	38.8 (97%)
High Dose	0.505 0.537 0.448	0.538 0.570 0.525	15	11.0	9.8	9.24 8.69 10.41	8.68 8.19 8.88	77.4 (111%)	78.7 (112%)

a. The mean minute volume to body weight ratio (L/min/kg) for each male and female group was used for these calculations. Minute volume determinations were performed near the start of dosing (12/14-12/16/99) and 7 additional times during the study (2/8-2/9/00, 3/21-3/23/00, 4/26/00, 5/25/00, 6/22-6/23/00, 7/25/00, and 8/30/00). Only the first 3 sets of minute volume determinations (12/1999, 2/2000, and 3/2000) were used for calculation of target concentrations. The mean body weight of each group (by sex) determined closest to the dates of the minute volume determinations were used for calculations. The effective dates for calculated target concentrations and mean minute volume/body weight values were 12/16/99, 2/10/00, and 3/24/00. The sponsor considered measurements collected after March 2000 to be inappropriate for use, because very high minute volume values were obtained for some animals that were apparently highly stressed and/or low minute volume values were obtained from other animals that were most likely inaccurate.

b. Actual exposure concentrations of free base were determined by chemical analysis of aerosol samples collected on filters. For each exposure (male and female), one aerosol sample of the atmosphere was collected on a 25-mm glass fiber filter held in an in-line filter holder. The filter holder was connected to the nose-only mask dedicated to atmosphere sampling. The mass of formoterol free base on each filter was determined using an HPLC method. The actual exposure concentration (as free base) was calculated by dividing the analytically determined mass of free base by the sample volume.

Aerosol particle size determinations were to be conducted for the low and high concentrations of R,R-Formoterol. Aerosol particle size determinations were conducted using a seven-stage cascade impactor. Glass-fiber filters were used as collection substrates. Formoterol free base collected on the substrates was chemically analyzed by HPLC. Particle size was calculated based on impactor stage cut-offs. A particle size determination could not be conducted for the low concentration due to insufficient amounts of material collected from the upper stages of the cascade impactor.

Aerosol particle size in test atmosphere.

Dose	MMAD	GSD
70 µg/kg/day	0.7	1.62

Target, estimated, and deposited doses (µg/kg/day) for dogs exposed to low, mid, and high doses of (R,R)-Formoterol.

Target Dose µg/kg/day	Estimated Inhaled Dose µg/kg/day		Deposited Dose[a] µg/kg/day	
	Male	Female	Male	Female
5	4.4	6.2	0.9	1.2
40	42.6	38.8	8.5	7.8
70	77.4	78.7	15.5	15.7

a. The deposited dose was calculated from the estimated inhaled dose using a deposition factor of 0.20.

The control group was exposed to an aerosol of the vehicle, 0.9% Sodium Chloride (USP), at a level approximately equivalent to the saline concentration in the test atmosphere for the high dose group.

Route, form, volume, and infusion rate: Nose-only aerosol inhalation

Animals of the same sex were exposed by group using mask-type, nose-only exposure systems operated under dynamic conditions to maintain a minimum O_2 content of 19% and an evenly distributed exposure atmosphere. During exposures, each animal was restrained in canvas sling apparatus. A mask-type, nose-only exposure apparatus was secured over the animal's muzzle and a flexible airline was attached to the mask. To provide a seal around the dog's snot and prevent dilution by room air, a flexile rubber diaphragm with a hole for the snout was attached to the open side of the mask. To allow for acclimation to restraint and the normalization of the breathing pattern, each dog was exposed to an aerosol of saline for at least 5 min (15 min during ECG evaluations) prior to exposure to the test article. Three exposure material-dedicated generation and exposure systems, consisting of one system for the control group, one system for low and mid dose groups, and one system for the high dose group.

For test article exposures of treatment groups, liquid droplet aerosol atmospheres were generated by jet nebulization from saline solutions of the test article. A modified ⊏ ⊐ collision nebulizer was used to produce aerosols with particle sizes <2 µm. A diagram of the exposure system is shown in the review of the 13-week inhalation study with (R,R)-formoterol in dogs. b(4)

Observations and times:
Clinical signs: Animals were observed twice daily for mortality and moribundity. Animals were monitored for clinical signs of toxicity during the time of exposure and approximately 1-2 hr after exposure. Physical examinations were conducted once per week. Animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and electrocardiogram.
Body weights: Body weights were measured weekly.
Food consumption: Individual food consumption was recorded daily and weekly averages were reported.
Ophthalmoscopy: Ophthalmic examinations were conducted on all animals prior to the start of treatment (week -1) and during week 38.

EKG: Modified lead 1, 2, 3 electrocardiograms (ECGs) for evaluation of heart rate and/or cardiac rhythm were recorded for each dog using a [redacted] prior to the start of treatment (week -1), at 6 months of exposure (week 26), and during the last week of exposure (week 38). "The purpose of these ECGs was to permit a standard evaluation for persistent effects on heart rate and/or cardiac rhythm of long-term exposure to the test article." In addition, ECGs were also recorded using a [redacted] The [redacted] ECGs, which were used only for heart rate evaluation, were recorded for 3-5 min and were collected near the end of the saline and test article exposures during weeks 0 and 38. The ECGs during week 0 were collected during and following the first exposure for each animal. Also during week 0 (following the first exposure for each animal) and 38, ECGs were recorded using the three-channel [redacted] The [redacted] ECGs were recorded at 2, 4, and 24 hr after completion of exposure. For the control group, only the 2 hr time point was collected (i.e., the 4 and 24 hr time points were omitted). b(4)

Hematology: Blood samples for determination of hematology parameters were collected prior to test article exposure (week -3), at 6 months of dosing (week 25), and prior to scheduled necropsy (week 38).

Clinical chemistry: Blood samples for determination of serum chemistry parameters were collected prior to test article exposure (week -3), at 6 months of dosing (week 25), and prior to scheduled necropsy (week 38).

Urinalysis: Urine samples for determination of urinalysis parameters were collected prior to test article exposure (week -3), at 6 months of dosing (week 25), and prior to scheduled necropsy (week 38).

Gross pathology: Necropsy examinations were conducted on all animals. Three animals were found dead. Remaining animals were sacrificed at the scheduled termination.

Organs weighed: Absolute and relative organ weights were determined for the brain, kidneys, liver, lungs, heart, spleen, thymus, uterus, ovaries (without oviducts), testes with epididymides, thyroid with parathyroid, and adrenals.

Histopathology: Tissues were processed into paraffin blocks, sectioned at 5-8 µm, mounted on glass microscope slides, stained with hematoxylin and eosin, and examined by light microscopy. Tissues were examined for animals found dead and all animals sacrificed at the scheduled necropsy.

Toxicokinetics: Blood for measurement of plasma drug levels were collected from 4 dogs/sex/group after the first exposure and during the last week of exposure (week 38). Samples were collected at 0.5, 1, 2, 6, and 24 hr after completion of exposure. Samples were analyzed by [redacted] Plasma concentrations of formoterol and the desformoterol/formoterol ratios were measured using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The validated quantitation range of formoterol was 2.50 to 200 pg/mL using a 1-mL sample volume. Because the assay method lacks chiral specificity, concentration data were expressed as formoterol. Although, the method was not validated to quantify desformoterol, a metabolite of formoterol, its multiple reaction monitoring (MRM) mass channel was determined and acquired for qualitative purposes only. The signal for the desformoterol MRM mass channel was assumed to be approximately the same as that for formoterol. Toxicokinetic analysis was conducted by [redacted] b(4)

Results:

Mortality: One control male dog (#6896) and two male dogs (#6901 and #6888) in the high dose group were found dead during exposures on days 244, 35, and 151, respectively. Causes of death could not be determined from gross pathological or histopathological examinations of tissues; however, the two deaths in the high dose group are assumed to be treatment-related based upon observations of mortality at this dose level in the 13-week study. The sponsor speculated that these two deaths could have resulted from an excessive exposure or a series of excessive exposures to the test article. Dogs that become agitated and hyperventilate during restraint and inhalation exposures may receive an inhaled drug dose in excess of the target level.

One control male dog (#6896) died during exposure on day 244. Histopathological changes identified for this animal were as follows. Multifocal hemorrhage was observed in the brain. Unilateral, multifocal medullary mineralization was observed in one kidney. Focal mononuclear infiltrate was observed in the liver. Focal hemorrhage was observed in the submandibular lymph node. Multifocal congestion was observed in the lungs. Multifocal, subacute inflammation was observed in the submucosa of the larynx. A cyst was observed in the parathyroid gland. Multifocal congestion was observed in the salivary glands. Multifocal hemorrhage was observed in the cervical region of the spinal cord. Multifocal lymphoid depletion was observed in the spleen. Unilateral, multifocal, multinucleated giant cells were observed in one testis. Multifocal hemorrhage was observed in the thymus gland. The sponsor did not assert a cause of death for this animal, although, multifocal hemorrhage observed in the brain and cervical region of the spinal cord may suggest that a traumatic injury occurred during exposure.

Two male dogs, #6901 and #6888, in the high dose group died during exposures on days 35 and 151, respectively.

Histopathological changes in tissues identified for male dog #6901 that died during exposure on day 35 were as follows. Multifocal gliosis was observed in the brain. Multifocal mononuclear infiltration was observed in the submucosa of the gall bladder. Bilateral, multifocal medullary mineralization was observed in the kidneys. Multifocal mononuclear infiltration was observed in the liver. Multifocal congestion and serous exudate were observed in the lungs. Multifocal, subacute inflammation was observed in the submucosa of the larynx. Multifocal hemorrhage was observed in the medullary lymph node. Multifocal, subacute inflammation was observed in the submucosa of the septum and turbinates (middle turbinate for level 4) for nasal levels 4 and 5. Bilateral, multifocal, multinucleated giant cells were observed in the testes. Multifocal hemorrhage was observed in thymus. The cause of death could not asserted.

Histopathological changes in tissues identified for male dog #6888 that died during exposure on day 151 were as follows. Multifocal congestion was observed in the duodenum, jejunum, ileum, cecum, colon, and rectum. Unilateral, focal, subacute inflammation was observed in one epididymis. Multifocal, mononuclear infiltration was observed in the submucosa of the gall bladder. Bilateral, multifocal medullary mineralization was observed in the kidneys. Multifocal brown pigment was observed in the submandibular lymph node. Multifocal, subacute inflammation was observed in the

submucosa of the larynx. Multifocal, subacute inflammation was observed in the ventral turbinates of nasal level 4. A multifocal cyst was observed in the pars distalis of the pituitary gland. Multifocal, subacute inflammation was observed in the hair follicles of the skin. Multifocal, lymphoid depletion was observed in the spleen. Bilateral, multifocal, multinucleated giant cells were observed in the testes. Multifocal hemorrhage was observed in the thymus gland. The cause of death could not asserted, although, congestion was evident throughout the small and large intestines.

Clinical signs:

Treatment-related clinical signs, consisting of flushing of the body surface and facial area, reddened ears, and reddened gums, were observed throughout the treatment period at all dose levels. These clinical signs were noted primarily at 1-hr post-exposure. In many cases, the incidence of clinical signs were higher at the mid dose as compared to the high dose.

There were additional clinical signs noted at 1-hr post-exposure for treatment groups; however, dose-response relationships were weak or the increased incidences of selected findings were primarily confined to one sex. Hypoactivity was increased for male and female treatment groups. Partial closure of the right and left eyes was increased for male and female treatment groups. Hyper-reactivity to touch was increased for female treatment groups. The incidence of injected sclera for the right and left eyes was increased for male and female treatment groups, although, it was more predominant for female treatment groups. The incidence of clear discharge, right and left eyes, was increased for male and treatment groups. The incidence of soft feces and diarrhea were increased for male treatment groups.

Clinical findings at the time of exposure (total occurrence/# of animals).

Clinical signs	Sex	Control	Low dose	Mid dose	High dose
Behavior/CNS					
-partial closure, right eye	M	1/1	1/1	0/0	0/0
	F	0/0	0/0	3/1	3/2
-partial closure, left eye	M	1/1	1/1	0/0	0/0
	F	0/0	0/0	5/1	3/2
-hyper-reactivity to touch	M	16/3	8/1	1/1	0/0
	F	2/2	0/0	1/1	17/2
Body/Integument					
-body flushed	M	1/1	4/3	1/1	4/2
	F	2/2	2/1	0/0	5/4
-flushed facial area	M	2/2	5/3	5/3	5/2
	F	2/2	7/2	1/1	11/2
Eyes/Ears/Nose					
-reddened right ear	M	9/3	18/4	8/4	8/4
	F	12/4	5/2	1/1	24/4
-reddened left ear	M	9/3	17/4	7/4	7/4
	F	12/4	4/2	1/1	22/4
-injected sclera, left eye	M	37/4	84/4	60/4	39/3
	F	33/4	45/4	52/4	73/4
-injected sclera, right eye	M	37/4	84/4	62/4	38/3
	F	31/4	45/4	52/4	72/4
-clear discharge, right eye	M	3/3	46/3	163/4	14/3
	F	159/3	270/3	327/4	226/4
-clear discharge, left eye	M	2/2	54/3	42/4	14/3
	F	151/3	226/3	275/4	236/4
Oral/Dental					
-gums reddened	M	0/0	0/0	1/1	3/3
	F	1/1	2/2	1/1	3/2
Excreta					
-soft feces	M	3/1	49/4	85/4	60/4
	F	33/4	25/4	11/4	19/4
-diarrhea	M	1/1	8/3	35/2	43/2
	F	1/1	3/1	1/1	4/1

Clinical findings at 1 hr after exposure (total occurrence/# of animals).

Clinical signs	Sex	Control	Low dose	Mid dose	High dose
Behavior/CNS					
-hypoactivity	M	0/0	13/3	9/3	13/4
	F	0/0	2/2	2/1	2/2
-partial closure, right eye	M	0/0	1/1	3/1	3/1
	F	1/1	0/0	5/2	4/3
-partial closure, left eye	M	0/0	1/1	4/1	4/2
	F	2/1	0/0	5/2	4/3
-hyper-reactivity to touch	M	3/1	46/1	13/3	3/1
	F	2/1	6/3	15/3	23/2
Body/Integument					
-body flushed	M	6/3	370/4	961/4	666/4
	F	5/3	438/4	846/4	1008/4
-flushed facial area	M	47/4	270/4	469/4	444/4
	F	8/2	289/4	479/4	628/4
Eyes/Ears/Nose					
-reddened right ear	M	7/4	327/4	647/4	509/4
	F	23/3	314/4	492/4	743/4
-reddened left ear	M	7/4	332/4	642/4	502/4
	F	19/3	310/4	481/4	740/4
-injected sclera, left eye	M	140/4	267/4	268/4	216/4
	F	150/4	260/4	320/4	421/4
-injected sclera, right eye	M	146/4	252/4	280/4	204/4
	F	146/4	264/4	314/4	422/4
-clear discharge, right eye	M	4/2	71/4	218/4	28/4
	F	209/4	277/4	385/4	262/4
-clear discharge, left eye	M	3/2	84/4	69/4	27/4
	F	201/4	247/4	333/4	273/4
Oral/Dental					
-wet clear material round mouth	M	7/4	24/4	15/4	14/4
	F	5/2	16/4	8/3	12/4
-gums reddened	M	18/3	272/4	573/4	438/4
	F	28/4	381/4	487/4	661/4
Excreta					
-soft feces	M	9/4	44/4	50/4	40/3
	F	24/4	20/3	4/4	19/4
-diarrhea	M	0/0	7/3	27/2	15/2
	F	6/3	1/1	0/0	0/0

Body weights: There were no treatment related effects on body weight gain. Body weights for male controls at weeks 0 and 39 were 7.4 and 10.4 kg, respectively, yielding a 40.5% increase of initial body weights. For male dogs in the low, mid, and high dose groups, body weights at week 39 were increased by 59.7, 45.8, and 48.6% of initial body weights at week 0, respectively. Body weights for female controls at weeks 0 and 39 were 5.8 and 8.4 kg, respectively, yielding a 44.8% increase of initial body weight. For female dogs in the low, mid, and high dose groups, body weights at week 39 were increased by 50.9, 46.6, and 41.7% of initial body weights at week 0; respectively.

Food consumption: There were no treatment-related effects on food consumption.

Ophthalmoscopy: No treatment-related effects were identified from ophthalmic examinations conducted with each animal during week 38.

Electrocardiography: Heart rate and electrocardiogram were evaluated on day 0 and during weeks 26 and 38. The evaluation at week 26 was conducted only prior to dosing. On day 0 at 2- and 4-hr post-exposure, elevated heart rates (and sinus tachycardia) were evident for male and female dogs at all dose levels. At 4-hr post-exposure, ventricular tachycardia was evident for 1 female dog in the high dose group. At 24-hr post-exposure, ventricular tachycardia and R on T depolarizations were evident for this dog. Further at 24-hr post-exposure for 1 male dog in the high dose group, ventricular premature beat, slow couplets, rapid premature tachycardia, and bigeminy were observed. During week 38, atrial (supraventricular) premature depolarization was observed for 1 female dog in the mid dose group prior to dosing, 1 female dog in the low dose group at 2-hr post-exposure, and for 1 female dog in the high dose group at 24-hr post-exposure. Exercise periods were discontinued at the beginning of the study due to concerns regarding drug-induced changes of heart rate and rhythm.

Week 0: At 2- and 4-hr post-exposure, sinus tachycardia was evident for male and female dogs at all dose levels. At 2- and 4-hr post-exposure, ventricular tachycardia was observed for 1 female dog (#6910) in the high dose group. At 24-hr post-exposure, sinus tachycardia was evident for only 1 male dog in the high dose group. Further, ventricular tachycardia and R on T depolarizations were observed for this same female dog (#6910) in the high dose group. Ventricular premature depolarizations, periods of slow couplets, rapid premature tachycardia, and bigeminy were observed for 1 male dog (#6892) in the high dose group.

Ventricular tachycardia is a continuous series of three or more ventricular premature complexes (VPCs) (Miller, M.S. and Tilley, L.P., Electrocardiography, In: Canine and Feline Cardiology, Editor: Fox, P.R., New York, Churchill Livingstone, 1988, pages 43-89). It may be intermittent (e.g., paroxysmal) or persistent and sustained. Ventricular tachycardia is generally considered a serious and life-threatening tachyarrhythmia. The same conditions that cause VPCs also cause ventricular tachycardia. VPCs are impulses that arise from an ectopic ventricular focus. They spread through both ventricles with delay. VPCs in the dog comprise the most frequent arrhythmia after sinus tachycardia. Two possible mechanisms for VPCs are reentry and increased automaticity. The R on T depolarizations observed for 1 female dog in the high dose group indicate that ventricular depolarization (QRS complex) leading to

contraction initiated prior to the completion of ventricular repolarization (T wave). A couplet refers to two successive ventricular premature beats. Ventricular bigeminy is an arrhythmia consisting of the repeated sequence of one ventricular complex followed by one normal beat.

The sponsor suggested that the female dog in the high dose group with an apparent ventricular tachycardia might have actually had a wide QRS supraventricular tachycardia. While the tachycardia was regarded to pose no immediate risk, the ventricular ectopia occurring only at the highest dose was considered an undesirable and potentially serious finding. These findings were considered transient, being observed only during the first week of exposure. However, the sponsor ignored the occurrences of atrial (or supraventricular) premature depolarization at all dose levels during week 38.

ECG Evaluations with first drug exposure (week 0) at 2, 4, and 24 hr post-exposure.

Week 0: Time	Diagnosis	Control		Low Dose		Mid Dose		High Dose	
		M	F	M	F	M	F	M	F
2-hr post-exposure	Sinus tachycardia	0/4	0/4	**3/4**	**2/4**	**3/4**	**4/4**	**3/4**	**3/4**
	Ventricular tachycardia	0/4	0/4	0/4	0/4	0/4	0/4	0/4	**1/4** (6910)
4-hr post-exposure	Sinus tachycardia	-	-	**3/4**	**4/4**	**4/4**	**4/4**	**4/4**	**3/4**
	Ventricular tachycardia	-	-	0/4	0/4	0/4	0/4	0/4	**1/4** (6910)
	Not Collected	4/4	4/4	-	-	-	-	-	-
24-hr post-exposure	Sinus tachycardia	-	-	0/4	0/4	0/4	0/4	**1/4** (6888)	04
	Ventricular tachycardia	-	-	0/4	0/4	0/4	0/4	0/4	**1/4** (6910)
	Ventricular premature beat	-	-	0/4	0/4	0/4	0/4	**1/4** (6892)	04
	R on T depolarizations	-	-	0/4	0/4	0/4	0/4	0/4	**1/4** (6910)
	Slow couplet	-	-	0/4	0/4	0/4	0/4	**1/4** (6892)	0/4
	Rapid premature ventricular tachycardia	-	-	0/4	0/4	0/4	0/4	**1/4** (6892)	0/4
	Bigeminy	-	-	0/4	0/4	0/4	0/4	**1/4** (6892)	0/4
	Not Collected	4/4	4/4	-	-	-	-	-	-

Heart rate was significantly increased for all male and female treatment groups during exposure and at 2- and 4-hr post-exposure. By 24-hr post-exposure, heart rate had returned to levels observed prior to exposure except for 1 male dog (#6888) in the high dose group.

Heart Rate-Week 0 (Values in parentheses are percent of saline exposure).

Time	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
During saline exposure	158	115	127	140	128	159	116	138
During test article exposure	164 (104%)	116 (101%)	153 (120%)	**197** (141%)	**217** (169%)	**230** (145%)	**222** (191%)	**231** (167%)
2 hr post-exposure	138 (87%)	102 (89%)	**215** (169%)	**214** (153%)	**200** (156%)	208 (131%)	**207** (178%)	**223** (162%)
4 hr post-exposure	-	-	177 (139%)	178 (127%)	**210** (164%)	**217** (136%)	**215** (185%)	**215** (156%)
24 hr post-exposure	-	-	132 (104%)	142 (101%)	130 (102%)	142 (89%)	150 (129%)	152 (110%)

Week 26: During week 26 at 20-26 hr post-exposure, no treatment-related effects on heart rate or electrocardiogram were evident.

Week 38: At 20-26 hr post-exposure, atrial (or supraventricular) premature depolarization was evident for one female dog (#6926) in the mid dose group. Sinus tachycardia was evident for 1 female dog (#6913) in the high dose group. At 2-hr post-exposure, atrial premature depolarization was evident for 1 female dog (#6923) in the low dose group. Sinus tachycardia was evident for 1 female dog (#6909) at the mid dose and 1 female dog (#6910) at the high dose. At 4-hr post-exposure, the only findings were sinus tachycardia for 1 female dog (#6926) at the mid dose and 1 female dog (#6910) at the high dose. At 24-hr post-exposure, atrial premature depolarization was observed for 1 female dog (#6918) in the high dose group. The sponsor stated that atrial (or supraventricular) premature depolarization might be an exaggerated sinus arrhythmia.

