Dogs

Study title: **Three-Month Inhalation Toxicity Study of (R,R)-Formoterol and (R,R)-Desformoterol in Dogs (with a One-Month Recovery).**

Key study findings:

• In a 13-week inhalation toxicology study, dogs were exposed to 40 µg/kg/day (R,R)-formoterol in the presence or absence of 2.8 µg/kg/day (R,R)-desformoterol. Pulmonary deposited doses for (R,R)-formoterol and (R,R)-desformoterol were 8.50-9.50 µg/kg/day and 0.52-0.60 µg/kg/day, respectively. A concurrent negative control group received citrate-buffered isotonic saline on a comparable regimen. Additional dogs were included in treatment groups for a 4-week recovery period, although no concurrent control dogs were included.

• The secondary objective of this study was to assess the toxicity of potential leachables from (R,R)-formoterol unit dose vials (UDV). A solution of (R,R)-formoterol from UDVs that had been stored for an extended time was used to prepare the test article formulation with appropriate concentrations of bulk (R,R)-formoterol and (R,R)-desformoterol. No specifications of leachables were provided.

• Increased heart rates were observed in both the formoterol only and formoterol + desformoterol groups, although there was no evidence of ectopia as observed in the 13-week and 9-month inhalation toxicology studies with dogs. It should be noted that ECG measurements were not conducted on the first day of exposure as done in the 13-week and 9-month inhalation toxicology studies with dogs.

• Histopathological findings were observed in the heart, epididymides, testes, lung, and mesenteric lymph node, although these findings were not observed in the 13-week and 9-month inhalation toxicology studies with dogs that received (R,R)-formoterol alone at comparable and higher doses. Findings were generally comparable between the formoterol only and formoterol + desformoterol groups.

• There were concerns about the design of this study given that only one dose of (R,R)-desformoterol was tested and the cardiac toxicity of (R,R)-formoterol in dogs might confound interpretation of the study. There were no apparent differences in toxicity between the (R,R)-formoterol alone and (R,R)-formoterol + [redacted] (R,R)-desformoterol groups. b(4)

Study no.: Sepracor Document Number 090-836
Conducting laboratory and location: [redacted]

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Date of study initiation: October 11, 2002
GLP compliance: Yes

QA report: yes (X) no ( )
Drug, lot #, and % purity:

Identification	Quantity Received	Physical Description
[redacted] of(R,R)-formoterol (L)-tartrate Lot No. 053 0001 Retest Date: [redacted] [redacted] Log No. 5502B]	200 Bottles	Off-white fine powder
[redacted] of (R,R)-formoterol (L)-tartrate, 32.4 mg of (R,R)-desformoterol (L)-tartrate Lot No. 054 0001 Retest Date: [redacted] [redacted] Log No. 5503B]	200 Bottles	Off-white fine powder

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b(4)

For Group 3, (R,R)-Formoterol + (R,R)-Desformoterol

The vehicle for the (R,R)-formoterol and (R,R)-desformoterol was (R,R)-formoterol 60 mcg clinical unit dose vial (UDV), and was received from [redacted] on October 8, 2002, as follows:

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(R,R)-formoterol [redacted] Lot No. 00601E Exp. Date: 05/07/03 [redacted] Log No. 5504B]	9000 pouches (12 ampoules/pouch)	Clear colorless liquid

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Purity and stability data for the vehicle were the responsibility of the sponsor. A Certificate of Analysis was provided by the sponsor and is presented in Appendix A. The UDV was refrigerated, and was considered stable under this condition. A reserve sample (one ampule) was collected and stored in the Archives of [redacted]

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For Groups 1 and 2,

4.1.3. VEHICLE IDENTIFICATION FOR (R,R)-FORMOTEROL

The dosing solution for the vehicle control (Group 1) and the vehicle for the (R,R)-formoterol (Group 2) was citrate-buffered isotonic saline and was received from Sepracor Inc., Marlborough, Massachusetts, as follows:

Identification	Quantity Received	Identification	Date of Receipt
Citrate-buffered isotonic saline [Log No. 5501B]	14 Bottles	White crystalline powder	October 3, 2003
Citrate-buffered isotonic saline [Log No. 5501C]	8 Bottles	White crystalline powder	December 4, 2002
Citrate-buffered isotonic saline [Log No. 5501D]	7 Bottles	White crystalline powder	December 17, 2002

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Methods

Doses: See doses of (R,R)-formoterol and (R,R)-desformoterol in the table below. Values are shown as male/female.

Group	Test article	Free base concentration µg/L		Target Inhaled Dose µg/kg/day		Achieved Inhaled Dose µg/kg/day	
		(R,R)-Formoterol	(R,R)-Desformoterol	(R,R)-Formoterol	(R,R)-Desformoterol	(R,R)-Formoterol	(R,R)-Desformoterol
1	Vehicle control	-	-	0	0	0	0
2	(R,R)-formoterol	6.75/6.05	0	40	0	34.0/36.3	0
3	(R,R)-formoterol and (R,R)-desformoterol	6.42/6.76	0.36/0.39	40	[ ]	37.9/40.5	2.1/2.4

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Group	Pulmonary deposited dose, µg/kg/day	
	(R,R)-Formoterol*	(R,R)-Desformoterol*
1	0	0
2	8.5	0
3	9.5	0.52/0.60

* A deposition factor of 25% was used.

Liquid droplet aerosols of the test articles were generated using a Collision jet nebulizer. Three exposure systems, one for each test article group and one for the vehicle control group, were used for the study. Each exposure was performed by restraining the dog in a sling and fitting an exposure mask over its muzzle. The treatment setup allowed 4 to 6

dogs of one sex from each group to be exposed simultaneously using a 4- or 6-port exposure system. The exposure period was 15 min per day, 7 days per week for 3 months (until the day prior to the primary necropsy). Exposure concentrations were determined by chemical analyses of test atmosphere samples by HPLC that were collected on glass-fiber filters during the 15-min exposure periods. The actual exposure concentrations for each group (by sex) was used with the exposure duration of 15 min and the group mean minute volume to calculate an inhaled dose (estimated dose level; µg/kg/day).

All vehicle-control group animals were exposed to citrate-buffered isotonic saline daily prior to proceeding with exposure of Group 2 and 3 animals.

Aerosol particle size determinations were conducted for the (R,R)-formoterol and (R,R)-formoterol + (R,R)-desformoterol groups using a 7-stage cascade impactor during weeks 5 and 9 of the exposure period. Glass-fiber filters were used as collection substrates. (R,R)-formoterol collected on the filters was measured by chemical analysis using a HPLC method for concentration and particle size calculation based on impactor stage cut-offs. Due to the absence of quantifiable amounts of formoterol on the upper stages, the particle size determinations for Groups 2 and 3 in the 9th week could not be calculated. After termination of all animal exposures, a particle size determination was conducted for both exposure groups during the 17th week of the study. The exposure duration was increased to 40 min to obtain quantifiable particle size.

	Group 2 (R,R)-formoterol		Group 3 (R,R)-formoterol and (R,R)-desformoterol	
Date	MMAD	GSD	MMAD	GSD
10/19/02	0.5	2.32	0.6	2.37
12/02/02	0.4	1.27	NA	NA
11/28/02	NA	NA	0.6	1.61
02/18/03	0.5	2.36	0.5	2.21
Mean	0.5	1.98	0.6	2.06

NA = Not Applicable
MMAD = mass median aerodynamic diameter
GSD = geometric standard deviation

Species/strain: Male and female purpose-bred beagle dogs were received from [redacted] b(4) Animals were allowed regular opportunities for exercise and social interaction in accordance with standard operating procedures, except during the dosing phase of the study.

Number/sex/group or time point (main study): 4 dogs/sex/group

Route, formulation, volume, and infusion rate: Exposures were conducted using muzzle-only (nose-only) exposure systems operated under dynamic conditions to maintain a minimum O_2 content of 19% and an evenly distributed exposure atmosphere.

Satellite groups used for toxicokinetics or recovery: Treatment groups included an additional 2 dogs/sex/group for a 4-week recovery period; however, no concurrent vehicle-control dogs were included for comparison.

Age: At the start of treatment, dogs were 7 to 8 months old.

Weight: At the start of treatment, body weight ranges were 7.3 to 10.7 kg for males and 5.8 to 7.8 kg for females.
Unique study design or methodology (if any): The objective of this study was to evaluate the potential toxicity of (R,R)-formoterol containing an expected level of the degradant, (R,R)-desformoterol. (R,R)-formoterol and (R,R)-desformoterol were administered to dogs by inhalation (muzzle only using a mask) exposure once daily for 15 min, seven days per week, over 91 days at target exposure levels of 40 and 0 µg/kg/day (R,R)-formoterol and (R,R)-desformoterol, respectively, in Group 2, and 40 and 2.8 µg/kg/day (R,R)-formoterol and (R,R)-desformoterol, respectively, in Group 3. A concurrent negative control group received citrate-buffered isotonic saline on a comparable regimen. Additionally, the recovery of any potential observed effects was assessed during a 1-month recovery period. During the prolonged storage of clinical unit dose vials (UDV) of (R,R)-formoterol, there exists a possibility of a leaching process. The secondary objective of this study was to assess the toxicity of potential leachables from (R,R)-formoterol UDV. A solution of (R,R)-formoterol from UDVs that had been stored for an extended time was used as the vehicle to prepare the test article formulation with appropriate concentrations of bulk (R,R)-formoterol and (R,R)-desformoterol. However, no specifications for leachables were provided. The target (R,R)-formoterol dose was equivalent in groups with or without (R,R)-desformoterol.

Observations and times:
Mortality: During the dosing phase, animals were observed twice daily for mortality/moribundity.
Clinical signs: During the dosing phase, animals were observed at 1 and 4 hr after dosing for clinical signs. Detailed physical examinations were conducted once per week. Rectal body temperatures were measured on day 0 following exposure.
Body weights: Body weights were measured once per week.
Food consumption: Food consumption was measured daily and calculated as g/animal/day.
Ophthalmoscopy: Not performed.
EKG: Multi-lead (I, II, III, aVR, aVL, aVF) electrocardiograms were recorded for all animals prior to the initiation of exposure (week -1), on day 7, during weeks 3 and 12, and prior to recovery necropsy. During the dosing phase, ECGs were recorded prior to inhalation and at 1 and 4 hr after exposure. QTc was assessed using the Van de Water's correction factor according to the equation, QTc = QT-87 (60/HR-1). Blood pressures were recorded using a [redacted] tail cuff device according to the same schedule as ECGs. Mean arterial pressure was calculated using the following equation, MAP = Pd + 1/3(Ps-Pd). It should be noted that ECG measurements were not conducted on the first day of exposure as done in the 13-week and 9-month inhalation toxicology studies with dogs. b(4)
Hematology: Blood samples for measurement of hematology parameters were collected at week -1 (prior to the start of treatment), day 3, near the end of the exposure period (week 12), and at recovery necropsy (week 16).
Clinical chemistry: Blood samples for measurement of serum chemistry parameters were collected at week -1 (prior to the start of treatment), day 3, near the end of the exposure period (week 12), and at recovery necropsy (week 16).

Urinalysis: Urine samples were collected at week -1 (prior to the start of exposure), near the end of the exposure period (week 12), and at recovery necropsy (week 16).
Gross pathology: Animals were sacrificed at the end of the treatment and recovery periods and submitted to gross necropsy examination. Tissues and organs were collected and placed in 10% neutral buffered formalin except the epididymides and testes which were fixed in Bouin's solution and the eyes with optic nerves which were placed in Davidson's solution. Bone marrow smears were obtained at necropsy.
Organ weights: Absolute and relative organ weights were determined for the adrenal glands, brain, epididymides, heart, kidneys, liver, lung, ovaries, pituitary, spleen, testes, thymus, thyroids with parathyroids, and uterus.
Histopathology: Organs and tissues were processed into paraffin blocks, sectioned at 4 to 8 µm, mounted on glass slides, and stained with hematoxylin and eosin. Microscopic examination of all tissues was performed at the primary necropsy. For recovery animals, examination was limited to potential target tissues (i.e., heart and respiratory tract including lung, larynx, trachea, pharynx, nasal cavity) and gross lesions.
Toxicokinetics: Blood samples for toxicokinetic evaluation were collected on study days 0, 29, and 86 at 0 hr (prior to exposure) and at 0.5, 1, 2, 6, and 24 hr after exposure. Plasma samples were shipped on dry ice by overnight courier to [redacted] for analysis. Plasma concentrations of (R,R)-formoterol and relative peak area ratios of (R,R)-desformoterol were measured using a non-chiral liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. The LLOQ was 5 pg/mL. It was reported that a total of only 30 samples produced measurable peak ratios of (R,R)-desformoterol, which were used for qualitative purposes only. (R,R)-desformoterol was measured on glass fiber filters inserted in nose-only exposure systems during the treatment period. b(4)

Results

Mortality: None.

Clinical signs: Prominent clinical signs at 1- and 4-hr post exposure included body flushed, facial area flushed, reddened ears, reddened gums, and injected sclera in right and left eye. Rectal body temperature was unaffected on day 0 following exposure.

Clinical signs at 1-hr post-exposure during the treatment period (Total occurrence/number of animals)

Clinical signs	Males			Females		
	Vehicle-Control	Formoterol	Formoterol + Desform	Vehicle-Control	Formoterol	Formoterol + Desform
Body flushed	22/3	457/6	467/6	2/2	446/6	423/6
Facial area flushed	31/4	221/6	275/6	22/2	207/6	157/6
Reddened ears	16/3	403/6	387/6	4/1	360/6	331/6
Reddened gums	58/3	433/6	414/6	33/3	354/6	344/6
Injected sclera, left eye	72/4	273/6	206/6	70/4	110/6	83/5
Injected sclera, right eye	77/4	261/6	206/6	70/4	116/6	90/6
Clear discharge,	4/1	70/5	115/5	102/4	45/2	103/5

left eye						
Clear discharge, right eye	4/1	78/5	104/6	95/3	50/2	95/5
Increased heart rate	0	2/2	2/2	-	-	-
Pupil(s) dilated	1/1	17/6	17/6	0	20/6	15/6
Soft feces	6/3	28/4	33/6	6/2	32/6	45/6

Clinical signs at 4-hr post-exposure during the treatment period (Total occurrence/number of animals)

Clinical signs	Males			Females		
	Vehicle-Control	Formoterol	Formoterol + Desform	Vehicle-Control	Formoterol	Formoterol + Desform
Body flushed	28/4	182/6	218/6	4/3	156/6	186/6
Facial area flushed	32/3	59/6	139/6	15/3	61/6	58/6
Reddened ears	14/4	136/6	158/6	1/1	76/6	115/6
Reddened gums	50/4	161/6	189/6	19/4	105/6	156/6
Injected sclera, left eye	42/4	213/6	193/6	31/4	84/6	93/6
Injected sclera, right eye	41/4	213/6	186/6	29/3	90/6	95/6
Clear discharge, left eye	0	77/6	107/4	99/3	76/3	94/4
Clear discharge, right eye	0	120/6	98/4	88/3	72/4	83/4

Body weights: Body weight gains for male and female dogs in Groups 2 and 3 were significantly increased during the treatment period as compared to vehicle-control groups. Body weight gains for male dogs in Groups 2 and 3 were increased to 174.1 and 164.7% of the control, respectively. Body weight gains for female dogs in Groups 2 and 3 were increased to 162.7 and 145.6% of the control, respectively. Slight body weight losses were evident for male and female dogs in Groups 2 and 3 during the recovery period; however, a concurrent control group was not available for comparison.

Food consumption: Food consumption was slightly increased for male and female dogs in Groups 2 and 3 during the treatment period. Food consumption for males in Groups 2 and 3 were increased to 105.7 and 104.6% of the control (4133 g/animal), respectively. Food consumption for females in Groups 2 and 3 were increased to 116 and 108.6% of the control (3675 g/animal), respectively.

EKG: Electrocardiogram, heart rate, systolic blood pressure, diastolic blood pressure, and QTc interval were evaluated in dogs on day 7 and during weeks 3 and 12. Increased heart rate and/or sinus tachycardia were observed for male and female dogs in the formoterol only group and formoterol + desformoterol group on day 7 and during weeks 3 and 12 at 1 and/or 4 hr postdose. It was noted that increased discharge of the sinoatrial node could result in ectopia and, if sustained, could result in a mismatch between oxygen delivery and oxygen consumption by the myocardium leading to subendocardial and papillary muscle ischemia and necrosis. Slight decreases of systolic and diastolic blood pressure were observed for male and female dogs in the formoterol

only group and formoterol + desformoterol group on day 7 and during weeks 3 and 12 at 1 and/or 4 hr postdose. There were no treatment-related effects on QTc interval.

Electrocardiography evaluation at day 7 and during weeks 3, 12, and 16

Wk	ECG Finding	Males			Females		
		Cont	F	F + D	Cont	F	F + D
Day 7							
-1hr PD	Increased heart rate	0/4	6/6	0/6	0/4	6/6	6/6
-4hr PD	Increased heart rate	0/4	6/6	0/6	0/4	6/6	0/6
Wk 3							
-0 hr		0/4	0/6	0/6	0/4	0/6	0/6
-1 hr PD	Right ventricular premature depolarization	1/4	0/6	0/6	0/4	0/6	0/6
	Sinus tachycardia	0/4	3/6	0/6	0/4	0/6	0/6
-4 hr PD	Increased heart rate	0/4	6/6	6/6	0/4	6/6	6/6
Wk 12							
-0 hr		0/4	0/6	0/6	0/4	0/6	0/6
-1 hr PD	Sinus tachycardia	0/4	5/6	0/6	0/4	3/6	3/6
-4 hr PD	Sinus tachycardia	1/4	1/6	0/6	1/4	1/6	0/6
Wk 16 Recovery	Right axial deviation	-	0/2	0/2	-	1/2	0/2

Heart rate and blood pressure data at day 7 and weeks 3 and 12

Parameter	Wk	Males			Females		
		Control	F	F + D	Control	F	F + D
Heart rate bpm	Day 7 -0	129	122	112	148	118	127
	-1 hr PD	143	215* (150%)	196* (137%)	146	199* (136%)	190 (130%)
	-4hr PD	144	167	156	140	155 (111%)	160 (114%)
	Wk 3 -0	153	120* (78%)	112* (73%)	142	99* (70%)	118 (83%)
	-1 hr PD	160	230* (144%)	173* (108%)	163	160 (98%)	187 (115%)
	-4hr PD	159	180 (113%)	156	148	148	155
	Wk 12 -0	143	88	99	123	101	106
	-1 hr PD	136	184* (135%)	157* (115%)	138	170 (123%)	167 (121%)
	-4hr PD	148	151	139	144	143	136
Systolic Pressure mm Hg	Day 7 -0	141	156	164	133	148	145 (80%)

	-1 hr PD	163	139 (85%)	139 (85%)	152	137 (90%)	121 (80%)
	-4hr PD	154	150	156	143	149	140
	Wk 3						
	-0	163	175	157	160	162	166
	-1 hr PD	170	118* (69%)	139* (82%)	170	141 (83%)	137 (81%)
	-4hr PD	174	142* (82%)	164* (94%)	188	147* (78%)	151* (80%)
	Wk 12						
	-0	183	182	183	170	170	176
	-1 hr PD	172	149* (87%)	162 (94%)	183	149 (81%)	140* (77%)
	-4hr PD	167	169	188	165	161	181
Diastolic Pressure mm Hg	Day 7						
	-0	80	100	116	91	1021	100
	-1 hr PD	78	100	90	81	67 (83%)	66 (82%)
	-4hr PD	87	103	106	96	93	94
	Wk 3						
	-0	77	93	96	97	94	114
	-1 hr PD	87	67 (77%)	89	114	93 (82%)	78* (68%)
	-4hr PD	99	90	107	102	98	100
	Wk 12						
	-0	123	119	124	120	98 (82%)	120
	-1 hr PD	110	90 (82%)	95 (86%)	131	94* (72%)	83* (63%)
	-4hr PD	111	111	127	107	106	96 (89.7%)
Mean Arterial Pressure mm Hg	Day 7						
	-0	100	119	132	105	117	115
	-1 hr PD	107	113	106 (80%)	105	91 (78%)	84 (73%)
	-4hr PD	109	119	123	112	112	109
	Wk 3						
	-0	105	121	117	118	116	131
	-1 hr PD	115	84 (69%)	106	132	109	98 (75%)
	-4hr PD	124	108	126	131	114	117
	Wk 12						
	-0	143	140	144	137	122	139
	-1 hr PD	131	110 (79%)	117 (81%)	148	112	102 (73%)
	-4hr PD	130	130	148	126	124	124

* $p < 0.05$ compared to the control

Hematology: Red blood cell counts, hemoglobin levels, and hematocrit during week 0 were slightly decreased for male and female dogs in the formoterol only group and formoterol + desformoterol group. However, no differences were evident at later time points.

Hematology parameters at weeks 0, 12, and 16

Parameter	Wk	Males			Females		
		Control	F	F + D	Control	F	F + D
Red blood cells $10^6/\mu L$	0	6.64	5.75* (87%)	5.84* (88%)	6.33	5.83 (92%)	5.52 (87%)
Hemoglobin g/dL	0	14.4	12.9 (90%)	13.1 (91%)	14.2	13.0 (92%)	12.5 (88%)
Hematocrit %	0	41.7	36.5* (88%)	36.7* (88%)	40.0	36.4 (91%)	35.1 (88%)

* $p < 0.05$ compared to the control

Clinical chemistry: There were no significant differences in changes of serum chemistry parameters between the formoterol only group and formoterol + desformoterol group.

Triglyceride levels were decreased for female treatment groups at weeks 0 and 12 and male treatment groups at week 12.

Urea nitrogen and creatinine levels were slightly increased for male and female treatment groups at week 12, although there were no treatment-related histopathological findings in the kidneys.

Potassium levels were increased for male treatment groups at week 12 and female treatment groups at weeks 0 and 12, although these changes are attributed to the pharmacological action of formoterol.

Albumin, total protein, and globulin levels were slightly decreased for male treatment groups at week 0; however, no changes were evident at later time points. Albumin and total protein levels were slightly decreased for females in the formoterol + desformoterol group at week 0, although there were no changes at later time points. The A/G ratio was slightly increased for males in the formoterol + desformoterol group at week 0, although no differences were evident at later time points.

Creatine kinase activity was increased for males in the formoterol + desformoterol group at week 12. This increase was primarily attributed to 1 male with a value of 1266 U/L as compared to the control range of 134 to 189 U/L.

Lactate dehydrogenase (LDH) activities were increased for male treatment groups at week 0 and males in the formoterol + desformoterol group at week 12 as well as female treatment groups at weeks 0 and 12. During week 0, values for 5 males in the

formoterol group ranged from 160.5 to 242.4 U/L and two males in the formoterol + desformoterol group had values of 239.3 and 278.5 U/L as compared to the control range of 105.1 to 137.7 U/L. One female in the formoterol group had a value of 354.0 U/L and one female in the formoterol + desformoterol group had a value of 341.5 U/L as compared to control range of 101.1 to 288.6 U/L. During week 12, one male in the formoterol group had a value of 177.5 U/L and three males in the formoterol + desformoterol group had values ranging from 149.9 to 234.5 U/L as compared to the control range of 79.7 to 118.8 U/L. One female in the formoterol group had a value of 302.9 U/L and three females in the formoterol + desformoterol group had values ranging from 144.2 to 171.4 U/L as compared to the control range of 82.5 to 132.1 U/L.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were increased for males in the formoterol + desformoterol group at week 12. One male (#9435) in the formoterol + desformoterol group had ALT and AST values of 77 and 73 U/L, respectively, as compared to the control range of 29 to 56 U/L and 24 to 42 U/L.

Alkaline phosphatase (ALP) activities were increased for females in the formoterol only and formoterol + desformoterol groups at week 0. Two females in the formoterol only group has values of 145 and 148 U/L and five females in the formoterol + desformoterol group had values of 129, 185, 183, 135, and 129 U/L as compared to the control range of 87 to 117 U/L.

Serum chemistry parameters at weeks 0, 12, and 16

Parameter	Wk	Males			Females		
		Control	F	F + D	Control	F	F + D
Triglyceride mg/dL	0				36	32 (90%)	27 (75%)
	12	37	28* (76%)	28* (76%)	38	29 (76%)	27* (71%)
	16				-	25	28
Urea nitrogen mg/dL	0	12.2	9.7 (80%)	7.7* (63%)			
	12	12.9	19.9* (154%)	18.7* (145%)	16.4	18.5 (113%)	19.5 (119%)
	16	-	15.1	17.3	-	19.1	21.1
Creatinine mg/dL	0						
	12	0.6	1.0* (167%)	1.0* (167%)	0.7	1.0* (143%)	1.0* (143%)
	16	-	15.1	17.3	-	0.9	0.9
Potassium mEq/L	0				4.68	4.88 (104%)	4.93 (105%)
	12	4.27	5.06 (119%)	5.46* (128%)	4.50	5.18* (115%)	5.32* (118%)
Albumin g/dL	0	3.4	3.1*	3.1*	3.5	3.3	3.1*

			(91%)	(91%)			(89%)
Total Protein g/dL	0	5.8	5.3* (91%)	5.1* (88%)	5.7	5.3	5.3 (93%)
Globulin g/dL	0	2.4	2.3	2.1* (88%)			
A/G Ratio	0	1.44	1.39	1.50* (104%)			
Creatine kinase U/L	12 16	154 -	224 (145%) 611	428 (278%) 185			
Lactate dehydrogenase g/dL	0 12	121.9 97.6	175.9 (144%) 93.0	162.2 (133%) 140.2 (144%)	164.4 109.6	179.6 (109%) 124.2 (113%)	192.2 (117%) 134.0 (122%)
ALT U/L	12 16	39 -	42 50	56 (144%) 46			
AST U/L	12 16	33 -	31 40	42 (127%) 36			
ALP U/L	0				103	117 (114%)	143 (139%)

* $p < 0.05$ compared to the control

Urinalysis: Urinary volumes at week 12 for male treatment groups were increased to 197.2 and 196.3% of the control (91.3 mL), respectively. Urinary volumes at week 12 for female treatment groups were increased to 144.6 and 142.9% of the control (76.3 mL), respectively.