ECG Evaluations during week 38 at 2, 4, and 24 hr post-exposure. Animal number(s) effected are designated in parentheses. One control male and two males in the high dose group died prior to evaluation.

Week 38: Time	Diagnosis	Control		Low Dose		Mid Dose		High Dose	
		M	F	M	F	M	F	M	F
20-26 hr post-exposure	Sinus tachycardia	-	-	04	0/4	0/4	04	0/2	**1/4** (6913)
	Atrial premature depolarization	-	-	0/4	0/4	0/4	**1/4** (6926)	0/2	0/4
	Not Collected	3/3	4/4	-	-	-	-	-	-
2-hr post-exposure	Sinus tachycardia	0/3	0/4	0/4	0/4	0/4	**1/4** (6909)	0/2	**1/4** (6910)
	Atrial premature depolarization	0/3	0/4	0/4	**1/4** (6923)	0/4	0/4	0/2	0/4
4-hr post-exposure	Sinus tachycardia	-	-	0/4	0/4	0/4	**1/4** (6926)	0/2	**1/4** (6910)
	Not Collected	3/3	4/4	-	-	-	-	-	-
24-hr post-exposure	Atrial premature depolarization	-	-	0/4	0/4	0/4	0/4	0/4	**1/4** (6918)
	Not Collected	3/3	4/4	-	-	-	-	-	-

Atrial premature depolarization or complexes (APCs) arise from ectopic atrial foci (Miller, M.S. and Tilley, L.P., Electrocardiography, In: Canine and Feline Cardiology, Editor: Fox, P.R., New York, Churchill Livingstone, 1988, pages 43-89). APCs are frequently caused by cardiac disease and may progress to atrial tachycardia, atrial flutter, or atrial fibrillation. Rare APCs can be a normal finding in very aged dogs and cats. APCs are often caused by atrial enlargement from acquired chronic valvular insufficiency, primary myocardial diseases, congenital heart diseases, right atrial hemangiosarcoma, hyperthyroidism, digitalis toxicity, general anesthesia, and various drugs, chemicals, and noxious stimuli. Adrenergic stimulants are known to precipitate APCs (Josephson, M.E. and Zimetbaum, P., Chapter 230 The Tachyarrhythmias. In: Harrison's Principles of Internal Medicine 15th Edition, Editors: Braunwald, E. et al., McGraw-Hill, Washington, D.C., 2001, pages 1292-1309). Spontaneous atrial premature beats were observed in 14 of 3000 (0.47%) dogs examined, although, the type of dog was not specified (Detweiler, D.K. The Dog Electrocardiogram: A Critical Review, In: Comprehensive Electrocardiography Volume 2, Editors: MacFarlane, P.W. and Veitch Lawrie, T.D., New York, Pergamon Press, 1989, pages 1268-1329).

Heart rate was generally elevated for all dose groups during test article exposure and at 2-hr post-exposure. Heart rate was elevated for the high dose group at 4-hr post-exposure.

Heart Rate-Week 38 (Values in parentheses are percent of saline exposure).

Time	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
20-26 hr post-exposure	-	-	95	150	81	98	77	106
During saline exposure	107	108	85	103	82	74	79	79
During test article exposure	109 (102%)	108 (100%)	108 (127%)	**147** (143%)	105 (128%)	**163** (220%)	**146** (185%)	**148** (187%)
2 hr post-exposure	138 (129%)	122 (113%)	**140** (165%)	**168** (163%)	**132** (161%)	**165** (223%)	**137** (173%)	**164** (208%)
4 hr post-exposure	-	-	120 (141%)	135 (131%)	108 (132%)	137 (185%)	117 (148%)	**152** (192%)
24 hr post-exposure	-	-	102 (120%)	112 (109%)	87 (106%)	97 (131%)	94 (119%)	102 (129%)

Hematology:

Week 25: Red blood cell counts, hemoglobin levels, and hematocrit for male dogs in the high dose group were decreased to 87.1, 86.7, and 85.9% of control values (7.66 x 10^6/µL, 17.3 g/dL, and 50.2%), respectively.

Week 38: Red blood cell counts, hemoglobin levels, and hematocrit for male dogs in the high dose group were decreased to 83.1, 83.9, and 81.1% of control values (7.52 x 10^6/µL, 17.4 g/dL, and 48.2%), respectively. Monocyte percentages for male dogs in the mid and high dose groups were increased to 142.9 and 228.6% of the control (7%), respectively. Monocyte counts for male dogs in mid and high dose groups were increased to 128.6 and 185.7% of the control (0.7 x 103/µL), respectively. Monocyte percentages for female treatment groups were elevated to 180-200% of the control (5%). Monocyte counts for female treatment groups were increased to 150-200% of the control (0.4 x 10^3/µL).

Clinical chemistry: Amylase activities were elevated for male dogs in the high dose group at weeks 25 and 38. Lipase activities were generally elevated for male and female treatment groups during weeks 25 and 38. Changes in other parameters (i.e., creatine kinase activity, urea nitrogen, creatinine, cholesterol, triglyceride, potassium, globulin, A/G ratio) were evident, although, changes were generally small and their toxicological significance was unclear.

Week 25: Amylase activity for male dogs in the high dose group was elevated to 169% of the control (499 U/L). Lipase activities for male dogs in mid and high dose groups were increased to 191.6 and 263.5% of the control (249 U/L), respectively. Lipase activities for female dogs in low, mid, and high dose groups were increased to 198.2, 185.3, and 135.8% of the control (394 U/L), respectively. Creatine kinase activity for female dogs in the high dose group was elevated to 142.6% of the control (319 U/L), respectively. Urea nitrogen levels for male dogs in low, mid, and high dose group were increased to 115.2, 115.2, and 136.7% of the control (15.8 mg/dL), respectively. Urea nitrogen levels for female dogs in mid and high dose groups were increased to 114.8 and 115.4% of the control (18.2 mg/dL), respectively. Creatinine levels for male dogs in the high dose group were elevated to 137.5% of the control (0.8 mg/dL). Creatinine levels for female treatment groups were increase to 128.6-142.9% of the control (0.7 mg/dL). Cholesterol levels for male dogs in mid and high dose groups were decreased

to 89.5 and 88.3% of the control (162 mg/dL), respectively. Potassium levels for male dogs in low, mid, and high dose groups were increased to 105.3, 106.4, and 114.3% of the control (4.69 mEq/L), respectively. Triglyceride levels for male and female dogs in the high dose group were decreased to 56.8 and 83.3% of control values (37 and 36 mg/dL), respectively.

Week 38: Amylase activity for male dogs in the high dose group was elevated to 139.4% of the control (512 U/L). Lipase activities for male dogs in low, mid, and high dose groups were increased to 123, 136.3, and 174.2% of the control (422 U/L), respectively. Lipase activities for female dogs in low and mid dose groups were increased to 156.6 and 139.5% of the control (394 U/L), respectively, although, no change was evident for the high dose group. Creatine kinase activity for male dogs in the low dose group was elevated to 404% of the control (203 U/L), although, no changes were evident for mid and high dose groups. Creatine kinase (MB) activities for male treatment groups were elevated to 130-190% of the control (5.0 U/L), although, there was no dose response relationship. Globulin levels for male dogs in mid and high dose groups were increased to 88 and 84% of the control (2.5 g/dL), respectively. Albumin to globulin (A/G) ratios for male dogs in mid and high dose groups were increased to 114.7 and 116% of the control (1.50), respectively. The A/G ratio for female dogs in the high dose group was increased to 114.2% of the control (1.55). Urea nitrogen levels for male dogs in low, mid, and high dose groups were increased to 110.9, 115.8, and 122.4% of the control (16.5 mg/dL), respectively. Creatinine levels for male dogs in the high dose group were elevated to 125% of the control (0.8 mg/dL). Creatinine levels for female treatment groups were increased to 128.6% of the control (0.7 mg/dL). Cholesterol levels for male dogs in mid and high dose groups were decreased to 76.3 and 78.6% of the control (173 mg/dL), respectively. Triglyceride levels for male and female dogs in the high dose group were decreased to 59.4 and 82% of control values (32 and 39 mg/dL), respectively. Potassium levels for female dogs in low, mid, and high dose groups were increased to 109.5, 110.1, and 107.6% of the control (4.75 mEq/L), respectively.

Urinalysis: During week 38, urinary bilirubin levels for male treatment groups were elevated for the low dose (4 at 1+), mid dose (1 negative and 3 at 1+), and high dose (2 at 1+) as compared to the control (4 negative). The toxicological significance of this change was unclear. There were no treatment-related changes in other urinalysis parameters.

Organ weights: Changes in absolute and relative weights were evident for several organs (i.e., spleen, thymus, lungs, and liver), although, there were no correlations to histopathological findings.

Spleen: Absolute spleen weights for male dogs in low, mid, and high dose groups were decreased to 77.8, 68.9, and 77.8% of the control (75.31 g), respectively. Relative spleen weights for male dogs in low, mid, and high dose groups were decreased to 71.1, 69.4, and 75.9% of the control (0.731%), respectively. Absolute spleen weights for female dogs in low, mid, and high dose groups were decreased to 76.8, 84.7, and 80.2% of the control (49.83 g), respectively. Relative spleen weights for

female dogs in low, mid, and high dose groups were decreased to 72.9, 82.2, and 78.5% of the control (0.628%), respectively.

Thymus: Absolute and relative thymus weights for male dogs in the high dose group were increased to 222.9 and 214% of control values (5.03 g and 0.050%), respectively. Absolute thymus weights for male dogs #6892 and #6898 in the high dose group were 10.95 and 11.46 g, respectively, as compared to a control range of 4.30-5.46g. Relative thymus weights for male dogs #6892 and #6898 in the high dose group were 0.103 and 0.110%, respectively, as compared to a control range of 0.044-0.054%. Absolute and relative thymus weights for female dogs in the high dose group were decreased to 74 and 71.2% of control values (4.65 g and 0.059%), respectively.

Lungs: Absolute lung weights for male dogs in mid and high dose groups were decreased to 79.1 and 80.5% of the control (105.83 g), respectively. Relative lung weights for male dogs in low, mid, and high dose groups were decreased to 83.9, 76.6, and 77% of the control (1.050%), respectively.

Liver: Absolute and relative liver weights for male treatment groups were increased to 111.6-126.8% and 108.8-113.8% of control values (254.31 g and 2.512%), respectively.

Gross pathology: There were no apparent treatment-related gross pathological findings. White areas in the lung were evident for single animals in the male mid and high dose groups and the female low and mid dose groups. For the high dose group, there were single findings of reddened mucosa in the ileum, reddened mesenteric lymph node, cyst(s) in the oviduct.

Gross pathological findings following 273 days of consecutive treatment in dogs that received (R,R)-formoterol at target inhaled doses of 5, 40, and 70 µg/kg/day (deposited doses of 0, 1, 8, and 16 µg/kg/day). This table includes only animals sacrificed at scheduled termination during week 39.

Tissue/Organ	Sex	Control	Low Dose	Mid Dose	High Dose
Heart					
-AV valve(s) thickened	M	0/3	1/4	0/4	0/2
	F	0/4	0/4	0/4	0/4
-AV valves hematocyst(s)	M	0/3	1/4	0/4	0/2
	F	0/4	0/4	0/4	0/4
-white areas	M	0/3	0/4	0/4	0/2
	F	0/4	0/4	1/4	0/4
Lungs					
-white areas	M	0/3	0/4	1/4	1/2
	F	0/4	1/4	1/4	0/4
-firm	M	0/3	0/4	0/4	0/2
	F	0/4	0/4	1/4	0/4
-raised areas	M	0/3	0/4	0/4	0/2
	F	0/4	0/4	1/4	0/4
Oviduct					
-cyst(s)	F	0/4	0/4	0/4	1/4
Ileum					
-reddened mucosa	M	0/3	0/4	0/4	0/2
	F	0/4	0/4	0/4	1/4
Mesenteric lymph nodes					
-reddened	M	0/3	0/4	0/4	0/2
	F	0/4	0/4	0/4	1/4

Histopathology: There were no apparent target organs of toxicity. Increased incidences of subacute inflammation of the lacrimal glands and heart mineralization were observed for male and female dogs in the high dose group. Incidences of interstitial lung fibrosis, subacute inflammation in the salivary glands, cyst in the thyroid gland, mineralization in the adrenal cortex, sinus dilatation in the mesenteric lymph nodes, cyst in the parathyroid gland, and squamous hyperplasia in the stomach were increased for male and/or female treatment groups, although, no definitive treatment relationships were evident.

Histopathological findings following 273 days of consecutive treatment in dogs that received (R,R)-formoterol at target inhaled doses of 5, 40, and 70 µg/kg/day (deposited doses of 0, 1, 8, and 16 µg/kg/day). This table includes only animals sacrificed at scheduled termination during week 39.

Tissue/Organ	Sex	Control	Low Dose	Mid Dose	High Dose
Lacrimal glands					
-inflammation, subacute, minimal	M	0/2	0/3	0/3	2/2
	F	0/4	0/4	0/4	1/4
Heart					
-mineralization, minimal	M	0/3	0/4	0/4	1/2
	F	0/4	0/4	0/4	1/4
-endocardiosis, valvular, mild	M	0/3	1/4	0/4	0/2
	F	0/4	0/4	0/4	0/4
Lungs					
-inflammation, subacute, minimal	M	3/3	3/4	0/4	1/2
	F	1/4	1/4	2/4	3/4
-ossification, minimal	M	1/3	0/4	0/4	0/2
	F	0/4	0/4	0/4	0/4
-inflammation, granulomatous, minimal	M	0/3	1/4	2/4	0/2
	F	1/4	0/4	1/4	1/4
-fibrosis, interstitial, minimal-moderate *(only notable finding)*	M	0/3	1/4	1/4	1/2
	F	0/4	1/4	1/4	0/4
-inflammation, acute, mild	M	0/3	1/4	0/4	0/2
	F	0/4	0/4	0/4	0/4
-hemorrhage, mild	M	0/3	1/4	0/4	0/2
	F	0/4	0/4	0/4	0/4
Salivary gland					
-inflammation, subacute, minimal	M	0/3	2/4	0/4	1/2
	F	1/4	2/4	1/4	0/4
Thyroid glands					
-cyst, mild	M	0/3	0/4	0/4	1/2
	F	0/4	0/4	0/4	0/4
Adrenal cortex					
-mineralization, minimal	M	0/3	0/4	0/4	1/2
	F	0/4	0/4	0/4	0/4
Mesenteric lymph node					
-dilatation, sinus, minimal-mild	M	1/3	2/4	1/4	1/2
	F	0/4	0/4	0/4	1/4
-congestion, minimal	M	0/3	0/4	0/4	0/2
	F	0/4	0/4	0/4	1/4
-histiocytosis, sinus, minimal-mild	M	1/3	0/4	0/4	1/2
	F	2/4	0/4	0/4	1/4

Parathyroid gland -cyst, minimal	M F	0/2 0/4	0/3 1/4	0/3 0/4	0/2 1/4
Stomach -hyperplasia, squamous, minimal	M F	0/3 0/4	0/4 0/4	0/4 0/4	0/2 1/4

Toxicokinetics:

AUC and C_{max} values for formoterol on day 1 and during week 38 increased with elevating doses in male and female dogs. Increases in AUC and C_{max} were approximately dose proportional for female dogs, although, increases were not dose proportional for male dogs. Systemic exposure at the high dose on day 1 and during week 38 was greater for male dogs than female dogs, although, at the lose dose, exposure was greater for female dogs than male dogs.

Comparison of AUC values for formoterol on day 1 and during week 38 would suggest no accumulation of drug except possibly for male dogs at the high dose. Plasma desformoterol/formoterol ratios in male and female dogs from low, mid, and high dose groups at day 0 and during week 38 were ≤0.062 and 0.0283, respectively.

(R,R)-Formoterol Inhalation Dosage Level (µg/kg/day)	Study Interval	$AUC_{0.5\text{-}24hr}$ (pg•hr/mL)		C_{max} (pg/mL)		T_{max} (hr)	
Male Dogs		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
5	Day 1	1070	357	194	68.7	0.88	0.25
	Week 38	1280	419	160	82.7	0.75	0.29
40	Day 1	17900	13700	2400	1580	0.5	0.0
	Week 38	16300	10300	1730	1310	1.13	0.63
70	Day 1	26300	7450	4300	1580	0.88	0.75
	Week 38	50200	35700	4530	2590	12.3	16.62
Female Dogs		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
5	Day 1	2200	1120	316	163	0.88	0.25
	Week 38	2290	1690	308	263	0.88	0.25
40	Day 1	16700	4140	1440	216	1.88	2.75
	Week 38	12200	3640	1940	664	0.63	0.25
70	Day 1	20900	7950	3180	2030	0.63	0.25
	Week 38	23500	18800	2830	1750	1.13	0.63

Summary of individual study findings:

In a 9-month nose-only aerosol inhalation toxicology study, male and female beagle dogs (4 dogs/sex/group) received (R,R)-formoterol at target inhaled dose of 5, 40, or 70 µg/kg/day (deposited doses of 0, 1, 8, and 16 µg/kg/day, respectively).

A NOAEL was not established in this study. Atrial (supraventricular) premature depolarization was observed at all doses during week 38. Based upon histopathology, there were no target organs of toxicity.

Apparent treatment-related mortality occurred at the high dose. One control male dog and two male dogs in the high dose group were found dead during exposures on days 244, 35, and 151, respectively. Causes of death could not be determined from gross pathological and histopathological examinations; however, the two deaths in the high dose group are assumed to be treatment-related based upon mortality at this dose level in the 13-week study.

Treatment-related clinical signs, consisting of flushing of the body surface and facial area, reddened ears, and reddened gums, were observed throughout the treatment period at all dose levels. These clinical signs were noted primarily at 1-hr post-exposure. Amylase activities were elevated for male dogs in the high dose group at weeks 25 and 38. Lipase activities were generally elevated for male and female treatment groups during weeks 25 and 38.

Heart rate and electrocardiogram were evaluated on day 0 and during weeks 26 and 38. On day 0 at 2- and 4-hr post-exposure, elevated heart rates (and sinus tachycardia) were evident for male and female dogs at all dose levels. At 4-hr post-exposure, ventricular tachycardia was evident for 1 female dog in the high dose group. At 24-hr post-exposure, ventricular tachycardia and R on T depolarizations were evident for this dog. Further at 24-hr post-exposure for 1 male dog in the high dose group, ventricular premature beat, slow couplets, rapid premature tachycardia, and bigeminy were observed for 1 male dog in the high dose group. During week 38, atrial (supraventricular) premature depolarization was observed for 1 female dog in the mid dose group prior to dosing, 1 female dog in the low dose group at 2-hr post-exposure, and for 1 female dog in the high dose group at 24-hr post-exposure. Histopathological examination of the heart tissue found no evidence of injury.

Toxicology summary:

In a 6-month inhalation toxicology study, rats were exposed to (R,R)-formoterol at target doses of 100, 400, and 800 µg/kg/day. Deposited doses were 10, 40, and 77 µg/kg/day, respectively. The sponsor's dose selection complied with reviewer recommendations (see reviews dated December 20, 1999 and May 2, 2000). The NOAEL was identified as 10 µg/kg/day due to treatment-related mortality observed with doses of 40 and 77 µg/kg/day. Decreased levels of glucose and amylase activity in male and female treatment groups appeared to be related to the pharmacological activity of (R,R)-formoterol. There was no apparent target organ of toxicity. However, an increased incidence of thymic hemorrhage was observed at 77 µg/kg/day. The sponsor considered thymic hemorrhage a sporadic finding related to the euthanasia or tissue

collection processes and not a direct test article-related effect. Thymic hemorrhage was not observed in the 28-day inhalation toxicology study with rats. AUC values for (R,R)-formoterol at the NOAEL for male and female rats were 2920 and 4010 pg·hr/mL, respectively.

In a 13-week nose-only aerosol inhalation toxicology study, beagle dogs received (R,R)-formoterol at total inhaled doses of 0, 5, 40, and 70/100 µg/kg/day. Using a deposition factor of 0.20, deposited doses were calculated as 0, 1, 8, and 14/20 µg/kg/day, respectively. Due to clinical effects including respiratory depression and cyanosis (during exposure) for two male dogs and morbidity for 1 female dog, the high dose was lowered from 100 to 70 µg/kg/day on day 3 for males and day 22 for females. A NOAEL was not established due to electrocardiographic abnormalities (i.e., ventricular ectopic patterns) consisting of premature ventricular beat and/or ventricular escape beat or rhythm, which were observed after the first exposure to R,R-formoterol at all dose levels. Based upon histopathology, there were no target organs of toxicity.

In this study, treatment-related deaths occurred at the high dose for one male and one female, which were both euthanized in extremis on days 87 and 21, respectively. Treatment-related clinical signs, consisting of flushing of the body surface and facial area, reddened ears, and reddened gums, were observed throughout the treatment period at all dose levels.

Elevated heart rates were evident at all dose levels on the first day of exposure and at weeks 3 and 12, during exposure and at 2- and 4-hr post-exposure (note: the sponsor designated the first week of treatment as week 0). Sinus tachycardia was observed following the first exposure to R,R-formoterol at all dose levels at 2- and 4-hr post-exposure. Electrocardiographic abnormalities consisting of ventricular ectopic patterns were also observed following the first exposure at all dose levels at 4 and 24 hr after dosing. These ectopic patterns (i.e., ventricular escape beat, ventricular escape rhythm, and ventricular premature beat) were clearly treatment-related as they could be attributed to known pharmacological effects of β-adrenergic agonists, although, dose-response relationships were not evident. These ECG abnormalities could be characterized as transient as they were not evident during weeks 3 and 12; however, this is unclear given that in the 9-month study, ectopic changes were observed late in the study. Histopathological examination of the heart revealed no evidence of treatment-related myocardial injury. These ventricular ectopic patterns appeared to be tolerated, although, they were clearly undesirable effects, which could lead to potentially serious adverse events. Animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm.