Gross pathology: At the end of the 13-week treatment period, there were gross necropsy findings observed in the heart, lung, pituitary, testes, ovaries, and mesenteric lymph nodes. The finding of a heart hematocyst was confirmed by histopathological examination. Dark red areas in the lung correlated with histopathological findings of hemorrhage. Findings of small testes appeared to correlate with histopathological findings of degeneration of seminiferous tubules and multinucleated spermatids. There were no apparent correlations of gross pathological findings in the ovaries and mesenteric lymph nodes to histopathological findings.

For the necropsy at week 17, there was no concurrent control group for comparison to the two treatment groups. Findings at the end of the 13-week treatment period were not generally observed at the end of the 4-week recovery period. Findings of dark red discoloration in the mesenteric lymph nodes correlated with findings of hemorrhage.

Week 13 necropsy

Organ/Tissue	Sex	Control	Formoterol	Formoterol + Desformoterol
Heart				
-hematocyst(s)	M	0/4	0/4	1/4
	F	0/4	0/4	0/4
Lungs				
-area(s), dark red	M	0/4	0/4	1/4
	F	0/4	0/4	0/4
Pituitary				
-small	M	0/4	1/4	0/4
	F	0/4	0/4	0/4
Testes				
-small	M	0/4	1/4	0/4
Ovaries				
-enlarged	F	0/4	0/4	¼
Mesenteric LN				
-discoloration, white	M	0/4	0/4	0/4
	F	0/4	1/4	0/4

Week 17 necropsy

Organ/Tissue	Sex	Formoterol	Formoterol + Desformoterol
Mesenteric LN			
-discoloration, dark red	M	0/2	1/2
	F	0/2	0/2

Organ weights: Absolute and relative organ weights were determined at weeks 13 and 17; however, a concurrent control group was not included at week 17. Thus, it is not possible to assess recovery at week 17. Organ weights at week 17 are not shown in the table.

Differences in organ weights between control and treatment groups were observed for the heart, epididymides, thymus, thyroid/parathyroid, spleen, pituitary, liver, and uterus. Histopathological changes were observed for the heart, epididymides, testes, lung, and mesenteric lymph nodes, although correlations to organ weight changes were unclear.

Heart weights were slightly increased for male and female treatment groups, which have been previously observed following treatment with formoterol. Epididymides weights were increased for male treatment groups, although it is not clear if this difference was due formoterol treatment or variations in sexual maturity. Thymus weights were decreased for males in the formoterol + desformoterol group and females in both treatment groups. Thyroid/parathyroid weights were decreased for males in the formoterol + desformoterol group; however, increases were observed for female treatment groups. Spleen weights were decreased for males in the formoterol + desformoterol group and female treatment groups. Pituitary weights were decreased for male treatment groups. Liver weights were increased for male treatment groups. Uterus weights were decreased for female treatment groups, although it is not clear if this difference was due formoterol treatment or variations in sexual maturity.

Organ weights at week 13 and 17 necropsies

Organ/Tissue	Wk	Males			Females		
		Control	F	F + D	Control	F	F + D
Heart g	13	87.56	98.28 (112%)	90.63 (104%)	63.69	73.87 (116%)	71.90 (113%)
Heart %BrW					89.131	109.881* (123%)	99.061* (111%)
Epididymides g	13	1.80	3.10 (172%)	3.38* (188%)			
Epididymides %BW	13	0.017	0.026 (153%)	0.030 (177%)			
Epididymides %BrW	13	2.420	3.947 (163%)	4.477* (185%)			
Thymus g	13	8.29	8.83	6.08 (73%)	7.34	7.93 (108%)	6.05 (82%)
Thymus %BW	13	0.073	0.072	0.054 (74%)	0.098	0.088 (90%)	0.073 (75%)
Thymus %BrW	13	10.955	11.524	8.140 (74%)	10.359	11.781 (114%)	8.356* (81%)
Thyroid/Parathyroid g	13	0.8386	0.7817 (93%)	0.7013 (84%)	0.5871	0.7068 (120%)	0.7451 (127%)
Thyroid/Parathyroid %BrW	13	1.115	1.000	0.933 (84%)	0.821	1.052 (128%)	1.028 (125%)
Spleen g	13	60.95	61.85	52.69 (86%)	48.11	40.53 (84%)	40.36 (84%)
Spleen %BW	13	0.565	0.506 (90%)	0.442 (78%)	0.636	0.450 (71%)	0.491 (77%)
Spleen %BrW	13	79.970	79.328	69.543 (87%)	67.310	59.800 (89%)	55.584 (83%)
Pituitary g	13	0.0766	0.0505 (66%)	0.0607 (79%)			
Pituitary %BrW	13	0.099	0.065 (66%)	0.081 (82%)			
Liver g	13	272.05	345.57 (127%)	305.61 (112%)			
Liver %BrW	13	360.104	441.808 (123%)	405.572 (113%)			
Uterus g	13				7.67	6.53 (85%)	5.96 (78%)
Uterus %BW	13				0.105	0.069 (66%)	0.067 (64%)
Uterus %BrW	13				11.023	9.195 (83%)	8.155 (74%)

*p <0.05 compared to the control

Histopathology: There were no significant differences in histopathological findings between the formoterol only group and formoterol + desformoterol group. There was no concurrent control group for the histopathological examination of organs and tissues after the 4-week recovery period.

Examination of the heart found a hematocyst (i.e., cyst containing blood) in 1 of 4 male dogs from Group 3. The heart is known target organ of toxicity for formoterol, although it is not clear if the finding of hematocyst has any relationship to treatment.

Examination of the testes found an increased incidence of multinucleated spermatids for male dogs from Groups 2 and 3. In addition, degeneration of seminiferous tubules was observed for 1 of 4 male dogs in Group 2. Testicular toxicity has been observed in rats treated with formoterol.

Examination of the epididymides found luminal cellular debris for male dogs from Groups 2 and 3. Epididymal toxicity has been observed in rats treated with formoterol.

There were several histopathological findings in the lung, although findings were generally common to Groups 2 and 3. An increased incidence of granulomatous inflammation was observed for male and female dogs in Group 3. Acute inflammation was observed for male dogs in Groups 2 and 3. Increased incidences of subacute inflammation were observed for male and female dogs in Groups 2 and 3. Hemorrhage was observed for 1 male dog in Group 3. After the 4-week recovery period, subacute inflammation and granulomatous inflammation were still evident for dogs in Groups 2 and 3.

Examination of the mesenteric lymph node found an increased incidence of hemorrhage for male and female dogs from Groups 2 and 3. After the 4-week recovery period, hemorrhage was only evident for 1 dog in Group 3.

There were additional histopathological findings in the pancreas, tongue, kidneys, eyes/optic nerve, uterus, and nasal level 1 with generally low incidence and unclear relationship to treatment.

Week 13 microscopic findings

Organ/Tissue	Sex	Control (Group 1)	Formoterol (Group 2)	Formoterol + Desformoterol (Group 3)
Heart				
hematocyst	M	0/4	0/4	1/4
	F	0/4	0/4	0/4
Testes				
-spermatids, multinucleated, minimal-mild	M	1/4	3/4	2/4
-degeneration, seminiferous tubules, mild	M	0/4	1/4	0/4
Epididymides				
-luminal debris, cellular, minimal-mild	M	0/4	3/4	2/4
Lungs				
-inflammation, granulomatous, minimal-mild	M	0/4	0/4	1/4
	F	1/4	0/4	2/4

-inflammation, acute, minimal-mild	M F	0/4 0/4	1/4 0/4	2/4 0/4
-inflammation, subacute, minimal-mild	M F	2/4 2/4	3/4 3/4	3/4 4/4
-hemorrhage, mild	M F	0/4 0/4	0/4 0/4	1/4 0/4
Mesenteric LN -hemorrhage, minimal-mild	M F	1/4 1/4	3/4 3/4	4/4 2/4
Liver -inflammation, granulomatous, minimal	M F	0/4 0/4	0/4 0/4	1/4 0/4
Pancreas -atrophy, acinar, moderate	M F	0/4 0/4	1/4 0/4	1/4 0/4
Tongue -inflammation, acute, minimal	M F	0/4 0/4	1/4 0/4	0/4 0/4
Kidneys -cyst, medullary, minimal	M F	0/4 0/4	0/4 0/4	1/4 0/4
-basophilic tubules, minimal	M F	0/4 0/4	1/4 0/4	0/4 0/4
Eyes/Optic nerve -dysplasia, retinal, minimal	M F	1/4 0/4	0/4 0/4	0/4 2/4
Uterus -adenomyosis, mild	F	0/4	0/4	1/4
Nasal Level 1 -hyperplasia, epithelial, mild	M F	0/4 0/4	0/4 1/4	0/4 0/4

Week 17 microscopic findings

Organ/Tissue	Sex	Formoterol	Formoterol + Desformoterol
Lung -inflammation, subacute, minimal	M F	0/2 1/2	1/2 0/2
-inflammation, granulomatous, minimal	M F	0/2 1/2	1/2 1/2
Medullary LN -hemorrhage moderate	M F	- -	1/1 -

Toxicokinetics: (R,R)-formoterol was detected in plasma samples from control and treatment groups on days 0, 29, and 86. Plasma levels of (R,R)-formoterol in the control group were highest on day 0 as compared to days 29 and 86; however, plasma levels were significantly lower than those observed in treatment groups. A detailed discussion of (R,R)-formoterol in plasma samples from control animals is provided below.

Systemic exposure to (R,R)-formoterol in treatment groups increased as treatment progressed suggesting drug accumulation; however, C_{max} values were relatively comparable. AUC and C_{max} values for the formoterol only group were relatively comparable between males and females. However, AUC and C_{max} values for the formoterol + desformoterol group were greater for males as compared to females.

Table 3. Mean ±SD (R,R)-Formoterol Pharmacokinetic Parameters Following the Inhalation Administration of the Vehicle Control or 40 µg base/kg/day (R,R)-Formoterol (With or Without [redacted] (R,R)-Desformoterol) in Male and Female Dogs

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(R,R)-Formoterol Inhalation Dose (µg base/kg/day)	Male Dogs			Female Dogs		
	AUC (0-24h) (pg•h/mL)	Cmax (pg/mL)	tmax (h)	AUC (0-24h) (pg•h/mL)	Cmax (pg/mL)	tmax (h)
40						
Day 0	8660±9980	1570±1660	1.17±0.68	8640±5660	1460±895	0.67±0.26
Day 29	7620±1930	1240±232	0.50±0.00	8670±1760	1340±338	0.50±0.00
Day 86	10600±2610	1380±132	0.58±0.20	10600±4760	1520±710	0.83±0.61
40 + [redacted] DES[a]						
Day 0	6000±2150	1240±483	0.58±0.20	5160±2000	897±380	0.50±0.00
Day 29	12100±2600	2010±559	1.00±0.77	8820±1100	1170±144	0.92±0.58
Day 86	16300±6360	1540±563	1.33±0.75	9960±2410	1470±368	0.67±0.26
Vehicle Control						
Day 0	155±5.81	8.40±1.02	1.25±0.50	144±7.08	10.0±3.81	0.63±0.48
Day 29	6.29±7.74	3.85±1.09	1.25±0.87	4.28±7.57	1.48±1.71	2.00±0.00
Day 86	10.6±10.7	3.66±3.14	2.83±2.84	0.158±0.315	0.630±1.26	0.50± ---[b]

[a] DES = (R,R)-Desformoterol.

[b] Only one observation; no standard deviation calculated.

There was evidence that control animals were exposed to R,R-formoterol. The applicant provided the following information of explain the presence of the test article in control animals. The configuration of the combined exposure/housing room and procedures used during the pre-study and exposure periods apparently led to some exposure (inhalation and/or oral) to (R,R)-formoterol prior to the exposure period and to a lesser extent during the exposure phase. A single room (approximately 58.5 ft. L x 31.5 ft. W x 8.5 ft. H) was used for animal housing and conducting exposures. Cages were placed along the longer walls and exposure exhaust hoods and systems were centrally located to provide separation from the housing areas. The two high airflow exhaust hoods (one each for the two test articles) were integral to the room HVAC, providing the only exhaust sources for facility air, and were designed so that the head and exposure mask of each dog could be placed under the hood. This arrangement was intended to maintain containment of any test article aerosol and prevent leakage from the exposure system to the surrounding vivarium. However, during the pre-study period when the generation and exposures systems were being validated, naïve dogs were allowed to exercise in a fenced area near the middle of the room. Based upon detection of trace levels of (R,R)-formoterol in the plasma collected prior to the first actual test article exposures (pre-dose) in all animals from control and treated groups on study day 0, it appeared that some aerosol containing (R,R)-formoterol escaped from the containment system and either contaminated breathing air in the exercise area and/or was inadvertently transferred to the dogs by personnel during handling. The level of (R,R)-formoterol was fairly uniform at each sample time up to 24 hours after the first exposure

in the vehicle-control animals. (R,R)-formoterol levels were not detected in almost all pre-dose samples from study days 29 and 56 in vehicle-control animals, although occasional residual levels were observed in some dogs during the post-exposure period on study days 29 or 56. These observations suggest that all dogs were most likely to have been exposed to (R,R)-formoterol at low levels during the pre-study period and consistently on study day 0, but thereafter the separation of the exposure system and the housing area was generally maintained. Some of the residual levels of (R,R)-formoterol observed after the first day may have been attributed to unexplained environmental effects.

Study title: **Qualitative Evaluation of Electrocardiograms Recorded for Three Inhalation Toxicity Studies of (R,R)-Formoterol in Dogs.**

Key study findings:

• Pretest and post-exposure electrocardiograms recorded for dogs in the 14-day, 13-week, and 9-month inhalation toxicology studies were re-evaluated for the presence of cardiac rhythm changes by two veterinary cardiologists.

• Sinus tachycardia was a common findings in dogs treated with (R,R)-formoterol at doses ≥5 µg/kg/day. Various types of ECG abnormalities (i.e., ectopic findings) were also observed in dogs treated with (R,R)-formoterol at doses ≥5 µg/kg/day.

• The applicant noted that NOAEL could not be identified for ectopic activity with particular regard to the 13-week and 9-month inhalation toxicology studies; however, they have asserted that an exposure threshold for ectopic findings can be identified within the low dose groups that received 5 µg/kg/day.

• Using C_{max} or AUC data from the study week 0 toxicokinetic evaluations from the 13-week and 9-month studies, the five 5 µg/kg/day group animals with ectopic findings (i.e., Male #6845, Male #6848, and Female #6864 in the 13-week study and Female #6915 and Female #6924 in the 9-month study) had C_{max} values >250 pg/mL and AUC values >2000 pg·hr/mL.

• The applicant's designation of a threshold for ectopic activity within the 5 µg/kg/day groups of the 13-week and 9-month inhalation toxicology studies appears potentially arbitrary. As noted in original study reviews, ECG monitoring periods were short and could have missed ectopic activity. Further, animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm. AUC and C_{max} data is known to vary from day to day (or measurement to measurement), which could significantly shift the designated threshold. Exposure margins between animals with and without ectopic activity were small. All dogs were young with no pre-existing cardiac problems, which minimized potential adverse effects of ectopic activity. The designation of threshold may have little value for the COPD patient population, which generally have pre-existing cardiac problems.

Study no.: Sepracor Document number 090-841
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: May 20, 2004
GLP compliance: No. The applicant had two cardiologists re-evaluate ECG recordings from the 14-day, 13-week, and 9-month inhalation toxicology studies with dogs. The original studies were GLP compliant.
QA report: yes ( ) no (X)
Drug, lot #, and % purity: (R,R)-formoterol-L-tartrate, Lot number 010799A

Methods
Doses:
(R,R)-Formoterol doses used in the 14-day inhalation dose range finding study were 70, 100, and 200 µg/kg/day.
(R,R)-Formoterol doses used in the 13-week inhalation toxicology study were 5, 40, and 70/100 µg/kg/day.
(R,R)-Formoterol doses used in the 9-month inhalation toxicology were 5, 40, and 70 µg/kg/day.
Species/strain. Male and female purpose-bred beagle dogs were obtained from [redacted] Animals were approximately 5 to 6 months old when received. See original study reviews for further details. b(4)
Number/sex/group or time point (main study). For the 14-day inhalation toxicology study, there were 2 dogs/sex/group. For the 13-week inhalation toxicology study, there were 6 dogs/sex/group in the control and high dose groups and 4 dogs/sex/group in the low and mid dose groups. For the 9-month inhalation toxicology study, there were 4 dogs/sex/group.
Route, formulation, volume, and infusion rate. (R,R)-formoterol was formulated in 0.9% sodium chloride for inhalation administration of nebulized aerosols. See original study reviews for further details.
Unique study design or methodology (if any). Pretest and post-exposure electrocardiograms recorded for dogs in the 14-day, 13-week, and 9-month inhalation toxicology studies were re-evaluated for the presence of cardiac rhythm changes by two veterinary cardiologists, [redacted] For the original evaluations of these ECGs, the recordings were evaluated by interval (i.e., all animals at pretest, then all animals at study week 0, etc.). For the re-evaluations, the ECGs were to be examined animal by animal. ECGs for an individual dog, from pretest to the final ECG recording, were to be evaluated before ECGs from another animal were examined. The objective of this approach was to provide the clearest possible picture of any changes within each individual animal and whether changes were related to the (R,R)-formoterol exposure or were possibly spontaneous. b(4)

Observations and times:

EKG: Pretest and post-exposure electrocardiograms recorded for dogs in the 14-day, 13-week, and 9-month inhalation toxicology studies were re-evaluated for the presence of cardiac rhythm changes. The [redacted] three-channel electrocardiograph was used to record ECGs at post-exposure time points from these three studies. The 28-day inhalation toxicology study with dogs was not included in this re-evaluation study, because post-exposure ECGs were recorded using the [redacted] b(4)
[redacted] The format and quality of the ECG recordings form the [redacted] instrument were not considered appropriate for comparison. A [redacted] was used to produce short recordings. Instruments used to record heart rate and ECGs in these three studies are listed in the tables below. See original study reviews for further details.

14-day inhalation toxicology study with dogs

ECG System	Schedule	Purpose
[redacted]	Pretest Only	ECG complex and parameter screening for group assignment
[redacted]	During acclimation to vehicle aerosol (pretest) and during exposures on days 0 and 13	Heart rate, not evaluated by study cardiologist
[redacted]	At 2, 4 and 24 hrs post-exposure on days 0 and 13	Heart rate and qualitative evaluation

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13-week inhalation toxicology study with dogs

ECG System	Schedule	Purpose
[redacted]	Pretest	ECG complex and parameter screening for group assignment
[redacted]	On weeks 3 and 12 (@20-26 hrs after an exposure[a]) and prior to recovery necropsy (week 16)	ECG complex and parameter evaluation for persistent toxic effects
[redacted]	During exposures on one day in weeks 0, 3 and 12[b]	Heart rate, not evaluated by study cardiologist
[redacted]	At 2, 4 and 24 hours post-exposure on one day in weeks 0, 3 and 12 (only 2 hours post-exposure for controls)[b]	Heart rate and qualitative evaluation

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[a]20-26 hrs after an exposure is equivalent to "predose"
[b]All week 0 ECGs were recorded during or following exposure on day 0

9-month inhalation toxicology study with dogs

ECG System	Schedule	Purpose
[redacted]	Pretest	ECG complex and parameter screening for group assignment
[redacted]	On weeks 26 and 38 (@20-26 hrs after an exposure[a])	ECG complex and parameter evaluation for persistent toxic effects
[redacted]	During exposures on one day in weeks 0 and 38	Heart rate, not evaluated by study cardiologist
[redacted]	At 2, 4 and 24 hours post-exposure on one day in weeks 0 and 38 (only 2 hours post-exposure for controls)[b]	Heart rate and qualitative evaluation

b(4)

[a]20-26 hrs after an exposure is equivalent to "predose"
[b]All week 0 ECGs were recorded during or following exposure on day 0

Results

EKG: Sinus tachycardia (heart rate >170 beats per minute) was a common findings in dogs treated with (R,R)-formoterol at doses ≥5 µg/kg/day in the three studies used for re-evaluation of ECG findings. Various types of ECG abnormalities (i.e., ectopic findings), as detailed in tables below and original study reviews, were also observed in dogs treated with (R,R)-formoterol at doses ≥5 µg/kg/day. NOAELs for these ECG abnormalities were not established in the three studies under consideration. These ectopic patterns were clearly treatment-related as they could be attributed to known pharmacological effects of β-adrenergic agonists. These ectopic findings 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.

The applicant agreed that NOAEL could not be identified for ectopic activity with particular regard to the 13-week and 9-month inhalation toxicology studies; however, they have asserted that an exposure threshold for ectopic findings can be identified within the low dose groups that received 5 µg/kg/day. Findings of sinus tachycardia were removed from consideration as this is an expected finding with β-adrenergic agonists. They contend that most ectopic activity was observed during week 0, although the data indicated ectopic activity at later time points. Using C_{max} or AUC data from the study week 0 toxicokinetic evaluations from the 13-week and 9-month studies, the five 5 µg/kg/day group animals with ectopic findings (i.e., Male #6845, Male #6848, and Female #6864 in the 13-week study and Female #6915 and Female #6924 in the 9-month study) had C_{max} values >250 pg/mL and AUC values >2000 pg·hr/mL. Nine 5 µg/kg/day group animals with no ectopic activity had C_{max} values <250 pg/mL and AUC values <2000 pg·hr/mL. However, it is noted that Male #6871 in the 5 µg/kg/day group of the 13-week study had second degree atrioventricular (AV) block during week 3 at 2 hr after dosing. It is possible that second degree AV block may be a common finding for young dogs given that these animals are known to have a high vagal tone; however, this is an unusual finding for a dogs treated with a β-adrenergic agonist. Male #6890 in the 5 µg/kg/day group from the 9-month study was not observed with ectopic activity, but had a C_{max} value of 271 pg/mL exceeding the proposed threshold. Female #6916 in the 5 µg/kg/day group from the 9-month study was not observed with ectopic activity, but had C_{max} and AUC values of 313 pg/mL and 2198.3 pg·hr/mL, respectively, exceeding the threshold.

The applicant's designation of a threshold for ectopic activity within the 5 µg/kg/day groups of the 13-week and 9-month inhalation toxicology studies appears potentially arbitrary and inappropriate. All animals were considered at risk for ectopic activity. As noted in original study reviews, ECG monitoring periods were short and could have missed ectopic activity. Further, animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm. AUC and C_{max} data is known to vary from day to day (or measurement to measurement), which could significantly shift the designated threshold. Exposure margins between animals with and without ectopic activity were small. All dogs were young with no pre-existing cardiac problems, which minimized potential adverse effects of ectopic activity. The designation of threshold may have little value for the COPD patient population, which generally have pre-existing cardiac problems.

Toxicokinetic data from the 13-week inhalation toxicology study with dogs

Text Table 3. Study Week 0 Exposure to (R,R)-Formoterol in 5 µg/kg/day group [redacted] 312055

Animal No.	Sex	Day 1 $AUC_{0.5-24h}$ (pg·h/ml)	Day 1 C_{max} (pg/ml)	Week 0 ECG Findings
6840	M	1082.5	227	Sinus Tachycardia
6845	M	3414.2	357	Sinus Tachycardia PVCs/Ventricular Ectopic Beats
6848	M	2422.8	333	Sinus Tachycardia Junctional Escape Beats/Ventricular Ectopic Beats
6854	M	817.6	100	Slight increase in sinus discharge (but not sinus tachycardia)
6864	F	2116.2	270	Sinus Tachycardia Idioventricular rhythm Ventricular Ectopic Beats
6871	F	1523.1	192	Sinus Tachycardia
6872	F	1744.3	194	Sinus Tachycardia
6883	F	974.0	124	Sinus Tachycardia

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Toxicokinetic data from the 9-month inhalation toxicology study with dogs

Text Table 5. Week 0 Exposure to (R,R)-Formoterol in 5 µg/kg/day group in [redacted] 312056

Animal No.	Sex	Day 1 $AUC_{0.5-24h}$ (pg·h/ml)	Day 1 C_{max} (pg/ml)	Week 0 ECG Findings
6887	M	1179.6	181	Sinus Tachycardia
6890	M	1526.7	271	Sinus Tachycardia
6904	M	760.9	107	Sinus Tachycardia
6905	M	810.6	216	Sinus Tachycardia
6915	F	3612.5	513	Sinus Tachycardia Ventricular extrasystoles/PVCs
6916	F	2198.3	313	Sinus Tachycardia
6923	F	883.6	115	Sinus Tachycardia
6924	F	2105.3	324	Sinus Tachycardia Ventricular extrasystoles/PVCs

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13-Week Inhalation Toxicology Study with Dogs

Dose	Male#	Pretest	Week 0			Week 3				Week 12				Week 16
		Pretest	2 hr	4 hr	24 hr	Prior to exp.	2 hr	4 hr	24 hr	Prior to exp.	2 hr	4 hr	24 hr	Recovery
0 µg/kg	6841	WNL	WNL	NR	NR	SA (WNL)	WNL	NR	NR	WNL	WNL	NR	NR	WNL
	6844	WNL	SA (WNL)	NR	NR	SA (WNL)	SA (WNL)	NR	NR	WNL	WNL	NR	NR	WNL
	6847	WNL	SA (WNL)	NR	NR	WNL	WNL	NR	NR	WNL	WNL	NR	NR	-
	6849	WNL	WNL	NR	NR	WNL	WNL	NR	NR	WNL	WNL	NR	NR	-
	6855	WNL	WNL	NR	NR	WNL	WNL	NR	NR	WNL	WNL	NR	NR	-
	6856	WNL	WNL	NR	NR	WNL	WNL	NR	NR	WNL	WNL	NR	NR	-
5 µg/kg	6840	WNL	ST (WNL)	ST (WNL)	WNL	WNL	ST	WNL	WNL	WNL	ST	WNL	WNL	-
	6845	WNL	ST	ST	IVR,VPC (VEB,	WNL	ST	WNL (ST)	WNL	WNL	WNL	WNL	WNL	-
	6848	WNL	ST	ST	JEB (VEB)	SA (WNL)	ST	ST (WNL)	SA (WNL)	WNL	ST	WNL	WNL	-
	6854	SA (WNL)	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	-
40 µg/kg	6842	WNL	ST (WNL)	ST	IVR (VEB,VER)	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	-
	6843	WNL	ST	ST	WNL	WNL	WNL (ST)	WNL	WNL	WNL	ST	WNL	WNL	-
	6858	WNL	ST	ST	IVR,AVB (VEB)	WNL	WNL	WNL	SA (WNL)	SA (WNL)	ST (WNL)	WNL	WNL	-
	6861	WNL	ST	WNL	WNL	WNL	WNL	WNL	WNL	SA (WNL)	WNL	WNL	SA (WNL)	-
70/100 µg/kg	6837	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	SA (WNL)	ST (WNL)	WNL	SA (WNL)	-
	6838	WNL (ST)	ST	C,ST (ST,VEB,	VPC,JEB (VEB	WNL	WNL	ST (WNL)	WNL	WNL	ST (WNL)	WNL	WNL	WNL
	6851	WNL	ST	ST	VPC,JEB (VEB	SA (WNL)	WNL	WNL	WNL	SA (WNL)	WNL	WNL	SA (WNL)	-
	6852	WNL	ST	ST	WNL	WNL	ST	ST	WNL	WNL	ST	WNL	WNL	-
	6853	WNL	NC	NC	NC	WNL	WNL	WNL	WNL	WNL	WNL	WNL	SA (WNL)	-
	6857	WNL	NC	NC	NC	SA (WNL)	ST (WNL)	WNL	WNL	SA (WNL)	ST	WNL	WNL	WNL

Parentheses denote where there were differences in diagnosis between the two cardiologists.

Abbreviations used in tables: WNL = within normal limits; NC = not collected; NR = not required; SA = sinus arrhythmia; ST = sinus tachycardia; VT = ventricular tachycardia; VR = ventricular rhythm; C = couplets; VEB = ventricular ectopic beats; VER = ventricular extrasystole rhythm; IVR = idioventricular rhythm; SVT = supraventricular tachycardia; VPC = : JEB = junctional escape beats; AVB = atrioventricular block; 2°AVB = second degree atrioventricular block; PJB = premature junction beat; VES = ventricular extrasystoles; RBBB = right bundle branch block; VPC = ventricular premature contraction; QRS LA = QRS low amplitude; PAC = premature atrial contraction; and PJC = premature junction contractions.

13-Week Inhalation Toxicology Study with Dogs

Dose	Female#	Week 0 Pretest	Week 3 2 hr	Week 3 4 hr	Week 3 24 hr	Week 3 Prior to exp.	Week 3 2 hr	Week 3 4 hr	Week 3 24 hr	Week 12 Prior to exp.	Week 12 2 hr	Week 12 4 hr	Week 12 24 hr	Week 16 Recovery
0 µg/kg	6867	WNL	WNL	NR	NR	WNL	WNL	NR	NR	WNL	SA (WNL)	NR	NR	-
	6868	WNL	WNL	NR	NR	WNL	WNL (VEB)	NR	NR	WNL	VPC (VES)	NR	NR	-
	6880	WNL	WNL	NR	NR	WNL	WNL	NR	NR	WNL	AVB (WNL)	NR	NR	WNL
	6881	WNL	WNL	NR	NR	SA (WNL)	SA (WNL)	NR	NR	WNL	SA (WNL)	NR	NR	WNL
	6884	SA (WNL)	WNL	NR	NR	SA (WNL)	SA (WNL)	NR	NR	WNL	SA (WNL)	NR	NR	-
	6886	WNL	WNL	NR	NR	WNL	WNL	NR	NR	WNL	WNL	NR	NR	-
5 µg/kg	6864	WNL	ST	ST	IVR (VEB)	WNL	ST	ST	WNL	WNL	ST	WNL	WNL	-
	6871	WNL	ST	ST	WNL	SA (WNL)	2°AVB, ST	ST (WNL)	WNL	WNL	ST	WNL	WNL	-
	6872	WNL	ST	ST	WNL	WNL	ST	ST (WNL)	WNL	SA (WNL)	WNL	ST (WNL)	WNL	-
	6883	SA (WNL)	ST	WNL	WNL (ST)	WNL	WNL (ST)	WNL	WNL	WNL	WNL	WNL	WNL	-
40 µg/kg	6862	WNL	ST	VPC,ST (VEB,S	IVR,VPC (VEB)	SA	WNL	WNL	WNL	SA (WNL)	SA (WNL)	WNL	WNL	-
	6863	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	SA (WNL)	WNL	WNL	VPC (VEB)	-
	6866	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	SA (WNL)	ST (WNL)	WNL	WNL	-
	6875	WNL	ST	ST	WNL	WNL	ST (WNL)	WNL	WNL	WNL	WNL	WNL	WNL	-
70/100 µg/kg	6870	WNL	IVR,ST, PJB (S	ST	WNL	SA (WNL)	ST (WNL)	ST (WNL)	WNL	SA (WNL)	ST (WNL)	WNL	WNL	SA (WNL)
	6873	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	-
	6877	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	-
	6878	WNL	ST	ST	WNL	SA (WNL)	WNL (2°AVB)	WNL (2°AVB)	WNL (2°AVB)	WNL	WNL	WNL	WNL	-
	6879	WNL	ST	ST	IVR (VEB)	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6885	WNL	ST	ST	C (VEB)	Died	Died	Died	Died	Died	Died	Died	Died	-

Parentheses denote where there were differences in diagnosis between the two cardiologists.

Abbreviations used in tables: WNL = within normal limits; NC = not collected; NR = not required; SA = sinus arrhythmia; ST = sinus tachycardia; VT = ventricular tachycardia; VR = ventricular rhythm; C = couplets; VEB = ventricular ectopic beats; VER = ventricular extrasystole rhythm; IVR = idioventricular rhythm; SVT = supraventricular tachycardia; VPC = : JEB = junctional escape beats; AVB = atrioventricular block; 2°AVB = second degree atrioventricular block; PJB = premature junction beat; VES = ventricular extrasystoles; RBBB = right bundle branch block; VPC = ventricular premature contraction; QRS LA = QRS low amplitude; PAC = premature atrial contraction; and PJC = premature junction contractions.

9-Month Inhalation Toxicology Study

Dose	Male#	Pretest	Week 0 Pretest	Week 0 2 hr	Week 0 4 hr	Week 0 24 hr	Week 26 20-26 hr	Week 38 Prior to exp.	Week 38 2 hr	Week 38 4 hr	Week 38 24 hr	Week 38 20-26 hr
0 µg/kg	6891	WNL	NR	NR	NR	NR	WNL	WNL	WNL	NR	NR	NR
	6894	ST (WNL)	NR	NR	NR	NR	WNL	WNL	WNL	NR	NR	NR
	6896	ST (WNL)	NR	NR	NR	NR	WNL	Died	Died	NR	NR	NR
	6906	WNL	NR	NR	NR	NR	WNL	SA	WNL	NR	NR	NR
5 µg/kg	6897	WNL	ST (WNL)	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6890	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6904	WNL	ST	ST	WNL	WNL	WNL	WNL	ST	WNL	WNL	WNL
	6905	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	SA (WNL)	SA (WNL)	WNL
40 µg/kg	6889	WNL	ST	ST	C (WNL)	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6895	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6900	WNL	ST	ST	WNL	WNL	WNL	WNL	ST	WNL	WNL	WNL
	6902	WNL	ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
70 µg/kg	6888	ST (WNL)	ST	ST	ST	Died	Died	Died	Died	Died	Died	Died
	6892	WNL	ST	ST	C,IVR (VPC,VE	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6898	ST (WNL)	ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6901	WNL	ST	ST	WNL	Died	Died	Died	Died	Died	Died	Died

Parentheses denote where there were differences in diagnosis between the two cardiologists.

Abbreviations used in tables: WNL = within normal limits; NC = not collected; NR = not required; SA = sinus arrhythmia; ST = sinus tachycardia; VT = ventricular tachycardia; VR = ventricular rhythm; C = couplets; VEB = ventricular ectopic beats; VER = ventricular extrasystole rhythm; IVR = idioventricular rhythm; SVT = supraventricular tachycardia; VPC = : JEB = junctional escape beats; AVB = atrioventricular block; 2°AVB = second degree atrioventricular block; PJB = premature junction beat; VES = ventricular extrasystoles; RBBB = right bundle branch block; VPC = ventricular premature contraction; QRS LA = QRS low amplitude; PAC = premature atrial contraction; and PJC = premature junction contractions.

9-Month Inhalation Toxicology Study

Dose	Female#	Pretest	Pretest	Week 0 2 hr	4 hr	24 hr	Week 26 20-26 hr	Week 38 Prior to exp.	2 hr	4 hr	24 hr	
0 µg/kg	6907	WNL		SA (WNL)	NR	NR	WNL	WNL	SA (WNL)	NR	NR	NR
	6912	WNL		SA (WNL)	NR	NR	WNL	WNL	SA (WNL)	NR	NR	NR
	6921	WNL		WNL	NR	NR	WNL	WNL	WNL	NR	NR	NR
	6922	WNL		WNL	NR	NR	WNL	WNL	WNL	NR	NR	NR
5 µg/kg	6915	WNL		ST	ST	VPC,IVR (VPC;	WNL	WNL	ST	WNL	WNL	WNL
	6916	WNL		ST	ST	WNL	WNL	WNL	ST (WNL)	WNL	WNL	WNL
	6923	WNL		ST	ST	WNL	WNL	WNL	ST	WNL	SA (WNL)	SA (WNL)
	6924	WNL		ST	ST	VPC,IVR (VPC,	WNL	WNL	ST	ST (WNL)	WNL	WNL
40 µg/kg	6909	WNL		ST	ST	VPC,ST (VPC,(	WNL	WNL	ST	WNL	WNL (VPC)	WNL
	6919	WNL		ST	ST	WNL	SA (WNL)	WNL	WNL	WNL	WNL	WNL
	6925	WNL		ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6926	WNL		ST	ST (WNL)	WNL	WNL	WNL	ST	ST	SA (WNL)	WNL
70 µg/kg	6910	RBBB (WNL)		SVT,ST (ST)	SVT,ST (ST)	ST,SVT (VPC,\	IVCD (WNL)	IVCD,SVT (WN	IVCD,SVT (ST)	IVCD,SVT (ST)	IVCD,SVT (WN	IVCD,SVT (WN
	6913	WNL		ST	ST	ST (WNL)	WNL	WNL	SVT (WNL)	WNL	SA (WNL)	WNL
	6918	WNL		ST	ST	WNL	WNL	WNL	WNL	WNL	WNL	WNL
	6920	WNL		ST	ST	VPC,IVR,C (VP	WNL	WNL	WNL	WNL	SA (WNL)	SA (WNL)

Parentheses denote where there were differences in diagnosis between the two cardiologists.
Abbreviations used in tables: WNL = within normal limits; NC = not collected; NR = not required; SA = sinus arrhythmia; ST = sinus tachycardia; VT = ventricular tachycardia; VR = ventricular rhythm; C = couplets; VEB = ventricular ectopic beats; VER = ventricular extrasystole rhythm; IVR = idioventricular rhythm; SVT = supraventricular tachycardia; VPC = : JEB = junctional escape beats; AVB = atrioventricular block; 2°AVB = second degree atrioventricular block; PJB = premature junction beat; VES = ventricular extrasystoles; RBBB = right bundle branch block; VPC = ventricular premature contraction; QRS LA = QRS low amplitude; PAC = premature atrial contraction; and PJC = premature junction contractions.

14-Day Inhalation Toxicology Study with Dogs

Dose	Male#	Day 0			Day 13		
		2 hr	4 hr	24 hr	2 hr	4 hr	24 hr
0 µg/kg	6594	WNL	WNL	WNL	WNL	WNL	WNL
	6598	WNL	SA (WNL)	WNL	WNL	WNL	WNL
70 µg/kg	6591	ST	ST	WNL	ST	ST	SA (WNL)
	6595	ST	ST	IVR,C (VES,VE	ST	WNL	SA (WNL)
100 µg/kg	6590	ST	VPC,ST (ST,VE	VPC,ST (VES)	ST	WNL	SA (WNL)
	6596	VPC, APC, SVT,IVR,ST (VT)	SVT,ST (ST)	C (VEB, VER)	WNL	WNL	SA (WNL)
200 µg/kg	6588	VPC,ST (ST)	ST	ST (WNL)	VPC (WNL)	WNL	SA (WNL)
	6597	ST	VPC,ST (VES)	IVR,ST (VER)	WNL	WNL	WNL

Dose	Female#	Day 0			Day 13		
		2 hr	4 hr	24 hr	2 hr	4 hr	24 hr
0 µg/kg	6600	WNL	WNL	WNL	WNL	WNL	WNL
	6605	WNL	WNL	WNL	SA (WNL)	WNL	WNL
70 µg/kg	6606	ST	ST	WNL	ST	ST	SA (WNL)
	6607	ST	ST	IVR (VES)	WNL	AVB (WNL)	AVB (WNL)
100 µg/kg	6601	ST	ST	WNL	ST	ST	SA (WNL)
	6608	ST	ST	IVR (VES,VER)	ST (WNL)	WNL	SA (WNL)
200 µg/kg	6603	ST	IVR,ST (ST,VE	WNL	WNL	WNL	WNL
	6604	ST	ST	IVR,C (VES,VE	ST (QRS LA)	?,ST (QRS LA)	? (QRS LA)

Parentheses denote where there were differences in diagnosis between the two cardiologists.
Abbreviations used in tables: WNL = within normal limits; NC = not collected; NR = not required; SA = sinus arrhythmia; ST = sinus tachycardia; VT = ventricular tachycardia; VR = ventricular rhythm; C = couplets; VEB = ventricular ectopic beats; VER = ventricular extrasystole rhythm; IVR = idioventricular rhythm; SVT = supraventricular tachycardia; VPC = : JEB = junctional escape beats; AVB = atrioventricular block; 2°AVB = second degree atrioventricular block; PJB = premature junction beat; VES = ventricular extrasystoles; RBBB = right bundle branch block; VPC = ventricular premature contraction; QRS LA = QRS low amplitude; PAC = premature atrial contraction; and PJC = premature junction contractions.

Histopathology inventory (optional)

Study	13-week study with desformoterol	13-week study with desformoterol	13-week study with formoterol + desformoterol
Species	Rats (090-840)	Rats (090-844)	Dogs
Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow smear	Not examined	Not examined	X
Bone (femur)			X (bone with marrow)
Brain	X*	X*	X*
Cecum	X	X	X
Cervix			X (w/uterus)
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X*	X*
Esophagus	X	X	X
Eye	X	X	X (w/optic nerve)
Fallopian tube			
Gall bladder			X
Gross lesions	X	X	
Harderian gland	X	X	
Heart	X*	X*	X*
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lacrimal gland	X	X	X
Larynx	X (3 levels)	X (3 levels)	X
Liver	X* (2 lobes)	X* (2 lobes)	X* (2 lobes)
Lungs	X* (all lobes)	X* (all lobes)	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Lymph nodes, tracheobronchial	X	X	
Lymph nodes, mediastinal			X
Mammary Gland	X[a] (inguinal)	X[a] (inguinal)	X (females only)
Nasal cavity	X (3 levels)	X (3 levels)	X (5 levels)
Optic nerves	X[a]	X[a]	X (w/eyes)
Ovaries	X*		X*
Pancreas	X	X	X
Parathyroid	X*[a] (w/thyroid)	X[a] (w/thyroid)	X* (w/thyroid)
Peripheral nerve			
Pharynx	X	X	

Pituitary	X*	X*	X*
Prostate	X	X*	X
Rectum	Not examined	Not examined	X
Salivary gland	X	X	X (submandibular)
Sciatic nerve	X	X	X
Seminal vesicles	X	X	
Skeletal muscle	X	X	X
Skin	X (inguinal)	X (inguinal)	X
Spinal cord	X	X	X
Spleen	X*	X*	X*
Sternum	X (bone and marrow)	X (bone and marrow)	X (bone with marrow)
Stomach	X	X	X
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid	X* (w/ parathyroid)	X (w/ parathyroid)	X* (w/ parathyroid)
Tongue	X	X	X
Trachea	X	X	X
Tracheal bifurcation (w/mainstem bronchi)	X	X	
Urinary bladder	X	X	X
Uterus	X		X* (w/cervix)
Vagina	X		X
Vas deferens			X
Zymbal gland			

X, histopathology performed
*, organ weight obtained
a. The optic nerves, parathyroid glands, and mammary gland were only examined if found in routine sections of eyes, thyroid lobes, and skin, respectively.

2.6.6.4 Genetic toxicology

See attached reviews of studies submitted under IND 55,302.

2.6.6.5 Carcinogenicity

See attached reviews of studies submitted under IND 55,302 and NDA 21-912 Review #01.

2.6.6.6 Reproductive and developmental toxicology

See attached reviews of studies submitted under IND 55,302.

Embryofetal development

Rabbits

Study title: **A Study of the Effects of (R,R/S,S)-Formoterol and (R,R)-Formoterol on Embryo/Fetal Development in Rabbits.**

Key study findings:

• In an embryofetal development study, artificially-inseminated female rabbits received either 160 mg/kg/day R,R/S,S-formoterol, 80 mg/kg/day (R,R)-formoterol, or 0.5% carboxymethylcellulose from gestation days 7 to 22.

• The incidence of decreased defecation was increased in the 160 mg/kg/day R,R/S,S-formoterol group.

• Post-implantation losses were significantly increased in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. This resulted in significant decreases in numbers of viable pups in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Gravid uterus weights and fetal weights were also decreased in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups.

• Incidences of visceral malformations, malpositioned kidney, bulbous aorta, and interventricular septal defect, were increased in both the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Incidences of external malformations, ectrodactyly and brachydactyly, were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Single incidences of spina bifida were observed in both treatment groups. Incidences of skeletal malformations, sternebrae fused, vertebral anomaly with or without associated rib anomaly, and sternebra(e) malaligned, were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Sternebra(e) malaligned was observed in both treatment groups.

• Increased incidences of skeletal variations, sternebra(e) malaligned (slight or moderate), 13th full rib, and sternebra(e) with thread-like attachment, were increased in both the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Skeletal variations, 27 presacral vertebrae and hyoid body and/or arch(es) unossified, were observed in the 80 mg/kg/day (R,R)-formoterol group.

• R,R/S,S-formoterol at 160 mg/kg/day and (R,R)-formoterol at 80 mg/kg/day were teratogenic in rabbits.

Study no.: Sepracor Document Number 090-834
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: June 28, 2002
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity
(R,R/S,S)-Formoterol Fumarate, Lot number 019 9936 (Purity, 99.6%)
(R,R)-Formoterol, Lot number 021 0006 (Purity, 99.3%)

Methods

Doses: R,R/S,S-Formoterol was administered at an oral dose of 160 mg/kg/day. (R,R)-formoterol was administered at an oral dose of 80 mg/kg/day. A vehicle-control group received 0.5% carboxymethylcellulose.

Species/strain: Sexually mature, virgin female New Zealand White rabbits were obtained from [redacted]. Selected females were approximately 6 months old at the time of insemination (gestation day 0) and had a body weight range of 2946 to 3367 g. b(4)

Number/sex/group: 22 artificially-inseminated female rabbits/group

Route, formulation, volume, and infusion rate: Vehicle or drug suspensions were administered by oral gavage using a dose volume of 4 mL/kg.

Satellite groups used for toxicokinetics: 4 rabbits/group that received either 160 mg/kg/day R,R/S,S-formoterol or 80 mg/kg/day (R,R)-formoterol

Study design: Artificially-inseminated female rabbits received either 160 mg/kg/day R,R/S,S-formoterol, 80 mg/kg/day (R,R)-formoterol, or 0.5% carboxymethylcellulose from gestation days 7 to 22.

Dose selection was based upon a dose range finding embryofetal development study in which 6 artificially-inseminated rabbits/group received either racemic formoterol at oral doses of 5, 10, 20, 40, 80, and 160 mg/kg/day or (R,R)-formoterol at oral doses of 10, 20, or 40 mg/kg/day from gestation days 7 to 20. A vehicle-control group received 0.5% carboxymethylcellulose. One female in the 160 mg/kg/day racemic formoterol group aborted on gestation day 24. Incidences of decreased defecation were increased for females receiving racemic formoterol or (R,R)-formoterol at doses ≥10 mg/kg/day. Decreased defecation for the 80 and 160 mg/kg/day racemic formoterol groups was attributed to decreases of food consumption. Gravid uterus weights were decreased for dams in the 160 mg/kg/day racemic formoterol group. Numbers of viable pups/dam were decreased for dams in the 160 mg/kg/day racemic formoterol group. Resorptions (early, late, and total) and post-implantation losses were increased for dams in the 160 mg/kg/day racemic formoterol group. Combined fetal weights were decreased for the 80 and 160 mg/kg/day racemic formoterol groups and the 40 mg/kg/day (R,R)-formoterol group. External examination of pups found 1 pup (1/26 = 3.8%) in the 40 mg/kg/day (R,R)-formoterol group with a cleft palate.

In the present study, there were 22 rabbits per group in the main study. All rabbits were observed twice daily for moribundity/mortality. Animals were observed for signs of toxicity at 1 hr postdose. Body weights were measured on gestation days 0, 7-21 (daily), 24, and 29. Food consumption was measured daily. All maternal rabbits were euthanized on day 29. The uterus and ovaries were removed. The number of corpora lutea on each ovary was recorded. The uterus was weighed and opened, and the numbers of implantation sites were recorded. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal to the left proximal uterine horn. The location of each fetus was identified. For each fetus, the viability and weight were determined. A detailed external examination of each fetus was conducted to include, but was not limited to, an examination of the eyes, palate, and

external orifices. Non-viable fetuses were examined, weighed, and the crown-rump length was measured. For late resorptions, crown-rump measurements and the degree of autolysis were recorded. Each viable fetus was subjected to a visceral examination using a modification of the Stuckhardt and Poppe fresh dissection technique. The sex of each fetus was determined by internal examination. Heads from all fetuses were examined by a mid-coronal slice. A skeletal examination of each fetus was conducted after staining with Alizarin Red S. External, visceral and skeletal findings were recorded as developmental variations or malformations. A toxicokinetic phase was conducted in conjunction with the main study. Two groups of 4 rabbits each received either 160 mg/kg/day R,R/S,S-formoterol or 80 mg/kg/day (R,R)-formoterol from gestation days 7 to 20. On gestation days 7 and 20, blood samples for measurements of plasma concentrations of (R,R)-formoterol and (S,S)-formoterol were collected at 0.5, 1, 2, 6, and 24 hr postdose. Plasma concentrations of (R,R)-formoterol and (S,S)-formoterol were determined by ⊏ ⊐ using a validated chiral LC/MS/MS method. b(4)

Parameters and endpoints evaluated: Potential maternal and developmental toxicity of R,R/S,S-formoterol and (R,R)-formoterol were evaluated in rabbits.

Results

Mortality (dams): None.

Clinical signs (dams): Clinical signs consisting of decreased defecation, soft stool, and mucoid feces were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Brown material at the base of the tail were increased in the 160 mg/kg/day R,R/S,S-formoterol group and 80 mg/kg/day (R,R)-formoterol group.

Daily clinical examination (total occurrence/number of animals)

Clinical sign	Control	160 mg/kg/day R,R/S,S-formoterol	80 mg/kg/day (R,R)-formoterol
Sent to necropsy, gestation day 29	22/22	22/22	22/22
Decreased defecation	3/1	13/4	3/2
Soft stool	2/2	8/4	2/2
Mucoid feces	0/0	2/1	0/0
Head tilt	0/0	14/1	0/0
Dried brown material, base of tail	4/3	14/5	11/6
Hair loss, urogenital area	1/1	11/2	8/3

Body weight (dams): There were no treatment-related effects on body weight gain from gestation days 7 to 21.

Food consumption (dams): There were no treatment-related effects on food consumption from gestation days 7 to 21.

Toxicokinetics: Exposure to (R,R)-formoterol was greater in the 160 mg/kg/day (R,R)-formoterol group as compared to the 80 mg/kg/day (R,R)-formoterol group. For the 80

mg/kg/day (R,R)-formoterol group, there was no evidence of exposure to (S,S)-formoterol. AUC and C_{max} values for (R,R)-formoterol and (S,S)-formoterol were greater on gestation day 20 as compared to gestation day 7 suggesting potential accumulation.

Table 2. Mean ±SD (R,R)- and (S,S)-Formoterol Toxicokinetic Parameters Following Oral Administration of 160 mg/kg/day (R,R/S,S)-Formoterol or 80 mg/kg/day (R,R)-Formoterol in Pregnant Rabbits

	Pregnant Rabbits		
Oral Gavage Dose/ Evaluation Period	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)
	(R,R)-Formoterol Results		
160 mg/kg/day (R,R/S,S)-Formoterol			
Gestation Day 7	1630±912	520±479	1.1±0.6
Gestation Day 20	1840±328	1410±313	0.5±0.0
80 mg/kg/day (R,R)-Formoterol			
Gestation Day 7	643±144	117±36.2	1.0±0.7
Gestation Day 20	1050±461	780±493	0.50±0.0
	(S,S)-Formoterol Results		
160 mg/kg/day (R,R/S,S)-Formoterol			
Gestation Day 7	1740±922	548±518	0.63±0.25
Gestation Day 20	2330±425	1580±251	0.50±0.0
80 mg/kg/day (R,R)-Formoterol			
Gestation Day 7	0.0±0.0	0.0±0.0	N/A
Gestation Day 20	0.0±0.0	0.0±0.0	N/A

Terminal and necroscopic evaluations: Post-implantation losses were significantly increased in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. This resulted in significant decreases in numbers of viable pups in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Gravid uterus weights and fetal weights were also decreased in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. There were no treatment-related gross necropsy findings in dams.

Fetal data at scheduled necropsy

Parameter	Control	160 mg/kg/day R,R/S,S-Formoterol	80 mg/kg/day (R,R)-Formoterol
Females examined at scheduled necropsy	22	22	22
Nongravid	3	2	4
Gravid (with viable fetuses)	19	20	18
Viable fetuses	5.9 (112/19)	4.9 (97/20)	4.9 (88/22)
Viable fetuses, %	93.1%	66.7%	78.7%
Resorptions (%) -early -late	 4 (3.4%) 4 (3.5%)	 32 (20.0%) 20 (13.2%)	 14 (12.9%) 9 (8.4%)
Post-implantation loss (%)	8 (6.9%)	52 (33.3%)	23 (21.3%)
Gravid uterus weight, g	384.7	345.2 (89.7%)	321.8 (83.6%)
Fetal weight, g	48.4	41.6* (86%)	43.1* (89%)

Offspring (malformations, variations, etc.): Incidences of visceral malformations, malpositioned kidney, bulbous aorta, and interventricular septal defect, were increased in both the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Incidences of external malformations, ectrodactyly and brachydactyly, were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Single incidences of spina bifida were observed in both treatment groups. Incidences of skeletal malformations, sternebrae fused, vertebral anomaly with or without associated rib anomaly, and sternebra(e) malaligned, were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Sternebra(e) malaligned was observed in both treatment groups.