In the 13-week dog study, the sponsor speculated that the presence of ventricular escape beats or rhythms observed at the low and mid doses was a physiological compensatory response, probably as a result of blood pressure elevation and vagal slowing of the SA node, and was not considered to be toxicologically significant. Further, this form of ectopy was not regarded as hazardous, because it supposedly occurs commonly in response to agents possessing mixed α- and β-adrenergic agonistic properties. However, the presence of left ventricular premature

beats in single electrocardiographs of three dogs in the mid and high dose groups were considered by the sponsor to be potentially adverse findings since such ectopic activity may lead to more serious arrhythmias. It should be noted that as a safety consideration, ectopic changes, consisting of ventricle escape beat, ventricle escape rhythm, and ventricular premature beat, that were collectively observed in 5 male dogs and 1 female dog from the low, mid, and high dose groups cannot be separated. These three types of ectopic patterns were caused by reentry and/or increased automaticity, and all were regarded as treatment-related and may have potentially deleterious consequences. The reason for the loss of sensitivity to test article-related effects on cardiac rhythm at later time points (i.e., weeks 3 and 12) is unclear given the findings of atrial premature depolarization in the last week of the 9-month study. It is possible these events may have been difficult to detect given that the level of monitoring on designated days was no more than 9-15 min per 24 hr (0.625-1%; ECG monitoring occurred at 2-, 4-, and 24-hr post-exposure for 3-5 min per time point).

In a 9-month nose-only aerosol inhalation toxicology study, male and female beagle dogs (4 dogs/sex/group) received (R,R)-formoterol at target inhaled dose of 5, 40, or 70 µg/kg/day (deposited doses of 0, 1, 8, and 16 µg/kg/day, respectively). A NOAEL was not established in this study. Atrial (supraventricular) premature depolarization was observed at all doses during week 38. Apparent treatment-related mortality occurred at the high dose. One control male dog and two male dogs in the high dose group were found dead during exposures on days 244, 35, and 151, respectively. Causes of death could not be determined from gross pathological and histopathological examinations; however, the two deaths in the high dose group are assumed to be treatment-related based upon mortality at this dose level in the 13-week study. Treatment-related clinical signs, consisting of flushing of the body surface and facial area, reddened ears, and reddened gums, were observed throughout the treatment period at all dose levels. These clinical signs were noted primarily at 1-hr post-exposure. Amylase activities were elevated for male dogs in the high dose group at weeks 25 and 38. Lipase activities were generally elevated for male and female treatment groups during weeks 25 and 38. Heart rate and electrocardiogram were evaluated on day 0 and during weeks 26 and 38. On day 0 at 2- and 4-hr post-exposure, elevated heart rates (and sinus tachycardia) were evident for male and female dogs at all dose levels. At 4-hr post-exposure, ventricular tachycardia was evident for 1 female dog in the high dose group. At 24-hr post-exposure, ventricular tachycardia and R on T depolarizations were evident for this dog. Further at 24-hr post-exposure for 1 male dog in the high dose group, ventricular premature beat, slow couplets, rapid premature tachycardia, and bigeminy were observed for 1 male dog in the high dose group. During week 38, atrial (supraventricular) premature depolarization was observed for 1 female dog in the mid dose group prior to dosing, 1 female dog in the low dose group at 2-hr post-exposure, and for 1 female dog in the high dose group at 24-hr post-exposure. Histopathological examination of the heart tissue found no evidence of injury. There were no target organs of toxicity.

Toxicology conclusions:

In the 13-week and 9-month dog studies with (R,R)-formoterol, electrocardiographic (ECG) examinations revealed ventricular and/or atrial ectopic patterns at all dose levels. There is concern that these ectopic patterns could lead to

potentially serious or fatal consequences. Thus, NOAELs could not be established for either study. Histopathological examinations of heart tissue in both studies revealed no evidence of lesions. Further, there was some discordance in the findings of ECG abnormalities between the 13-week and 9-month studies, which used similar doses. In the 13-week study, ventricular ectopic patterns were observed at all dose levels following the first exposure to (R,R)-formoterol; however, there were no findings at later time points (i.e., weeks 3 and 12). In the 9-month study, ventricular ectopic patterns were observed for only the high dose level following the first exposure to (R,R)-formoterol; however, during week 39 (i.e., last week of treatment), atrial ectopic patterns were evident at all dose levels.

In the conduct of these toxicology studies, it should be noted that ECG monitoring occurred at selected time points (i.e., 2-, 4-, and 24-hr post-exposure) for short periods of approximately 3-5 min per time point (this is an assumption based upon information provided), which amounts to less than 1% per 24-hr period. Many of these ectopic events may be very brief and difficult to detect with such limited monitoring. Further, even though these studies were conducted with relatively young adult dogs with no pre-existing cardiac damage, exercise periods were discontinued during treatment periods due to concerns regarding drug-induced heart rate increases and ECG abnormalities.

b(4)

The ventricular and/or atrial ectopic patterns observed in dogs during the 13-week and 9-month toxicology studies are most likely due to the pharmacological effects of (R,R)-formoterol, a β_2-adrenergic agonist. These effects on the heart have been well characterized for adrenergic agonists, such as epinephrine and isoproterenol. In the 13-week dog study, it is noted that AUC values of 1.12 and 1.49 ng·hr/mL at the low dose for male and female dogs are 33 to 44-fold, respectively, greater than the AUC of 0.0338 ng·hr/mL at the human clinical dose of 48 µg/day (cited by sponsor from Study number 091-004); however, the low dose was not a NOAEL and the margin of safety, if any, is unknown. Given that NOAELs were not established for these ectopic events in

either the 13-week or 9-month toxicology studies with (R,R)-formoterol in dogs, safety concerns remain.

Histopathology Inventory for IND # 55,302

Study	6-month inhalation study (090-827)	13-week inhalation study (090-830)	9-month inhalation study
Species	Rat	Dog	Dog
Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow smear	X (femur)	X	X (from rib)
Bone with marrow	X (sternebrae)	X (rib-costochondral junction)	X (rib-costochondral junction)
Brain	X*	X*	X*
Cecum	X		
Cervix		X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X*	X*
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder		X	X
Gross lesions	X	X	X
Harderian gland			
Heart	X*	X*	X*
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland		X	X
Larynx	X	X	X
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph nodes, cervical			
Lymph nodes, mandibular			
Lymph nodes, mesenteric	X	X	X
Lymph nodes, bronchial	X		
Lymph nodes, mediastinal	X	X	X
Lymph nodes, submandibular		X	X
Mammary Gland	X	X (females only)	X (females only)
Nasal cavity	X	X (five levels)	X (five levels)
Optic nerves	X	X	X
Ovaries	X*	X*	X*
Pancreas	X	X	X
Parathyroid	X*	X*	X*
Peripheral nerve (sciatic)	X	X	X
Pharynx	?	?	?

Pituitary	X	X	X
Prostate	X	X	X
Rectum	X	X	X
Salivary gland	X	X	X
Sciatic nerve	X (Peripheral nerve)	X (Peripheral nerve)	X (Peripheral nerve)
Seminal vesicles	X		
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X (cervical)	X (cervical)
Spleen	X*	X*	X*
Sternum			
Stomach	X	X	X
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid	X*	X*	X*
Tongue	X		
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X*	X*	X*
Vagina	X	X	X
Zymbal gland			
Standard List			

X, histopathology performed
*, organ weight obtained

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

(R,R)-Formoterol, a β_2-adrenergic agonist, is under development for treatment of [redacted] chronic obstructive pulmonary disease (COPD). In the present amendments in support of the continued development of (R,R)-formoterol, the sponsor has provided a 6-month inhalation toxicology study in rats and 13-week and 9-month inhalation toxicology studies in beagle dogs. b(4)

The sponsor's proposed Phase III program for the development of (R,R)-formoterol consists of two pivotal studies in subjects (≥35 years old) with COPD. (R,R)-Formoterol will be administered at inhaled doses of 15 µg BID, 25 µg BID, and 50 µg QD using a nebulizer. The proposed treatment period is 12 weeks. Subjects that successfully complete the clinical trial with (R,R)-formoterol at 25 µg BID or 50 µg QD will be offered the opportunity to enroll in a chronic safety study. The proposed treatment period is 9 months, which will result in a total treatment time of 12 months. In an EOP2 meeting on September 5, 2001, the Division informed the sponsor that the 6-month inhalation study in rats and the 13-week inhalation study in dogs should be submitted prior to initiation of proposed 12-week trials. Assuming adequate safety

margins were established in both studies, which were review issues, there may be adequate support for the initiation of the proposed clinical trial. The sponsor was also informed that prior to extension of the proposed clinical trials beyond 12 weeks of treatment, the 9-month inhalation study in dogs should be submitted.

In a 6-month inhalation toxicology study, rats were exposed to (R,R)-formoterol at target doses of 100, 400, and 800 µg/kg/day. Deposited doses were 10, 40, and 77 µg/kg/day, respectively. The sponsor's dose selection complied with reviewer recommendations (see reviews dated December 20, 1999 and May 2, 2000). The NOAEL was identified as 10 µg/kg/day due to treatment-related mortality observed with doses of 40 and 77 µg/kg/day. Decreased levels of glucose and amylase activity in male and female treatment groups appeared to be related to the pharmacological activity of (R,R)-formoterol. There was no apparent target organ of toxicity. However, an increased incidence of thymic hemorrhage was observed at 77 µg/kg/day. The sponsor considered thymic hemorrhage a sporadic finding related to the euthanasia or tissue collection processes and not a direct test article-related effect. Thymic hemorrhage was not observed in the 28-day inhalation toxicology study with rats. AUC values for (R,R)-formoterol at the NOAEL for male and female rats were 2920 and 4010 pg·hr/mL, respectively.

In a 13-week nose-only aerosol inhalation toxicology study, beagle dogs received (R,R)-formoterol at total inhaled doses of 0, 5, 40, and 70/100 µg/kg/day. Using a deposition factor of 0.20, deposited doses were calculated as 0, 1, 8, and 14/20 µg/kg/day, respectively. Due to clinical effects including respiratory depression and cyanosis (during exposure) for two male dogs and morbidity for 1 female dog, the high dose was lowered from 100 to 70 µg/kg/day on day 3 for males and day 22 for females. A NOAEL was not established due to electrocardiographic abnormalities (i.e., ventricular ectopic patterns) consisting of premature ventricular beat and/or ventricular escape beat or rhythm, which were observed after the first exposure to R,R-formoterol at all dose levels. Based upon histopathology, there were no target organs of toxicity.

In this study, treatment-related deaths occurred at the high dose for one male and one female, which were both euthanized in extremis on days 87 and 21, respectively. Treatment-related clinical signs, consisting of flushing of the body surface and facial area, reddened ears, and reddened gums, were observed throughout the treatment period at all dose levels.

Elevated heart rates were evident at all dose levels on the first day of exposure and at weeks 3 and 12, during exposure and at 2- and 4-hr post-exposure (note: the sponsor designated the first week of treatment as week 0). Sinus tachycardia was observed following the first exposure to R,R-formoterol at all dose levels at 2- and 4-hr post-exposure. Electrocardiographic abnormalities consisting of ventricular ectopic patterns were also observed following the first exposure at all dose levels at 4 and 24 hr after dosing. These ectopic patterns (i.e., ventricular escape beat, ventricular escape rhythm, and ventricular premature beat) were clearly treatment-related as they could be attributed to known pharmacological effects of β-adrenergic agonists, although, dose-response relationships were not evident. These ECG abnormalities could be characterized as transient as they were not evident during weeks 3 and 12; however,

this is unclear given that in the 9-month study, ectopic changes were observed late in the study. Histopathological examination of the heart revealed no evidence of treatment-related myocardial injury. These ventricular ectopic patterns appeared to be tolerated, although, they were clearly undesirable effects, which could lead to potentially serious adverse events. Animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm.

In the 13-week dog study, the sponsor speculated that the presence of ventricular escape beats or rhythms observed at the low and mid doses was a physiological compensatory response, probably as a result of blood pressure elevation and vagal slowing of the SA node, and was not considered to be toxicologically significant. Further, this form of ectopy was not regarded as hazardous, because it supposedly occurs commonly in response to agents possessing mixed α- and β-adrenergic agonistic properties. However, the presence of left ventricular premature beats in single electrocardiographs of three dogs in the mid and high dose groups were considered by the sponsor to be potentially adverse findings since such ectopic activity may lead to more serious arrhythmias. It should be noted that as a safety consideration, ectopic changes, consisting of ventricle escape beat, ventricle escape rhythm, and ventricular premature beat, that were collectively observed in 5 male dogs and 1 female dog from the low, mid, and high dose groups cannot be separated. These three types of ectopic patterns were caused by reentry and/or increased automaticity, and all were regarded as treatment-related and may have potentially deleterious consequences. The reason for the loss of sensitivity to test article-related effects on cardiac rhythm at later time points (i.e., weeks 3 and 12) is unclear given the findings of atrial premature depolarization in the last week of the 9-month study. It is possible these events may have been difficult to detect given that the level of monitoring on designated days was no more than 9-15 min per 24 hr (0.625-1%; ECG monitoring occurred at 2-, 4-, and 24-hr post-exposure for 3-5 min per time point).

In a 9-month nose-only aerosol inhalation toxicology study, male and female beagle dogs (4 dogs/sex/group) received (R,R)-formoterol at target inhaled dose of 5, 40, or 70 µg/kg/day (deposited doses of 0, 1, 8, and 16 µg/kg/day, respectively). A NOAEL was not established in this study. Atrial (supraventricular) premature depolarization was observed at all doses during week 38. Apparent treatment-related mortality occurred at the high dose. One control male dog and two male dogs in the high dose group were found dead during exposures on days 244, 35, and 151, respectively. Causes of death could not be determined from gross pathological and histopathological examinations; however, the two deaths in the high dose group are assumed to be treatment-related based upon mortality at this dose level in the 13-week study. Treatment-related clinical signs, consisting of flushing of the body surface and facial area, reddened ears, and reddened gums, were observed throughout the treatment period at all dose levels. These clinical signs were noted primarily at 1-hr post-exposure. Amylase activities were elevated for male dogs in the high dose group at weeks 25 and 38. Lipase activities were generally elevated for male and female treatment groups during weeks 25 and 38. Heart rate and electrocardiogram were evaluated on day 0 and during weeks 26 and 38. On day 0 at 2- and 4-hr post-exposure, elevated heart rates (and sinus tachycardia) were evident for male and

female dogs at all dose levels. At 4-hr post-exposure, ventricular tachycardia was evident for 1 female dog in the high dose group. At 24-hr post-exposure, ventricular tachycardia and R on T depolarizations were evident for this dog. Further at 24-hr post-exposure for 1 male dog in the high dose group, ventricular premature beat, slow couplets, rapid premature tachycardia, and bigeminy were observed for 1 male dog in the high dose group. During week 38, atrial (supraventricular) premature depolarization was observed for 1 female dog in the mid dose group prior to dosing, 1 female dog in the low dose group at 2-hr post-exposure, and for 1 female dog in the high dose group at 24-hr post-exposure. Histopathological examination of the heart tissue found no evidence of injury. Exercise periods were discontinued at the start of the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm. There were no target organs of toxicity.

General Toxicology Issues:

In the 13-week and 9-month dog studies with (R,R)-formoterol, electrocardiographic (ECG) examinations revealed ventricular and/or atrial ectopic patterns at all dose levels. There is concern that these ectopic patterns could lead to potentially serious or fatal consequences. Thus, NOAELs could not be established for either study. Histopathological examinations of heart tissue in both studies revealed no evidence of lesions. Further, there was some discordance in the findings of ECG abnormalities between the 13-week and 9-month studies, which used similar doses. In the 13-week study, ventricular ectopic patterns were observed at all dose levels following the first exposure to (R,R)-formoterol; however, there were no findings at later time points (i.e., weeks 3 and 12). In the 9-month study, ventricular ectopic patterns were observed for only the high dose level following the first exposure to (R,R)-formoterol; however, during week 39 (i.e., last week of treatment), atrial ectopic patterns were evident at all dose levels.

In the conduct of these toxicology studies, it should be noted that ECG monitoring occurred at selected time points (i.e., 2-, 4-, and 24-hr post-exposure) for short periods of approximately 3-5 min per time point (this is an assumption based upon information provided), which amounts to <1% per 24-hr period. Many of these ectopic events may be very brief and difficult to detect with such limited monitoring. Further, even though these studies were conducted with relatively young adult dogs with no pre-existing cardiac damage, exercise periods were discontinued during treatment periods due to concerns regarding drug-induced heart rate increases and ECG abnormalities.

b(4)

The ventricular and/or atrial ectopic patterns observed in dogs during the 13-week and 9-month toxicology studies are most likely due to the pharmacological effects of (R,R)-formoterol, a β_2-adrenergic agonist. These effects on the heart have been well characterized for adrenergic agonists, such as epinephrine and isoproterenol (Dhalla, N.S. et al., Cardiotoxicity of Catecholamines and Related Agents. In: Cardiovascular Toxicology, Second Edition, Editor: Acosta, D., Raven Press, New York, 1992, pages 239-282). However, given that NOAELs were not established for these ectopic events in either the 13-week or 9-month toxicology studies with (R,R)-formoterol in dogs, safety concerns remain, given that this drug will be used in patients with chronic obstructive pulmonary disease, where the average age is >45 years and there may be a high incidence of pre-existing cardiac disease. Drug-induced ventricular and/or atrial ectopic events could initiate potentially serious or fatal sequelae in these patients.

It is noted that AUC values of 1.12 and 1.49 ng·hr/mL at the low dose for male and female dogs, respectively, in the 13-week toxicology study are 33 to 44-fold greater than the AUC of 0.0338 ng·hr/mL at the human clinical dose of 48 µg/day (cited by sponsor from Study number 091-004). However, given the lack of a NOAEL in this study, the margin of safety, if any, is unknown.

In a meeting with Drs. Ray Anthracite, Robin Huff, and Tim Robison on January 29, 2002, the preclinical findings of ventricular and/or atrial ectopic patterns observed in the 13-week and 9-month toxicology studies with R,R-formoterol in dogs were discussed. It was noted that these effects were most likely due to the pharmacological effects of (R,R)-formoterol, which is a β_2-adrenergic agonist. However, given that NOAELs for these cardiac ectopic events were not established in either study, there was concern that a margin of safety for these drug-induced effects in clinical subjects is not known. Further, this drug will be used in COPD patients, which are generally older and have a high incidence of pre-existing heart disease. Therefore, there is a safety concern that these drug-induced ectopic effects could result in serious or life threatening events. It was a consensus that the sponsor should vigorously pursue cardiac monitoring in clinical trials with (R,R)-formoterol. Performing additional short-term studies in dogs to define the lower bound of the NOAEL would not necessarily allow the NOAEL to be defined with confidence, given the sporadic timing of events seen in both the 13-week and 9-month studies.

Recommendations:

1. In the 13-week and 9-month dog studies with (R,R)-formoterol, electrocardiographic examinations revealed ventricular and/or atrial ectopic patterns at all dose levels. Thus, NOAELs could not be established for either study. A margin of safety for these cardiac

effects in clinical subjects is not known. In a clinical setting, there is concern that these ectopic patterns could potentially lead to serious events. For the planned 12-week clinical trials (Protocol numbers 091-050 and 091-51) and 9-month chronic safety study, cardiac monitoring of clinical subjects should be vigorously pursued.

Labeling with basis for findings: None.

Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Robin Huff, Ph.D.
Non-Concurrence - _____
(see memo attached)

cc: list:
OstroffC
AnthraciteR
HuffR
RobisonT

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Timothy Robison
2/6/02 11:10:52 AM
PHARMACOLOGIST

Robin Huff
2/6/02 04:52:53 PM
PHARMACOLOGIST
I concur.

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

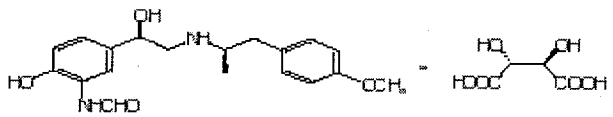
IND number: 55,302
Review number: #12
Sequence number/date/type of submission: #046/October 1, 2001/Amendment
#052/December 18, 2001/Amendment

Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Sepracor Inc.
111 Locke Drive
Marlborough, MA 01752
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: February 13, 2002

Drug:

Trade name:
Generic name (list alphabetically): (R,R)-Formoterol-L-tartrate
Code name:
Chemical name: (R,R)-(-)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide-(R,R)-2,3-dihydroxybutanedioate (1:1 salt)
CAS registry number:
Mole file number:
Molecular formula/molecular weight: $C_{23}H_{30}N_2O_{10}$ / MW 494.5
Structure:



Relevant INDs/NDAs/DMFs: ⊏ ⊐
⊏ ⊐ NDA 20-831 (Formoterol, Novartis). b(4)

Drug class: β_2-Adrenergic Agonist

Indication: ⊏ ⊐ Chronic Obstructive Pulmonary Disease (COPD)

Clinical formulation: Not provided.

Route of administration: Oral Inhalation

Proposed clinical protocol: See review of Amendments #045 and #050 for a description of the sponsor's proposed Phase 3 program.

Previous clinical experience: See review of Amendment #043 for a listing of clinical studies conducted with (R,R)-formoterol.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:

(R,R)-Formoterol, a β_2-adrenergic agonist, is under development for treatment of [redacted] COPD. The formoterol molecule contains two chiral centers and therefore, two pairs of enantiomers (i.e., (R,R)-, (S,S)-, (R,S)-, and (S,R)-). It has been suggested that the (S,S)-formoterol enantiomer lacks therapeutic effects and contributes only to adverse reactions. The (R,R)-formoterol enantiomer is apparently twice as potent as racemic formoterol based on unit weight, [redacted] b(4)

The sponsor's proposed Phase III studies consist of two pivotal studies in subjects (≥35 years old) with COPD. (R,R)-Formoterol will be administered at inhaled doses of 15 µg BID, 25 µg BID, and 50 µg QD using a nebulizer. The proposed treatment period is 12 weeks. Subjects that successfully complete the clinical trial with (R,R)-formoterol at 25 µg BID or 50 µg QD will be offered the opportunity to enroll in a chronic safety study. The proposed treatment period is 9 months. .

In support of the proposed Phase III program, the sponsor submitted a 6-month inhalation toxicology study in rats and 13-week and 9-month inhalation toxicology studies in dogs.

Studies reviewed within this submission:

STUDY	Sepracor Document #	Amendment
ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:		
Pharmacokinetics/Toxicokinetics		
Mice		
Toxicokinetics of formoterol during a 2-year oral oncogenicity study of (R,R)-formoterol in mice.	090-539	#046
Rabbits		
Toxicokinetics of (R,R)- and (S,S)-formoterol after oral administration of racemic formoterol during an embryo/fetal development study in rabbits.	090-523	#046
Distribution		
Rats		
Placental transfer studies of [^{3}H]-(R,R)-formoterol during and after repeated oral administration to pregnant rats.	090-526	#052
Secretion of radioactivity in milk following administration of [^{3}H]-(R,R)-formoterol as single oral doses to lactating rats.	090-531	#052
Rabbits		

Placental transfer studies of [^{3}H]-(R,R)-formoterol during and after repeated oral administration to pregnant rabbits.	090-525	#052
Metabolism		
In Vitro		
(R,R)- and (R,R/S,S)-formoterol -- potential inhibition of cytochrome P450 in human liver microsomes.	090-538	#052

Note: The 6-month inhalation toxicology study in rats and 13-week inhalation toxicology study in dogs were submitted in Amendment #046 and the 9-month inhalation toxicology study in dogs was submitted in Amendment #052. These studies were evaluated in Review #011.

b(4)

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TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

Appears This Way
On Original

PHARMACOLOGY/TOXICOLOGY REVIEW

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Mice

Study Title: **Toxicokinetics of Formoterol During a 2-Year Oral Oncogenicity Study of (R,R)-Formoterol in Mice, in Support of — 312063.**
Study no: Sepracor Document number 090-539, 2001
Volume #, and page #: Volume 9, Pages 1-22
Conducting laboratory and location: b(4)
Test Facility: ⌐

Toxicokinetic Interpretation: ⌐ b(4)

Date of study initiation: January 31, 2000
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol L-tartrate, Lot number 0215012 (Purity, 100.1%).
Formulation/vehicle: The vehicle was deionized water.

Dosing:
Species/strain: Male and female ⌐ ¬ CD-1® (ICR)BR mice b(4)
#/sex/group: 15 to 25 mice/sex/group/period
Age: Mice were 8-weeks old at the start of dosing.
Weight: Male and female mice had a body weight range of 20-40 g at the start of dosing.
Doses in administered units and Route: The drug solution was administered by oral gavage at doses of 1, 5, and 25 mg/kg/day using a dose volume of 10 mL/kg.

Methods: Toxicokinetic parameters for formoterol were assessed in mice that received (R,R)-formoterol at oral doses of 1, 5, and 25 mg/kg/day for periods up to 9 months. This study is intended to support the 24-month oral carcinogenicity study with (R,R)-formoterol in mice. On day 28, blood samples were collected from mice in the 5 mg/kg/day group at 0.5, 1, and 2 hr after dosing. At 9 months, blood samples were collected from mice in the 1, 5, and 25 mg/kg/day groups at 0.5, 1, 2, 6, and 24 hr after dosing. Blood was collected from up to 3 mice/sex/time point for each dose group. Plasma concentrations of formoterol and desformoterol/formoterol ratios were quantified using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The quantitation range for formoterol was 2.50-200 pg/mL. The assay method lacked chiral specificity, and concentration data were expressed as formoterol. The assay method was not validated to quantify the metabolite, desformoterol; however, its corresponding multiple reaction monitoring (MRM) mass channel was

acquired and presented as a ratio with respect to the MRM signal of formoterol for qualitative purposes only. The MRM signal for an equal quantity of desformoterol was assumed to be approximately equivalent to that for formoterol.

Results: AUC and C_{max} values for formoterol measured at month 9 increased with elevating dose; however, dose proportionality was not observed. AUC values for formoterol were higher in males than females. C_{max} values observed with doses of 5 and 25 mg/kg/day were also higher in males than females. With a dose of 5 mg/kg/day, AUC values at month 9 were greater than those observed on day 28, which might be suggestive of drug accumulation, although, termination of blood sampling at 2 hr after dosing on day 28 might also be responsible for the observed differences. Desformoterol/formoterol MRM ratios for male and female mice in the 1, 5, and 25 mg/kg/day groups were ≤0.0266 suggesting low desformoterol exposure following administration of (R,R)-formoterol.

Formoterol pharmacokinetic parameters following administration of (R,R)-formoterol to male and female mice at oral doses of 1, 5, and 25 mg/kg/day.

Dose mg/kg/day	Time point	$AUC_{(0.5\text{-}24hr)}$ pg·hr/mL		C_{max} pg/mL		T_{max} hr	
		Male	Female	Male	Female	Male	Female
1	Day 28[a]	-	-	-	-	-	-
	Month 9	16300	4800	1750	1710	0.5	2.0
5	Day 28	14400[b]	7000[b]	21000	6740	0.5	0.5
	Month 9	27800	13000	22000	4540	1.0	1.0
25	Day 28[a]	-	-	-	-	-	-
	Month 9	295000	73100	112000	34200	1.0	0.5

a. The 1 and 25 mg/kg/day groups were not sampled on day 28.
b. AUC values for the 5 mg/kg/day group on day 28 were truncated at 2 hr when blood sampling was stopped.

Rabbits

Study Title: Toxicokinetics of (R,R)- and (S,S)-Formoterol After Oral Administration of Racemic Formoterol During an Embryo/Fetal Development Study in Rabbits, in Support of [redacted]-312049.
Study no: Sepracor Document number 090-523, 2001
Volume #, and page #: Volume 9, Pages 1-18
Conducting laboratory and location:
Test Facility: [redacted] b(4)

Toxicokinetic Interpretation: [redacted] b(4)

Date of study initiation: September 20, 1999
Drug, lot #, radiolabel, and % purity: Racemic formoterol hemifumarate, Lot number BX9041 (Purity, >99%).

Formulation/vehicle: The vehicle was 0.5% carboxymethylcellulose.

Dosing:
Species/strain: Female New Zealand White [HRA(NZW)SPF] rabbits
#/sex/group: 3 rabbits/group
Age: Animals were approximately 6 months old at the start of dosing.
Weight: The body weight range at the start of dosing was 2.9-4.8 kg.
Doses in administered units and Route: Racemic formoterol was administered by oral gavage at a dose of 20 mg/kg/day.

Methods: Toxicokinetic parameters of (R,R)-formoterol and (S,S)-formoterol were determined in pregnant female rabbits that received racemic formoterol at an oral dose of 20 mg/kg/day from days 7 to 20 of gestation. Blood samples for measurement of plasma drug levels were collected on days 7 and 20 of gestation prior to dosing and at 0.5, 1, 2, 6, and 24 hr after dosing. Plasma concentrations of (R,R)-formoterol and (S,S)-formoterol were measured using a chiral-specific LC/MS/MS method. The limit of quantitation for (R,R)- and (S,S)-formoterol was 0.5 ng/mL. The quantitation range of (R,R)- and (S,S)-formoterol was 0.5 to 50 ng/mL.

Results: Pregnant female rabbits were exposed to both (R,R)-formoterol and (S,S)-formoterol following oral administration of racemic formoterol. AUC and C_{max} values for (R,R)-formoterol were higher than those for (S,S)-formoterol. It might be expected that levels of the two enantiomers would be approximately equal, although, it is unclear if enantiomer interconversion occurred. The sponsor claimed that the sum of plasma (R,R)- and (S,S)-formoterol concentrations obtained from rabbits dosed with racemic formoterol were generally comparable to total formoterol concentrations previously reported using an achiral separation technique. There was no evidence of drug accumulation as AUC values on day 20 were lower than those observed on day 7.

Mean (R,R)- and (S,S)-formoterol pharmacokinetic parameters following oral administration of 20 mg/kg/day racemic formoterol to pregnant rabbits on days 7 through 20 of gestation.

Enantiomer	$AUC_{(0.5\text{-}24hr)}$ ng.hr/mL		C_{max} ng/mL		T_{max} hr	
	Day 7	Day 20	Day 7	Day 20	Day 7	Day 20
(R,R)-Formoterol	427	275	136	133	1.33	0.83
(S,S)-Formoterol	286	211	89.6	98.2	1.33	0.83

Distribution:

Rats

Study Title: Placental Transfer Studies of [^{3}H]-(R,R)-Formoterol During and After Repeated Oral Administration to Pregnant Rats.

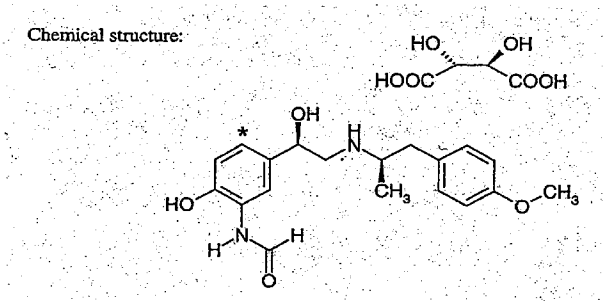
Study no: Sepracor Document number 090-526
Volume #, and page #: Volume 9, Pages 1-75
Conducting laboratory and location: ⌐

b(4)

1

Date of study initiation: May 11, 2001
Drug, lot #, radiolabel, and % purity: [^{3}H]-(R,R)-Formoterol L-tartrate, batch number 146-208-0065 (Radiochemical purity, 97.1%) and (R,R)-Formoterol L-tartrate, lot number 021-0006 (Purity, 99.4%). The asterisk denotes the position of the radiolabel.

Chemical structure:



Formulation/vehicle: 0.5% carboxymethylcellulose

Dosing:
Species/strain: Pregnant female Sprague-Dawley CD (albino) rats were obtained from ⌐ ¬ The rats were obtained on day 5 of gestation. b(4)
#/sex/group: 3 pregnant female rats/time point. A total of 17 pregnant female were used in this study with animals divided into two phases. Phase A consisted of 15 animals and Phase B consisted of 2 animals. Animals in Phase B served as replacements for animals in Phase A.
Age: Animals were approximately 10 weeks old at the start of dosing.
Weight: The body weight range for animals was 241-314 g at the start of dosing.
Doses in administered units and Route: [^{3}H]-(R,R)-Formoterol L-tartrate and (R,R)-Formoterol L-tartrate were mixed such that a dose of 10 mg/kg containing 10 µCi (designated as batch SR100501) was administered by oral gavage to each rat using a dose volume of 5 mL/kg.

Methods: Tissue distribution and placental transfer of radioactivity were assessed in pregnant female Sprague-Dawley rats that received [^{3}H]-(R,R)-formoterol from days 7 to 17 of gestation (i.e., a total of 11 doses). Three rats/time point (from Phase A) were sacrificed on gestation day 12 (6th dose) at 2 hr after dosing, on gestation day 14 (8th dose) at 2 hr after dosing, and on gestation day 17 (11th dose) at 2, 24, and 48 hr after dosing. Following sacrifice, the following organs, tissues, or fluids were removed or sampled: blood, amniotic fluid, brain, fetuses, heart, kidneys, liver, lungs, mammary glands, ovary, placenta, and uterus. Plasma and blood cells were obtained from whole blood. Samples were processed for liquid scintillation counting by direct or combustion methods.

Results: There were no clinical signs of toxicity or moribundity/mortality in pregnant female rats treated with [^{3}H]-(R,R)-formoterol at an oral dose of 10 mg/kg/day from days 7 to 17 of gestation. Radioactivity levels in tissues, plasma, whole blood, blood cells, and amniotic fluids at 2 hr after the 6th, 8th, and 11th doses were relatively comparable implying that a steady-state may have been achieved. At 2 hr after the 11th dose, radioactivity levels were highest in the kidneys and liver, which have an excretory function. Levels of radioactivity in the kidneys and liver were 224 and 214% of the plasma level, respectively. Levels of radioactivity in amniotic fluid and fetuses were both approximately 31% of the plasma level (1.180 µg/g) implying that (R,R)-formoterol and/or metabolites had crossed the placental barrier. It is estimated that radioactivity levels in the amniotic fluid and fetuses were approximately 0.05 and 0.16% of the administered dose, respectively. At 24 and 48 hr after the 11th dose, radioactive levels in most tissues as well as plasma, whole blood, blood cells, and amniotic fluid had declined to approximately 20 and 16%, respectively, of levels at 2 hr after the 11th dose. Levels were slightly higher in kidneys, liver, and placenta. Radioactivity levels in tissue, whole blood, blood cells, and amniotic fluid were approximately equivalent to plasma levels at 24 and 48 hr after the 11th dose.

TABLE 1

Mean concentrations of radioactivity in tissues of pregnant rats during and after repeated oral administration of [^{3}H]-(R,R)-formoterol L-tartrate (10 mg/kg/day)

Concentrations are expressed as µg equivalents free base/g and are means for 3 rats at each sacrifice time

Sample	Sacrifice time									
	2 hours after dose 6		2 hours after dose 8		2 hours after dose 11		24 hours after dose 11		48 hours after dose 11	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Plasma	0.942	0.159	0.778	0.107	1.180	0.143	0.247	0.010	0.184	0.025
Whole blood	0.668	0.091	0.585	0.084	0.861	0.103	0.234	0.016	0.183	0.030
Blood cells	0.321	0.034	0.293	0.039	0.378	0.041	0.214	0.016	0.179	0.033
Brain	0.229	0.044	0.242	0.018	0.319	0.033	0.222	0.009	0.165	0.033
Heart	0.447	0.069	0.410	0.008	0.524	0.051	0.246	0.003	0.202	0.036
Kidney	2.254	0.714	1.732	0.094	2.600	0.227	0.311	0.024	0.229	0.041
Liver	2.448	0.556	2.344	0.164	2.500	0.105	0.368	0.031	0.270	0.048
Lungs	0.633	0.097	0.593	0.103	0.773	0.078	0.263	0.003	0.206	0.033
Amniotic fluid	0.286	0.037	0.289	0.015	0.368	0.033	0.246	0.006	0.205	0.041
Foetuses	0.293	0.022	0.278	0.022	0.364	0.033	0.245	0.005	0.194	0.044
Mammary glands	0.414	0.057	0.368	0.065	0.444	0.084	0.192	0.011	0.149	0.010
Ovaries	0.450	0.033	0.426	0.037	0.568	0.076	0.245	0.020	0.186	0.005
Placentae	0.579	0.072	0.446	0.044	0.658	0.058	0.320	0.023	0.240	0.026
Uterus	0.600	0.090	0.545	0.041	0.863	0.088	0.282	0.013	0.203	0.025

sd standard deviation

Percentage of the administered dose in amniotic fluid and fetuses at 2 hr after the 11th dose. The administered dose of [^{3}H]-(R,R)-formoterol to each rat (mean BW ~ 300 g) was estimated to be 3 mg equivalent or 31228177 dpm. These calculations assume no drug accumulation.

Amniotic fluid	0.368 µg/g x 4.2230 g (mean weight) = 1.55 µg or 0.05% 4239.8 dpm/g x 42230 g (mean weight) = 17905 dpm or 0.06%
Fetuses	0.364 µg/g x 13.379 g (mean weight) = 4.87 µg or 0.16% 4197 dpm/g x 13.379 g (mean weight) = 56151 dpm or 0.18%

Study Title: Secretion of Radioactivity in Milk Following Administration of [^{3}H]-(R,R)-Formoterol as Single Oral Doses to Lactating Rats.

Study no: Sepracor Document number 090-531
Volume #, and page #: Volume 9, Pages 1-50
Conducting laboratory and location: ⌐

b(4)

⌙

Date of study initiation: June 7, 2001

Drug, lot #, radiolabel, and % purity: [^{3}H]-(R,R)-Formoterol L-tartrate, batch number 146-208-0065 (Radiochemical purity, 97.1%) and (R,R)-Formoterol L-tartrate, lot number 021-0006. The position of the radiolabel was the same as that described in Sepracor Document number 090-526.
Formulation/vehicle: 0.5% aqueous carboxymethylcellulose solution

Dosing:
Species/strain: Female Sprague-Dawley CD (albino) rats were obtained from [redacted] b(4)
#/sex/group: 3 rats per time point
Age: Animals were approximately 10-15 weeks old on the day of dosing
Weight: The body weight range was 297-376 g on the day of dosing.
Doses in administered units and Route: [^{3}H]-(R,R)-Formoterol L-tartrate and (R,R)-Formoterol L-tartrate were mixed such that a dose of 10 mg/kg was administered by oral gavage to each rat using a dose volume of 5 mL/kg.

Methods: Distribution of drug into maternal milk was assessed in lactating female Sprague-Dawley rats that received [^{3}H]-(R,R)-formoterol at an oral dose of 10 mg/kg/day. [^{3}H]-(R,R)-formoterol was administered at approximately 10 days after parturition. Rats (3 per time point) were sacrificed at 0.5, 1, 3, 6, and 24 hr after the single dose for collection of milk and blood. Approximately 15 min before sacrifice, rats received an intraperitoneal dose of oxytocin at 1 unit/kg. Total weights of milk were measured and recorded. Duplicate weighed aliquots of milk and plasma were combusted and radioactivity was measured with a liquid scintillation counter.

Results: AUC_{24} values for radioactivity in plasma and milk were 11.15 and 7.95 µg equivalents·hr/g, respectively. T_{max} values for radioactivity in plasma and milk were 0.5 and 6 hr after dosing, respectively. The milk to plasma ratio was 0.713. The sponsor calculated that the maximum daily dose of radioactivity likely to be ingested by a rat pup was 0.649 µg equivalents [^{3}H]-(R,R)-formoterol or 0.03% of the dose administered to the dam.

Rabbits

Study Title: Placental Transfer Studies of [^{3}H]-(R,R)-Formoterol During and After Repeated Oral Administration to Pregnant Rabbits.
Study no: Sepracor Document number 090-525
Volume #, and page #: Volume 9, Pages 1-81
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: May 23, 2001
Drug, lot #, radiolabel, and % purity: [^{3}H]-(R,R)-Formoterol L-tartrate, batch number 146-208-0065 (Radiochemical purity, 95.9-97.1%) and (R,R)-Formoterol L-tartrate, lot number 021-0006 (Purity, 100.1%). The position of the radiolabel was the same as that described in Sepracor Document number 090-526.
Formulation/vehicle: 0.5% carboxymethylcellulose

Dosing:
Species/strain: Pregnant female New Zealand White rabbits were obtained from [redacted] The rabbits were received on May 24, 2001, which was day 1 of gestation. b(4)
#/sex/group: 3 rabbits/time point (There were a total of 18 pregnant female rabbits with 3 animals serving as potential replacements).
Age: Animals were approximately 18 months old at the start of treatment.
Weight: The body weight range was 3096-3721 g at the start of treatment.
Doses in administered units and Route: [^{3}H]-(R,R)-Formoterol L-tartrate and (R,R)-Formoterol L-tartrate were mixed such that a dose of 10 mg/kg containing 25 µCi (designated as batch SR250501) was administered by gastric intubation to each rabbit using a dose volume of 5 mL/kg.

Methods: Tissue distribution and placental transfer of radioactivity were assessed in pregnant female New Zealand White rabbits that received [^{3}H]-(R,R)-formoterol from days 7 to 19 of gestation (i.e., a total of 13 doses). Three rabbits/time point were sacrificed on gestation day 12 (6th dose) at 2 hr after dosing, on gestation day 15 (9th dose) at 2 hr after dosing, and on gestation day 19 (13th dose) at 2, 24, and 48 hr after dosing. Following sacrifice, the following organs, tissues, or fluids were removed or sampled: amniotic fluid, brain, heart, kidneys, liver, lungs, mammary glands, ovary, pancreas, placenta, spleen, uterus, fetus (intact), fetal blood, fetal brain, fetal carcass, fetal kidney, fetal liver, and fetal lung. At sacrifices on gestation days 12 and 15, only whole fetuses were collected. At sacrifices on gestation day 19 (i.e., 2, 24, and 48 hr), three fetuses from each rabbit were retained intact, while fetal tissues/organs were collected from remaining fetuses and pooled by litter. Plasma and blood cells were obtained from whole blood. Animal 16F replaced animal 10F at the 24 hr sacrifice on gestation day 19, because animal 10F contained only 1 fetus. Samples were processed for liquid scintillation counting by direct or combustion methods.

Results: There were no clinical signs of toxicity or moribundity/mortality in pregnant female rabbits treated with [^{3}H]-(R,R)-formoterol at an oral dose of 10 mg/kg/day from days 7 to 19 of gestation. Radioactivity levels in maternal tissues, plasma, whole blood, and blood cells as well as amniotic fluid and the intact fetus were generally highest at 2 hr after the 9th dose (i.e., day 15 of gestation). At 2 hr after the 6th, 9th, or 13th dose, radioactivity levels were highest in the maternal kidney. Levels of radioactivity in the kidneys at 2 hr after the 6th, 9th, or 13th dose were 843, 512.6, and 332% of the plasma levels (4.682-5.683 µg/g), respectively. Levels of radioactivity in amniotic fluid and intact fetuses at 2 hr after the 13th dose were approximately 76.5 and 75.3% of the maternal plasma level (4.848 µg/g) implying that (R,R)-formoterol and/or metabolites had crossed the placental barrier. Further, radioactivity levels in fetal blood, brain, carcass, kidney, liver, and lung were 72.5, 71.7, 70, 36.5, 68, and 71% of the maternal plasma (4.848

µg/g), respectively. It is estimated that radioactivity levels in the amniotic fluid and intact fetus were approximately 0.1 and 0.07% of the administered dose, respectively. Further, radioactivity levels in the fetal brain, carcass, kidney, liver, and lung were 0.006, 0.07%, 0.0002, 0.005, and 0.0015% of the administered dose, respectively. At 24 hr after the 13th dose, radioactive levels in most maternal and fetal tissues declined to approximately 40-60% of levels at 2 hr after the 13th dose. However, disparity was evident between radioactivity levels at 24 and 48 hr after dosing, as levels at 48 hr were higher than those observed at 24 hr.