In an earlier rabbit teratology study, teratogenic effects were evident in fetuses obtained from does treated with (R,R)-formoterol at doses ≥20 mg/kg/day. Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol. Adactyly, syndactyly, and umbilical herniation of the intestine were observed at 80 mg/kg/day. Brachydactyly and short tails were observed at 40 and 80 mg/kg/day. Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Malpositioned kidney at 20 mg/kg/day occurred independently of maternal toxicity except for decreased defecation. Heart and/or great vessel anomalies were observed at 40 and 80 mg/kg/day. Lobular dysgenesis of the lungs was observed at 80 mg/kg/day.

Several malformations were observed in fetus #36516-5 from the 160 mg/kg/day R,R/S,S-formoterol group that included craniorachischisis, microglossia, open eyelid (bilateral), micromelia, carpal and/or tarsal flexure, gastroschisis, maxillary micrognathia, ectrodactyly, microphthalmia and/or anophthalmia, supernumerary testis, and hydrocephaly.

Fetuses with malformations (Fetuses/Litters)

Parameter	Control	160 mg/kg/day R,R/S,S-Formoterol	80 mg/kg/day (R,R)-Formoterol	Historical control incidence
Total malformations -External -Soft tissue -Skeletal	 0/0 0/0 1/1	 7/6 14/10 8/6	 1/1 11/5 1/1	
Number examined externally	112/19	97/20	88/18	
Spina bifida	0/0	1 (1%)/1[b]	1 (1%)/1	8/4708 (0.17%)
Short tail	0/0	1 (1%)/1[b]	0/0	4/4708 (0.08%)
Ectrodactyly	0/0	4 (4.1%)/3	0/0	
Brachydactyly	0/0	2 (2.1%)/2	0/0	
Craniorachischisis	0/0	1 (1%)/1[a]	0/0	1/4708 (0.02%)
Macroglossia	0/0	1 (1%)/1[a]	0/0	
Open eyelid	0/0	1 (1%)/1[a]	0/0	
Micromelia	0/0	1 (1%)/1[a]	0/0	
Carpal and/or tarsal flexure	0/0	1 (1%)/1[a]	0/0	7/4708 (0.15%)
Gastroschisis	0/0	1 (1%)/1[a]	0/0	2/4708 (0.04%)
Maxillary micrognathia	0/0	1 (1%)/1[a]	0/0	3/4708 (0.06%)
Microphthalmia and/or anophthalmia	0/0	1 (1%)/1[a]	0/0	4/4708 (0.08%)
Number examined viscerally	112/19	97/20	88/18	
Malpositioned kidney	0/0	8 (8.2%)/4[b]	8 (9.1%)/3[d]	
Bulbous aorta	0/0	3 (3.1%)/3[c]	4 (4.5%)/2[d]	
Interventricular septal defect	0/0	3 (3.1%)/3[c]	1 (1%)/1	1/4708 (0.02%)
Lungs - lobular agenesis	0/0	0/0	1 (1%)/1	
Supernumerary testis	0/0	1 (1%)/1[a]	0/0	
Hydrocephaly	0/0	1 (1%)/1[a]	0/0	8/4708 (0.17%)
Fused kidney	0/0	1 (1%)/1	0/0	
Number examined skeletally	112/19	97/20	88/18	
Sternebrae fused	1/1	3 (3.1%)/2	0/0	7/4708 (0.15%)
Vertebral anomaly with or without associated rib anomaly	0/0	3 (3.1%)/3[b]	0/0	61/4708 (1.3%)
Sternebra(e) malaligned (severe)	0/0	2 (2.1%)/2[c]	1 (1%)/1	2/4708 (0.04%)
Sternoschisis	0/0	1 (1%)/1	0/0	1/4708 (0.02%)

a. Fetus #36516-5 was observed with craniorachischisis, microglossia, open eyelid (bilateral), micromelia, carpal and/or tarsal flexure, gastroschisis, maxillary micrognathia, ectrodactyly, microphthalmia and/or anophthalmia, supernumerary testis, and hydrocephaly.

b. Fetus #36422-7 was observed with spina bifida, short tail, malpositioned kidney, and vertebral anomaly with or without associated rib anomaly.

c. Fetus #36520-6 was observed with bulbous aorta, interventricular septal defect, and sternebra(e) malaligned.
d. Fetuses #36464-3 and #36480-6 were observed with malpositioned kidney and bulbous aorta.

Increased incidences of skeletal variations, sternebra(e) malaligned (slight or moderate), 13th full rib, and sternebra(e) with thread-like attachment, were increased in both the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Skeletal variations, 27 presacral vertebrae and hyoid body and/or arch(es) unossified, were observed in the 80 mg/kg/day (R,R)-formoterol group.

Fetuses with variations (Fetuses/Litters)

Parameter	Control	160 mg/kg/day R,R/S,S-Formoterol	80 mg/kg/day (R,R)-Formoterol	Historical control incidence
Number examined externally	112/19	97/20	88/18	
Number examined viscerally	112/19	97/20	88/18	
Renal papilla(e) not developed and/or distended ureter(s)	0/0	0/0	2/1	1/4708 (0.02%)
Number examined skeletally	112/19	97/20	88/18	
Sternebra(e) malaligned (slight or moderate)	1/1	2/2	3/3	60/4708 (1.3%)
13th full ribs	44/17	53/17	56/16	1935/4708 (41%)
27 presacral vertebrae	24/12	24/11	34/14	805/4708 (17.1%)
Sternebra(e) with thread-like attachment	1/1	9/6	10/7	88/4708 (1.9%)
Hyoid body and/or arch(es) unossified	0/0	0/0	1/1	6/4708 (0.1%)

Prenatal and postnatal development

Study title: **Study of the Effects of (R,R)-Formoterol on Pre- and Postnatal Development Including Maternal Function in the Rat.**

Key study findings:

• Time-mated F_0 female rats received (R,R)-formoterol at oral doses of 0, 1, 5, and 10 mg/kg/day from gestation day 6 to postnatal day 20.

• One female in the 10 mg/kg/day group was euthanized on gestation day 21 due to complications during parturition. This female had total litter loss.

• Lengths of gestation for F_0 female rats in the 1, 5, and 10 mg/kg/day groups were slightly prolonged.

• Survival was slightly decreased for F_1 pups in the 5 and 10 mg/kg/day groups on postnatal days 0 and 1 and birth to postnatal day 4. No effects on survival were observed at later time points.

• Umbilical hernia, a malformation observed in teratology studies with (R,R)-formoterol, was observed for 1 pup in the 10 mg/kg/day group. Incidences of missing (presumed cannibalized), subcutaneous hemorrhage, pale in color, and cyanotic were increased for pups in all (R,R)-formoterol treatment groups.

• Clinical observations of F_1 males and females during the post-weaning period found increased incidences of prominent annular rings over the entire length of the tail for the 5 and 10 mg/kg/day groups on postnatal days 35, 42, 49, and 56; however, this finding did not persist.

• During the post-weaning period, absolute body weights for F_1 male and female pups in the mid and high doses group were generally lower than corresponding control values; however, body weight gains for F_1 male rats from postnatal day 21 to week 22 and F_1 female rats from postnatal day 21 to week 17 were unaffected by treatment.

• Acquisition of balanopreputial separation appeared to be delayed for male pups receiving the high dose of 10 mg/kg/day, although all male pups from treatment groups acquired separation. Acquisition of vaginal patency appeared to be delayed for female pups receiving the high dose of 10 mg/kg/day, although all female pups from treatment groups acquire vaginal patency.

• Measurements of acoustic startle responses found that mean V_{max} values were increased for F_1 male pups in the 5 and 10 mg/kg/day groups on postnatal day 20 and F_1 males in the 1, 5, and 10 mg/kg/day groups on postnatal day 60. V_{max} values were increased for F_1 females in all treatment groups on postnatal days 20 and 60; however, lack of dose-response relationships, particularly on postnatal day 60, suggests that there was no treatment-related effect.

• Differences of swimming ability, mean times to escape from the Biel maze and mean numbers of errors committed during trials 1-10 (days 2-6 of the Biel maze procedure), and/or when probed for memory, mean time to escape and mean number of errors (day 7 of the Biel maze procedure) were observed between control and treatment groups; however, toxicological significance was unclear.

• Male and female F_1 rat mating and fertility indexes were unaffected by treatment. No treatment-related effects were observed for the F_2 generation fetuses.

• Delays of prenatal and postnatal development were potentially present for (R,R)-formoterol high dose group that received 10 mg/kg/day.

Study no.: Sepracor Document No. 090-832
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: February 4, 2000
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (R,R)-Formoterol-L-Tartrate, Lot number 010799A

Methods
Doses: 0, 1, 5, and 10 mg/kg/day
Species/strain: Female Sprague-Dawley [redacted] CD®(SD)IGS BR rats were obtained from [redacted] Animals were paired for mating in the home cage of the resident male (untreated). Following positive identification of mating, the females were individually housed in plastic maternity cages with nesting material. Female rats were approximately 12 weeks old when paired for breeding. The body weight range on gestation day 0 was 220 to 284 g. b(4)
Number/sex/group: 25 time-mated female rats/group
Route, formulation, volume, and infusion rate: Vehicle (0.5% carboxymethylcellulose) and (R,R)-formoterol suspensions were administered by oral gavage using a dose volume of 5 mL/kg. Formulations for all groups were prepared weekly and were stored refrigerated.
Satellite groups used for toxicokinetics: None.
Study design: Time-mated F_0 female rats received (R,R)-formoterol at oral doses of 0, 1, 5, and 10 mg/kg/day from gestation day 6 to postnatal day 20.

F_0 rats were observed twice daily for moribundity/mortality. Clinical observations for F_0 rats were recorded daily prior to dosing and approximately 1 hr after dosing. F_0 maternal body weights and food consumption were measured on gestation days 0, 6, 9, 12, 15, 18, and 20 and lactation days 1, 4, 7, 10, 14, and 21.

F_0 females were allowed to deliver naturally and rear their young to weaning (postnatal day 21). During the period of expected parturition, females were observed twice daily for initiation and completion of parturition and for signs of dystocia. The day of delivery was designated postnatal day 0. When parturition was complete, numbers of stillborn and live F_1 pups in each litter were counted. F_1 pups were sexed and examined for gross malformations. The duration of gestation was determined for each F_0 rat. F_0 females that did not deliver were sacrificed on post-mating day 25 and submitted to gross necropsy examination. F_0 females with total litter loss were sacrificed and submitted to gross necropsy examination. All surviving females with viable F_1 pups on lactation day 21 were sacrificed and submitted to gross necropsy examination.

F_1 litters were examined daily for survival and all deaths were recorded. Intact offspring dying from postnatal days 0 to 4 were necropsied using a fresh dissection technique. For F_1 pups dying after postnatal day 4, a detailed gross necropsy was performed. On

postnatal day 4, four F_1 pups/sex/litter were randomly selected. Culled F_1 pups were weighed, sacrificed, and subsequently discarded.

F_1 litters were examined daily for any adverse changes in appearance or behavior. F_1 pups received detailed physical examinations on postnatal days 1, 4, 7, 10, 14, 17, and 21 and at weekly intervals thereafter until necropsy. F_1 pups were weighted on postnatal days 1, 4, 7, 10, 14, 17, and 21, and at weekly intervals thereafter until necropsy. F_1 pups were individually sexed on postnatal days 0, 4, and 21.

When F_1 pups were between 15 and 20 days of age, 25/sex/group were randomly selected for the F_1 generation. A minimum of 1 F_1 pup/sex/litter was selected for evaluation of attainment of developmental landmarks and neurobehavioral evaluations. Randomly selected subsets (10/sex/group) of pups were selected for each evaluation of sensory function and behavioral testing. All selected pups were evaluated for attainment of landmarks of sexual maturity. All other F_1 pups not selected for further examination were sacrificed and necropsied on postnatal day 21. Each dam and litter remained together until weaning on postnatal day 21. Following weaning on postnatal day 21, pups were housed by litter in plastic maternity cages with nesting material until postnatal day 28. Beginning on postnatal day 28, offspring were housed individually.

F_1 post-weaning developmental landmarks, sensory function, and neurobehavioral testing were performed as described below. Each F_1 male pup was observed daily for balanopreputial separation beginning on postnatal day 35 and the day that separation was evident was recorded. Each F_1 female pup was observed daily for vaginal perforation beginning on postnatal day 25 and the day on which the vaginal lumen was first observed to open was recorded for each pup. An auditory startle response test was performed on 10 rats/sex/group on postnatal days 20 and 60. Motor activity was assessed for 10 rats/sex/group at each time point on postnatal days 21 and 61. Swimming ability and learning and memory were assessed for 10 rats/sex/group using a water-filled eight-unit T-maze (Biel Maze swimming trials). Each testing interval consisted of three phases that were conducted over 7 consecutive days. Phase 1 was an evaluation of swimming ability and motivation to escape from the maze and was performed on day 1 of the Biel maze procedure. Phase 2 of the Biel maze procedure evaluated sequential learning. This evaluation was conducted on days 2-6 of the Biel maze procedure. Phase 3 of the Biel maze procedure probed the animal for its memory to solve the maze and was conducted on day 7.

F_1 rats were a minimum of 92 days old when paired on a 1 to 1 ratio during the mating interval for each treatment group. Vaginal smears were prepared to assess the regularity and duration of the estrous cycle of each F_1 female for 10 consecutive days before pairing and continuing until evidence of mating was observed or to the end of the breeding period. All F_1 females with evidence of mating were housed in wire cages throughout gestation and were necropsied on gestation day 20. Females with no evidence of mating were necropsied following completion of the breeding period. For F_1 females with evidence of mating, body weights were measured on gestation days 0, 6, 9, 12, 16, and 20. All F_1 maternal animals were sacrificed on gestation day 20 and

submitted to gross necropsy examination. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. The uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantations were recorded. Each fetus was weighed, sexed, sacrificed, and detailed examinations were conducted. Approximately one-half of the fetuses were prepared for possible future soft tissue examination and the remaining one-half were prepared for possible future skeletal examination. External malformations and variations were recorded. When F_1 female laparohysterectomies were completed, F_1 males were sacrificed and submitted to gross necropsy examination.

Parameters and endpoints evaluated. Potential adverse effects on pregnancy, parturition, and lactation in the maternal (F_0) generation and to evaluate growth, viability development, and reproductive performance of the offspring (F_1) generation.

Results

F_0 in-life:
Female #38972 in the 10 mg/kg/day group was euthanized on gestation day 21 due to complications during parturition. This female was hypoactive and delivered 7 dead pups and 5 late resorptions on postnatal day 0. In addition, 1 late resorption was found in the vaginal canal at necropsy. Necropsy examination did not indicate a cause of death.

One control female #38990 exhibited prolonged parturition or dystocia with duration of 3 days. Seven pups from this dam were found dead during lactation days 1-2. However, 8 of 18 pups were viable on lactation day 21.

Incidences of several nonspecific clinical signs (i.e., hair loss on the right and left forelimbs and scabbing on the right and left forelimbs) were increased for females in the 5 and 10 mg/kg/day groups (see table below). The relationships of these clinical findings to treatment were unclear.

F_0 female rats: Clinical findings from gestation day 6 to postnatal day 21 (total occurrence/number of animals)

Clinical sign	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Hypoactive	1/1	0	0	1/1
Hair loss, right forelimb	92/4	97/6	212/12	257/9
Hair loss, left forelimb	93/4	93/5	187/12	255/9
Scabbing, right forelimb	0	0	8/3	28/6
Scabbing, left forelimb	0	0	1/1	29/5
Firm, moveable mass, right lateral abdominal area 10 mm x 10 mm x 10 mm	0	0	0	5/1
Firm, moveable mass, right lateral abdominal area 20 mm x 15 mm x 15 mm	0	0	0	2/1

Body weight gains from gestation days 6 to 20 for F_0 females in the 1, 5, and 10 mg/kg/day groups were increased to 107.9, 103.7, and 107.8% of the control, respectively. Body weight gains from lactation days 1 to 21 for F_0 females in the 1, 5, and 10 mg/kg/day groups were increased to 214.7, 184, and 183.5% of the control, respectively. Increased body weight gain is attributed to the pharmacological action of (R,R)-formoterol. There were no treatment-related changes of food consumption from gestation days 6 to 20 or lactation days 1 to 21.

Lengths of gestation for the 1, 5, and 10 mg/kg/day groups were slightly prolonged to 22.1* (22-23), 22.2* (22-24), and 22.1* (22-23) days, respectively, as compared to control (21.8 days, Range of 21-22 days; $p < 0.05$ or 0.01). Prolonged parturition or dystocia has been reported with high oral doses of racemic formoterol and attributed to the pharmacological action of β-adrenergic agonists to relax uterine musculature. Ranges for the 1, 5, and 10 mg/kg/day groups slightly exceeded the historical control range (21.5-22.8 days) for the testing laboratory.

Incidences of total litter loss appeared to be increased for the 5 and 10 mg/kg/day group. For 1 control female (#38935) and 2 females in the 5 mg/kg/day group (#38925 and #38963) were observed with total litter loss between postnatal days 0 and 4. Further, 1 female in the 10 mg/kg/day group (#38972) sacrificed on gestation day 21 (lactation day 0) was found with total litter loss (see above). Lengths of gestation were prolonged for females #38925 and #38963 in the 5 mg/kg/day group to 23 and 24 days, respectively, as compared to the mean gestation length of 22.2 day for that group.

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TABLE 1 (F0)
STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS
SUMMARY OF MATERNAL SURVIVAL AND PREGNANCY STATUS

PAGE 1

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25	
FEMALES THAT DIED	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES EUTHANIZED IN EXTREMIS	0	0.0	0	0.0	0	0.0	1-A	4.0
FEMALES ALLOWED TO DELIVER	25		25		25		25-A	
GRAVID	23	92.0	23	92.0	23	92.0	23-A	92.0
FEMALES WITH TOTAL LITTER LOSS	1	4.3	0	0.0	2	8.7	1-A	4.3
FEMALES WITH VIABLE PUPS	22	95.7	23	100.0	21	91.3	22	95.7
NONGRAVID	2	8.0	2	8.0	2	8.0	2	8.0
TOTAL FEMALES GRAVID	23	92.0	23	92.0	23	92.0	23-A	92.0

1- 0 MG/KG/DAY 2- 1 MG/KG/DAY 3- 5 MG/KG/DAY 4- 10 MG/KG/DAY
A = FEMALE NO. 38972 WAS EUTHANIZED IN EXTREMIS DURING PARTURITION (TOTAL LITTER LOSS); INCLUDED IN CALCULATIONS

b(4)

F_0 necropsy:
For 1 control female (#38935) and 2 females in the 5 mg/kg/day group (#38925 and #38963) with observations of total litter loss between postnatal days 0 and 4 as well as 1 female in the 10 mg/kg/day group (#38972) sacrificed on gestation day 21 (lactation day 0) and found with total litter loss, there were no gross necropsy findings (i.e., internally normal) to indicate causes of total litter loss.

For 2 females in each of the control and treatment groups that failed to deliver (i.e., nongravid), there were no gross pathological findings in these 8 females (i.e., internally normal and nongravid).

For females sacrificed on lactation day 21 (i.e., 22 control females, 23 females in the 1 mg/kg/day group, 21 females in the 5 mg/kg/day group, and 22 females in the 10 mg/kg/day group), there were no gross necropsy findings.

F_1 physical development:

Birth:
Twenty-three F_0 females in each group were gravid. At birth, live litter sizes were decreased in the 5 and 10 mg/kg/day groups as shown in the table below. There were no treatment-related effects on numbers of implantation sites, number born, numbers of unaccounted sites, and sex at birth.

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Postnatal day 0 litter data for F_0 dams

Parameter	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Live litter size, postnatal day 0	14.9	14.6	12.9 (86.6%)	13.5 (90.6%)

Weaning period (Postnatal days 0 to 21):
Survival was slightly decreased for F_1 pups in the 5 and 10 mg/kg/day groups on postnatal days 0 and 1 and birth to postnatal day 4. No effects on survival were observed at later time points. These decreases of survival were primarily attributed to 2 females in the 5 mg/kg/day group (#38925 and 38963) and 1 female in the 10 mg/kg/day group (#38972) with total litter loss.

F_1 postnatal survival, percentage per litter

Period	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Postnatal day 0 (relative to number born)	98.9	96.4	91.1	90.7
Postnatal days 0 to 1	98.6	97.6	92.7	93.8
Birth to Postnatal day 4 (Pre-selection)	91.6	93.2	86.1	84.7

Incidences of several pre-weaning observations were increased in treatment groups as compared to the control. Umbilical hernia was observed for 1 pup in the high dose group. This treatment-related malformation was observed in rat teratology studies with (R,R)-formoterol. Incidences of missing (presumed cannibalized), subcutaneous hemorrhage, pale in color, and cyanotic were increased for pups in all treatment groups. Incidences of left and right forelimb swollen, left hindlimb swollen, firm moveable mass, and scabbing were increased for pups in the mid and high dose groups. Incidences of body cool to touch and right hindlimb swollen were increased for pups in the high dose group.

F_1 pre-weaning observations: summary of pup clinical observations (total number of findings/number of pups with finding)

Observation	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Euthanized in extremis	0	0	1/1	0
Scheduled euthanasia (postnatal day 21)	125/125	132/132	112/112	124/124
Culled on scheduled day (postnatal day 4)	139/139	142/142	115/115	116/116
Missing	1/1	3/3	14/14	13/13
Umbilicial hernia (malformation)	0	0	0	3/1
Subcutaneous hemorrhage	0	2/2	2/2	6/5
Pale in color	0	1/1	1/1	8/8
Body cool to touch	0	1/1	0	4/4
Left forelimb swollen	0	0	5/4	13/8

Right forelimb swollen	0	0	6/5	6/4
Right hindlimb swollen	0	0	0	4/3
Left hindlimb swollen	0	0	1/1	4/2
Cyanotic	0	4/4	12/12	3/3
Firm moveable mass	0	0	1/1	3/1
Scabbing	0	0	1/1	1/1

Numbers of pups that were found dead, sacrificed in a moribund condition, and/or late resorptions that were delivered during the pre-weaning, postnatal period (postnatal days 0-21) were relatively comparable between control and treatment groups as shown in the table below. As noted in the table above, incidences of missing pups (presumed cannibalized) were increased in treatment groups as compared to the control group. Necropsy examinations of pups found dead indicated that numbers of pups with no milk present were increased in the 5 and 10 mg/kg/day groups. Numbers of pups with milk present were increased for all treatment groups.

Necropsy findings for pups found dead (pups/litters)

Parameter	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Number examined viscerally	32/8	22/9	34/8	38[a]/13
Stomach				
-milk not present	6/5	6/5	20/6	13/7
-milk present	0	4/3	2/2	5/5

a. includes 5 late resorptions from the litter of dam #38972 that were delivered and one late resorption that was found in the vaginal canal of this dam. Late resorptions were not submitted to visceral examination.

Absolute body weights for male and female pups in the and mid high dose groups were decreased on postnatal days 1, 14, 17, and 21 as compared to control values (see table below); however, body weight gains for F_1 male and female pups from postnatal days 0 to 21 (i.e., lactation or weaning period) were unaffected.

PROJECT NO. [redacted]-312040 TABLE 20 (F1 - PRE-WEANING) PAGE 1
SPONSOR:SEPRACOR INC. STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS
SUMMARY OF MEAN OFFSPRING WEIGHTS (GRAMS)
(LITTER AS EXPERIMENTAL UNIT)

DOSE GROUP:		0 MG/KG/DAY	1 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY
PND 1					
MALES	MEAN	7.0	6.9	6.7	6.4**
	S.D.	0.57	0.57	0.69	0.74
	N	23	23	21	22
FEMALES	MEAN	6.6	6.6	6.4	6.0**
	S.D.	0.51	0.50	0.67	0.69
	N	23	23	21	22

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There were no treatment-related gross necropsy findings in pups culled on postnatal day 21.

Post-Weaning Period:
One F_1 male in each of the 1, 5, and 10 mg/kg/day groups and two F_1 females in the 5 mg/kg/day group were found dead or euthanized in extremis during the study. Male #38924-07 in the 1 mg/kg/day group was found dead on postnatal day 82, approximately 61 days after discontinuation of test article administration. Male #38946-04 in the 10 mg/kg/day group was found dead on postnatal day 96 (approximately 75 day after discontinuation of exposure to test article), one day after pairing for the reproduction study. There were no apparent treatment-related gross necropsy findings in these animals. Female #38961-10 and male #38995-02 in the 5 mg/kg/day group were both sacrificed in a moribund condition on postnatal days 51 and 52, respectively. Female #38936-10 in the 5 mg/kg/day group was found dead on postnatal day 86 (approximately 65 days after discontinuation of exposure to the test article). Findings for female #38961-10 in the 5 mg/kg/day included a dome-shaped head (hydrocephalic with increased cavitation of the lateral (bilateral) and third ventricles), lethargy, dried red material around the nose and dried yellow material in the urogenital area. Findings for male #38995-02 in the 5 mg/kg/day included a dome shaped head (hydrocephalic with increased cavitation of lateral (bilateral and third ventricles), prominent annular rings over the entire length of the tail, body cool to touch, dried red material around the eyes, forelimbs, and nose, and dried yellow material in the urogenital area. Findings for female #38936-10 in the 5 mg/kg/day group consisted of distended kidneys, ureters, and urinary bladder as well as redden and enlarged mediastinal lymph nodes. These findings in the 5 mg/kg/day group do not appear to be treatment-related based upon lack of similar findings in the 10 mg/kg/day group.

Clinical observations of F_1 males and females during the post-weaning period found increased incidences of prominent annular rings over the entire length of the tail for the 5 and 10 mg/kg/day groups on postnatal days 35, 42, 49, and 56. This finding did not persist and its relationship to treatment was unclear. Other clinical observations were nonspecific and their relationships to treatment were unclear.

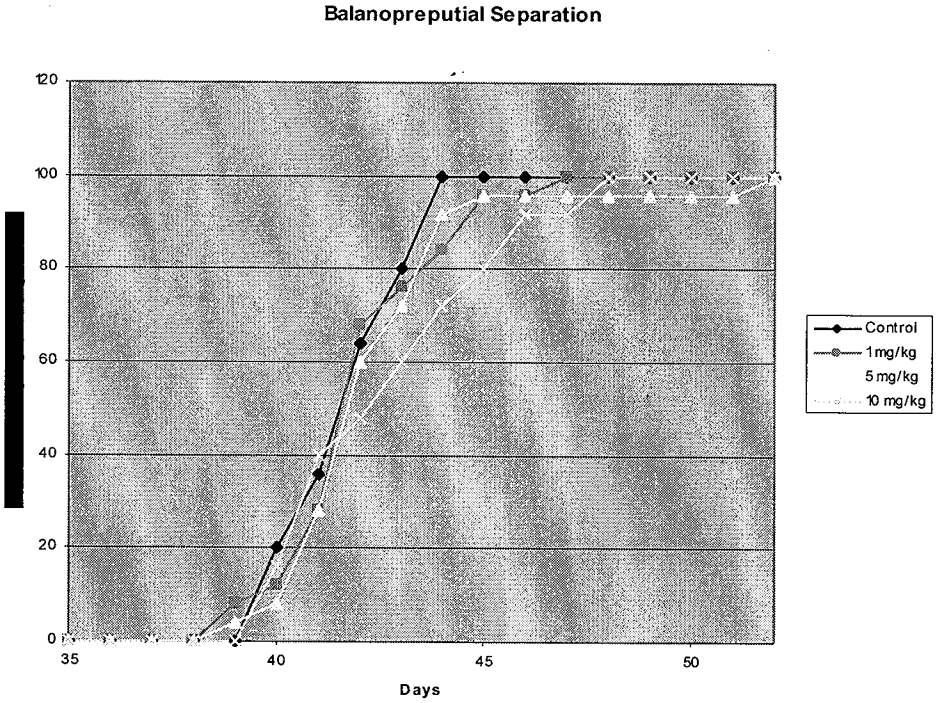
F_1 post-weaning observations: summary of pup clinical observations (total number of findings/number of pups with finding)

Observation	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
No remarkable observations	346/50	335/50	318/50	319/50
Prominent annular rings entire length of tail	0	0	6/4	15/8
Hair loss right forelimb	1/1	2/1	6/4	4/3
Hair loss left forelimb	1/1	2/1	5/3	7/3

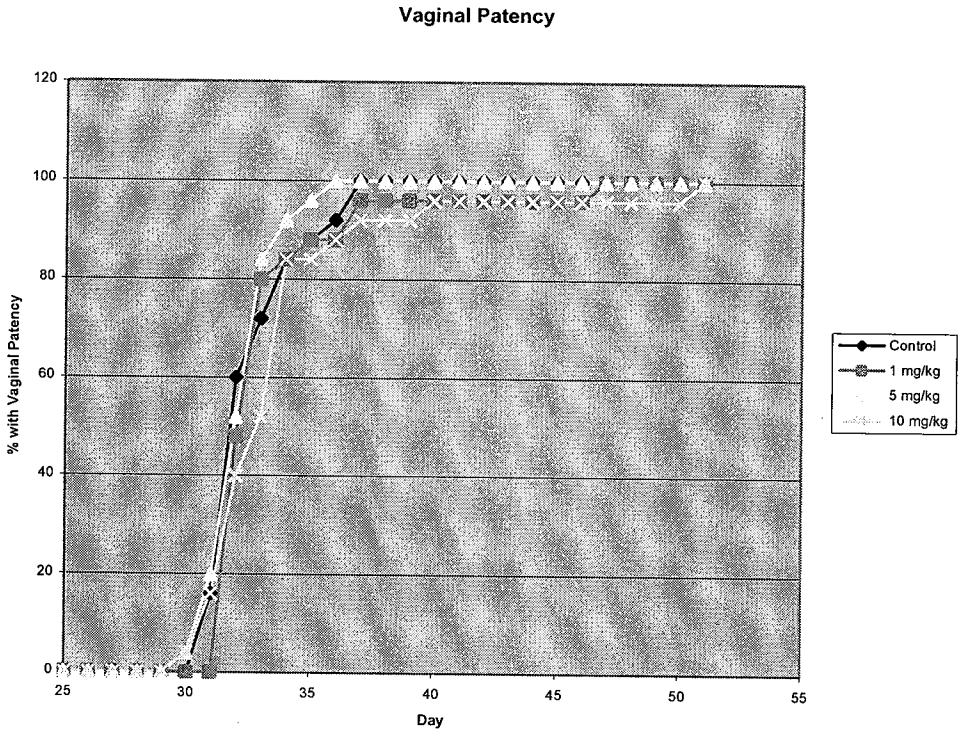
During detailed physical examinations of F_1 male and female rats, a number of nonspecific clinical findings (i.e., hair loss on the right and left forelimb, red ocular discharge from the right and left eyes, hair loss around the right and left eyes, dried red material around the right and left eye) were found to be increased in incidence for male treatment groups, although primarily for the high dose group. There were no similar findings for female treatment groups. The relationships of these nonspecific clinical findings to treatment were unclear.

Absolute body weights for F_1 male and female pups in the mid and high doses group were generally lower than corresponding control values during the post-weaning period; however, body weight gains for F_1 male rats from postnatal day 21 to week 22 and F_1 female rats from postnatal day 21 to week 17 were unaffected by treatment.

Acquisition of balanopreputial separation appeared to be delayed for male pups receiving the high dose of 10 mg/kg/day as compared to the concurrent control, although all males acquired separation within the historical control range (41.6-49.0 days) for the testing laboratory (see figure below). Separation also appeared to be slightly delayed for males in the 1 and 5 mg/kg/day groups as compared to the concurrent control. One male in the 5 mg/kg/day group acquired separation outside the historical control range. It should be noted that all male pups from treatment groups acquired balanopreputial separation.



Acquisition of vaginal patency appeared to be delayed for female pups receiving the high dose of 10 mg/kg/day as compared to the concurrent control. Two females in the 10 mg/kg/day acquired vaginal patency outside the historical control range (31.9-38.8 days) for the testing laboratory. It should be noted that all female pups from treatment groups did acquire vaginal patency.



F_1 behavioral evaluation: Measurements of acoustic startle responses found that mean V_{max} values were increased for F_1 male pups in the 5 and 10 mg/kg/day groups on postnatal day 20 and F_1 males in the 1, 5, and 10 mg/kg/day groups on postnatal day 60, although observed increases of V_{max} for the 1 mg/kg/day group were small. V_{max} values were increased for F_1 females in all treatment groups on postnatal days 20 and 60; however, lack of dose-response relationships, particularly on postnatal day 60, suggests that there was no treatment-related effect.

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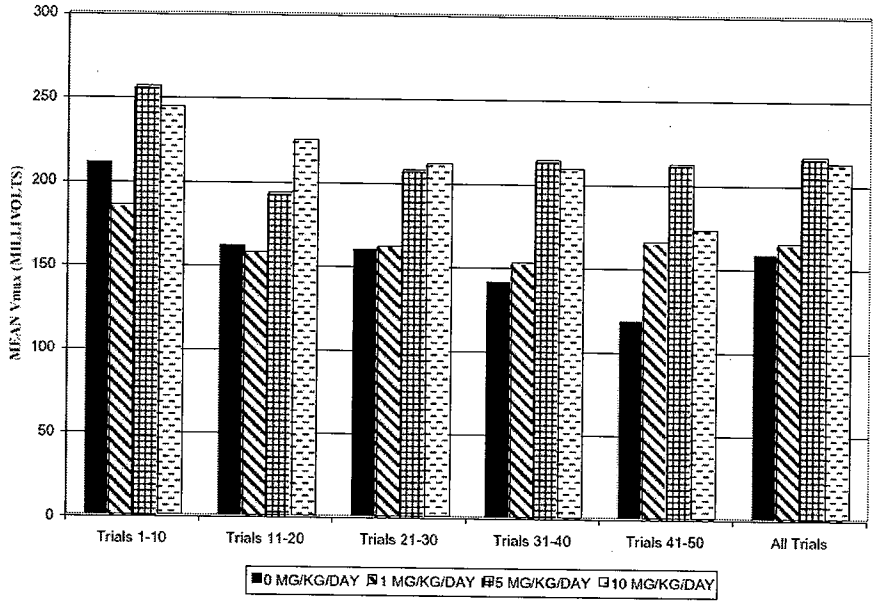
FIGURE 1 (F1 MALES - PND 20)
STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS
MEAN PEAK STARTLE RESPONSE



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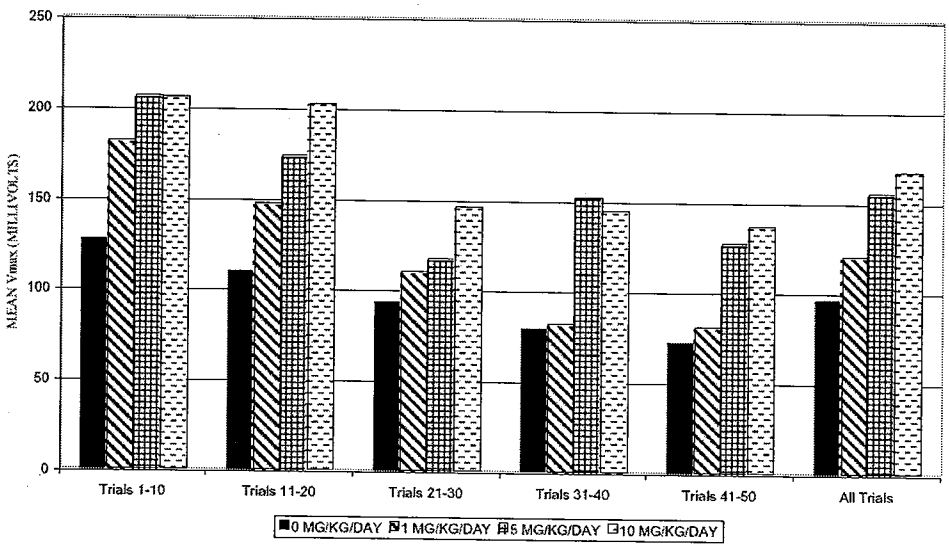
PROJECT NO. -312040
SPONSOR:SEPRACOR INC.

FIGURE 3 (F1 MALES - PND 60)
STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS
MEAN PEAK STARTLE RESPONSE



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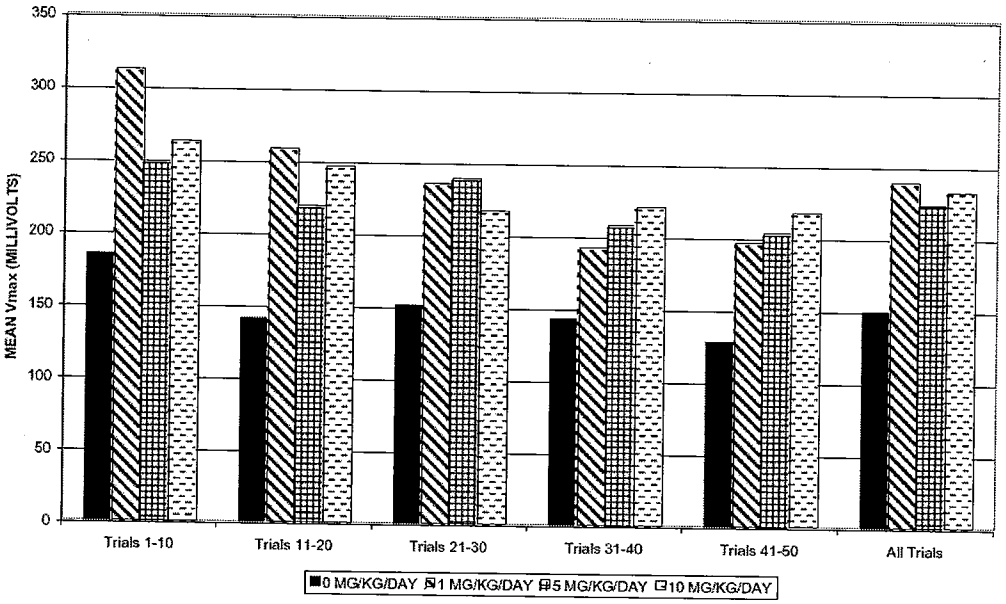
PROJECT NO [redacted]-312040
SPONSOR:SEPRACOR INC

FIGURE 2 (F1 FEMALES - PND 20)
STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS
MEAN PEAK STARTLE RESPONSE



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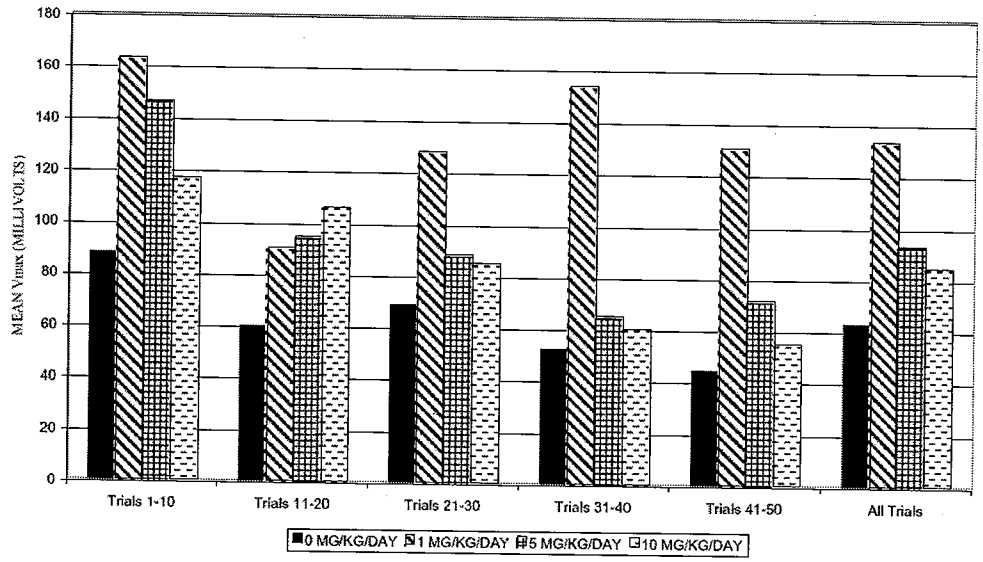
PROJECT NO.:312040
SPONSOR:SEPRACOR INC.

FIGURE 4 (F1 FEMALES - PND 60)
STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS
MEAN PEAK STARTLE RESPONSE



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Total and ambulatory motor counts on postnatal days 21 and 61 were increased for F_1 males in the 1 and 5 mg/kg/day groups; however, counts in the 10 mg/kg/day group were relatively comparable to the control. Total and ambulatory motor counts were increased for F_1 female pups in all treatment groups on postnatal days 21 and 61; however, dose-response relationships were not present. Increases for F_1 female pups in

the 1 mg/kg/day group were generally greater than those for F_1 female pups in the 5 and 10 mg/kg/day groups. The lack of dose-response relationships suggested that observed differences between control and treatment groups were not related to (R,R)-formoterol.

Swimming ability was slightly impaired for F_1 males in the 10 mg/kg/day group on postnatal day 22. Mean times to escape from the Biel maze and mean numbers of errors committed during trials 1-10 (days 2-6 of the Biel maze procedure) were increased for F_1 males in all treatment groups on postnatal day 22 (see table below). When probed for memory, mean time to escape and mean number of errors (day 7 of the Biel maze procedure) were increased for F_1 males in the 5 and 10 mg/kg/day groups on postnatal day 22. Swimming ability was unaffected for F_1 males on postnatal day 62 and F_1 females on postnatal days 22 and 62. Mean times to escape from the Biel maze and mean numbers of errors committed during trials 1-10 (days 2-6 of the Biel maze procedure) and when probed for memory (day 7 of the Biel maze procedure) were similar for F_1 males in control and treatment groups on postnatal day 62 and F_1 females in control and treatment groups on postnatal days 22. Mean times to escape from the Biel maze and mean numbers of errors committed during trials 1-10 (days 2-6 of the Biel maze procedure) were increased for F_1 females in all treatment groups on postnatal day 62 (see table below); however, when probed for memory, mean time to escape and mean number of errors (day 7 of the Biel maze procedure) were unaffected for F_1 female treatment groups on postnatal day 62. The toxicological significance of these differences between F_1 control and treatment groups in terms of swimming ability, motivation, learning, and memory are unclear. Data measured in the current study were generally within the historical control range of the testing laboratory.

Biel maze swimming trials: F_1 male pups in control and treatment groups on postnatal day 22

Test	Male Rats – Postnatal Day 22			
	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Day 1 swimming ability, seconds	9.73	9.01	10.36	11.14 (114.5%)
Overall Biel (Trials 1-10) Mean time, seconds	71.31	83.29 (116.8%)	92.70 (130%)	90.92 (127.5%)
Overall Biel (Trials 1-10) Mean number of errors	13	16 (123%)	17 (130.8%)	17 (130.8%)
Overall Probe (Trials 11-12) Mean time, seconds	55.09	48.86	61.00 (110.7%)	73.06 (132.6%)
Overall Probe (Trials 11-12) Mean number of errors	15	12	18 (120%)	19 (126.7%)

Biel maze swimming trials: F_1 female pups in control and treatment groups on postnatal day 62

Test	Female Rats – Postnatal Day 62			
	Control	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Overall Biel (Trials 1-10) Mean time, seconds	49.03	69.17 (141%)	65.71 (134%)	60.42 (123%)
Overall Biel (Trials 1-10) Mean number of errors	9	12 (133%)	12 (133%)	11 (122%)

F_1 reproduction: Estrous cycle duration for female F_1 rats (4.0-4.1 days) was unaffected by treatment. Male and female F_1 rat mating and fertility indexes were unaffected by treatment. Body weight gains for female F_1 rats from gestation days 0 to 20 were unaffected by treatment. There were no treatment-related gross pathological findings for F_1 females sacrificed on gestation day 20. There were no treatment-related gross pathological findings for F_1 males sacrificed after completion of female laparohysterectomies.

TABLE 35 (F1)

PROJECT NO -312040A STUDY OF (R,R)-FORMOTEROL ON PRE- AND POSTNATAL DEV. IN RATS PAGE 3
SPONSOR:SEPRACOR INC. SUMMARY OF REPRODUCTIVE PERFORMANCE

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
MALE MATING INDEX	25/25	100.0	24/24	100.0	21/23	91.3	22/24	91.7
FEMALE MATING INDEX	25/25	100.0	24/25	96.0	22/23	95.7	24/25	96.0
MALE FERTILITY INDEX	24/25	96.0	24/24	100.0	19/22-G	86.4	22/23-G	95.7
FEMALE FERTILITY INDEX	24/25	96.0	24/25	96.0	20/23	87.0	24/25	96.0
MEAN PRE-COITAL INTERVALS (DAYS)	2.9	NA	3.1	NA	2.6	NA	2.9	NA
S.D.	1.61	NA	1.42	NA	2.06	NA	1.76	NA
N	25		24		21		23	

$$\text{MALE (FEMALE) MATING INDEX (\%)} = \frac{\text{NO. OF MALES (FEMALES) WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)}}{\text{TOTAL NO. OF MALES (FEMALES) USED FOR MATING}} \times 100$$

$$\text{MALE FERTILITY INDEX (\%)} = \frac{\text{NO. OF MALES SIRING A LITTER}}{\text{TOTAL NO. OF MALES USED FOR MATING}} \times 100$$

$$\text{FEMALE FERTILITY INDEX (\%)} = \frac{\text{NO. OF FEMALES WITH CONFIRMED PREGNANCY}}{\text{TOTAL NO. OF FEMALES USED FOR MATING}} \times 100$$

1- 0 MG/KG/DAY 2- 1 MG/KG/DAY 3- 5 MG/KG/DAY 4- 10 MG/KG/DAY
NOTE: MALES WERE CONSIDERED TO HAVE SIRED A LITTER IF THE PAIRED FEMALE WAS GRAVID
G - DOES NOT INCLUDE PAIRINGS FOR WHICH SIRE COULD NOT BE DETERMINED
NA - NOT APPLICABLE

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F_2 findings: For the F_2 generation, numbers of viable fetuses (male and females), dead fetuses, early and late resorptions, post-implantation loss, implantation sites, corpora lutea, pre-implantation loss, and fetal body weights were unaffected by treatment. Percentage F_2 male and female fetuses were unaffected by treatment. For F_2 fetuses, there were no treatment-related external malformations or variations.

2.6.6.7 Local tolerance

Not Applicable.

2.6.6.8 Special toxicology studies

Not Applicable.

2.6.6.9 Discussion and Conclusions

Findings in toxicology studies with (R,R)-formoterol were generally similar to those with racemic formoterol, although differences were noted, possibly due to differences in doses and routes of administration used in studies.

In a 28-day inhalation toxicity study with (R,R)-formoterol in rats, target organs of toxicity were identified as the heart, kidneys, lungs, nasal cavity, testes, and epididymides. The incidence of cardiomyopathy, characterized by multifocal myofiber degeneration and infiltration of mononuclear cells, was increased for male rats at in the high dose group. The incidence of lymphoid infiltration in the kidneys was increased for females in the high dose group. The incidence of vascular mineralization in the lung was increased for males and females combined in the high dose group. Incidences of non-suppurative inflammation in the nasal cavity level 2 were increased for all treatment groups, although this finding might be considered monitorable. Incidence of degeneration in the testes and intratubular cell debris in the epididymides were increased for males in the high dose group. These findings in the heart, kidneys, lungs, nasal cavity, testes, and epididymides were not confirmed in the 6-month inhalation toxicology study with rats that received (R,R)-formoterol at deposited doses up to 77 µg/kg/day.

In a 6-month inhalation toxicology study with rats that were exposed to (R,R)-formoterol, there was no apparent target organ of toxicity. However, an increased incidence of thymic hemorrhage was observed at the high dose. Thymic hemorrhage was potentially considered a sporadic finding related to the euthanasia or tissue collection processes and not a direct test article-related effect.

In a 14-day inhalation range-finding study with dogs (2/sex/group), the target of toxicity was the heart. There were findings of increased heart rate, sinus tachycardia, and ectopic ventricular activity, and degeneration of the myocardium in all treatment groups. A NOAEL was not identified based upon histopathological findings and ectopic activity in the heart at all doses. Lower doses of (R,R)-formoterol were used in subsequent studies of longer duration.

In a 28-day inhalation toxicity study with (R,R)-formoterol in dogs, histopathological changes observed for male dogs at the high dose included dilatation of the medullary renal tubules in the kidneys, cytoplasmic vacuolation of the periportal region in the liver, non-suppurative inflammation in the nasal cavity (Level 2), and vacuolation of the germinal epithelium in the testes. There were no significant histopathological findings in female treatment groups. These histopathological findings were not confirmed in the 13-week or 9-month inhalation toxicology studies with dogs that received (R,R)-formoterol at comparable and higher doses.

In 13-week and 9-month inhalation toxicology studies with beagle dogs that received (R,R)-formoterol, there were no target organs of toxicity. However, increased heart rate,

sinus tachycardia, and ventricular ectopic arrhythmias were observed. There were no corresponding histopathological findings in the heart.

The carcinogenic potential of (R,R)-formoterol was evaluated in a 2-year oral carcinogenicity study with mice and a 2-year inhalation carcinogenicity study with rats. In mice, there were findings of uterine and cervical endometrial stromal polyps. In rats, there were findings of thyroid C-cell adenoma and carcinoma. Treatment with β-adrenergic agonists, such as racemic formoterol, is known to produce tumors in the female rodent genital tract (i.e., uterine leiomyomas and leiomyosarcomas), although the tumor findings with (R,R)-formoterol appear to differ from that observed with racemic formoterol.

(R,R)-formoterol was found to be teratogenic in rats and rabbits. A number types of malformation and variations were observed in studies with rats and rabbits that received (R,R)-formoterol. In addition for rats, numbers of viable fetuses and fetal body weights were decreased with high doses of (R,R)-formoterol. For rabbits, post-implantation loss was increased and consequent decreased pup viability were observed with high doses of (R,R)-formoterol. Distribution studies with pregnant rats and rabbits indicated that (R,R)-formoterol and/or its metabolites could cross the placenta and into distribute into the amniotic fluid and fetal tissues.

2.6.6.10 Tables and Figures

In the 6- and 9-month inhalation toxicology studies with rats and dogs, respectively, no target organs of toxicity were identified. However, in the dog study, there were findings of increased heart rate, sinus tachycardia, and various types of ECG abnormalities (i.e., ectopic findings) at all doses tested. Findings in dogs were considered to be monitorable in a clinical setting.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not provided by the applicant.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Arformoterol Tartrate Inhalation Solution is an inhalation drug product containing the long acting β_2-adrenergic agonist, (R,R)-formoterol. It is indicated for twice daily (morning and evening) long-term maintenance treatment of bronchoconstriction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. The recommended dosage of Arformoterol Tartrate Inhalation Solution for COPD patients is 15 µg administered twice a day (morning and evening) by nebulization. Sepracor has conducted an extensive array of nonclinical studies to characterize the pharmacological and toxicological properties of (R,R)-formoterol.

Pharmacology: Formoterol is a potent, selective, and long-acting β_2-adrenergic agonist that relaxes smooth muscle and exerts bronchodilatory effects in animals and humans.

Through G-protein coupling, binding of formoterol to beta receptors activates adenylate cyclase and results in an increase of intracellular cAMP which in turn causes bronchodilation. Formoterol has two chiral centers, which results in four enantiomers (i.e., R,R-, R,S-, S,R-, and S,S-). The active ingredient of Foradil® Aerolizer™ is R,R/S,S-formoterol, which is composed of equal proportions of the (R,R)- and (S,S)-enantiomers. In the present application, Sepracor has proposed to market the (R,R)-enantiomer of formoterol. (R,R)-formoterol has a K_d for the β_2 receptor of 2.9 nM as compared to 3100 nM for (S,S)-formoterol. (R,R)-formoterol has a nearly 40-fold greater selectivity for the human β_2-receptor as compared to the β_1-receptor. (R,R)-formoterol was equipotent to isoproterenol in stimulating the generation of intracellular cAMP, whereas (S,S)-formoterol had little activity.