TABLE 1

Mean concentrations of radioactivity in tissues of pregnant New Zealand White Rabbits during and after repeated oral administration of [^{3}H]-(R,R)-formoterol L-tartrate (10 mg/kg/day)

Concentrations are expressed as µg equivalents free base/g and are means for 3 rabbits at each sacrifice time

Sample	Sacrifice time					
	2 hours after dose 6		2 hours after dose 9		2 hours after dose 13	
	Mean	sd	Mean	sd	Mean	sd
Plasma	4.682	1.061	5.683	1.285	4.848	1.004
Whole blood	3.722	0.902	4.710	1.043	4.214	0.911
Blood cells	2.358	0.687	3.272	1.072	2.925	0.820
Brain	2.361	0.861	3.373	1.157	3.156	0.926
Heart	2.897	0.903	3.995	0.923	3.500	0.859
Kidney	39.490	28.220	29.130	5.940	16.100	3.351
Liver	3.814	1.197	4.450	0.718	4.125	1.119
Lungs	3.481	0.806	4.263	1.068	3.874	0.986
Pancreas	2.381	1.283	2.747	0.244	2.873	0.940
Spleen	3.231	1.034	4.709	1.438	3.976	1.007
Amniotic fluid	2.827	0.969	4.082	1.414	3.709	1.012
Foetal blood	NS	NS	NS	NS	3.513	0.625
Foetal brain	NS	NS	NS	NS	3.476	1.036
Foetal carcass	NS	NS	NS	NS	3.396	0.951
Foetal kidney	NS	NS	NS	NS	1.768	1.667
Foetal liver	NS	NS	NS	NS	3.296	0.990
Foetal lung	NS	NS	NS	NS	3.445	0.994
Foetus (intact)	2.370	1.041	3.847	1.343	3.650	0.934
Mammary glands	2.613	0.555	3.513	0.746	3.448	1.220
Ovaries	3.662	1.809	4.060	1.094	3.577	0.956
Placentae	2.826	0.842	3.957	1.294	3.602	1.014
Uterus	3.241	1.202	4.225	1.130	3.789	1.007
Plasma (freeze-dried)	2.085	0.697	1.944	0.500	1.474	0.542

sd standard deviation
NS no sample

TABLE 1

(continued)

Concentrations are expressed as µg equivalents free base/g and are means for 3 rabbits at each sacrifice time

Sample	Sacrifice time			
	24 hours after dose 13		48 hours after dose 13	
	Mean	sd	Mean	sd
Plasma	3.001	0.731	3.726	0.674
Whole blood	2.675	0.603	3.370	0.625
Blood cells	2.297	0.576	3.042	0.607
Brain	2.545	0.512	3.300	0.632
Heart	2.452	0.523	2.725	0.352
Kidney	3.416	0.958	4.219	0.598
Liver	2.731	0.583	3.440	0.587
Lungs	2.557	0.582	3.042	0.589
Pancreas	1.790	0.348	2.366	0.319
Spleen	2.740	0.644	3.704	0.694
Amniotic fluid	2.964	0.572	3.942	0.753
Foetal blood	2.646	0.549	3.610	0.740
Foetal brain	2.889	0.569	3.764	0.704
Foetal carcass	2.899	0.754	3.689	0.812
Foetal kidney	2.571	0.719	3.547	0.610
Foetal liver	2.678	0.520	3.440	0.792
Foetal lung	2.760	0.567	3.697	0.770
Foetus (intact)	2.789	0.572	3.774	0.755
Mammary glands	2.105	0.562	2.631	0.422
Ovaries	2.228	0.629	3.257	0.616
Placentae	2.733	0.571	3.570	0.821
Uterus	2.667	0.704	3.516	0.683
Plasma (freeze-dried)	0.076	0.067	0.078	0.067

sd standard deviation

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On Original

Percentage of the administered dose in the amniotic fluid and fetus at 2 hr after the 13th dose. The administered dose of [^{3}H]-(R,R)-formoterol to each rabbit (mean BW = 3655.67 g) was estimated to be 36.6 mg equivalent or 56910700 dpm. These calculations assume no drug accumulation.

Amniotic Fluid	3.709 µg/g x 9.6124 g = 36.56 µg or 0.0975% 8272 dpm/g x 9.6124 g = 79513.8 dpm or 0.14%
Fetus (Intact)	3.650 µg/g x 7.2338 g = 26.4 µg or 0.0722% 8142.2 µg/g x 7.2338 g = 58899 dpm or 0.1035%
Fetal Brain	3.476 µg/g x 0.6491 g = 2.26 µg or 0.006% 7753 dpm /g x 0.6491 g = 5032 dpm or 0.009%
Fetal Carcass	3.396 µg/g x 7.1600 g = 24.3 µg or 0.07% 7574.4 dpm/g x 7.1600 g = 54233 dpm or 0.095%
Fetal Kidney	1.768 µg/g x 0.0438 g = 0.077 µg or 0.0002% 5914.7 dpm/g x 0.0438 g = 259 dpm or 0.0005%
Fetal Liver	3.296 µg/g x 0.5529 g = 1.82 µg or 0.005% 7352 dpm/g x 0.5529 g = 4065 dpm or 0.007%
Fetal Lung	3.445 µg/g x 0.1562 g = 0.54 µg or 0.0015% 7684 dpm/g x 0.1562 g = 1200.2 dpm or 0.002%

Metabolism:

In Vitro

Study Title: (R,R)- and Racemic (R,R/S,S)-Formoterol: Potential Inhibition of Cytochrome P450 in Human Liver and Microsomes.
Study no: Sepracor Document number 090-538, 2001
Volume #, and page #: Volume 9, Pages 1-58
Conducting laboratory and location: ⌐

b(4)

Date of study initiation: June 22, 2001
Drug, lot #, radiolabel, and % purity:
(R,R)-Formoterol L-tartrate, Lot number 021-0006 (Purity, 99.4%)
Racemic (R,R/S,S)-Formoterol, Lot number XL018P9F

Methods: (R,R)-Formoterol and (R,R/S,S)-formoterol were assessed for potential inhibition of human cytochrome P450 isozymes, CYP1A2, CYP2A6, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11. (R,R)-Formoterol at 100 nM or (R,R/S,S)-formoterol at 200 nM were incubated with pooled human liver

microsomes, with or without a 15-min pre-incubation period, prior to addition of a substrate selective for one specific isoform of cytochrome P450. The effects of (R,R)-formoterol and (R,R/S,S)-formoterol on the metabolism of these substrates were compared with those elicited by a range of isoform-selective chemical inhibitors (see table below). Human liver microsomes were prepared from human donors (HHM-100, HHM-133, HHM-141, HHM-201, HHM-227, and HHM-228, designated as HHM-221100) by ⊏ ⊐ Activities of cytochrome 450 isozymes were assayed using standardized methods. b(4)

Activity	Related P450	Selective Inhibitor (concentration)
7-Ethoxyresorufin O-deethylase	CYP1A2	Furafylline (30 μM)
Coumarin 7-hydroxylase	CYP2A6	8-Methoxypsoralen (1 μM)
Diclofenac 4'-hydroxylase	CYP2C9/C10	Sulfaphenazole (20 μM)
S-Mephenytoin 4'-hydroxylase	CYP2C19	Tranylcypromine (100 μM)
Debrisoquine 4-hydroxylase	CYP2D6	Quinidine (5 μM)
Lauric acid 11-hydroxylase	CYP2E1	Diethyldithiocarbamate (300 μM)
Testosterone 6β-hydroxylase	CYP3A4/5	Troleandomycin (100 μM)
Lauric acid 12-hydroxylase	CYP4A9/11	Diethyldithiocarbamate (300 μM)*

*No selective chemical inhibitor of CYP4A9/11 has been established; the effects of this compound were evaluated incidental to its use as a selective inhibitor of CYP2E1 in the same assay.

Results: (R,R)-formoterol at 100 nM or (R,R/S,S)-formoterol at 200 nM, with or without pre-incubation, produced no significant inhibition (i.e., ≤7%) of the activities of cytochrome P450 isozymes (i.e., CYP1A2, CYP2A6, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11). The sponsor reported that concentrations of (R,R)-formoterol at 100 nM and (R,R/S,S)-formoterol at 200 nM were approximately 500-fold higher than the expected clinically relevant peak concentrations.

PK/TK summary:

Toxicokinetic parameters for formoterol were assessed in mice that received (R,R)-formoterol at oral doses of 1, 5, and 25 mg/kg/day for periods up to 9 months. This study is intended to support the 24-month oral carcinogenicity study with (R,R)-formoterol in mice. AUC and C_{max} values for formoterol measured at month 9 increased with elevating dose; however, dose proportionality was not observed. AUC values for formoterol were higher in males than females.

Toxicokinetic parameters of (R,R)-formoterol and (S,S)-formoterol were determined in pregnant female rabbits that received racemic formoterol at an oral dose of 20 mg/kg/day from days 7 to 20 of gestation. Pregnant female rabbits were exposed to both (R,R)-formoterol and (S,S)-formoterol following oral administration of racemic formoterol. AUC and C_{max} values for (R,R)-formoterol were higher than those for (S,S)-formoterol. It might be expected that levels of the two enantiomers would be approximately equal, although, it is unclear if enantiomer interconversion occurred. The sponsor claimed that the sum of plasma (R,R)- and (S,S)-formoterol concentrations obtained from rabbits dosed with racemic formoterol were generally comparable to total formoterol concentrations previously reported using an achiral separation technique.

In studies with [^{3}H]-(R,R)-formoterol administered by the oral route to pregnant, female Sprague-Dawley rats or New Zealand White rabbits, there was evidence that (R,R)-formoterol and/or metabolites had distributed across the placental barrier into the amniotic fluid and fetuses (0.05 and 0.16% of the administered dose to rats, respectively, and 0.1 and 0.07% of the administered dose to rabbits, respectively).

In studies with [^{3}H]-(R,R)-formoterol administered to lactating female Sprague-Dawley rats, there was evidence that (R,R)-formoterol and/or metabolites had distributed into the milk. AUC_{24} values for radioactivity in plasma and milk were 11.15 and 7.95 µg equivalents·hr/g, respectively. The milk to plasma ratio was 0.713. The sponsor calculated that the maximum daily dose of radioactivity likely to be ingested by a rat pup was 0.649 µg equivalents [^{3}H]-(R,R)-formoterol or 0.03% of the dose administered to the dam.

In in vitro studies with pooled human liver microsomes, (R,R)-formoterol at 100 nM, with or without pre-incubation, produced no significant inhibition (i.e., ≤7%) of the activities of cytochrome P450 isozymes (i.e., CYP1A2, CYP2A6, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11).

PK/TK conclusions: In studies with [^{3}H]-(R,R)-formoterol administered by the oral route to pregnant, female Sprague-Dawley rats or New Zealand White rabbits, there was evidence that (R,R)-formoterol and/or metabolites distributed across the placental barrier into the amniotic fluid and fetuses. In studies with [^{3}H]-(R,R)-formoterol administered to lactating female Sprague-Dawley rats, there was evidence that (R,R)-formoterol and/or metabolites distributed into the milk. In in vitro studies with pooled human liver microsomes, (R,R)-formoterol at 100 nM, with or without pre-incubation, produced no significant inhibition of the activities of cytochrome P450 isozymes (i.e., CYP1A2, CYP2A6, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11).

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

(R,R)-Formoterol, a β_2-adrenergic agonist, is under development for treatment of [redacted] chronic obstructive pulmonary disease (COPD). In the present amendments in support of the continued development of (R,R)-formoterol, the sponsor has provided a number of preclinical studies assessing the absorption, distribution, metabolism, and excretion of (R,R)-formoterol. b(4)

Toxicokinetic parameters for formoterol were assessed in mice that received (R,R)-formoterol at oral doses of 1, 5, and 25 mg/kg/day for periods up to 9 months. This study is intended to support the 24-month oral carcinogenicity study with (R,R)-formoterol in mice. AUC and C_{max} values for formoterol measured at month 9 increased with elevating dose; however, dose proportionality was not observed. AUC values for formoterol were higher in males than females.

Toxicokinetic parameters of (R,R)-formoterol and (S,S)-formoterol were determined in pregnant female rabbits that received racemic formoterol at an oral dose of 20 mg/kg/day from days 7 to 20 of gestation. Pregnant female rabbits were exposed to both (R,R)-formoterol and (S,S)-formoterol following oral administration of racemic formoterol. AUC and C_{max} values for (R,R)-formoterol were higher than those for (S,S)-formoterol. It might be expected that levels of the two enantiomers would be approximately equal, although, it is unclear if enantiomer interconversion occurred. The sponsor claimed that the sum of plasma (R,R)- and (S,S)-formoterol concentrations obtained from rabbits dosed with racemic formoterol were generally comparable to total formoterol concentrations previously reported using an achiral separation technique.

In studies with [^{3}H]-(R,R)-formoterol administered by the oral route to pregnant, female Sprague-Dawley rats or New Zealand White rabbits, there was evidence that (R,R)-formoterol and/or metabolites had distributed across the placental barrier into the amniotic fluid and fetuses (0.05 and 0.16% of the administered dose to rats, respectively, and 0.1 and 0.07% of the administered dose to rabbits, respectively).

In studies with [^{3}H]-(R,R)-formoterol administered to lactating female Sprague-Dawley rats, there was evidence that (R,R)-formoterol and/or metabolites had distributed into the milk. AUC_{24} values for radioactivity in plasma and milk were 11.15 and 7.95 µg equivalents·hr/g, respectively. The milk to plasma ratio was 0.713. The sponsor calculated that the maximum daily dose of radioactivity likely to be ingested by a rat pup was 0.649 µg equivalents [^{3}H]-(R,R)-formoterol or 0.03% of the dose administered to the dam.

In in vitro studies with pooled human liver microsomes, (R,R)-formoterol at 100 nM, with or without pre-incubation, produced no significant inhibition (i.e., ≤7%) of the activities of cytochrome P450 isozymes (i.e., CYP1A2, CYP2A6, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11).

General Toxicology Issues:

In studies with [^{3}H]-(R,R)-formoterol administered to lactating female Sprague-Dawley rats, there was evidence that (R,R)-formoterol and/or metabolites had distributed into the milk. This information should be conveyed in product labeling, unless studies are conducted in humans.

In studies with [^{3}H]-(R,R)-formoterol administered by the oral route to pregnant, female Sprague-Dawley rats or New Zealand White rabbits, there was evidence that (R,R)-formoterol and/or metabolites had distributed across the placental barrier into the amniotic fluid and fetuses. Teratology studies conducted with rats and rabbits demonstrated that (R,R)-formoterol was teratogenic in both species (see review of amendment #030). These distribution studies add supporting information to the teratology studies conducted in rats and rabbits.

Toxicokinetic parameters for formoterol were assessed in mice that received (R,R)-formoterol at oral doses of 1, 5, and 25 mg/kg/day for periods up to 9 months. This study is intended to support the 24-month oral carcinogenicity study with (R,R)-

formoterol in mice. AUC and C_{max} values for formoterol measured at month 9 increased with elevating dose; however, dose proportionality was not observed. AUC values for formoterol were higher in males than females. This information needs to be incorporated into the assessment of the carcinogenicity study with (R,R)-formoterol in mice after it has been submitted.

Recommendations:

1. With regard to the 9-month toxicokinetic study in mice (Sepracor Document number 090-539, 2001), metabolism of (R,R)-formoterol should be assessed in mice as was conveyed in the EOP2 meeting held on September 5, 2001. A comprehensive understanding of the metabolism of (R,R)-formoterol in mice and humans will be essential in the analysis of the carcinogenicity study with (R,R)-formoterol in mice. Similarly, a comprehensive understanding of the metabolism of (R,R)-formoterol in rats and humans will be essential in the analysis of the carcinogenicity study with (R,R)-formoterol in rats. These comments were conveyed to the sponsor during the EOP2 meeting.

Labeling with basis for findings: Product labeling should convey that in studies with [^{3}H]-(R,R)-formoterol administered to lactating female Sprague-Dawley rats, there was evidence that (R,R)-formoterol and/or metabolites distributed into the milk.

Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Robin Huff, Ph.D.
Non-Concurrence - _____
(see memo attached)

cc: list:
OstroffC
AnthraciteR
HuffR
RobisonT

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Timothy Robison
2/13/02 04:00:30 PM
PHARMACOLOGIST

Robin Huff
2/14/02 10:22:24 AM
PHARMACOLOGIST
I concur.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

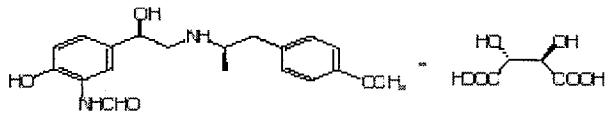
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 55,302
Review number: #13
Sequence number/date/type of submission: #347/October 21, 2004/Amendment
#349/November 3, 2004/Amendment
#367/March 14, 2005/Amendment
#369/March 18, 2005/Amendment
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Sepracor Inc.
111 Locke Drive
Marlborough, MA 01752
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: April 29, 2005

Drug:

Generic name (list alphabetically): Arformoterol, (R,R)-Formoterol-L-tartrate
Chemical name: (R,R)-(-)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide-(R,R)-2,3-dihydroxybutanedioate (1:1 salt)
Molecular formula/molecular weight: $C_{23}H_{30}N_2O_{10}$ / MW 494.5
Structure:



Relevant INDs/NDAs/DMFs: ⊏ ⊐ b(4)
⊏ ⊐ NDA 20-831 (Formoterol, Novartis).

Drug class: β_2-Adrenergic Agonist

Indication: ⊏ ⊐Chronic Obstructive Pulmonary Disease (COPD) b(4)

Clinical formulation: See earlier reviews.

Route of administration: Inhalation (nebulizer)

Proposed clinical protocol: Phase 3 studies conducted with COPD patients.

Previous clinical experience: See earlier reviews.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:
2-year mouse carcinogenicity study
2-year rat carcinogenicity study

Studies not reviewed within this submission: None.

TABLE OF CONTENTS

2.6.6 TOXICOLOGY

2.6.6.5 Carcinogenicity

Mice:

Study title: A 24-Month Oral (Gavage) Oncogenicity Study of (R,R)-Formoterol in Mice.

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: Mice received (R,R)-formoterol at oral doses of 0, 1, 5, and 25 mg/kg/day for periods up to 104 weeks. The sponsor did not have ECAC concurrence for dose selection. The sponsor did not contact the Division prior to early termination of groups.

Survival was significantly decreased for males and females in the 25 mg/kg/day group. Thus, the maximum tolerated dose (MTD) was exceeded at 25 mg/kg/day. Due to decreased survival for males in the 25 mg/kg/day group, the sponsor elected to sacrifice this group at week 77 when survival was 34%. The sponsor elected to sacrifice females in the 25 mg/kg/day group during week 92 when survival was 33%. The sponsor elected to sacrifice males in the 5 mg/kg/day group during week 95 when survival was 35%, although decreased survival was not statistically significant for this group.

Surviving females in control groups 1 and 2 and the 1 and 5 mg/kg/day groups were sacrificed during week 102 when survival was approximately 36, 39, 43, and 38%, respectively. Surviving males in control groups 1 and 2 and the 1 mg/kg/day group were sacrificed during week 104 when survival was approximately 42, 36, and 39%, respectively.

A maximum tolerated dose was achieved based upon decreased survival for males and females at 25 mg/kg/day; however, surviving males in the 25 mg/kg/day group were inappropriately sacrificed up to 6 months early and the duration of treatment appeared to be insufficient. The treatment period appeared to be sufficient for females at 25 mg/kg/day (sacrificed at week 92) and males at 5 mg/kg/day (sacrificed at week 95. Histopathological examination of tissues was complete for males at 5 and 25 mg/kg/day and females at 25 mg/kg/day.

Evaluation of tumor findings: For the mouse carcinogenicity study, treatment with (R,R)-formoterol ranged from 77 to 104 weeks. The incidences of uterine endometrial stromal polyps, combined incidences of uterine endometrial stromal polyps and stromal cell sarcoma, and combined incidences of uterine and cervical endometrial stromal polyps and stromal cell sarcoma were significantly increased for female treatment groups. It is noted that tumor incidences for the high dose group were lower than the low and mid dose groups due to decreased survival and early termination of surviving animals at the

high dose. Based upon tumor findings in the uterus and cervix combined, (R,R)-formoterol is tumorigenic in female mice.

The carcinogenic assessment of (R,R)-formoterol in male mice appears acceptable given that (1) there was evidence that the MTD was exceeded for males at 25 m/kg/day, although the treatment period was insufficient (2) the treatment period was adequate for males at 5 mg/kg/day and survival was reduced, although it did not reach a level of statistical significance, and (3) the incidence and severity of cardiomyopathy were increased for male treatment groups, although the correlation between cardiomyopathy and decreased survival was weak (i.e., for unscheduled deaths, cardiomyopathy was attributed as the cause of death in the control-1, control-2, 1 mg/kg/day, 5 mg/kg/day, and 25 mg/kg/day groups for 2 or 32, 1 of 39, 4 of 37, 7 of 40, and 6 of 40 animals, respectively). Systemic exposure to (R,R)-formoterol in males at 5 mg/kg/day was >25-fold of exposure at the clinical dose, although metabolism of (R,R)-formoterol in mice and humans have not been examined.

Study no.: Sepracor Document Number 090-833 — 312063)
Volume #, and page #: Amendment 347, Volumes 1-17, Pages 1-6102
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: December 15, 1999 (Start of treatment, January 31, 2000)
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: (R,R)-Formoterol tartrate, Lot number 021 5012 (Purity, 100.1%)
CAC concurrence: No

The sponsor conducted a 4-week oral dose range finding study with mice; however, the study report was not submitted to the Division (May 13, 2003) until after completion of the in-life phase of the carcinogenicity study. (R,R)-formoterol was administered at doses of 5, 15, 50, and 150 mg/kg/day. Target organs of toxicity were the heart, salivary gland, thymus, liver, and kidneys. Minimal to mild cardiomyopathy consisting of focal or multifocal lesions sequestered around the papillary muscles in the left ventricle was observed in males at 150 mg/kg/day and females at 50 and 150 mg/kg/day. Minimal to severe generalized hypertrophy of serous acinar cells in the mandibular and parotid salivary glands was observed at doses of 50 and 150 mg/kg/day. Thymic atrophy (i.e., decreased thickness of thymic cortex) was observed for males at 15, 50, and 150 mg/kg/day. Minimal to moderate glycogen deposition in the liver was observed at higher incidences in treatment groups. This appeared to correlate with increased liver weights for females. A higher incidence of minimal to mild basophilic cortical tubules was in the kidneys was observed in all male treatment groups.

Methods
Doses: 1, 5, and 25 mg/kg/day

Group	Treatment	Dose mg/kg/day	Number of mice at the start of treatment[a]		Number of mice for interim sacrifice after treatment for 25 weeks		Approximate number of mice scheduled for treatment up to 104 weeks	
			Males	Females	Males	Females	Males	Females
Toxicology Group — 312063M and —312063F)								
1	Vehicle	0	77	77	12	12	60	60
2	Vehicle	0	65	65	-	-	60	60
3	(R,R)-Formoterol	1	77	77	12	12	60	60
4	(R,R)-Formoterol	5	77	77	12	12	60	60
5	(R,R)-Formoterol	25	77	77	12	12	60	60
Toxicokinetic Groups — -312063A and —-312063B)								
6	(R,R)-Formoterol	1	15	15	-	-	-	-
7	(R,R)-Formoterol	5	25	25	-	-	-	-
8	(R,R)-Formoterol	25	15	15	-	-	-	-

b(4)

a. For toxicology groups, target group sizes were 72/sex and 60/sex in vehicle groups 1 and 2, respectively, and 72/sex in the (R,R)-formoterol groups. An additional 5 mice/sex/group were included to accommodate potential dosing accidents for smaller animals. After the first 4 weeks of treatment, the last 4 or 5 mice/sex/group were selected for elimination from the study such that there were no more than 72 mice/sex/group for vehicle control group 1 and test article-treated groups and 60 mice/sex/group for vehicle control group 2 group. The culled animals were subject to gross necropsy examination and tissues were collected. Body weights, food consumption, and clinical observations from these animals were included in the final report. Please note that some of these mice died during the treatment period and were submitted to histopathological examination with unscheduled deaths. The total number of animals examined for female control group 1 includes female number 9177, and the total number of animals examined in female control group 2 includes female number 8991. These two animals died prior to culling of extra animals at week 4 and were classified as accidental deaths. Since tissues from these two animals were examined microscopically, they were included in these two groups. After these deaths, female #9427 (control group 1) and 9419 (control group 2) remained on study (instead of being culled). Therefore, the total number of animals examined microscopically for female vehicle groups 1 and 2 were actually 73 and 61, respectively.
b. Groups 6, 7, and 8 were treated with solutions prepared in the same fashion as for Groups 3, 4, and 5, respectively.

Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: Male and female — CD-1®(ICR)BR mice were obtained from [redacted] b(4)

Number/sex/group (main study For toxicology groups, target group sizes were 72/sex and 60/sex in vehicle groups 1 and 2, respectively, and 72/sex in the (R,R)-formoterol groups. An additional 5 mice/sex/group were included to accommodate potential dosing accidents for smaller animals. After the first 4 weeks of treatment, the last 4 or 5 mice/sex/group were selected for elimination from the study such that there were no more than 72 mice/sex/group for vehicle control group 1 and test article-treated groups and 60 mice/sex/group for vehicle control group 2 group. After 25 weeks, 12 mice/sex/group were sacrificed for assessment. There were approximately 60 mice/sex/group for neoplastic assessment after treatment for periods up to 104 weeks.
Route, formulation, volume: The control and test article solutions were administered by oral gavage using a dose volume of 10 mL/kg. The vehicle was deionized water.
Frequency of dosing: Mice were treated with the vehicle or test article daily for periods up to 104 weeks.
Satellite groups used for toxicokinetics or special groups: For toxicokinetic assessment, there were 15 mice/sex/group that received (R,R)-formoterol at 1 or 25 mg/kg/day and 25 mice/sex/group that received (R,R)-formoterol at 5 mice/sex/group.
Age: Mice were approximately 8 weeks old at the start of treatment. For the carcinogenicity study, body weight ranges were 28.3-40.3 g for males and 21.0-34.2 g for females. For the toxicokinetic study, body weight ranges were 29.5-41.0 g for males and 24.3-30.3 g for females.
Animal housing: Animals were housed individually in wire-mesh cages suspended above cage-board.
Restriction paradigm for dietary restriction studies: No.
Drug stability/homogeneity: Dosing solutions were analyzed for homogeneity and stability using a HPLC method with UV detection.
Homogeneity of the 0.1 and 2.5 mg/mL dosing solutions were analyzed at the top, middle, and bottom. Averaged concentrations at the top, middle, and bottom for the 0.1 and 2.5 mg/mL solutions were 97.7 and 99.1% of nominal concentrations, respectively. Stability was assessed by collecting samples from the top and bottom of the 0.1 and 2.5 mg/mL solutions and analyzing solutions after 10 days of storage. Concentrations at the top and bottom of the 0.1 and 2.5 mg/mL solutions on the day of preparation and after 10 days of storage were 98.4-101% of nominal concentrations. Concentrations of dosing solutions were analyzed from January 28, 2000 through November 16, 2001 and are shown in the table below.

Formulation Date	Group 3&5 (0.1 mg/mL)	Group 4&7 (0.5 mg/mL)	Group 5&8 (2.5 mg/mL)
1/28/00	0.0989 (98.9)	0.509 (102)	2.60 (104)
2/4/00	0.0975 (97.5)	0.481 (96.2)	2.42 (97.0)
2/11/00	0.0992 (99.2)	0.486 (97.2)	2.44 (97.6)
2/18/00	0.0972 (97.2)	0.498 (99.7)	2.45 (98.2)
3/24/00	0.110 (110)	0.498 (99.7)	2.61 (104)
5/19/00	0.0921 (92.1)	0.468 (93.5)	2.37 (94.6)
8/18/00	0.101 (101)	0.495 (99.1)	2.43 (97.2)
11/17/00	0.0950 (95.0)	0.498 (99.6)	2.48 (99.3)
2/16/01	0.0991 (99.1)	0.490 (98.0)	2.41 (96.4)
5/18/01	0.101 (101)	0.494 (98.8)	2.44 (97.5)
8/17/01	0.0991 (99.1)	0.489 (97.8)	2.40 (96.0)
11/16/01	0.0989 (98.9)	0.480 (96.1)	-----

Dual controls employed: Yes.
Interim sacrifices: 12 mice/sex/group were sacrificed after treatment for 25 weeks.
Deviations from original study protocol:
Due to decreased survival for males in the 25 mg/kg/day group, the sponsor elected to sacrifice this group at week 77 when survival was 34%. No males in control group 1 or 2 were sacrificed for organ weight comparisons; however, this is not to imply that it is appropriate to sacrifice control animals for organ weight comparisons. The sponsor elected to sacrifice females in the 25 mg/kg/day group during week 92 when survival was 33%. Five females in control group 2 were also sacrificed for organ weight comparisons. The sponsor elected to sacrifice males in the 5 mg/kg/day group during week 95 when survival was 35%. Five males in control group 1 were sacrificed for organ weight comparisons.

Surviving females in control groups 1 and 5 and the 1 and 5 mg/kg/day groups were sacrificed during week 102 when survival was approximately 36, 39, 43, and 38%, respectively.

On May 14, 2000 (study day 104), control group 1 males and females in the 1 mg/kg/day received the vehicle (deionized water) and test article solution, respectively, that were designated for dosing of another 2-year carcinogenicity study in mice — 312052) that was being conducted concurrently with — 312063. These animals were dosed inadvertently with the incorrect dosing solutions on study day 104 only. Prior to this date and from day 105 onward, animals received their correct dosing solutions. Vehicles for both studies were identical and there was no impact on the study for the control group. The sponsor evaluated the toxicokinetic data for the test article from — 312052 (reported in —-312012) and concluded that a single dose of the test article from — 312052 at low dose levels would not have "a sufficient or long lasting effect" b(4)

on the study animals. "There were no clinical signs or pathologic effects from these animals that were indicative of additive effects of the test articles." "This incorrect dosing was not considered to be biologically significant or of a nature or magnitude that would effect the outcome of a 2-year study."

The test article was to be reanalyzed yearly by Sepracor. The analysis date on the Certificate of Analysis received with the test article was [redacted] Samples of the test article should have been sent on or about [redacted] for retest. Test article samples were sent for retest on [redacted] b(4)
[redacted] a 1-gram sample was sent for retest, [redacted]
a 10-gram sample was sent for retest. HPLC analyses dated [redacted]
February 19, 2002 indicated that purity was 110.7 and 99.6%, respectively.

Observation times

Mortality: Animals were observed twice daily for mortality and moribundity.

Clinical signs: Animals were observed for clinical signs at the time of dosing and 1 to 2 hr after dosing. Detailed physical examinations were conducted on all animals weekly. Animals were palpated for masses biweekly starting at week 28 and weekly from week 78 until completion of the study for the presence of palpable masses.

Body weights: Body weights were measured weekly.

Food consumption: Food consumption was measured weekly through week 12 and then monthly through week 100. Food consumption was measured on week 103 for surviving male groups.

Hematology: Blood samples for determination of hematology parameters were collected at the week 25 necropsy from 6 rats/sex/group. Blood smears were prepared from all animals at terminal necropsies and all animals sacrificed in a moribund condition. Blood smears (differential leukocyte counts) were not evaluated.

Serum chemistry: Blood samples for determination of serum chemistry parameters were collected at the week 25 necropsy from 6 rats/sex/group.

Organ weights: Absolute and relative organ weights during necropsies were measured for the brain, heart, kidneys, liver, pituitary, spleen, testes with epididymides, thymus, thyroids with parathyroid, and uterus.

Gross pathology: Complete necropsy examinations were conducted on all animals. Animals were euthanized with carbon dioxide from days 0 to 174 or isoflurane asphyxiation from days 175 to 729. Organs and tissues were collected and placed in 10% formalin except the testes and epididymides, which were placed in Bouin's solution and the eyes with optic nerves, which were placed in Davidson's solution,

Histopathology: Protocol-specified tissues were processed into paraffin blocks, sectioned at 4 to 8 µm, mounted on glass microscope slides, and stained with hematoxylin and eosin. On February 27, 2002, Masson's Trichrome stain was used inadvertently. Microscopic examinations were performed on all tissues in control group 1, control group 2, males in the 5 mg/kg/day group (sacrificed at week 95), and the 25 mg/kg/day group. All tissues were evaluated from animals that died or were sacrificed in a moribund condition from the 1 mg/kg/day group and females in the 5 mg/kg/day group. Target tissues consisting of the heart, liver, kidneys, uteri, cervices, and salivary glands as well as tissue masses/gross lesions were examined for all animals in the 1

mg/kg/day and females in the 5 mg/kg/day group. The sponsor indicated that microscopic examinations were also conducted on all tissues from animals in control group 1 and the 5 and 25 mg/kg/day groups sacrificed at week 25, although only the control and high dose groups were examined for some tissues. The study pathologist graded all neoplasms according to the method described by Peto. For animals that found dead or sacrificed in a moribund condition, tumors were graded according to their probability of causing death or moribundity (i.e., 1 = incidental, 2 = probably incidental, 3 = probably fatal, 4 = fatal, and 6 = incidental, scheduled sacrifice; a grade of 5 was not used). Final data reflected Peto scoring rather than severity scores. For statistical purposes, grades 1, 2, and 6 were grouped as not causing death and grades 3 and 4 were grouped as causing death. Microscopic examinations were performed by a [redacted] Senior Pathologist. A histopathology peer review was performed by an outside Pathologist. The histopathology peer review consisted of examination of all tumors, target tissues, and all protocol-specified tissues from 10% of the animals in the study. Findings in the report represent, where necessary, consensus between the study pathologist and the reviewing pathologist. b(4)

Toxicokinetics: Blood samples for measurement of plasma drug levels were collected during week 4 at 0.5, 1, and 2 hr after dosing for mice in the 5 mg/kg/day group and during week 38 at 0.5, 1, 2, 6, and 24 hr after dosing for mice in the 1, 5, and 25 mg/kg/day groups. Three mice/sex/group were assigned to each time point. Plasma drug concentrations were measured by [redacted] using a LC/MS/MS method. b(4)

Other: Sentinel animals (25 mice/sex) were sacrificed for evaluation of health status prior to the start of treatment. White blood cell counts were determined for 15 animals/sex. Serum antibody profiles were determined for 10 animals/sex. Data were provided in appendices.

An additional 15 mice/sex were assigned to a sentinel group and housed in the same room as animals in the main study to provide biological samples for diagnosis of possible disease conditions. For sentinel animals that survived to the end of the study or sacrificed in a moribund condition, blood samples were collected for possible serum analyses. Gross necropsies were performed on all sentinel animal and tissues were collected and preserved for possible microscopic examination. There were no indications that required follow up using sentinel animals.

Results

Mortality: All treatment groups had ≥50% survival through week 85 of the study except for males in the 25 mg/kg/day group. Survival was significantly decreased for males and females in the 25 mg/kg/day group (time-adjusted trend test for males, Cox p-value =0.0000 and Kruskal-Wallis p-value = 0.0000; for females, Cox p-value =0.0001 and Kruskal-Wallis p-value = 0.0001). Survival for males in the 5 mg/kg/day group was reduced; however, it did not reach a level of statistical significance (time-adjusted trend test excluding high dose group, Cox p-value =0.2414 and Kruskal-Wallis p-value = 0.2223). This data suggests that the MTD was exceeded was males and females at 25 mg/kg/day.

Due to decreased survival for males in the 25 mg/kg/day group, the sponsor elected to sacrifice this group at week 77 when survival was 34%. The sponsor elected to sacrifice females in the 25 mg/kg/day group during week 92 when survival was 33%. Five females in control group 2 were also sacrificed for organ weight comparisons. The sponsor elected to sacrifice males in the 5 mg/kg/day group during week 95 when survival was 35%. Five males in control group 1 were sacrificed for organ weight comparisons.

Surviving females in control groups 1 and 2 and the 1 and 5 mg/kg/day groups were sacrificed during week 102 when survival was approximately 36, 39, 43, and 38%, respectively. Surviving males in control groups 1 and 2 and the 1 mg/kg/day group were sacrificed during week 104 when survival was approximately 42, 36, and 39%, respectively.

The sacrifices of males at 5 and 25 mg/kg/day and females at 25 mg/kg/day appear to deviate from the Draft Guidance for Industry, Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May 2001); however, the duration of treatment may have been sufficient for males at 5 mg/kg/day and females at 25 mg/kg/day. There was no consultation with the Division prior to any of these sacrifices. The guidance states that if survival of the high dose group falls below 50% or 20-30 surviving animals after week 80, the study should be continued, either stopping dosing of animals in the high dose group or terminating only the high dose group, because the comparison of at least the control and low/middle doses would still be informative (the high dose comparison would depend on the situation). A study could be terminated early when the survival of the control or low dose group is reduced to 20-25% of the original number of animals.

The carcinogenic assessment of (R,R)-formoterol in male mice appears acceptable given that (1) there was evidence that the MTD was exceeded for males at 25 m/kg/day, although the treatment period was insufficient (2) the treatment period was adequate for males at 5 mg/kg/day and survival was reduced, although it did not reach a level of statistical significance, and (3) the incidence and severity of cardiomyopathy were increased for male treatment groups, although the correlation between cardiomyopathy and decreased survival was weak (i.e., for unscheduled deaths, cardiomyopathy was attributed as the cause of death in the control-1, control-2, 1 mg/kg/day, 5 mg/kg/day, and 25 mg/kg/day groups for 2 or 32, 1 of 39, 4 of 37, 7 of 40, and 6 of 40 animals, respectively). Systemic exposure to (R,R)-formoterol in males at 5 mg/kg/day was >25-fold of exposure at the clinical dose, although metabolism of (R,R)-formoterol in mice and humans have not been examined.

Text Table 1: Survival at the End of Study Weeks 25, 51, 76, 91, 94, 101 and 103 - Number and Percentage of Animals Surviving[a]

	MALES					FEMALES				
GROUP	1	2	3	4	5	1	2	3	4	5
DOSE (mg/kg/day)	0	0	1	5	25	0	0	1	5	25
Study Week										
25	60/60 100%	58/60 97%	59/59 100%	58/60 97%	47/60 78%	60/60 100%	60/60 100%	60/60 100%	58/60 97%	56/58 97%
51	58/60 97%	56/59 95%	57/59 97%	54/60 90%	39/60 65%	55/60 92%	57/60 95%	59/60 98%	55/60 92%	52/57 91%
76	47/60 78%	43/59 73%	50/59 85%	42/60 70%	20/59 34%	48/60 80%	45/60 75%	51/60 85%	44/60 73%	35/57 61%
80	45/60 75%	40/59 68%	47/59 80%	40/60 67%	NA	46/60 77%	41/60 68%	47/60 78%	44/60 73%	32/57 56%
91	36/60 60%	28/59 47%	36/59 61%	27/60 45%	NA	34/60 57%	36/60 60%	38/60 63%	31/60 52%	19/57 33%
94	32/60 53%	27/59 46%	34/59 58%	21/60 35%	NA	29/60 48%	30/55 55%	35/60 58%	30/60 50%	NA
101	23/55 42%	22/59 37%	24/59 41%	NA	NA	21/60 35%	21/55 38%	26/60 43%	23/60 38%	NA
103	23/55 42%	21/59 36%	23/59 39%	NA	NA	NA	NA	NA	NA	NA

[a] = Mortality data corrected for accidental deaths (*i.e.*, oral dosing intubation error), for animals that were removed from the study following six months of dosing and for control group 1 males (5) and control group 2 females (5) removed at study weeks 95 and 92, respectively. Culled and toxicokinetic animals excluded from calculation.

NA = Not Applicable

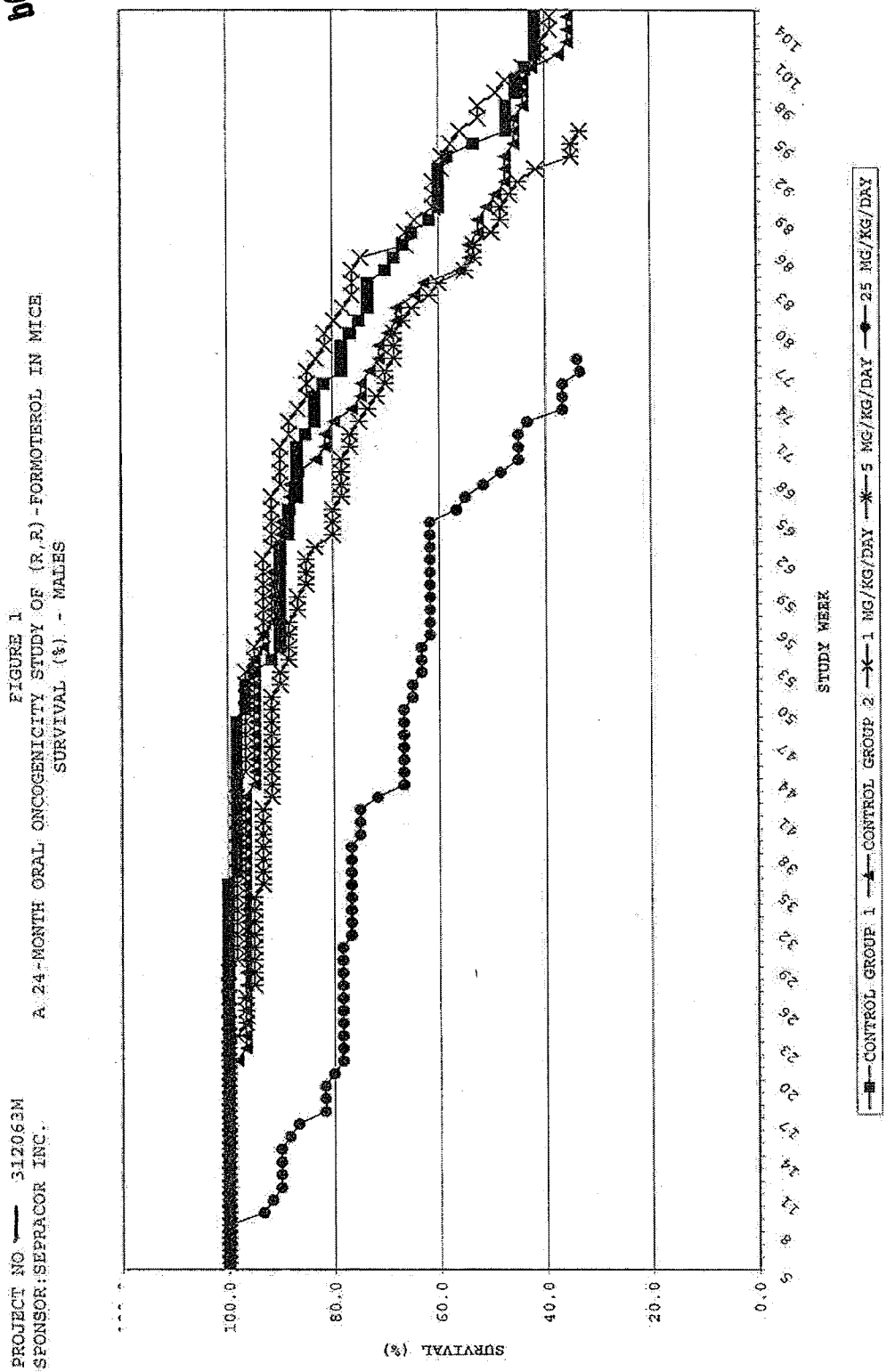
Disposition of male and female mice in the study

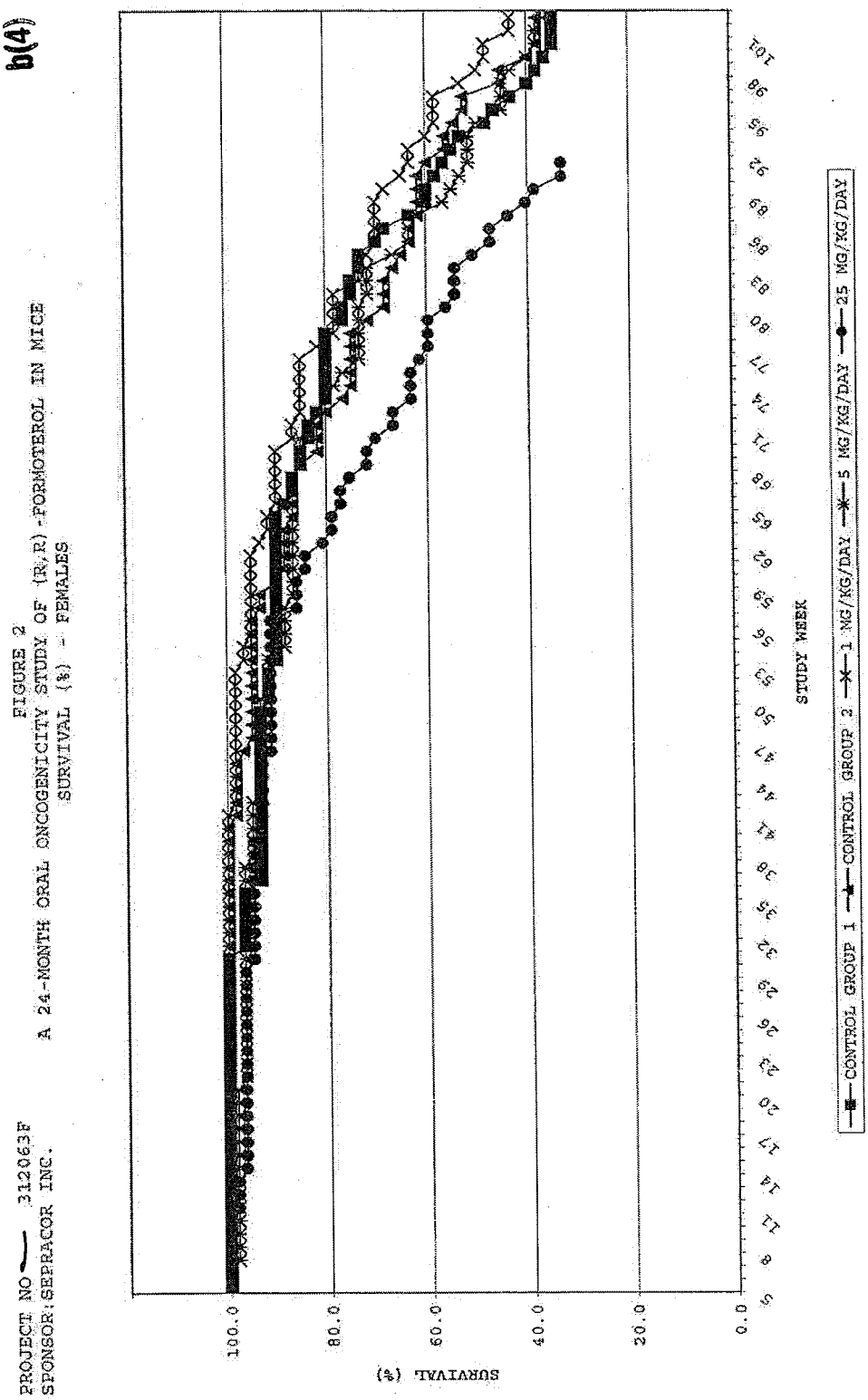
Disposition	Males					Females				
	0-1	0-2	1	5	25	0-1	0-2	1	5	25
Found dead	26	29	25	30	36	29	27	28	30	28
Euthanized in extremis	3	2	0	0	1	1	0	1	1	1
Culled, Week 4	5	5	5	5	5	4	4	5	5	5
Euthanized in extremis, physical condition	2	7	10	10	1	9	7	4	7	9
Euthanized in extremis-size, condition, masses	1	0	1	0	1	0	0	1	0	0
Interim necropsy, Wk 25	12	0	12	12	12	12	0	12	12	12
Interim necropsy, Wk 77	0	0	0	0	20	-	-	-	-	-
Interim necropsy, Wk 92	-	-	-	-	-	0	5	0	0	19
Interim necropsy, Wk 95	5	0	0	20	0	-	-	-	-	-
Primary necropsy, Wk 102	-	-	-	-	-	21	21	26	22	0
Primary necropsy, Wk 104	23	21	23	0	0	-	-	-	-	-
Accidental death	0	1	1	0	1	1	1	0	0	3

Unscheduled deaths - causes of death

Parameter	Males					Females				
	0-1	0-2	1	5	25	0-1	0-2	1	5	25
Dead/Euthanized	32	39	37	40	40	40	35	34	38	41
Undetermined cause of death	4	2	6	12	25	11	4	5	13	10
Thrombosis, atrial	2	1	4	7	6	0	0	0	0	2
Cardiomyopathy	2	1	4	7	6	0	0	0	0	4
Dilatation, ventricle	0	0	0	0	0	0	0	0	0	1

b(4)


PROJECT NO — 312063M
SPONSOR:SEPRACOR INC.
FIGURE 1
A 24-MONTH ORAL ONCOGENICITY STUDY OF (R,R)-FORMOTEROL IN MICE
SURVIVAL (%) - MALES
SURVIVAL (%)
100.0
80.0
60.0
40.0
20.0
0.0
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
1 MG/KG/DAY
5 MG/KG/DAY
25 MG/KG/DAY
% Survival was calculated for oncogenicity males only.