Pharmacokinetics/Toxicokinetics: Studies to assess the pharmacokinetic and toxicokinetic properties of (R,R)-formoterol were conducted in mice, rats, rabbits, and dogs using the intravenous, oral, and inhalation routes. In studies with lactating female rats that received [^{3}H]-(R,R)-formoterol, distribution of radioactivity into maternal milk was observed. The milk to plasma ratio was 0.713.

General Toxicology: Inhalation toxicology studies with duration up to 6 months in rats and 9 months in dogs were conducted to characterize the toxicological profile of (R,R)-formoterol.

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. Pulmonary deposited doses were estimated to be 10, 40, and 77 µg/kg/day, respectively. 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. An increased incidence of thymic hemorrhage was observed at 77 µg/kg/day. Thymic hemorrhage a sporadic finding related to the euthanasia or tissue collection processes and not a direct test article-related effect. There was no apparent target organ of toxicity.

Repeat-dose inhalation toxicology studies were conducted with dogs for duration up to 9 months. In the 14-day inhalation dose range finding study, doses were 70, 100, and 200 µg/kg/day. In the 13-week inhalation toxicology study, doses were 5, 40, and 70/100 µg/kg/day. In the 9-month inhalation toxicology study, doses were 5, 40, and 70 µg/kg/day. During these exposure periods, exercise programs were suspended due to time constraints resulting from the exposure regimen. In addition, formoterol, a β_2-adrenergic agonist, was known to produce to large increases of heart rate as well as arrhythmias. Continued exercise periods might have compromised the health of study animals. The impact of discontinued exercise periods on these studies was unclear.

Sinus tachycardia was a common findings in dogs treated with (R,R)-formoterol at doses ≥5 µg/kg/day in these three studies. Various types of ECG abnormalities (i.e., ventricular ectopic arrhythmias) were also observed in dogs treated with (R,R)-

formoterol at doses ≥5 µg/kg/day. NOAELs could not be identified for ectopic activity in these studies.

In the 14-day study, tachycardia was observed during and following exposure in all male and female treatment groups. Sinus tachycardia was observed at 2- or 4-hr postdose. Ectopic ventricular activity was generally observed at 24-hr postdose. Paroxysmal ventricular escape rhythms with or without paroxysmal ventricular tachycardia was observed for one male in the 70 µg/kg/day group and one male and one female each in the 100 and 200 µg/kg/day groups. In addition, one male in the 100 µg/kg/day group had ventricular escape beats. The target of toxicity was the heart. Degeneration of the myocardium was observed for 1 of 2 males and 2 of 2 females at 70 µg/kg/day, 2 of 2 males at 100 µg/kg/day, and 2 of 2 males and 1 of 2 females at 200 µg/kg/day. Incidences of mineralization of the myocardium were increased for males in the 70 and 200 µg/kg/day. A NOAEL was not identified based upon histopathological findings and ectopic activity in the heart at all doses.

In the 13-week study, 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. 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_2-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. A NOAEL was not identified based upon ectopic findings at all doses.

In the 9-month study, 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 postexposure, 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. A NOAEL was not identified based upon ectopic findings at all doses.

Pretest and post-exposure electrocardiograms recorded for dogs in the 14-day, 13-week, and 9-month inhalation toxicology studies were re-evaluated for the presence of cardiac rhythm changes by two veterinary cardiologists. The applicant noted that NOAEL could not be identified for ectopic activity with particular regard to the 13-week and 9-month inhalation toxicology studies; however, they have asserted that an exposure threshold for ectopic findings can be identified within the low dose groups that received 5 µg/kg/day. The applicant's designation of a threshold for ectopic activity within the 5 µg/kg/day groups of the 13-week and 9-month inhalation toxicology studies appears potentially arbitrary and inappropriate. As noted in original study reviews, ECG monitoring periods were short and could have missed ectopic activity. Further, animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm. AUC and C_{max} data is known to vary from day to day (or measurement to measurement), which could significantly shift the designated threshold. All dogs were young with no pre-existing cardiac problems, which minimized potential adverse effects of ectopic activity. The designation of threshold may have little value for the COPD patient population, which generally have pre-existing cardiac problems.

These cardiac effects were attributed to the pharmacological properties of (R,R)-formoterol, a β_2-adrenergic agonist. These effects were considered to be monitorable in a clinical setting. Thus, development of (R,R)-formoterol was allowed to proceed despite the lack of a NOAEL for cardiac effects in dogs.

Safety margins for the clinical dose of 15 µg BID based upon the 6- and 9-month inhalation toxicology studies with rats and dogs, respectively

Toxicology Studies			Clinical dose of 15 µg BID (AUC = 69 pg·hr/mL)
Study	NOAEL/LOAEL	AUC pg·hr/mL	
6-month rat	NOAEL = 100 µg/kg/day	2920/4010	42.3/58.1
9-month dog	LOAEL = 5 µg/kg/day	1280/2290	18.6/33.2

Genotoxicity: (R,R)-formoterol was negative in the in vitro bacterial reverse mutation assay, in vitro Chinese hamster ovary cell chromosomal aberration assay, and in vivo mouse micronucleus assay.

Carcinogenicity: Sepracor conducted an oral carcinogenicity study with mice and inhalation carcinogenicity study with rats.

Mice received (R,R)-formoterol at oral doses of 0, 1, 5, and 25 mg/kg/day for periods up 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 at oral doses ≥1 mg/kg. Systemic exposure to (R,R)-formoterol in

females at 1 mg/kg/day was 70-fold of exposure at the clinical dose of 15 µg BID. Given that (R,R)-formoterol was not genotoxic, observed tumors most likely developed through non-genotoxic mechanisms. Treatment with β_2-adrenergic agonists is known to produce tumors in the female rodent genital tract. In studies with albuterol, salmeterol, and racemic formoterol (R,R- and S,S-enantiomers), there were findings of uterine leiomyomas and/or leiomyosarcomas. (R,R)-formoterol produced tumors in the uterus and cervix as observed with other β_2-adrenergic agonists, although the tumor type differed. Leiomyomas and/or leiomyosarcomas are tumors derived from uterine smooth muscle (myometrial muscle), whereas endometrial stromal polyps and stromal cell sarcoma are derived from the endometrial stroma. There are no safety concerns for tumor findings in mice treated with (R,R)-formoterol given that β_2-adrenergic agonists are known to produce tumors in the female rodent genital tract.

In an inhalation carcinogenicity study, rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks. Sepracor did not have ECAC concurrence for dose selection. Further, they did not contact the Division prior to early termination of groups. The 400 µg/kg/day group was sacrificed early and considered invalid for assessment of carcinogenic potential. For the ovaries and oviducts, incidences of cyst(s) were significantly increased for female treatment groups at doses ≥40 µg/kg/day. For the thyroid gland, combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. The combined incidence of c-cell adenoma and carcinoma for females at 100 and 200 µg/kg/day exceeded mean incidences from the historical control background data of the testing laboratory. Increases at 100 and 200 µg/kg/day were significant using trend analysis. However, only the increase at 200 µg/kg/day was statistically significant by pairwise comparison. Based upon increased incidences of thyroid C-cell adenoma and carcinoma in female treatment groups, (R,R)-formoterol is tumorigenic in rats. Given that (R,R)-formoterol was not genotoxic, observed tumors most likely developed through non-genotoxic mechanisms. Systemic exposure in rats that received 40 µg/kg/day (R,R)-formoterol, where there were no treatment-related tumor findings, was approximately 35.9 to 55.5 times greater than exposure with a clinical dose of 15 µg BID. There are no safety concerns for tumors findings in rats treated with (R,R)-formoterol given that there is a sufficient safety margin.

Reproductive Toxicology: A fertility and reproductive performance study in rats, several teratology studies in rats and rabbits, and a pre- and post-natal development study in rats with (R,R)-formoterol were conducted. The oral route of administration was used in all studies.

Fertility and reproductive parameters were evaluated in male and female rats that received (R,R)-formoterol at oral doses of 0, 1, 5, and 10 mg/kg/day. (R,R)-formoterol at oral doses ≤10 mg/kg/day had no effects on fertility or reproductive performance in male and female rats.

(R,R)-formoterol was teratogenic in studies with both rats and rabbits. External and skeletal malformations were evident in studies conducted with rats. Omphalocele (i.e.,

several loops of the intestine protruded through an opening in the umbilicus, and remnants of a membranous sac were discernable) was observed in two studies with combined doses of 1, 5, 10, 60, and 120 mg/kg/day. Maternal toxicity was evident at 60 and 120 mg/kg/day. Omphalocele occurred independently of maternal toxicity. A NOAEL was not established for this finding (i.e., there was no threshold dose). On gestation days 6 or 17, the dose of 1 mg/kg/day produced an AUC that was 895.6 and 736.2 times the AUC observed with the clinical dose of 15 µg BID, respectively; however, it should be noted that 1 mg/kg/day is not a NOAEL.

In a teratology study, (R,R)-formoterol was administered by oral gavage at doses of 0, 20, 40, and 80 mg/kg/day to pregnant female rabbits per group from days 7 to 20 of gestation. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥20 mg/kg/day. Three female rabbits at 80 mg/kg/day and one female at 40 mg/kg/day aborted. It is noted that abortion is a fairly common observation for pregnant female rabbits (range of 0 to 33.3%). Decreased defecation was observed in a dose-related manner for all treatment groups. Post-implantation loss at 80 mg/kg/day was increased when compared to the control. This increase was attributed to higher early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control. Total malformations (i.e., external, visceral, and skeletal) at 20, 40, and 80 mg/kg/day were increased in a dose-related manner to 10(5), 11(9), and 26(12), respectively, as compared to 3 fetuses (2 litters) for the control. Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol. Adactyly was observed at 80 mg/kg/day. Brachydactyly was observed at 40 and 80 mg/kg/day. Adactyly was observed at a dose of 80 mg/kg/day in the dose range finding study. Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Bulbous aorta was observed at 40 and 80 mg/kg/day. Lobular dysgenesis of the lungs and interventricular septal defect were observed at 80 mg/kg/day. Cysts in the liver were observed in fetuses at 40 and 80 mg/kg/day. In a second study with doses of 2, 10, and 20 mg/kg/day, there were no teratogenic findings. The NOAEL could be considered 10 mg/kg/day. The dose of (R,R)-formoterol at 10 mg/kg/day produced an AUC on gestation days 7 and 20 that was 6217.4 and 4927.5 times the AUC observed with a clinical dose of 15 µg/day BID, respectively.

In a pre- and post-natal development study, time-mated F_0 female rats received (R,R)-formoterol at oral doses of 0, 1, 5, and 10 mg/kg/day from gestation day 6 to postnatal day 20. One female in the 10 mg/kg/day group was euthanized on gestation day 21 due to complications during parturition. This female had total litter loss. Lengths of gestation for F_0 female rats in the 1, 5, and 10 mg/kg/day groups were slightly prolonged. Survival was slightly decreased for F_1 pups in the 5 and 10 mg/kg/day groups on postnatal days 0 and 1 and birth to postnatal day 4. No effects on survival were observed at later time points. Umbilical hernia, a malformation observed in teratology studies with (R,R)-formoterol, was observed for 1 pup in the 10 mg/kg/day group. Incidences of missing (presumed cannibalized), subcutaneous hemorrhage, pale in color, and cyanotic were

increased for pups in all (R,R)-formoterol treatment groups. Absolute body weights for male and female pups in the and mid high dose groups were decreased on postnatal days 1, 14, 17, and 21 as compared to control values; however, body weight gains for F_1 male and female pups from postnatal days 0 to 21 (i.e., lactation or weaning period) were unaffected. During the post-weaning period, absolute body weights for F_1 male and female pups in the mid and high doses group were generally lower than corresponding control values; however, body weight gains for F_1 male rats from postnatal day 21 to week 22 and F_1 female rats from postnatal day 21 to week 17 were unaffected by treatment. Acquisition of balanopreputial separation appeared to be delayed for male pups receiving the high dose of 10 mg/kg/day. Acquisition of vaginal patency appeared to be delayed for female pups receiving the high dose of 10 mg/kg/day. Male and female F_1 rat mating and fertility indexes were unaffected by treatment. No treatment-related effects were observed for the F_2 generation fetuses. Potential delays of prenatal and postnatal development were observed for (R,R)-formoterol high dose group that received 10 mg/kg/day.

(R,R)-Desformoterol (A Degradation Product formed from (R,R)-formoterol in the Drug Product): Desformoterol is a degradation product of (R,R)-formoterol that is found in the Arformoterol Tartrate Inhalation Solution. It is formed from (R,R)-formoterol by the loss of the formyl moiety from its formamide group. It was shown to bind to beta-adrenergic receptors. The applicant conducted two 13-week inhalation toxicology studies with rats that received isolated (R,R)-desformoterol and a 13-week inhalation toxicology study with dogs that received (R,R)-desformoterol spiked into formoterol.

(R,R)-desformoterol contains an [redacted] (R,R)-desformoterol was negative in the in vitro Chinese hamster ovary cell chromosomal aberration assay and in vivo mouse micronucleus assay. (R,R)-Desformoterol was present at level of 0.17% of (R,R)-formoterol in mouse and rat carcinogenicity studies. Thus, the structural alert in (R,R)-desformoterol is not a safety concern. b(4)

In a 13-week nose-only inhalation toxicology study, rats were exposed to (R,R)-desformoterol at target inhaled doses of 3.1, 8.6, and 26.9 µg/kg/day. Using a deposition factor of 0.1, pulmonary deposited doses were calculated to be 0.3, 0.86, and 2.7 µg/kg/day, respectively. Histopathological findings were observed in the cecum, heart, adrenal gland, and prostate. Increased incidences of minimal to slight inflammation with resulting secondary and reactive hyperplasia were observed in the cecal mucosa for all male and female treatment groups. The incidence and severity of this finding displayed no dose-response relationship. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696). The incidence of mononuclear infiltration in the heart was increased for male and female rats in the high dose group. Lower dose groups were not examined to establish a NOAEL for this finding. Incidence of cortical vacuolation in the adrenal glands and inflammation in the prostate were increased for males in the high dose group as compared to the concurrent control. There was no evidence of local toxicity in the respiratory tract

tissues. A NOAEL was not established in the present study based upon an increased incidence of mononuclear cell infiltration in the heart for male and female rats in the high dose group and no histopathological examination of lower dose groups.

In a 13-week inhalation toxicology study with rats, (R,R)-desformoterol, a degradant of arformoterol, was administered at total inhaled doses of 1.02 and 3.65 µg/kg/day. Deposited doses were estimated to be 0.1 and 0.37 µg/kg/day, respectively. This study was conducted as a follow up to study 090-840 to explain various histopathological findings, particularly for male treatment groups. Histopathological findings of potential significance were observed in the cecum, colon, jejunum, and lung. In the cecum, incidences of minimal hemorrhage were increased for both treatment groups. The relationship of this finding to treatment was unclear given that there were no similar observations in study 090-840. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696). Hemorrhage was observed in the colon, jejunum, and lung. The relationships of these findings to treatment were unclear given that there were no similar observations from study 090-840. Consideration of the present study along with results of study 090-840 where (R,R)-desformoterol was administered at total inhaled doses up to 30 µg/kg/day suggests that findings in the cecum, colon, jejunum, and lung were unrelated to treatment and the NOAEL could be identified as the high dose. The deposited dose of 0.37 µg/kg/day for the high dose group provides a [redacted] safety margin for level of (R,R)-desformoterol [redacted] found in the clinical dose of arformoterol. b(4)

In a 13-week inhalation toxicology study, dogs were exposed to 40 µg/kg/day (R,R)-formoterol in the presence or absence of [redacted] (R,R)-desformoterol [redacted] Pulmonary deposited doses for (R,R)-formoterol and (R,R)-desformoterol were 8.50-9.50 µg/kg/day and 0.52-0.60 µg/kg/day, respectively. A concurrent negative control group received citrate-buffered isotonic saline on a comparable regimen. Additional dogs were included in treatment groups for a 4-week recovery period, although no concurrent control dogs were included. The secondary objective of this study was to assess the toxicity of potential leachables from (R,R)-formoterol unit dose vials (UDV). A solution of (R,R)-formoterol from UDVs that had been stored for an extended time was used to prepare the test article formulation with appropriate concentrations of bulk (R,R)-formoterol and (R,R)-desformoterol. No specifications of leachables were provided. Increased heart rates were observed in both the formoterol only and formoterol + desformoterol groups, although there was no evidence of ectopia as observed in the 13-week and 9-month inhalation toxicology studies with dogs. It should be noted that ECG measurements were not conducted on the first day of exposure as done in the 13-week and 9-month inhalation toxicology studies with dogs. Histopathological findings were observed in the heart, epididymides, testes, lung, and mesenteric lymph node, although these findings were not observed in the 13-week and 9-month inhalation toxicology studies with dogs that received (R,R)-formoterol alone at comparable and higher doses. Findings were generally comparable between the formoterol only and formoterol + desformoterol groups. Administration of desformoterol in the presence of formoterol produced no additional toxic effects. There were concerns b(4)

about the design of this study given that only one dose of (R,R)-desformoterol was tested and the cardiac toxicity of (R,R)-formoterol in dogs might confound interpretation of the study. There were no apparent differences in the toxic profiles between the (R,R)-formoterol alone and (R,R)-formoterol +[redacted] (R,R)-desformoterol groups. b(4)

These studies qualify levels of the degradant, desformoterol[redacted] in the drug product. b(4)

Conclusions: The applicant has a complete nonclinical pharmacology and toxicology program for (R,R)-formoterol, which supports the safety of the proposed clinical dose of 15 µg BID.

Unresolved toxicology issues (if any): None.

Recommendations: From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval.

Evaluation of labeling:
Systemic exposure (AUC) data was available for most nonclinical studies and has been used to determine exposure multiples between the clinical dose of 15 µg BID and observed nonclinical findings. Adverse findings were observed in the pre- and post-natal developmental toxicology study with rats; however, AUC data was not available for this study. AUC data from identical doses in the pivotal teratology study was used to approximate differences in exposure multiples between the clinical dose and adverse findings in the pre- and post-natal development study.

The labeling for the mouse and rat carcinogenicity studies has been revised to reflect the conclusions and recommendations of the Executive CAC. For the mouse study, incidences of endometrial stromal polyps and stromal cell sarcoma in the uterus and cervix were combined. In the rat study, the 400 µg/kg/day group was terminated early. The duration of treatment was considered inadequate and the group was considered invalid for analyses of study results. For the rat study, c-cell adenoma and carcinoma in the thyroid gland were combined.

In teratology studies with rats and rabbits, only teratogenic findings that were not associated with maternal toxicity have been listed in the revised labeling. Some adverse findings in the pre- and post-natal developmental toxicology study have also been listed in this section.

4 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 1

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

Supervisor signature: Concurrence - _____
C. Joseph Sun, Ph.D.,

cc: list:
NDA 21-912, HFD-570
JafariL, HFD-570
DurmowiczA, HFD-570
SunC, HFD-570
RobisonT, HFD-570

APPENDIX/ATTACHMENTS

Appendix 1	Minutes of ECAC Meeting dated September 7, 1999
Appendix 2	Minutes of ECAC Meeting dated May 10, 2005
Appendix 3	Minutes of ECAC Meeting dated June 13, 2006
Appendix 4	IND 55,302 Review #01 dated April 16, 1998
Appendix 5	IND 55,302 Review #04 dated December 20, 1999
Appendix 6	IND 55,302 Review #07 dated June 13, 2001
Appendix 7	IND 55,302 Review #10 dated November 26, 2001
Appendix 8	IND 55,302 Review #11 dated February 6, 2002
Appendix 9	IND 55,302 Review #12 dated February 13, 2002
Appendix 10	IND 55,302 Review #13 dated April 29, 2005
Appendix 11	NDA 21-912 Review #01 dated June 6, 2006

Pharmacokinetic parameters for the proposed clinical dose of (R,R)-formoterol at 15 µg BID in COPD patients.
AUC_{0-24} = 69 pg·hr/mL

Steady State Plasma Arformoterol Pharmacokinetic Parameters After Administration of Arformoterol 15 mcg BID for 14 Days in COPD Patients

Parameter	Arformoterol 15 mcg BID
Mean C_{max} (pg/mL)	4.3
Mean AUC_{0-12} (pg*hr/mL)	34.5
Median t_{max} (hr)	0.57
Mean $t_{1/2}$ (hr)	25.6

Drug: **R,R-Formoterol**

	age	µg/dose	# daily doses	µg/day	pg*hr/mL
Adult		15	2	30	69.000

Study	route	µg/kg/d	pg*hr/mL	Exposure margin Adults
Carcinogenicity:				
mouse M	Oral/F	1000	16300	236.23
mouse F	Oral/F	1000	4800	69.57
mouse M	Oral/F	5000	27800	402.90
mouse F	Oral/F	5000	13000	188.41
mouse M	Oral/F	25000	3E+05	4275.36
mouse F	Oral/F	25000	73100	1059.42
rat M	IH/F	40	2480	35.94
rat F	IH/F	40	3830	55.51
rat M	IH/F	100	7660	111.01
rat F	IH/F	100	5870	85.07
rat M	IH/F	200	11600	168.12
rat F	IH/F	200	8740	126.67
rat M	IH/F	400	67700	981.16
rat F	IH/F	400	22900	331.88
Reproduction and Fertility:				
rat	Oral/F	1000		0.00
rat	Oral/F	5000		0.00
rat	Oral/F	10000		0.00
Teratogenicity:				
rat	Oral/F	1000	25400	368.12
rat	Oral/F	5000	75800	1098.55
rat	Oral/F	10000	2E+05	2391.30
rabbit	Oral/F	2000	63700	923.19
rabbit	Oral/F	10000	3E+05	4927.54
rabbit	Oral/F	20000	6E+05	8434.78
6-month inhalation study:				
rat M	IH/F	100	2920	42.32
rat F	IH/F	100	4010	58.12
rat M	IH/F	400	13700	198.55
rat F	IH/F	400	19700	285.51
rat M	IH/F	800	21000	304.35
rat F	IH/F	800	32900	476.81
9-month inhalation study:				
dog M	Oral/F	5	1280	18.55
dog F	Oral/F	5	2290	33.19
dog M	Oral/F	40	16300	236.23
dog F	Oral/F	40	12200	176.81
dog M	Oral/F	70	50200	727.54
dog F	Oral/F	70	23500	340.58

Drug: **R,R-Formoterol**

	age	µg/dose	# daily doses	µg/day	kg	µg/kg	factor	µg/m²
Adult		15	2	30	50	0.6000	37	22.20

	route	µg/kg/d	conv. factor	µg/m²	Dose Ratio Adults	Rounded Dose Ratio Adults
Carcinogenicity:						
mouse	Oral/F	1000	3	3000	135.14	140
mouse	Oral/F	5000	3	15000	675.68	680
mouse	Oral/F	25000	3	75000	3378.38	3400
rat	IH/F	40	6	240	10.81	10
rat	IH/F	100	6	600	27.03	30
rat	IH/F	200	6	1200	54.05	50
Reproduction and Fertility:						
rat	Oral/F	1000	6	6000	270.27	270
rat	Oral/F	5000	6	30000	1351.35	1400
rat	Oral/F	10000	6	60000	2702.70	2700
Teratogenicity:						
rat	Oral/F	1000	6	6000	270.27	270
rat	Oral/F	5000	6	30000	1351.35	1400
rat	Oral/F	10000	6	60000	2702.70	2700
rabbit	Oral/F	20000	12	2E+05	10810.81	11000
rabbit	Oral/F	40000	12	5E+05	21621.62	22000
rabbit	Oral/F	80000	12	1E+06	43243.24	43000
rabbit	Oral/F	2000	12	24000	1081.08	1100
rabbit	Oral/F	10000	12	1E+05	5405.41	5400
rabbit	Oral/F	20000	12	2E+05	10810.81	11000
Overdosage:						
mouse	IN/F	400	3	1200	54.05	50
mouse	IN/F	800	3	2400	108.11	110
mouse	IN/F	1600	3	4800	216.22	220
rat	Oral/F	100000	6	6E+05	27027.03	27000
rat	IH/F	33	6	198	8.92	10
rat	IH/F	165	6	990	44.59	45
rat	IH/F	435	6	2610	117.57	120
rat	IH/F	1649	6	9894	445.68	450
rat	IH/F	1719	6	10314	464.59	460
rat	IV	1000	6	6000	270.27	270
rat	IV	50000	6	3E+05	13513.51	13500
rat	IV	75000	6	5E+05	20270.27	20000
rat	IV	100000	6	6E+05	27027.03	27000
dog	IV	3	20	60	2.70	3

Conversion, Correction, and Rounding Factors:

luman Age (yr)	Weight (kg)	Factor (kg/m²)	Species	Factor (kg/m²)	Exposure greater than x-times human	Round to nearest
0	3	25	dog	20	1	1
1	10	25	guinea pig	8	10	5
2	12	25	hamster	4	100	10
4	16	25	monkey	12	1000	100
6	20	25	mouse	3	10000	1000
12	50	37	rabbit	12		
			rat	6		

Appendix 1 Minutes of ECAC Meeting dated September 7, 1999

Appears This Way
On Original

Executive CAC
Date of Meeting: September 7, 1999

Committee: James Farrelly, Ph.D., HFD-530, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
Andrea Weir, Ph.D., HFD-550, Alternate Member
Robin Huff, Ph.D., HFD-570, Team Leader
Luqi Pei, Ph.D., HFD-570, Presenting Reviewer

Author of Minutes: Luqi Pei, D.V.M., Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND #: 55,302
Drug Name: R,R-Formoterol
Sponsor: Sepracor Inc., Marlborough, MA

Rat Dose Selection

R,R-formoterol is a beta 2 adrenergic agonist that is non-genotoxic. Sepracor proposed to conduct a 2-year inhalation study in rats to evaluate the carcinogenicity potential of the drug. The proposed doses were 40, 100, and 200 :g/kg/day (inhaled dosed) for the low, mid and high dose, respectively.

Dose selection was based on plasma AUC ratios between rats and humans. The high dose male and female rats are predicted to achieve AUC's approximately 80 and 60 times the human AUC, based on Day 26 values in a 28-day inhalation study in rats and a single dose study in healthy human volunteers.

Available supporting data included general toxicity studies with the longest treatment duration of 4 weeks in rats, pharmacokinetic and protein binding data in rats and humans, and *in vitro* metabolism data in humans. The 4-week toxicity study in rats showed NOAEL values of 400 :g/kg/day in males and at least 800 :g/kg/day in females. Metabolism data in rats are not available.