* Survival was calculated for oncogenicity animals only.

Clinical signs: Non-specific clinical signs at 1-hr post-dosing were generally most prominent with doses of 5 and 25 mg/kg/day. It should be noted that treatment was longer at 1 and 5 mg/kg/day as compared to 25 mg/kg/day.

Prominent clinical signs at 25 mg/kg/day included animals at front of cage, respiration labored, respiration rate increased, rales, respiration deep, swollen neck (attributed to salivary gland hypertrophy), circling (females), wet clear material around the mouth, ventral neck, forelimbs, and ventral trunk, wet yellow material around mouth and ventral neck, dried yellow material around mouth, ventral neck, and forelimbs (females), dried clear material around ventral neck, and wet yellow material around forelimb(s).

Prominent clinical signs at 5 mg/kg/day included head tilt (males), red vaginal discharge (females), extremities pale (males), body pale, respiration shallow, and wet yellow material around the hindlimbs (females).

Unkempt appearance was evident for males at 1 and 5 mg/kg/day and all female treatment groups. For all female treatment groups, there were increased incidences of dried yellow material around the urogenital area, ventral trunk, and anogenital area.

For males at 1 and 5 mg/kg/day, there were increased incidences of hypoactivity, body cool to the touch, and dried yellow material around the anogenital area, hindlimbs, and forelimbs. For females at 1 and 5 mg/kg/day, there were increased incidences of wet yellow material around the urogenital area, ventral trunk, and anogenital area.

Body weights: There were no treatment-related adverse effects on absolute body weight for male and female treatment groups throughout the course of the study.

Body weight and body weight gain

Parameter	Male mice					Female mice				
	0-1	0-2	1	5	25	0-1	0-2	1	5	25
Wk 0	33.9	34.3	33.7	33.5	33.2	25.8	25.4	26.1	25.8	25.6
Wk 25	42.7	43.7	43.8	44	43.8	33.1	32.9	33.4	34.5	35.7
BW, % of Control			101.4	101.9	101.4			101.2	104.6	108.2
BW gain Wk_{0-25}, % of control			112.3	117.5	119.7			96.7	116.6	136.5
Wk 51	42.6	44	44.1	44.1	44.5	34.7	34.6	35.3	35.9	38.2
BW, % of Control			101.9	101.9	102.8			101.9	103.6	110.3
BW gain Wk_{0-51}, % of control			114.4	117.3	126.2			99.7	110.7	139.2
Wk 77	42.8	44.8	44.5	43.6	43.5	36.1	35.4	36	36.2	37.8
BW, % of Control			101.6	99.5	99.3			100.7	101.3	105.7
BW gain Wk_{0-77}, % of control			112.7	106.0	109.1			133.4	141.8	167.6
Wk 95/92	42.3	43	43.8	43	NA	37.3	36.5	36.2	36.1	37
BW, % of Control			102.7	100.8				100.7	101.3	105.7
BW gain $Wk_{0-95/92}$, % of control			119.5	113.1				133.4	141.8	167.6
Wk 104/102	41.3	42.3	42.9	NA	NA	35.5	35.7	37.5	36.2	NA
BW, % of Control			102.6					105.3	101.7	
BW gain $Wk_{0-104/102}$, % of control			120.9					111.8	103.2	

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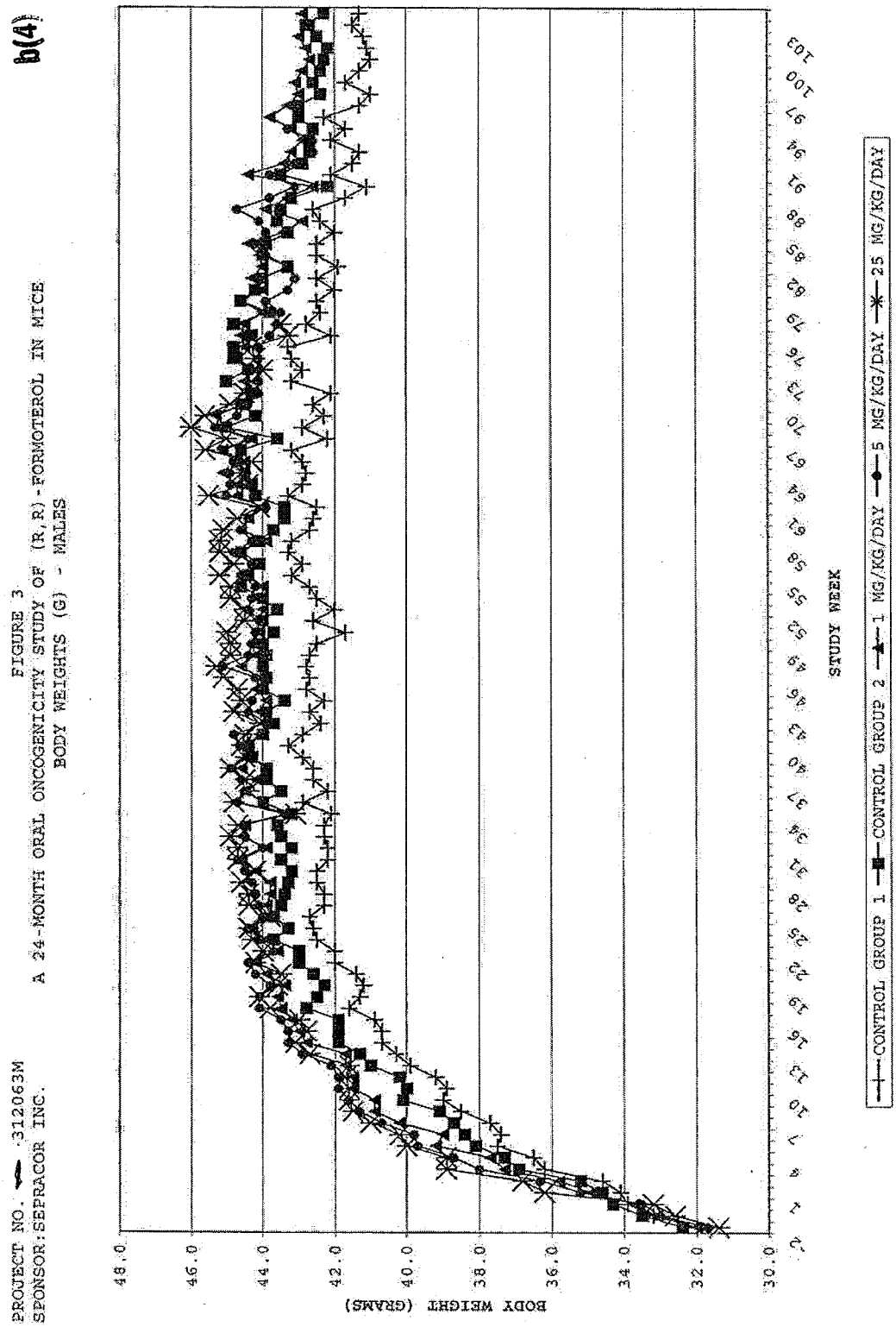
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PROJECT NO. - 312063M
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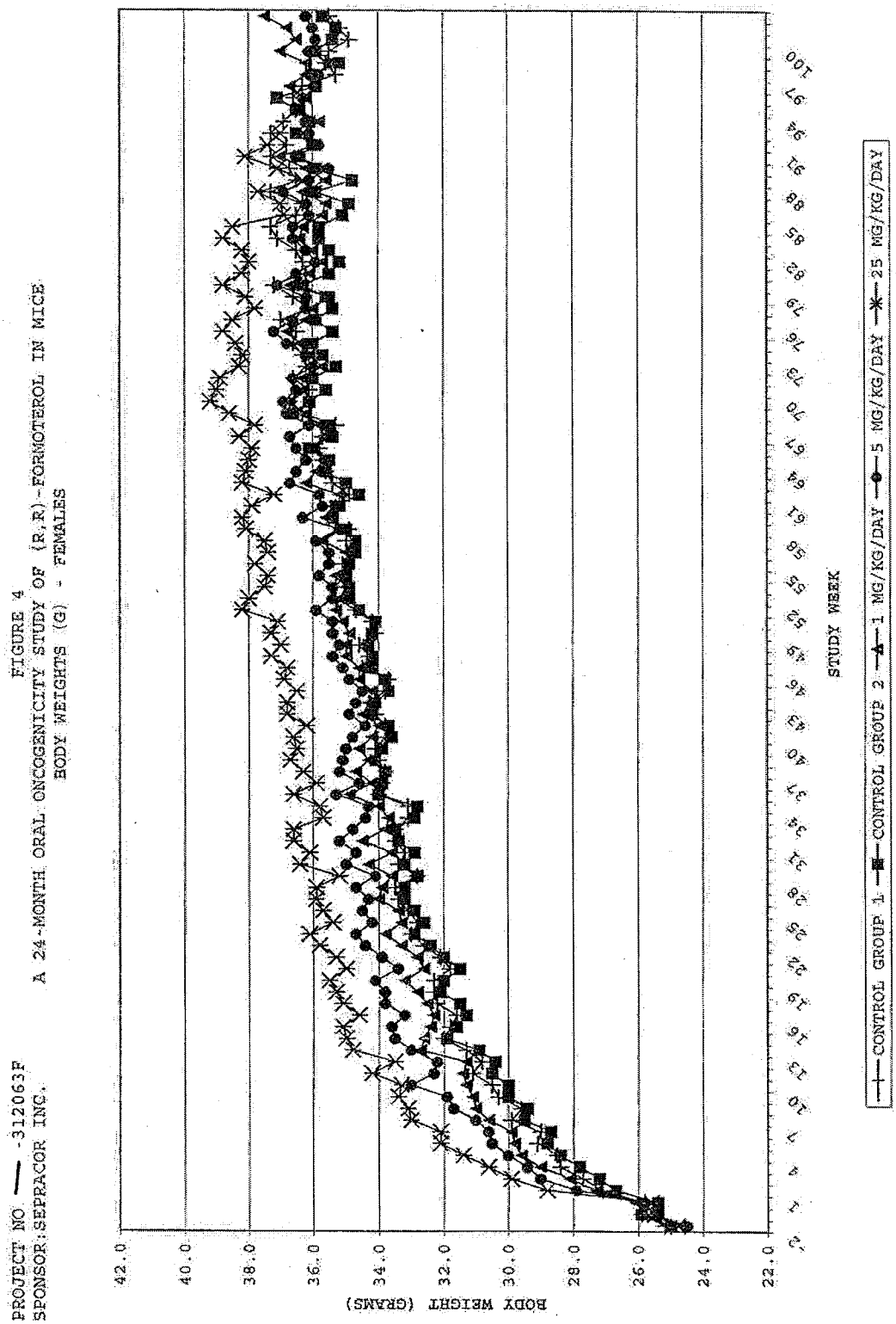
FIGURE 3
A 24-MONTH ORAL ONCOGENICITY STUDY OF (R,R)-FORMOTEROL IN MICE
BODY WEIGHTS (G) - MALES
BODY WEIGHT (GRAMS)
48.0
46.0
44.0
42.0
40.0
38.0
36.0
34.0
32.0
30.0
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
1 MG/KG/DAY
5 MG/KG/DAY
25 MG/KG/DAY

b(4)


PROJECT NO — -312063F
SPONSOR:SEPRACOR INC.
FIGURE 4
A 24-MONTH ORAL ONCOGENICITY STUDY OF (R,R)-FORMOTEROL IN MICE
BODY WEIGHTS (G) - FEMALES
BODY WEIGHT (GRAMS)
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
1 MG/KG/DAY
5 MG/KG/DAY
25 MG/KG/DAY

Food consumption: Food consumption for male mice at 25 mg/kg/day through week 77 of treatment was increased to 108.5% of pooled male controls (5.85 g/animal/day), which might be attributed to pharmacological effects of the β_2-adrenergic agonist, (R,R)-formoterol. There were no significant differences in food consumption for female treatment groups.

Hematology (Week 25): Alterations of leukocyte and red blood cell counts were observed for male and female treatment groups at week 25; however, these changes appeared to have little or no toxicological significance. White blood cell and lymphocyte counts for males at 25 mg/kg/day were decreased to 66.7 and 61.5% of control values ($5.1 \times 10^3/\mu L$ and $3.9 \times 10^3/\mu L$), respectively. Red blood cell counts, hemoglobin levels, and hematocrit for males at 25 mg/kg/day were increased to 112.2, 109.9, and 110.2% of controls ($8.83 \times 10^6/\mu L$, 14.2 g/dL, and 42.2%), respectively. The neutrophil percentage for males at 25 mg/kg/day was increased to 135% of the control (20%). White blood cell counts for females at 1, 5, and 25 mg/kg/day were increased to 115.2, 148.5, and 139.4% of the control ($3.3 \times 10^3/\mu L$), respectively. Neutrophil counts for females at 5 and 25 mg/kg/day were increased to 183.3 and 200% of the control ($0.6 \times 10^3/\mu L$), respectively. The neutrophil percentage for females at 25 mg/kg/day was increased to 142.9% of the control (21%).

Clinical chemistry (Week 25): Urea nitrogen levels were increased for males at 25 mg/kg/day and females at 5 and 25 mg/kg/day, which may correlate with histopathological findings of nephropathy. ALP activities were increased for males at 25 mg/kg/day and females at 1, 5, and 25 mg/kg/day, although these increases were not toxicologically significant. There were no corresponding histopathology findings in the liver. Glucose levels were decreased for male treatment groups. Cholesterol levels were decreased for males at 5 and 25 mg/kg/day. Phosphorus levels were decreased for males at 25 mg/kg/day. Globulin levels were increased for females at 25 mg/kg/day. ALT activities were increased for females at 1 and 5 mg/kg/day; however, no change was evident at 25 mg/kg/day.

Clinical chemistry values for male and female treatment groups at week 25.

Parameter	Males				Females			
	0-1	1	5	25	0-1	1	5	25
Urea nitrogen, mg/dL	29.4	28.7	26.1	39.0 (133%)	21.0	22.2	30.1 (143%)	28.7* (137%)
ALP, U/L	35	41	45	70* (200%)	73	90 (123%)	86 (118%)	104 (143%)
Glucose, mg/dL	213	181 (85%)	155* (73%)	161* (76%)				
Cholesterol, mg/dL	134	134	112 (84%)	103 (77%)				
Phosphorus, mg/dL	10.7	11.4	12.1	8.9 (83%)				
Globulin, g/dL					1.8	1.7	1.9	2.4 (133%)
ALT, U/L					33	78	48	34

						(236%)	(148%)	

Organ weights:

Week 25 Necropsy: Following treatment with (R,R)-formoterol for 25 weeks, organ weight differences were observed for heart, spleen, pituitary, liver, kidneys, uterus, and thyroid/parathyroid. Potential corresponding histopathological changes were evident for heart, spleen, and uterus. Cardiomyopathy was observed for males and females at 5 and 25 mg/kg/day. Extramedullary hematopoiesis was observed in the spleen for females at 25 mg/kg/day; however, lower doses were not examined. Increased incidences of adenomyosis were observed in the uterus for female treatment groups. Increased incidences of nephropathy were observed for males at 5 and 25 mg/kg/day; however, incidences were comparable for female control and treatment groups where organ weight differences were evident.

Absolute and relative organ weights at necropsies during weeks 25,.

Organ/Tissue	Males				Females			
	0-1	1	5	25	0-1	1	5	25
Heart g	0.2008	0.2170 (108%)	0.2167 (108%)	0.2371* (118%)	0.1547	0.1672 (108%)	0.1790* (116%)	0.1906* (123%)
Heart g/100g BW	0.468	0.483	0.479	0.534* (114%)				
Heart g/100g BrW	39.083	42.139	42.935	46.556* (119%)	28.878	31.876 (110%)	34.063* (118%)	36.108* (125%)
Spleen g	0.0987	0.1294* (131%)	0.1283* (130%)	0.1101 (112%)	0.1038	0.1223 (118%)	0.1433* (138%)	0.1673* (161%)
Spleen g/100g	0.230	0.287* (125%)	0.284* (124%)	0.249 (108%)	0.315	0.362 (115%)	0.397 (126%)	0.442* (140%)
Spleen g/100g BrW	19.200	25.152* (131%)	25.336* (132%)	21.650 (113%)	19.302	23.248 (120%)	27.316* (142%)	31.643* (164%)
Pituitary g	0.0024	0.0026	0.0023	0.0017 (71%)				
Pituitary g/100 g BrW	0.466	0.505	0.453	0.338 (73%)				
Liver g					1.4106	1.4818 (105%)	1.6526* (117%)	1.8748* (133%)
Liver g/100g BW					4.304	4.396	4.580	5.009* (116%)
Liver g/100g BrW					263.192	282.100 (107%)	314.108* (119%)	355.987* (135%)
Kidneys g					0.4168	0.4275	0.4735 (114%)	0.5755* (138%)
Kidneys g/100g BW					1.274	1.270	1.313	1.554 (122%)
Kidneys g/100g BrW					77.836	81.503 (105%)	90.021 (116%)	109.815* (141%)
Uterus g					0.4043	0.3920	03926	0.4574 (113%)
Uterus g/100g BrW					75.205	74.922	74.841	87.494 (116%)
Thyroid/Parathyroid					0.0051	0.0051	0.0051	0.0064*

g								(126%)
Thyroid/Parathyroid g/100g BrW					0.956	0.966	0.964	1.202* (126%)

Necropsies from Weeks 77 thru 104: Organ weights were provided males at 25 mg/kg/day that were sacrificed during week 77; however, there were no concurrent controls.

Organ weight differences observed at necropsies conducted from weeks 92 thru 104 were evident for the heart, uterus, thymus, kidneys, pituitary, thyroid/parathyroid, and spleen as shown in the tables below. Potential corresponding histopathological changes were evident for the heart, uterus, kidneys, and spleen. Cardiomyopathy and ventricular dilatation were evident for all male treatment groups and females at 5 and 25 mg/kg/day. Increased incidences of adenomyosis were evident in the uterus for all female treatment groups. Casts and lymphocyte infiltration were observed in the kidneys for all male treatment groups and females at 5 and 25 mg/kg/day. Lymphoid necrosis in the spleen was observed for male treatment groups and females at 5 and 25 mg/kg/day.

Absolute and relative organ weights at necropsy during week 92 for female mice at 25 mg/kg/day and week 95 for males at 5 mg/kg/day

Organ/Tissue	Males, Week 95		Females, Week 92	
	0-1 (n = 5)	5 (n = 20)	0-1 (n = 5)	25 (n = 19)
Heart g/100g BW			0.586	0634 (108%)
Heart g/100g BrW			42.002	46.412 (111%)
Uterus g			1.2852	0.8077 (63%)
Uterus g/100g BW			3.530	2.190 (62%)
Uterus g/100g BrW			253.116	162.075 (64%)
Thymus g			0.0212	0.0156 (74%)
Thymus g/100g BW			0.056	0.041 (73%)
Thymus g/100g BrW			4.042	3.097 (77%)
Kidneys g/100g BW			1.839	1.559 (85%)
Kidneys g/100g BrW			131.325	114.503 (87.2%)
Spleen g	0.1259	0.1760 (140%)		
Spleen g/100g BW	0.300	0.398 (133%)		
Spleen g/100g BrW	24.396	35.071 (144%)		
Pituitary g	0.0020	0.0026 (130%)	0.0032	0.0020 (63%)

Pituitary g/100g BW			0.009	0.005 (56%)
Pituitary g/100g BrW	0.382	0.512 (134%)	0.621	0.400 (64%)
Thyroid/Parathyroid g			0.0048	0.0065 (135%)
Thyroid/Parathyroid g/100g BW			0.013	0.018 (139%)
Thyroid/Parathyroid g/100g BrW			0.923	1.296 (140%)

Absolute and relative organ weights at necropsies during week 104 for male control and treatment groups and week 102 for female control and treatment groups

	Males, Week 104			Females, Week 102			
	0-1 (n=23)	0-2 (n=21)	1 (n=22)	0-1 (n=21)	0-2 (n=21)	1 (n=26)	5 (n=22)
Heart g				0.1901	0.1901	0.2157* (114%)	0.2110 (111%)
Heart g/100g BW				0.544	0.536	0.583 (108%)	0.594 (110%)
Heart g/100g BrW				37.729	36.009	41.721 (113%)	41.898 (114%)
Uterus g				2.1560	2.3268	10.5268 (68%)	1.2599 (56%)
Uterus g/100g BW				6.084	6.233	4.028 (65%)	3.526 (57%)
Uterus g/100g BrW				427.590	444.120	294.788 (68%)	252.651 (59%)
Thymus g				0.0213	0.0234	0.0281	0.0168 (75%)
Thymus g/100g BW				0.061	0.065	0.075	0.047 (75%)
Thymus g/100g BrW				4.218	4.457	5.397	3.334 (77%)
Spleen g	0.1342	0.1562	0.1126 (78%)				
Spleen g/100g BW	0.320	0.378	0.263 (75%)				
Spleen g/100g BrW	25.876	30.153	22.137 (79%)				
Thyroid/Parathyroid g/100g BrW				1.233	1.4397	1.679* (126%)	1.545 (116%)

Gross pathology: Gross pathological findings were reported for unscheduled deaths, the week 25 interim necropsy, the week 77 interim necropsy of males at 25 mg/kg/day, the week 92 interim necropsy of females at 25 mg/kg/day (plus 5 females from Control Group 2), the week 95 interim necropsy of males at 5 mg/kg/day (plus 5 males from Control Group 1), the primary necropsy during week 102 of the female 0-1, 0-2, 1, and 5 mg/kg/day groups, and the primary necropsy during week 104 of the male 0-1, 0-2, and 1 mg/kg/day groups. The only gross necropsy finding that corresponded with a histopathological finding was enlarged salivary glands for 1 of 12 males at 5 mg/kg/day and 4 of 12 males at 25 mg/kg/day during the week 25 interim necropsy and 1 of 19

females at 25 mg/kg/day during the week 92 interim necropsy. The corresponding histopathological finding for the salivary gland was hypertrophy (i.e., cytoplasmic enlargement of serous acinar cells). The sponsor noted that enlargement of the salivary glands may not have been identified correctly at necropsy in many cases.