Executive CAC Recommendations and Conclusions:

Rat study: The Committee could not concur with the dose selection, because all necessary data were not provided. Specifically, the sponsor needs to submit the following to support the protocol:

- Comparative metabolism data in rats and humans.
- Pharmacokinetic data from repeat dose trials in patients, so as to have a steady state AUC value.

The Committee would prefer a 13-week toxicity study as the dose-ranging study in order to increase confidence that a dose selected on the basis of AUC would not exceed the maximum tolerated dose.

The following comments were not discussed in the Committee meeting, but the review division would like to see them incorporated into the protocol during its revision:

1. Define the acceptable range of particle size. Report exposure data as inhaled dose, total body burden and pulmonary/bronchial dose (deposited doses) based on aerodynamic aerosol particle size.
2. In addition to organs already listed in the protocol, record organ weights of heart, uterus, thymus and spleen as they are potential target organs of toxicity of the drug.
3. Given the complexity of the exposure system of the inhalation toxicity studies, assess systemic exposure by determining plasma drug levels at middle and end of study.
4. Consider EKG monitoring.
5. In statistical analysis, tests should be run with each control group and the means of the two control groups combined, rather than solely against Group 1.

James Farrelly, Ph.D.
Acting Chair, Executive CAC

cc:\ IND 55,302/Division File, HFD-570
Robin Huff/Team Leader, HFD-570
Luqi Pei/Reviewer, HFD-570
Parinda Jani/PM, HFD-570
Adele Seifried, HFD-024

Appendix 2 Minutes of ECAC Meeting dated May 10, 2005

Appears This Way
On Original

Executive CAC
Date of Meeting: May 10, 2005

Committee: Abigail Jacobs, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Adebayo Laniyonu, Ph.D., HFD-160, Alternate Member
C. Joseph Sun, Ph.D., HFD-570, Team Leader
Tim Robison, Ph.D., Presenting Reviewer

Author of Draft: Tim Robison, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND# 55,302
Drug Name: Arformoterol or R,R-formoterol
Sponsor: Sepracor Inc.

Background: The sponsor did not have prior FDA concurrence on doses for either the mouse or rat carcinogenicity studies and did not consult with the FDA prior to early termination of treatment groups in either study.

Mouse Carcinogenicity Study:
Mice received (R,R)-formoterol at oral doses of 0, 1, 5, and 25 mg/kg/day for periods up to 104 weeks.

Survival was significantly decreased for males and females in the 25 mg/kg/day group. Due to decreased survival for males in the 25 mg/kg/day group, the sponsor elected to sacrifice this group at week 77. The sponsor elected to sacrifice females in the 25 mg/kg/day group during week 92 and males in the 5 mg/kg/day group during week 95.

Surviving females in control groups 1 and 2 and the 1 and 5 mg/kg/day groups were sacrificed during week 102. Surviving males in control groups 1 and 2 and the 1 mg/kg/day group were sacrificed during week 104.

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.

The combined incidences of uterine and cervical endometrial stromal polyps and stromal cell sarcoma were significantly increased for female treatment groups.

Rat Carcinogenicity Study:
Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group.

The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91-92. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90-91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100-101. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were sacrificed during week 104.

Decreases (approximately 10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period, suggesting a maximum tolerated dose was also achieved at this dose.

Surviving males and females in the 400 µg/kg/day group were sacrificed up to 3 months early. Sacrifice of these animals was inappropriate and it appears that the treatment period was insufficient. There was evidence that a maximum tolerated dose was obtained for males and females in the 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively; however, histopathological examinations of organs and tissues were incomplete for these animals.

Results regarding the carcinogenic potential of R,R-formoterol in rats are considered inconclusive due to incomplete examinations of tissues for the 200 µg/kg/day group and possibly lower dose groups.

Executive CAC Recommendations and Conclusions:

Mouse:

1. The sponsor sacrificed males in the 25 mg/kg/day group approximately 6 months early and the duration of treatment was considered inadequate for that group. However, the duration of treatment was considered adequate for females in the 25 mg/kg/day group and males in the 5 mg/kg/day. The Committee found that the study was acceptable for males and females.

2. The study was positive for drug-related incidences of uterine and cervical endometrial stromal polyps and stromal cell sarcoma. It was noted that the tumor incidences for the high dose group were lower than the low and mid dose groups, probably due to decreased survival and early termination of the surviving animals at the high dose group. The Committee concluded that the uterine and cervical neoplasms were drug-related.

Rat:

1. Surviving males and females in the 400 µg/kg/day group were sacrificed up to 3 months early. Sacrifice of these animals was inappropriate and the treatment period was judged to be inadequate for that group.

2. There was evidence that a maximum tolerated dose was obtained for males and females in the 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively, based upon decreased absolute body weights; however, histopathological examinations of organs and tissues were incomplete for these animals. The findings of the study were judged to be inconclusive because of incomplete histopathological examinations of organs and tissues for males and females in the 200 µg/kg/day group and lower dose groups.

a. The sponsor should complete the histopathological evaluations of organs and tissues for males and females in the 200 µg/kg/day group and lower dose groups if appropriate. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the males and females in the 200 µg/kg/day group, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group.

b. The Committee also noted that the controls need to be reexamined by the pathologist along with drug treatment groups in order to have a valid comparison.

c. A study report of histopathological examinations of organs and tissues needs to be reviewed by the Division and evaluated by the Executive CAC before the FDA could find the study to be adequate.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

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PHARMACOLOGIST

Appendix 3 Minutes of ECAC Meeting dated June 13, 2006

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Executive CAC
Date of Meeting: June 13, 2006

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Tim McGovern, Ph.D., DPAP, Alternate Member
C. Joseph Sun, Ph.D., DPAP, Team Leader
Tim Robison, Ph.D., DPAP, Presenting Reviewer

Author of Draft: Tim Robison, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA# 21-912
Drug Name: Arformoterol or R,R-formoterol
Sponsor: Sepracor Inc.

Background: For the rat carcinogenicity study with R,R-formoterol, the sponsor did not have prior FDA concurrence on dose selection and did not consult with the FDA prior to early termination of treatment groups. This study was first presented to the ECAC on May 10, 2005.

The committee on May 10, 2005 concluded that:

1. Surviving males and females in the 400 µg/kg/day group were sacrificed up to 3 months early. Sacrifice of these animals was inappropriate and the treatment period was judged to be inadequate for that group.

2. There was evidence that a maximum tolerated dose was obtained for males and females in the 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively, based upon decreased absolute body weights; however, histopathological examinations of organs and tissues were incomplete for these animals. The findings of the study were judged to be inconclusive because of incomplete histopathological examinations of organs and tissues for males and females in the 200 µg/kg/day group and lower dose groups.

Based upon conclusions and recommendations from the ECAC meeting dated May 10, 2005, the sponsor requested that study pathologists examine all remaining (unexamined) tissues for the animals from the 40, 100, and 200 µg/kg/day groups (i.e., animals from these groups that survived to the terminal primary necropsy and females in the 100 µg/kg/day group sacrificed during week 92).

Rat Carcinogenicity Study:
Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group. All surviving males in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 91-92. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90-91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100-101. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were sacrificed during week 104.

Decreases (approximately 10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period, indicating that a maximum tolerated dose was also achieved at this dose.

For the thyroid gland, combined incidences of c-cell adenoma and carcinoma were increased significantly for female treatment groups using trend analysis and exceeded mean incidences from the historical control background data of the testing laboratory. However, only the increase at 200 µg/kg/day was statistically significant by pairwise comparison.

Based upon increased incidences of thyroid C-cell adenoma and carcinoma for females in the 200 µg/kg/day group, R,R-formoterol is tumorigenic in rats.

Executive CAC Recommendations and Conclusions:

1. The Committee agreed that the study was adequate.

2. The Committee found that the study was positive for thyroid C-cell adenomas and carcinomas in female rats.

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

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David Jacobson-Kram
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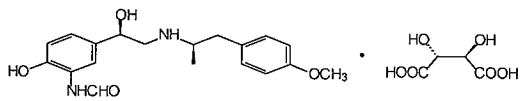
DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 1

Reviewer Name:	Luqi Pei, Ph.D.
Division Name:	HFD-570
Review Completion Date	April 16, 1998
IND No.	55,302
Serial Nos., Contents and Dates of Submission:	000, Original submission, February 20, 1998
Information to be conveyed to Sponsor:	Yes (X), No ( ).
Sponsor:	Sepracor Inc., Marlborough, MA
Manufacturer:	Sepracor Canada, Windsor, Nova Scotia, Canada
Drug:	
Code Name:	
Generic Name:	(R,R)-Formoterol-L-tartrate
Trade Name:	None
Chemical Names:	(R,R)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-ethylethyl]amino]ethyl]-phenyl]formamide, L-tartrate
CAS Registry No.	Not provided
Formula/Molecular Wt.:	$C_{23}H_{30}N_2O_{10}$, MW=494.5
Structure:	
Relevant INDs/NDAs/DMFs	IND ⊂ ⊐ b(4)
Class:	β_2-agonist
Indication:	⊂ ⊐

Clinical Formulation:

Component	mg/ml
R,R-formoterol-L-Tartrate	[redacted]
Citric acid, [redacted] USP	
Sodium citrate [redacted] USP	

[redacted]

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Route of Administration: Oral inhalation

Proposed Clinical protocol or use: Single dose cross-over study (6, 12, 24, 48, 72 μg) in asthmatic adults. (dosing interval > 5 days)

Previous clinical experience: Single dose trials: up to 72 μg in healthy subjects and at 48 μg in asthmatic patients.

Disclaimer: *Note some material may be taken directly from the sponsor's submission.*

Introduction and drug history:

Formoterol is a potent, highly selective, and long-acting β_2-adrenergic agonist that relaxes smooth muscle and exerts bronchodilatory effects in animals and humans. Inhalation formoterol fumarate has been approved to treat respiratory diseases in humans in several other countries. In the US, clinical trials are also underway. These marketed products formulations use a racemic mixture (R,R/S,S) of formoterol. [A formoterol molecule contains two chiral centers and, therefore, two pairs of enantiomers: (R,R), (S,S) and (R,S), (S,R)]. Recently, a few reports suggest that the S,S-formoterol enantiomer lacks therapeutic effects but contributes to the adverse reactions. This prompted Sepracor to pursue the R,R-formoterol tartrate only.

A pre-IND meeting was held between Sepracor and the Division to discuss the sponsor's developmental plan of R,R-formoterol on January 14, 1998. The proposed protocol in the current application was discussed among many other issues. The Division informed Sepracor that they may proceed with the protocol with minor modifications. This decision was based on available clinical data rather than the preclinical data. Sepracor has conducted 2 trials in Europe and results from these studies were available to the Agency for review. Preclinically, Sepracor had no adequate and appropriate data for us to evaluate safety of this drug in the protocol. Sepracor had not completed any inhalation toxicity studies although they did conduct a few oral toxicity studies.

90-415	-	Affinity to recombinant human β-receptor, capacity to stimulate cAMP production, and receptor desensitization	5	283
90-440	QTA0301	Effect of S,S-formoterol on responses of human bronchus to histamine	5	310
90-407	-	β-Receptor selectivity of formoterol and its isomers	5	337

Safety Pharmacology

Report#	Study#	Description	Vol.	Page
90-423	SEPBR-11105	Effects on cardiovascular system in conscious dogs (IV)	5	1

Pharmacokinetics

Report#	Author	Description	Vol.	Page
90-411	[redacted]	In vitro metabolism study bronchial epithelial cells and human liver cytosol - sulfation	7	279
90-425		in vitro metabolic interaction - sulfation - between albuterol and formoterol	7	288
90-424		in vitro human tissue metabolism of selective enantiomers	7	295
90-431	[redacted]	in vitro human tissue metabolism of selective enantiomers	7	295

b(4)

Toxicology

Report#	Study#	Description	Vol.	Page
90-800	[redacted] 402-SE-002-95	Acute oral toxicity study in rats	5	344
90-801	[redacted] 406-SE-002-95	Acute IV toxicity study in rats	5	360
90-808	[redacted] 2014-96	Acute IV toxicity study in dogs	6	1
90-802	[redacted] 40S23.01	7 day oral dose-ranging study in dogs	7	1
90-803	[redacted] 437DS23.002	14 day oral dose-ranging study in rats	6	76
90-806	[redacted] 437DS23.003	14 day oral toxicity study in dogs	7	92
90-807	[redacted] MG019-223	*S. Typhirmurium* reverse mutation assay	7	209

b(4)

Studies not reviewed in this submission:

Report#	Study#	Description	Vol.	Page
90-426	-	Method for resolution of the 4 stereoisomers.	7	228
90-441	-	Stability of formoterol in palsma	7	238
90-442		Stability of formoterol in gastric and intestinal fluid	7	258
Literature section		Clinical study reports and analytical assay development on formoterol racemic mixture.	7	321-469

Sepracor also plans to conduct oral toxicity studies to support their inhalation product in the future, provided that a planned 28-day inhalation toxicity study yields a similar toxicity profile as the 28-day oral toxicity study in rats. The Division informed Sepracor that such an approach is not acceptable. The divisional policy is that protocols of inhalation products in patients should be supported by inhalation toxicity studies in 2 species in animals (one study each).

Sepracor submitted their modified protocol on February 20, 1998 to open their new IND.

Studies reviewed in this submission:

Pharmacology

Report#	Study No.	Description	Vol.	Page
90-432	8929/8931	Lack of binding to H_1 receptor *In vitro*	4	1
90-403	663S810E	Binding to histamine and beta receptors *In vitro*	4	35
90-409	904S810E	Adrenoceptors binding *in vitro*	4	69
90-410	813S810E	Binding to PAF, TXA_2/PGH_2, Ca^{++} and K^+ channels	4	86
90-443	8933S810E	Binding to β_3, β_2, NK_1, NK_2 receptors *in vitro*	4	100
90-401	[redacted] 276-SEP-3-93	Reduction of acetylcholine induced bronchospasm in guinea pigs	4	128
90-412	SEPBR-4175	Reduction of histamine and ovalbumin-induced bronchospasm in sensitized guinea pigs (IV)	4	222
90-434	Phase IIA	Effect on antigen mediated airway hyperactivity (IH)	4	260
90-427	Phase IIB	Effect on antigen mediated airway hyperactivity (IH)	4	302
90-414	-	Lack of airway hypersensitivity in guinea pigs	4	333
90-405	SEPBR-5254	*In vitro* muscle relaxant and atrial effects of formoterol enantiomers in guinea pig tracheal strips	4	359
90-408	-	Effect of the enantiomers on guinea pig airways in vitro	4	404
90-446	-	Increase in ACh release and inhibition of ACh induced contraction of equine tracheal smooth muscles	5	52
90-406	-	Lack of effects on histamine release from human lymphocytes in vitro	5	86
90-450	[redacted] 90053	Receptor binding of desformoterol to human β-receptors in vitro	5	96
90-450	[redacted] 90041	Receptor binding of desformoterol to human β-receptors in vitro	5	135
90-451	[redacted] 90056	Receptor binding to human β-receptors in vitro	5	115
90-435	[redacted] 195	Lack of binding to M 1 -5 muscurinic receptor in vitro	5	153
90-404	[redacted] 696 S 500 E	Effect on lipoxygenase and cyclooxygenase activity	5	191
90-445	[redacted] 8924S500E	Lack of effect on IL-5 production by human peripheral blood mononuclear cells	5	211
90-416	[redacted] 8918S810E	Lack of affinity to recombinant human M_{1-3} muscurininc receptors	5	228
90-449	[redacted] 890-49S830E	Affinity of desformoterol to β-adrenoeceptors	5	264

b(4)

REVIEW:

PHARMACOLOGY:

I. Mechanism of Action

Formoterol is a potent, selective, and long-acting β_2-adrenergic agonist that relaxes smooth muscle and exerts bronchodilatory effects in animals and humans. Through G-protein coupling, binding of formoterol to beta receptors activates adenylyl cyclase and results in an increase of intracelluar cAMP which in turn causes bronchodilation.

II. Drug Activity Related to Proposed Indication

A. In vitro Studies:

1. Adrenergic Receptor Binding.

Racemic formoterol mixture (R,R/S,S) is know to bind selectively to the β2-adrenergic receptors. The sponsor here compared affinity of each formoterol enantiomer to β-adrenergic receptors and other types of receptors *in vitro*. (R,R)-formoterol was found to have nearly a 40-fold greater selectivity for the β_2-receptor than the β_1-receptor in cloned human β_1- and β_2-receptors expressed in yeast *Spodoptera frugiperda* 9 (SM) cells (Table 1). This selectivity, however, was less apparent in the rat heart and guinea pig lung preparations (β_1/β_2 = 1.6 - 4.7). No binding to β_3-receptors was observed.

Table 1. Receptor Affinity of Formoterol and its enantiomers

	K_d (nM)		β_2 Selectivity	Intrinsic
Formoterol	β_1	B_2	(β_1/β_2)	activity (cAMP)
R,R/S,S	192	5.2	36.9	0.94
R,R	113	2.9	39.0	1.02
S,R	2,500	75.0	33.0	0.91
R,S	133	103	1.3	0.65
S,S	6,800	3,100	2.2	0.18
Isoproterenol	24	37	0.6	1.00

At the tested concentrations, formoterol apparently did not bind to other receptor types: muscurinic, histamine, Platelet activating factor (PAF), thromboxane A2 (TXA_2), PGH_2, Bradykinnin B_2, NK1, NK2 receptors (Table 2).

Table 2. Affinity of Formoterol to Other Receptor Types[1]

Receptor	Tissue origin	Concentration tested (M)
Muscurinic (M_{1-3})	Recombinant human receptor in CHO cell	10^{-10} - 3 x 10^{-6}
Histamine (H_{1-3})	Guinea-pig lung, cerebellum (H_1), cerebral cortex (H_2), and rat cerebral cortex (H_3)	"
PAF	Isolated rabbit platelet	10^{-9} - 10^{-5}
TXA_2	isolated human platelet	"
Bradykinin B2	Human lung CCD-16 fibroblast	10^{-10} - 3 x 10^{-6}
NK1, NK2	Human U373MG glioblastome cells	"
L-type Ca^{++} channel	Rat cerebral cortex (DHP site only)	10^{-9} - 10^{-5}
K^+ Channel (ATP sensitive)	Rat cerebral cortex	"

[1] No apparent binding was detected in all the tested concentrations.

Desformoterol (R,S- or S,R-) is the residual of formoterol losing its formaldehyde moiety from its formamide group and thus, one of the formoterol metabolites. This compound was also found to bind to β-receptors. However, its affinity was much lower than the parent compound (Table 3).

Table 3. IC_{50} (nM) of formoterol and metabolite to β-receptors

Compound	IC50 (nM)	
	β_1	β_2
R,R/S,S- formoterol	344	4.9
R,R- formoterol	199	2.3
R,R-desformoterol	3,180	35.6
R,S-desformoterol	1,790	3,140
S,R-desformoterol	-#	1,830
S,S-desformoterol	64,710	> 10,000

IC_{50} could not be determined because only 22% inhibition was obtained at 10^{-5} M.

2. Functional assays

Beta-adrenergic agonists are known to exerting their bronchodilatory activity through increasing intracellualr cAMP levels. The intrinsic activities of formoterol and enantiomers were determined by its ability to generate of intracellular cAMP in human bronchial epithelial cells (BEAS-213) *in vitro*. The maximum activity of formoterol was comparable to isoproterenol. R,R-formoterol apparently contributed exclusively the pharmacological activity the racemic mixture (Table 1). The S,S-formoterol had very little activity.

Table 4. EC_{50} (nM) of formoterol on tracheal relaxation and heart rate

Formoterol	Tracheal relaxation	Atrial rate	Selective ratio
R,R/S,S	9.4	5.7	0.61
R,R,	2.5	6.3	2.5
S,S	3,463	> 10	> 11

The effects of formoterol on several other systems were also determined *in vitro*. R,R,-formoterol was found to augment the release acetylcholine (ACh) from tracheal preparations. In isolated human granulocytes, formoterol inhibited slightly PGE_4 release but lacked effect on interleuken-5 (IL-5) production. Formoterol did not affect the activity of the inducible nitric oxidase. S,S-formoterol had no effects on histamine-induced bronchial constriction in guinea pig lungs.

Table 5. Effect of formoterol in vitro

Report#	Species/route	Observations	Dose
90-446	Horse trachea, in vitro	Augmented ACh release by tracheal preparation upon electrical stimulation	10^{-8} - 3 x 10^{-5}
90-404	Human, in vitro	Week inhibition of PGE4 production (100 fold less than indomethasone) in human granulocytes	10^{-7} - 3 x 10^{-5}
90-404	"	Lack of effect on lipooxygenase activity	10^{-7} - 3 x 10^{-5}
90-455	Human, in vitro	Lack of effect of IL-5 production in human peripheral blood mononuclear	10^{-10} - 3 x 10^{-6}
90-440	" "	Desensitization of the β-receptors - no enzyme activity 12 hours after treatment	1 - 100x K_d
90-440	human, in vitro	S,S formoterol had did not attenuate histamine-induced bronchial muscle constriction trachea	100 μM

B. Effect in animal models of diseases:

Bronchodilatory effect of formoterol was tested in a sensitized guinea pig model. Guinea pigs were sensitized by intraperitoneal injection of ovalbumin about 4 weeks prior to antigen and histamine challenge (100 μg/kg each, IV). Bronchial constriction effect was measured by increases in airway resistance in the anesthetized animals. R,R-formoterol (0.1 - 10 μg/kg) was also given by intravenous injection prior to histamine and/or ovalbumin challenge. Formoterol was found to cause dose dependent attenuation of airway resistance induced by histamine (Fig. 1).

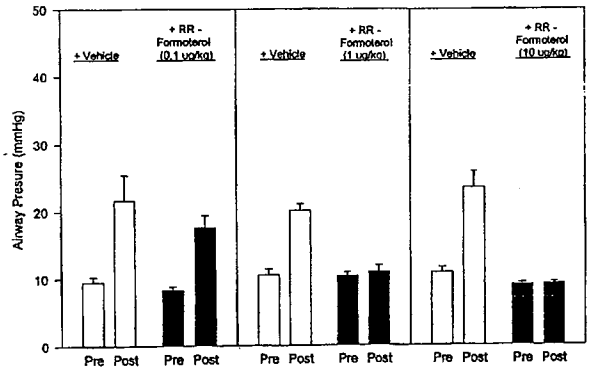

Fig. 1. Increase in airway pressure produced by intravenous administration of histamine (4 Ug/kg, IV) to sensitized, anesthetized guinea pigs treated with formoterol.

III. Ancillary Pharmacology Studies

Cardiovascular system: Effect of formoterol and its enantiomers on cardiovascular system was evaluated in conscious dogs. Intravenous administration of formoterol (racemic or R,R-formoterol 0.1 - 3 μg/kg) caused dose-related increases in heart rate and decreases in blood pressure. Larger doses (> 3 μg/kg) caused significant agitation. No significant changes were observed in the lower doses (< 0.1 μg/kg). The R,R-formoterol was as potent as the racemic mixture in increasing heart rate but more potent in reduction of blood pressure. In contrast, the S,S-isomer is almost ineffective although a slight increase in heart rates was seen at 300 μg/kg.

Respiratory system: Ability of formoterol to induce hyperractivity was evaluated in guinea pigs. Adolescent female Hartley guinea pigs were sensitized with aerosolized ovalbumin (OA) containing *B. Pertusis*, (report No. 90-414). OA challenges were administered twice weekly for 6 weeks. Aerosolized formoterol (0.32 μg/kg) was delivered two - three times daily, but not within 12 hours before or 4 hours after antigen challenge. Seventy-two hours after the last ovalbumin or formoterol exposure, pulmonary mechanics was performed in anesthetized animals. Then trachea was excised, and dose-response curves were determined for ACh and histamine induced contraction. In comparison with another β-agonist fornoterol, formoterol did not induce significant changes in airway resistance. Two other studies (90-434 and 90-427) for the hyperractivity of S,S-formoterol (6 days SC infusion) did not show any positive data either. However, these studies may be flawed due to the short induction period.

IV. Summary of Pharmacology

R,R-formoterol is a stereoisomer and the active component of the marketed formoterol in Europe and Japan. The market formoterol is a racemic mixture of R,R- and S,S-enantiomers (ratio of 1 to 1). The S,S-isomer is almost inert at the therapeutic dose of formoterol. This is indicated by the higher receptor affinity to the β_2-adrenergic receptor (60 fold) and higher intrinsic activity (6 fold) of receptor activation of the R,R-enantiomer. Through the production of

cAMP, formoterol induces relaxation of the bronchial smooth muscles and alleviate symptom of asthma. Its bronchodilatory effect was illustrated by the ability to attenuate histamine induced bronchospasms in sensitized guinea pigs. R,R-formoterol apparently has little or no activities in many other tapes of receptor including cholinergic (M), histamine, PAF, TXA_2, PGH_2, bradykinin B_2, NK1 and NK2 receptors.

Formoterol has been shown to cause increase in heart rate and decrease in blood pressure at therapeutic doses in dogs (0.1 - 0.3 μg/kg).

PHARMACOKINETICS

Absorption, distribution, and elimination of the R,R-formoterol has not been studies by the sponsor. This was primary due to [redacted] This application package did contain a few in vitro metabolic studies.

b(4)

Metabolism of formoterol enantiomers was study in these preparations: human liver cytosol and human bronchial epithelial cells in vitro. Radio-labeled (^{35}S)-phenolsulfotransferase was used as substrate for identify the occurrence of sulfation reactions. Sulfoconjugates were identified by HPLC assays. As with other beta agonists, sulfation also occurred with formoterol and was competitive between formoterol and albuterol. There was no apparent metabolic differences between these enantiomers.

Kinetic Parameter for Formoterol Enantiomers in human cells

Enantiomers	Km (μM)	Vmax (pmol/min/ mg protein)	Vmax/Km
a. Liver cytosol (n= 6)			
R,R/S,S	429 ± 68	64 ± 11	0.151 ± 0.021
S,S	348 ± 70	48 ± 9*	0.143 ± 0.023
R,R	1026 ± 362	50 ± 8	0.074 ± 0.021*
b. Bronchial epithelial cells (n = 3)			
R,R/S,S	170 ± 10	9463 ± 1365	56.1 ± 9
R,R	243 ± 29	9067 ±1288	39.3 ± 10*
S,S	615 ± 375	11468 ± 1165	32.3 ± 11*

* $P < 0.05$.