Histopathology:

Non-neoplastic:

25-Week Treatment: Following treatment with (R,R)-formoterol for 25 weeks, target organs of toxicity included the heart, salivary gland, parotid salivary gland, uterus, kidneys, and spleen. Cardiomyopathy was observed in male and female mice at 5 and 25 mg/kg/day as contrasted to no similar findings in controls. For the salivary gland and parotid salivary gland, hypertrophy was observed for male and female mice at 25 mg/kg/day. In addition for the parotid salivary gland, multinucleated giant cells were observed for male and female mice at 25 mg/kg/day. Incidences of uterine adenomyosis were increased in female treatment groups, although a dose-response relationship was not present. The incidence of nephropathy was increased for males at 5 and 25 mg/kg/day. There were findings in the vagina and cervix with uncertain relationships to treatment based upon no histopathological findings in these tissues from female mice treated with (R,R)-formoterol for periods greater than 92 weeks.

Microscopic Findings, Week 25 Necropsy

Organ/Tissue	Sex	0-1	1	5	25
Heart					
-cardiomyopathy, minimal	M	0/12	0/12	2/12	5/12
	F	0/12	0/12	1/12	1/12
Salivary gland					
-hypertrophy, minimal-moderate	M	0/12	0/12	0/12	8/12
	F	0/12	0/12	0/12	11/12
Salivary gland, Parotid					
-hypertrophy, minimal-moderate	M	0/12	-	-	8/12
	F	0/12	-	-	12/12
-giant cell, multinucleated, minimal	M	0/12	-	-	5/12
	F	0/12	-	-	5/12
Uterus					
-adenomyosis	F	0/12	4/12	7/12	4/12
Kidneys					
-nephropathy, minimal-mild	M	1/12	2/12	6/12	5/12
	F	3/12	4/12	2/12	2/12
Spleen					
-extramedullary hematopoiesis	M	1/12	-	-	2/12
	F	2/12	-	-	7/12
Vagina					
-exudate, suppurative, minimal	F	0/12	-	-	3/12
Cervix					
-hyperplasia, epithelial, minimal-mild	F	1/10	0/12	0/12	3/12
-exudate, suppurative, minimal	F	0/10	0/12	0/12	2/12

All Animals (excluding 25-week treatment): Following treatment with (R,R)-formoterol for periods greater than 77 weeks, target organs of toxicity included the heart, salivary glands, parotid salivary glands, kidneys, and uterus. There were additional findings in the liver, Harderian glands, thymus, spleen, and lung where the findings were of relatively low incidences or the incidences of findings only slightly exceeded controls.

For the heart, incidences of cardiomyopathy and ventricular dilatation were increased for males at 1, 5, and 25 mg/kg/day and females at 5 and 25 mg/kg/day. The severity of cardiomyopathy and ventricular dilatation increased in a dose-related manner. Cardiomyopathy was characterized by separation of myocytes within the myocardium by an increased amount of fibrous connective tissue. The left ventricular muscle was most commonly affected. Inflammation and myofiber degeneration did not appear to be associated with fibrosis. Cardiomyopathy in CD-1 mice is regarded as a spontaneous, progressive, and age-related degenerative condition of the heart. The sponsor stated that (R,R)-formoterol-related cardiomyopathy noted for animals sacrificed at terminal necropsies was difficult to discern from typical age-related changes due to the increased incidence of spontaneous cardiomyopathy in mice, most notably for males. However, the severity of cardiomyopathy increased in a dose-related manner. The earlier onset of cardiomyopathy is attributed to the pharmacological effects of β-adrenergic agonists such as (R,R)-formoterol.

For the salivary glands and parotid salivary glands, hypertrophy (i.e., cytoplasmic enlargement of serous acinar cells) was observed for males and females at 5 and 25 mg/kg/day. In addition for the parotid salivary gland, there were findings of multinucleated giant cells (i.e., acinar cells that contained multiple and variably sized nuclei) for males at 1, 5, and 25 mg/kg/day and females at 5 and 25 mg/kg/day. For the salivary glands, there were findings of multinucleated giant cells for 1 male and 1 female at the high dose. For the salivary glands and parotid salivary glands, the incidence of lymphocyte infiltration was increased for females at 25 mg/kg/day. Serous acinar cell hypertrophy is attributed to the pharmacological effects of the β-adrenergic agonists such as (R,R)-formoterol.

For the kidneys, incidences of casts and lymphocyte infiltration were increased for male and female mice at 5 and 25 mg/kg/day.

For the uterus, the incidence of adenomyosis was increased in a dose-related manner for female treatment groups. Adenomyosis was characterized by the presence of histologically normal endometrial glands and stroma within the myometrium (i.e., an in-growth of the endometrium into the uterine musculature).

Other observations of uncertain relationship to treatment were observed in the liver, Harderian glands, thymus, spleen, and lungs. In general, most of these findings were observed in animals found or sacrificed in a moribund condition.

Microscopic Findings, All Animals (Unscheduled deaths and animals sacrificed during weeks 77, 92, 95, 102, and 104; Animals from the week 25 necropsy have been removed)

Organ/Tissue	Sex	0-1	0-2	1	5	25
Heart						
-cardiomyopathy, minimal-moderate	M	34/60	28/60	41/60	48/60	43/60
	F	21/61	18/61	16/60	32/60	40/60
minimal		27:18	20:16	26:15	31:22	16:19
mild		5:3	6:1	11:1	12:8	18:13
moderate		2:0	2:1	4:0	5:2	9:7
severe		0:0	0:0	0:0	0:0	0:1
-dilatation, ventricle	M	1/60	0/60	3/60	4/60	5/60
	F	0/61	0/61	0/60	3/60	4/60
minimal		1:0	0:0	1:0	3:2	4:4
mild		0:0	0:0	1:0	1:1	1:0
moderate		0:0	0:0	1:0	0:0	0:0
Salivary glands						
-hypertrophy, minimal-moderate	M	0/60	0/60	0/60	1/60	40/60
	F	0/61	1/51	0/60	4/60	39/59
-giant cell, multinucleated, minimal	M	0/60	0/60	0/60	0/60	1/60
	F	0/61	0/61	0/60	0/60	1/59
-infiltrate, lymphocyte, minimal-moderate	M	25/60	24/60	32/60	30/60	21/60
	F	23/61	20/61	28/60	28/60	36/59
Salivary gland, parotid						
-hypertrophy, minimal-moderate	M	0/60	0/57	0/40	7/58	36/59
	F	0/59	0/60	0/44	12/60	38/60
-giant cell, multinucleated, minimal-mild	M	0/60	0/57	1/40	9/58	20/59
	F	0/59	0/60	0/44	6/60	28/60
-infiltrate, lymphocyte, minimal-mild	M	7/60	2/57	2/40	11/58	6/59
	F	7/59	7/60	6/44	10/60	14/60
Kidneys						
-casts, minimal-mild	M	7/60	5/60	16/60	21/60	24/60
	F	14/61	19/61	18/60	30/60	33/60
-infiltrate, lymphocyte, minimal-moderate	M	9/60	10/60	15/60	21/60	27/60
	F	20/61	21/61	16/60	28/60	37/60
Uterus						
-adenomyosis	F	9/61	12/61	20/60	29/60	32/60
Liver						
-necrosis, hepatocellular, centrilobular, minimal-moderate	M	0/60	0/30	3/60	1/60	6/60
	F	0/61	1/61	1/60	1/60	1/60
Harderian gland						
-pigment, minimal-moderate	M	35/60	44/60	34/40	39/60	50/60
	F	48/61	49/61	36/41	43/46	55/60
-infiltrate, lymphocyte, minimal-mild	M	23/60	13/60	9/40	20/60	17/60
	F	23/61	32/61	22/41	19/46	39/60
Thymus						
-necrosis, lymphoid, minimal-severe	M	3/57	1/51	0/31	0/52	10/58

	F	1/58	1/56	1/35	5/35	1/55
Spleen						
-necrosis, lymphoid, minimal-mild	M	0/60	0/59	3/38	1/59	5/60
	F	0/61	0/61	0/41	2/45	2/61
Lung						
-histiocytosis, alveolar, minimal-severe	M	14/60	12/60	11/43	17/60	12/60
	F	14/61	9/61	12/42	16/38	20/60
-inflammation, chronic, minimal-mild	M	2/60	8/60	6/43	10/60	6/60
	F	2/61	1/61	3/42	1/38	6/60

Unscheduled Deaths: For mice found dead or sacrificed in a moribund condition during the treatment period, target organs of toxicity included the heart, salivary glands, parotid salivary glands, kidneys, and uterus. Findings of uncertain relationship to treatment were observed in the liver, thymus, mesenteric lymph nodes, pancreas, Harderian glands, spleen, and lungs. These findings are similar to those described in the histopathology table for ALL animals.

The most prominent histopathological findings were evident in the heart. Incidences of cardiomyopathy and ventricle dilatation were increased for all male treatment groups and females at 5 and 25 mg/kg/day. The incidence of mineralization was increased for males at 5 mg/kg/day and females at 25 mg/kg/day. It should be noted that surviving males in the 25 mg/kg/day group were terminated during week 77. For unscheduled deaths, the correlation between cardiomyopathy and decreased survival appeared to be weak.

Microscopic Findings, Unscheduled Deaths

Organ/Tissue	Sex	0-1	0-2	1	5	25
N =		32	39	37	40	40
Heart						
-cardiomyopathy, minimal-severe	M	10/32	12/39	20/37	32/40	23/40
	F	11/40	8/35	4/34	16/38	25/41
-mineralization, minimal-mild	M	0/32	1/39	3/37	8/40	3/40
	F	2/40	1/35	5/34	4/38	6/41
-dilatation, ventricle, minimal-moderate	M	1/32	0/39	3/37	4/40	5/40
	F	0/40	0/35	0/34	3/38	4/41
Salivary glands						
-hypertrophy, minimal-moderate	M	0/32	0/39	0/37	1/40	20/40
	F	0/40	1/35	0/34	4/38	21/40
Salivary gland, parotid						
-infiltrate, lymphocyte, minimal-mild	M	0/32	1/36	2/37	5/38	3/39
	F	0/39	0/34	0/33	1/38	2/41
-hypertrophy, minimal-mild	M	0/32	0/36	0/37	3/38	16/39
	F	0/39	0/34	0/33	3/38	19/41
-giant cell, multinucleated, minimal-mild	M	0/32	0/36	1/37	5/38	19/39
	F	0/39	0/34	0/33	4/38	25/41

Kidneys						
-cast, minimal-mild	M	4/32	4/39	12/37	18/40	20/40
	F	9/40	11/35	12/34	20/38	20/41
-infiltrate, lymphocyte, minimal-mild	M	6/32	8/39	11/37	18/40	23/40
	F	12/40	12/35	3/34	15/38	24/41
Uterus						
-adenomyosis	F	7/40	4/35	7/34	12/38	18/41
Liver						
-necrosis, hepatocellular, centrilobular, minimal-moderate	M	0/32	0/39	3/37	1/40	6/40
	F	0/40	1/35	1/34	1/38	1/41
Thymus						
-necrosis, lymphoid, minimal-severe	M	3/30	1/32	0/30	0/34	10/38
	F	1/38	1/31	1/30	5/34	1/39
Mesenteric LN						
-necrosis, lymphoid, minimal-mild	M	1/31	1/38	2/35	1/40	4/40
	F	0/39	1/35	2/34	5/33	4/40
Pancreas						
-atrophy, islet cells, minimal-severe	M	4/32	1/38	1/37	8/40	4/40
	F	3/40	1/35	5/34	3/38	9/41
Harderian gland						
-infiltrate, lymphocyte, minimal-mild	M	7/32	7/39	7/37	12/40	7/40
	F	12/40	17/35	15/34	11/38	23/41
Spleen						
-necrosis, lymphoid, minimal	M	0/32	0/38	3/37	1/40	5/40
	F	0/40	0/35	0/34	2/38	2/41
Lungs						
-histiocytosis, alveolar, minimal-moderate	M	8/32	9/39	10/37	16/40	8/40
	F	10/40	7/35	12/34	16/38	16/41

Neoplastic: For the mouse carcinogenicity study, treatment with (R,R)-formoterol ranged from 77 to 104 weeks.

The incidences of uterine endometrial stromal polyps (p-value of pairwise comparison with mid dose group 0.0000<0.01) and combined incidences of uterine endometrial stromal polyps and stromal cell sarcoma (p-value of pairwise comparison with mid dose group 0.0000<0.01) were significantly increased for female treatment groups; however dose-response relationships were not present given that tumor incidences for the high dose group were lower than the low and mid dose groups due to decreased survival and early termination of surviving animals at the high dose. See the table below that shows that incidences of uterine endometrial stromal polyps and stromal cell sarcoma at the week 92 interim necropsy for the high dose group were lower than at the week 102 primary necropsy for low and mid dose groups. Trend tests were positive when the high dose group was excluded (i.e., uterine endometrial stromal polyps, 0.0000<0.005; uterine endometrial stromal polyps + stromal cell sarcoma, 0.0001<0.005); however, trend tests were negative when the high dose group was included (i.e., 0.0872>0.005 and 0.0440>0.005, respectively). These tumor incidences exceeded spontaneous incidences of uterine endometrial stromal polyps and sarcoma from the testing laboratory of 4.29 and 1.26%, respectively, [ ]

[ ] The combined incidences of uterine and cervical endometrial stromal b(4)

polyps and stromal cell sarcoma were significantly increased for female treatment groups (p-value of pairwise comparison with mid dose group 0.0000<0.01); however a dose-response relationship was not present for reasons noted above. The trend test was positive when the high dose group was excluded (0.0000<0.005); however, the trend test was negative when the high dose group was included (0.0348>0.005). Based upon tumor findings in the uterus and cervix combined, (R,R)-formoterol is tumorigenic in female mice.

Incidences of uterine leiomyoma and leiomyosarcoma and combined incidences of uterine leiomyoma and leiomyosarcoma displayed no relationship to treatment.

The incidences of hepatocellular adenoma and hepatocellular carcinoma and combined incidences of hepatocellular adenoma and carcinoma were increased for female treatment groups. These incidences exceed the spontaneous incidences of hepatocellular adenoma and carcinoma for female mice from the testing laboratory of 1.82 and 0%, respectively; however, they are within the ranges reported from [ ] Further, these incidences were not statistically significant. There were no treatment-related changes in the incidences of hepatocellular adenoma and carcinoma and combined incidences of hepatocellular adenoma and carcinoma for male treatment groups. The incidences of hepatocellular adenoma and carcinoma for male mice from the testing laboratory were 11.37 and 3.10%, respectively. b(4)

Neoplastic findings, all females (mice from 25-week sacrifice were removed). Data is displayed as number of animals with finding and the total number of animals examined (S = metastatic, B= benign, and M = malignant).

Organ/Tissue	0-1	0.2	1	5	25[a]	Historical Control[b]
N =	61	61	60	60	60	
Uterus						
NE	61	61	60	60	60	
B-polyp, endometrial stromal	5	5	15 (25%)	20 (35%)	9 (15%)	5.19%[4.29%][c]
B-polyp, endometrial stromal, multiple	0	0	0	0	2(3.3%)	(1.7-17.1%)
M-sarcoma, stromal cell	0	3	0	1 (1.7%)	1 (1.7%)	1.17% [1.26%]
Total (polyp, ES + sarcoma)	0	0	0	1 (1.7%)	0	(1.4-8.0%)
M-leiomyosarcoma	5	8	15 (25%)	22 (37%)	12(20%)	6.36%
B-leiomyoma	2	5	7	7	2	1.28% (0.9-6.0%)
B-leiomyoma, multiple	0	3	2	2	1	0.15%
M-leiomyosarcoma, multiple	0	0	0	1	0	(1.7-2.1%)
Total	0	0	1	0	0	
B-fibroma	2	8	10	10	3	
Cervix						
NE	0	0	0	1	0	
	57	54	56	59	58	

B-polyp, endometrial stromal	1	1	2	1	2	0.26% [NA] (1.15-3.33%)
M-sarcoma, stromal cell	0	0	0	1	0	0.22% [NA] (0.8-2.0%)
Total (polyp, ES + sarcoma)	1	1	2	0	2	0.48%
B-leiomyoma	0	0	1	2	1	0.44% (0.8-4.2%)
M-leiomyosarcoma	4	0	0	1	2	0.59% (1.5-4.2%)
Total	4	0	1	3	3	1.03%
Uterus + Cervix						
Total (polyp, ES + sarcoma)	6/61(9.8%)	9/61(15%)	16/60(27%)	24/60(40%)	14/60(23%)	
Liver						
NE	61	61	60	60	60	
M-carcinoma, hepatocellular	0	0	2 (3.3%)	1 (1.7%)	1 (1.7%)	0.66% [0%] (1.4-4.3%)
B-adenoma, hepatocellular	1	1	2 (3.3%)	4 (6.7%)	2 (3.3%)	0.99% [1.82%] (0.85-7.84%)
B-adenoma, hepatocellular, multiple	0	0	0	0	1 (1.7%)	
Total (adenoma + carcinoma)	1	1	4 (6.7%)	5 (3.0%)	4 (6.7%)	1.65%

a. Surviving females at 25 mg/kg/day were sacrificed during week 92.
b. Historical control data from ⊂ ⊐ (March 2000). b(4)
c. Historical control data from the testing laboratory are shown in brackets [ ].

Incidences of uterine endometrial stromal polyps and stromal cell sarcoma at the week 92 interim necropsy and week 102 primary necropsy.

Organ/Tissue	0-1	0-2	1	5[b]	25[a]
Week 92 Interim Necropsy					
Uterus					
NE	0	5	0	0	19
B polyp, endometrial stroma	-	0	-	-	4
M sarcoma, stromal cell	-	0	-	-	2
Week 102 Primary Necropsy					
Uterus					
NE	21	21	26	22	0
B polyp, endometrial stroma	2	2	7	10	-
M sarcoma, stromal cell	0	2	0	2	-

Neoplastic findings, all males (mice from 25-week sacrifice were removed). Data is displayed as number of animals with finding and the total number of animals examined (S = metastatic, B= benign, and M = malignant).

Organ/Tissue	0-1	0-2	1	5[b]	25[a]	Historical Control[c]
N =	60	60	60	60	60	
Liver						
NE	60	60	60	60	60	
B-adenoma, hepatocellular	4	4	3	5	4	10.46%[11.37%][d] (2.9-28%)
M-carcinoma, hepatocellular	2	1	6	3	1	5.29%[3.10%] (1.5-16.0%)
B-adenoma, hepatocellular, multiple	0	0	1	0	0	
Total	6 (10%)	5 (8%)	10(17%)	8 (13%)	5 (8%)	15.75%

a. Surviving males at 25 mg/kg/day were sacrificed during week 77.
b. surviving males at 5 mg/kg/day were sacrificed during week 95.
c. Historical control data from [redacted] (March 2000).
d. Historical control data from the testing laboratory are shown in brackets [ ].

b(4)

Toxicokinetics: C_{max} and AUC values for (R,R)-formoterol in male and female treatment groups increased in an approximate dose proportion manner. C_{max} and AUC values for (R,R)-formoterol were greater in male mice as compared to female mice. AUC values in male and female treatment groups ranged from 44 to 2706 times greater than exposure with a clinical dose of 50 µg/day. Comparison of AUC values at weeks 4 and 38 for male and female mice at 5 mg/kg/day suggests potential drug accumulation, although sampling at 4 weeks only extended to 2 hr after dosing.

Toxicokinetic parameters for (R,R)-formoterol in male mice

Dose mg/kg/day	Time point months	T_{max} hr	C_{max} pg/mL	$AUC_{0.5\text{-}24hr}$ pg·hr/mL	Exposure margin[a]
1	Week 4	-	-	-	-
	Week 38	0.5	1750	16300	150
5	Week 4	0.5	21000	14400[b]	132
	Week 38	1.0	22000	27800	255
25	Week 4	-	-	-	-
	Week 38	1.0	112000	295000	2706

a. Human exposure ($AUC_{0\text{-}24hr}$ = 109 pg·hr/mL) from 091-026 preliminary data (after 2 weeks of daily inhalation treatment of arformoterol in COPD subjects with a dose of 50 µg/day.
b. $AUC_{0.5\text{-}2hr}$ (No sampling after 2 hr time point).

Toxicokinetic parameters for (R,R)-formoterol in female mice

Dose µg/kg/day	Time point months	T_{max} hr	C_{max} pg/mL	$AUC_{0.5\text{-}24hr}$ pg·hr/mL	Exposure margin[a]
1	Week 4	-	-	-	-
	Week 38	2.0	1710	4800	44
5	Week 4	0.5	6740	7000[b]	64
	Week 38	1.0	4540	13000	119
25	Week 4	-	-	-	-
	Week 38	0.5	34200	73100	671