Summary of pharmacokinetics

Absorption, distribution and elimination of R,R-formoterol has not been studied by the sponsor. Metabolism of R,R-formoterol in human was studied with human liver cytosol and bronchial epithelium in vitro. Sulfation reaction occurred in both liver and epithelium, however, the latter possessed much higher (100 fold) activity based on unit of proteins. Desformoterol may also be a metabolite that possessed some beta adrenergic activity.

TOXICOLOGY

A. *Acute toxicity Studies*

1. Acute oral toxicity study in rats, Report No. 90-800, Vol. 5, Page 345.

Testing lab: [redacted]
Study number: [redacted]402-SE-002-95[redacted]
Study dates: 1/19/96 - 1/29/96; Report date: 11/24/97
GLP Statement: Yes.
Dose: 1000 mg/kg (R,R);
Batch No. QF-780-46

b(4)

Sprague-Dawley rats (3/group/sex) were given orally 1000 mg/kg of either R,R-, S,S- or R,R/S,S-formoterol to study their acute toxicity. Animals were observed for 14 days for clinical signs and were sacrificed at end of the observation period. Gross pathology were performed but microscopic examination was not done.

No mortality occurred during the observation period. Noticeable findings in the R,R-formoterol group included abnormal stance/gait (5/6), bright yellow urine (2/3♀), decreased activity and poor grooming (2/3♀), quivering (1/3♀), squinting (1/3♀), and decreased muscle tone (1/3♀). These signs lasted up to day 4 of the observation period.

The R,R/S,S-formoterol group showed abnormal stance/gait and bright yellow urine in the females only (2/3). The S,S group appeared to be normal except the bright yellow urine in the females (2/3).

Conclusion: Minimal lethal oral dose of formoterol is above 1000 mg/kg in rats.

2. Acute intravenous toxicity study in rats, Report No. 90-801, Vol. 5, Page 360.

Testing lab: [redacted]
Study number: [redacted]406-SE-002-95[redacted]
Study dates: 1/30/96 - 3/27/96; Report date: 11/24/97
GLP Statement: Yes.
Dose: 10, 50, 75, 100 mg/kg (R,R);
Batch No. QF-780-46

b(4)

Sprague-Dawley rats (3/group/sex) were given intravenously either R,R- or S,S-formoterol to study their acute toxicity. The respective dose levels were 10, 50, 75, and 100 for R,R-formoterol and 10, 50, 75 mg/kg for S,S-enantiomer. Animals were observed for 14 days for clinical signs. Animals were sacrificed at end of the observation period and gross pathology were performed. Note that microscopic examination was not done.

Mortality occurred in high dose groups in S,S- and R,R-formoterol treatment. The

incidences of death were 6/6 for 100 mg/kg R,R-enantiomer; and 4/6 for the 50 and 5/6 for 75 mg/kg of S,S S,S-formoterol. Clinical signs included labored breathing, body drop, prostration, vocalization, tremor and convulsion, in addition to the previous observations.

Conclusion: The median lethal intravenous dose of R,R,-formoterol is between 10 and 50 mg/kg in rats.

3. Single-dose intravenous toxicity study in Beagle dogs, Report No. 90-808, Vol. 6, Page 1.

Testing lab:	[redacted]
Study number:	2014-96 [redacted]
Study dates:	2/4/97 - 2/20/97; Report date: 12/5/97
GLP Statement:	Yes.
Dose:	3 μg/kg (RR); 6 μg/kg (RR/SS)
Batch No.	QF-780-46

b(4)

Beagle dogs (3/group/sex) were given intravenously a single dose of R,R-formoterol tartrate (3 μg/kg), R,R/S,S-formoterol fumarate (6 μg/kg), or vehicle (1 ml/kg/min). Animals were observed for 14 days for clinical signs. EKG was performed before, during and after the treatment. Serum potassium and glucose levels were monitored on the first day after injection. All animals were sacrificed at day 14 for gross and microscopic examinations. The sponsor indicated that blood samples were also taken for pharmacokinetic studies but such results were never reported.

The only noticeable findings were increases in heart rate during dosing (103 - 147%), the lower serum potassium levels (15 - 20%) and higher serum glucose levels (83 - 94%) in the formoterol treated groups. The degree of heart rate increases were similar between the two treatments (mean: 140%-RR vs 110%-RR/SS). Heart rate returned toward normal 5 minutes post-dosing, but were again elevated at 30 minutes and lasted till 120 minutes post-dosing. The decrease in serum potassium levels become evident 5 minutes after dosing and remained low at the last time point checked (2 hours post-dosing). The increase in glucose levels peaked at 15 minutes post-dosing but returned to normal 2 hours later.

There were no deaths or clinical signs of toxicity. Body weights and food consumption data were unremarkable. Similarly, no apparent abnormality were found upon the macroscopic and microscopic examinations, nor were the organ weight.

Conclusion: A single intravenous dose of 0.3 μg/kg of R,R-formoterol was well tolerated in dogs.

14-day oral toxicity study in rats. Report No. 90-803, Vol. 6, page 076 - 429.

Testing lab:	C [redacted]
Study number:	0437S23.002 C [redacted]
Study dates:	2/15/96 - 12/2/97; Report date: 12/02/97
GLP Statement:	Yes.
Dose:	0.1, 1.0, 10 mg/kg (R,R);
Batch No.	QF-780-46

b(4)

Sprague-Dawley rats (10/group/sex) were given by oral gavage either R,R-, S,S-, or R,R/S,S-formoterol to study their toxicity. The respective dose levels are listed in Table 7. The vehicle were 1.0% carboxymethylcellulose.

Table 7. Study Design of the 14-Day Oral Toxicity Study of Formoterol Enantiomers

	Dose level (mg/kg)			
Enantiomers	Vehicle	Low	Mid	High
R,R	0 (I)*	0.1 (II)	1.0 (III)	10.0 (IV)
S,S	-	-	-	10.0 (V)
R,R/S,S	-	-	2.0 (VI)	20.0 (VII)

* The Roman numerical numbers in parenthesis represent group numbers in the study.

Animals were observed for 14 days for clinical signs and sacrificed at end of the observation period. Gross pathology was done in all groups. Histopathology, with the exception of the spleen, was performed in the vehicle control and, mid and high dose R,R- and the S,S-formoterol groups. Spleen was evaluated histologically in all groups.

Clinical signs:	Daily
Body weight:	Twice a week
Food consumption:	Weekly
Ophthalmology:	Prior to treatment and sacrifice
Clinical pathology:	Terminal sacrifice
Pathology:	
Organ weights:	Adrenals, brain, kidneys, liver, testes, pituitary, and thyroid
Necropsy:	Terminal sacrifice
Histopathology:	Control and high dose groups only (Groups 1, VI, V and VII). See histology inventory table for tissues examined. For Groups II, III and VI, only spleen was examined.

Results:

Mortality: None.

Clinical signs: Redness of ears occurred sporadically in both males and females in the high dose groups.

Body weight: A dose-related loss in body weight was seen in all treated females and the mid and high dose males (Table 6).

Table 6. Body weight gain (g) in a 14-Day Oral Toxicity Study in Rats

Enantiomer	vehicle		R,R		S,S	R,R/SS	
Dose (mg/kg/day)	0	0.1	1.0	10.0	10.	2.0	20.0
Male: mean	107	118	129*	129*	100	129*	113
S.D	9.3	14.3	11.1	12.5	10.9	22.1	14.9
Female: mean	40	50	54*	66*	42	61*	66*
S.D.	6.6	8.9	11.7	10.2	11.6	9.7	10.6

* $P < 0.01$ by Dunnett's procedure.

Food consumption: A dose-related but non-statistically significant increase in food consumption was apparent but seen in both sexes.

Ophthalmology: No treatment-related effects were observed.

EKG: No treatment-related effects were observed.

Clinical pathology: The treated groups generally had lower serum glucose levels (Table 8) than the control groups. Also the hihg dose R,R- and the racemic mixture groups showed a slight increase in hematocrit (6%), and.

Table 7. Clinical pathological Findings in a 14-Day Oral Toxicity Study in Rats

Enantiomer	vehicle		R,R		S,S	R,R/SS	
Dose (mg/kg/day)	0	0.1	1.0	10.0	10.	2.0	20.0
Male: mean	107	118	129*	129*	100	129*	113
S.D	9.3	14.3	11.1	12.5	10.9	22.1	14.9
Female: mean	40	50	54*	66*	42	61*	66*
S.D.	6.6	8.9	11.7	10.2	11.6	9.7	10.6

* $P < 0.01$ by Dunnett's procedure.

Organ weights: No treatment-related effects were seen. Note that heart weight was not measured.

Pathology:

Necropsy: Noticeable gross pathological observations included small thymus in the mid and high dose animals (0/6-C, 0/6-LD, 1/6-MD, 4/6-HD).

Histopathology: Atrophy of the adrenal and lymphoid tissues was evident in the mid and high dose groups (Table 8). Thymus atrophy was seen in all treated groups.

Table 8. Microscopic findings in a 4-Week Inhalation Toxicity Study of Triamcinolone in Rats (n = 10/group)

Group	Sex	I	II	III	IV	V	VI	VII
Enantiomers		Vehicle	RR			SS	RR/SS	
Dose		0	0.1	1	10	10	2	20
Brain congestion:	M	1	-	-	4	2	-	3
	F	0	-	-	1	1	-	1
Heart, myocarditis, non-supparative multifical,	M	2	-	-	3	1	-	2
	F	1	-	-	1		-	
Kidney, mononuclear cells,	M	0	-	-	2	3	-	0
Nephritis, multifocal	F	1	-	-	3	2	-	1
Tubular regeneration	F	1	-	-	3	1	-	0
Pituitary cysts	M	0	-	-	5	0	-	4
	F	1	-	-	1	0	-	2
Spleen, capsulitis, multifocal,	M	0	2	4	3	1	6	2
	F	0	0	4	1	2	3	3
capsular thickening,	M	0	2	4	3	1	0	5
	F	0	0	4	1	2	3	3

Conclusion: The R,R-formoterol possessed similar toxicity profiles as the racemic mixture.

5. 7-day oral dose-ranging toxicity study in dogs. Report No. 90-802, Vol. 7, page 1 - 91.

Testing lab: ⊏ ⊐
Study number: 0440S23.001 ⊏ ⊐
Study dates: 1/22/96 - 2/11/96; Report date: 4/23/97
GLP Statement: No.
Dose: 0.1, 1.0, 10 mg/kg (R,R);
Batch No. QF-780-46

b(4)

Beagle dogs (1/sex) were orally administered in gelatin capsule R,R-formoterol at a raising dose schedule. Dosing schedule started with 0.1 mg/kg/day for 7 consecutive days, followed by 1.0 mg/kg/day for more 7 days, and finished with 10.0 mg/kg/day for another 7 days. Parameters observed included clinical signs, food consumption, hematology, serum chemistry, EKG and gross pathology examinations.

Clinical signs at 0.1 mg/kg included redness of the skin, gingiva, sclera and conjuctiva in both dogs. The male dogs also showed dryness of the mouth, clear ocular discharge, apparent increase in the abdominal surface temperature, and occasionally decreased activity. The female showed unsteady gait. These signs lasted for 1 - 7 hours post-dosing. These clinical signs were generally present, but more severe at the 1.0 mg/kg level. In addition, vocalization and tremors were seen in the male. More signs were detected in the third week (10.0 mg/kg dose level): panting, loose stools, emesis. The clinical pathology and EKG parameters were in the normal range. The only noticeable finding during gross pathology examination was the discoloration of the liver surface in the male dog.

Conclusion: Oral R,R-formoterol was tolerated in dogs for doses of up to 10 mg/kg/day for 7 days.

4. **6-day oral toxicity study in dogs. Report No. 90-806, Vol. 7, page 092 - 208.**

Testing lab: ⊏ ⊐
Study number: 0437S23.003
GLP Statement: No.
Dose: 0.1, 1.0, 10 mg/kg (R,R);

b(4)

Note: *This review refers to the study as 6-day study rather than the sponsor's title of 14-day toxicity study. This was based on the actual days of exposure. The study was originally designed for the 14 exposure duration; however, it was aborted at day 6 due to the early deaths and severe signs of toxicity.*

Beagle dogs (3/group/sex) were orally given in capsule either R,R-, S,S-, or R,R/S,S-formoterol to study their toxicity. The respective dose levels are listed in Table 7.

Table 7. Study Design of the 6-Day Oral Toxicity Study of Formoterol Enantiomers in Dogs

	Dose level (mg/kg)			
Enantiomers	Vehicle	Low	Mid	High
R,R	0 (I)[1]	0.1 (II)	1.0 (III)	$10.0^{2}{}_{♂:d1}$, 5_{d2}, 3_{d3-6} (IV)
S,S	-	-	-	10.0_{d1-6} (V)
R,R/S,S	-	-	2.0 (VI)	20.0_{d1-5}, 6_{d6} (VII)

1. The Roman numerical numbers in the parenthesis represent group numbers in the study.
2. The subscript indicates the days on that the actual dose (normal font) was given. These dose adjustments was triggered by mortality and severe toxicity.

Animals were intended to be dosed for 14 days. Due to early deaths of the animals, the experiment was aborted on day 6. Half of the high dose dogs (3/6) died on the first day of treatment. Clinical pathology parameters were obtained for the base line only. Clinical signs were similar to the 7-day dose-ranging study, but signs of toxicity in group VII were more severe. Increases in heart rate were seen in all the treated animals. No macroscopic and microscopic examinations were performed.

Conclusion: The oral dose of R,R-formoterol at 5 mg kg and above was lethal in dogs.

Summary of Toxicology

Toxicity of R,R-formoterol was evaluated by intravenous and oral administration in rats and dogs for a duration of up to 14 days. These studies are summarized in Table 9. The acute minimal lethal oral dose was more than 1,000 mg/kg in rats while the acute minimal intravenous dose was between 10 and 50 mg/kg. The signs of toxicity included labored breathing, tremor, convulsion, abnormal stance/gait, quivering, squinting, and decreased activity, even death. Dogs tolerated 0.3 μg/kg of single intravenous injection well.

The repeated dose toxicity studies were conducted in rats and dogs. In a 14-day study, Sprague-Dawley rats (10/sex/dose) were given by oral gavage 0.1, 1.0, and 10.0 mg/kg of R,R-formoterol in 0.1% carboxymethylcellulose (5 ml/kg/day). Histology, with the exception of the spleen that was done in all groups, was conducted in the high dose group only. Compared to the control, dose-related decreases in serum glucose (17 - 39%) were observed in all treated groups. Increases in body weight was observed in the control and high dose groups only. The high dose group also showed increases in the incidences of brain congestion (1/20-C vs 5/20-HD), nephritis (2/20-C vs 6/20-HD), pituitary cysts (1/20-C vs 6/20-HD), and renal tubular regeneration in the kidney (♀: 1/10-C vs 3/10). The highest incidence of multifocal caspulitis in the spleen was seen in the mid dose group (0/20-C, 2/20-LD, 8/20-MD, and 4/20-HD). No NOAEL was identified due to the failure to examine the low and mid dose groups.

Table 9. Toxicity Summary of R,R-Formoterol

Report#	Species	Route/ durat'n	Dose (mg/kg)	N/sex dose	Observations
90-800	Rat (SD)	oral, 1x	1,000	3	Abnormal stance/gait, bright yellow urine, poor grooming, decreased activity and muscle tone, quivering, squinting. Histopathology not performed
90-801	Rat (SD)	IV, 1x	10, 50, 75, 100	3	Mortality: 0/6 (< 75 mg/kg), 6/6 (HHD) Signs: same as above.
90-808	Dog (beagle)	IV, 1x	0.003	3	↑ heart rate (103 - 147%), ↓ serum K+ (15-20%), ↑ serum glucose (83 - 94%).
90-802	Dog (beagle)	Oral 7 day	0.1, 1, 10	1	Signs: ≥ 0.1mg/kg: redness of sclera, conjunctiva, dry mouth, gait; ≥ 1 mg/kg: tremors & vocalization; ≥ 10 mg/kg: panting, emesis
90-803	Rat (SD)	Oral, 14 day	0.1, 1, 10	10	HD: ↑ body weight (13%-HD), ↓ glucose (17%-LD, 32%-MD, 39%-HD).
90-806	Dog (beagle)	Oral 6 day	0.1, 1, 10_{d1} $/5_{d2}/3_{>d3}$	3	Mortality: 3/6-HD. Signs similar to dose ranging; also body weight loss (6%-MD, 8%-HD), No clinical pathology and histopathology was done.
"	"	"	$2, 20/6_{>d3}$	3	Racemic mixture: no mortality.

In a 7-day dose-ranging study, beagle dogs (1/sex) were given orally R,R-formoterol in gelatin capsules. The dosing schedule was 0.1 mg/kg/day for 7 days, followed by 1.0 mg/kg/day for 7 more days. Finally the dose was raised to 10 mg/kg/day for 7 days. During the experiment, clinical pathology and EKG was done weekly. Necropsy examinations were done 24 hours after last dosing. Clinical signs of toxicity were dose-related; redness of the sclera and conjunctiva, dryness of the mouse and gait were seen at 0.1 mg/kg; tremors and vocalization were observed at

≤ 1.0 mg/kg/day level; panting and emesis was noticed at ≤ 10 mg/kg/day. No abnormalities were seen at necropsy. Histology examination, however, was not done.

A 14-day oral study in dogs (3/sex/dose) was planned and initiated following the previous dose-ranging study. The same dose levels were adopted. The experiment, however, was aborted at day 6 of the treatment due to excessive mortality. Two of three high dose males died on day 1 of the treatment. Due to the deaths in the males, the females were given one half of the male dose (5 mg/kg). One of the three female dogs still died on day 1. The formoterol dose on day 2 (5 mg/kg) was further reduced to 3 mg/kg from day 3 and on. On day 6, the experiment was terminated due to excessive toxicity in the high dose group and no additional data was gathered.

During these toxicity studies, racemic and S,S-enantiomers were often used as reference controls. The respective dose levels were usually twice (racemic mixture) or equal (S,S-enantiomer) the R,R-enantiomers, based on mg/kg. Toxicity profile of the R,R-formoterol was generally similar to that of the racemic mixture and the S,S-enantiomer showed little activity.

Conclusion: Toxicology of R,R,-formoterol was not well characterized in laboratory animals by the sponsor.

Genotoxicity

1. ***Salmonella Typhimurium* reverse mutation assay (Ames test). Report No. 90-807. Vol. 7. P209-227.**

Testing lab:	[redacted]
Study number:	MG019-223 [redacted]
Study dates:	1/16-97-1/30/97; Report date: 2/20/97
GLP Statement:	Yes.
Batch No.	RH-924-96

b(4)

Mutagenicity of R,R-formoterol (5 mg/ml in sterile water) was tested in *Salmonella typhimurium* in the presence and absence of an externally supplied metabolic activation system (S-9). The bacterial strains were *S. typhimurium* TA 1535, TA1537, TA1538, TA98, TA100. The liver S9 fraction was from Aroclor 1254 induced rat. The respective positive controls were Dexon for test strains TA98, TA100 and TA 1537; sodium azide for strain TA1535; and 2-aminoantracene, strain TA1538. The negative control was sterile water. Criteria for the positive results were: 1) two fold increase in revertants over the mean vehicle control value, 2) dose-related increase in the mean revertants per plate of at least one test strain with minimum of three concentrations of test article, and 3) The above two criteria are met in any/all the testing strains. R,R-formoterol did not induce any positive responses in any strains tested.

Summary of genotoxicity: R,R-formoterol was tested negative in Ames test.

OVERALL SUMMARY AND EVALUATION

A. Summary:

A.1. *Pharmacology:* R,R-formoterol is a stereoisomer and the active component of the marketed formoterol in Europe and Japan. The market formoterol is a racemic mixture of R,R- and S,S-enantiomers (ratio of 1 to 1). The S,S-isomer is almost inert at the therapeutic dose of the racemic mixture. This is indicated by the higher receptor affinity to the β_2-adrenergic receptor (60 fold) and higher intrinsic activity (6 fold) of receptor activation of the R,R-enantiomer. Through the production of cAMP, formoterol induces relaxation of the bronchial smooth muscles and alleviates symptom of asthma. Its bronchodilatory effect was illustrated by the ability to attenuate histamine-induced bronchospasms in sensitized guinea pigs. R,R-formoterol apparently has little or no activities in many other tapes of receptor including cholinergic (M), histamine, PAF, TXA_2, PGH_2, bradykinin B_2, NK1 and NK2 receptors. Formoterol has been shown to cause an increase in heart rate and a decrease in blood pressure at therapeutic doses in dogs (0.1 - 0.3 μg/kg).

A.2. Pharmacokinetics: Absorption, distribution and elimination of R,R-formoterol has not been studied by the sponsor. Metabolism of R,R-formoterol in human was studied with human liver cytosol and bronchial epithelium *in vitro*. Sulfation reaction occurred in both liver and epithelium, however, the latter possessed much higher (100 fold) activity based on unit of proteins. Desformoterol may also be a metabolite that possessed some beta adrenergic activity.

A.3. *Toxicology:*

A.3.1. Acute Toxicity.

The minimal acute lethal oral dose of R,R-formoterol was 5 mg/kg in dogs and more than 1,000 mg/in rats and the median lethal intravenous dose was between 10 and 50 mg/kg in rats. Signs of toxicity included labored breathing, tremor, convulsion, abnormal stance/gait, quivering, squinting, and decreased activity, even death.

A.3.2. Repeated dose toxicity.

Limited data about the repeated dose toxicity of formoterol was available. There were a total of three studies for the duration of up to 14 days. One of the studies was a 14-day toxicity study in Sprague-Dawley rats. Animals (10/sex/dose) were given by oral gavage 0.1, 1.0, and 10.0 mg/kg of R,R-formoterol. Compared to the control, dose-related decreases in serum glucose (17 - 39%) were observed in all treated groups. Increases in body weight was observed in the high dose groups only. Histology was conducted in the high dose group only. Compared to the control, changes in the high dose group included increases in the incidences of brain congestion, nephritis and renal tubular regeneration in the kidney, pituitary cysts, and multifocal caspulitis in the. No NOAEL was identified due to the failure to examine the low and mid dose groups.

The other two studies were conducted in dogs for the duration of up to 7 days. These

studies are seriously flawed and no general conclusion can be drawn. The first study was a 7-day dose ranging study in beagle dogs. Two dogs (1/sex) were given formoterol (PO) for 0.1, 1.0 and 10 mg/kg for 7 days. Signs of toxicity were apparent even at the lowest dose. Histology, however, was not conducted. The second study was scheduled for 14 days and performed with the previous dose for only 6 days. Sadly, the study was aborted on day 6 because of the excessive mortality (3/6 on day 1) from the high dose. No clinical chemistry, gross pathology and histopathology was conducted in any of the animals.

Toxicity of R,R-formoterol has not been well studied in this application. Thus, target organs of toxicity have not been identified. The 14-day rat study suggests that heart, spleen and kidney may be potentially target of toxicity. Literature reports indicate that heart is the target organ of toxicity of racemic formoterol.

A.3.3. Special toxicity

A.3.3.1. Genetic toxicity

R,R-formoterol was tested negative in Ames test (S. typhimurium TA 1535, TA 151537, TA 1838, TA 98 and TA 100).

B. Evaluation:

Formoterol is a potent, selective, and long-acting β_2-adrenergic agonist and a bronchodilatory dilator. Inhalation formoterol fumarate has been approved to treat respiratory diseases in humans in several other countries. In the US, clinical trials are also underway. The approved and/or experienced dose of formoterol is 24 μg, bid. Chemically, formoterol molecule contains two chiral centers and, therefore, two pairs of enantiomers: (R,R), (S,S) and (R,S), (S,R). The marketed formoterol products use the racemic mixture (R,R/S,S) only. Recently, a few reports suggest that the S,S-formoterol enantiomer lacks therapeutic effects but may contribute to the adverse reactions. This prompted Sepracor to pursue the R,R-formoterol tartrate only.

Sepracor proposed a randomized, double-blind, placebo-controlled, single-dose six-way crossover study in 48 adult asthmatic patients study FVE1 reversibility. The subjects will receive these doses of formoterol tartrate: 12, 24, 48, or 72 μg and with a dose interval of 5 days between doses.

A pre-IND meeting was held between Sepracor and the Division to discuss the sponsor's developmental plan of R,R-formoterol tartrate on January 14, 1998. The proposed protocol in the current application was also discussed. Upon reviewing the information in the pre-IND package and based on available clinical data, the Division concluded that Sepracor may proceed with the protocol with minor modifications. Sepracor has conducted 2 clinical trials in Europe and results from these studies will be available to the Agency for review. Formoterol doses in these European trials are similar to the proposed doses in the protocol. The Division also informed the

sponsor Sepracor that their the preclinical data was inadequate to support the safety of this drug in the proposed protocol. Specifically, Sepracor has not completed any inhalation toxicity studies which are necessary to support the application for inhalation drug products. Sepracor did conduct a few oral toxicity studies but these studies were not sufficient to support their IND. This submission did not contain any significant and additional new information regarding to the preclinical data.

Despite the inadequacy of the preclinical data and as previously indicated, R,R-formoterol has been given to human healthy subjects and asthmatic patients for up to 72 μg (Table 10). Headache, nausea, abdominal pain and increase in QTc of EKG were reported in healthy subjects at 72 μg. This clinical experience may be sufficient to support the Sepracor's proposed trial.

Safety of the proposed high doses may be noteworthy, although no serious adverse reactions were identified at the highest proposed dose (72 μg) in humans. As previously pointed out, the clinical doses of racemic formoterol in other countries and the experienced dose in the US are 24 μg, bid. Pharmacological studies show that the R,R-formoterol is twice as potent as the racemic formoterol based on unit weight. A clinical study (091-001) revealed half of the healthy subjects receiving R,R-formoterol of 48 μg and 72 μg had tremors. A study (091-002) in asthmatic patients showed that incidences of tremors in R,R,-formoterol (48 μg) was higher than that of the racemic mixture Foradil (12 μg). Laboratory toxicology studies showed that quivering and tremors are signs of acute toxicity in animals at high doses. Also, large dose of formoterol and other β-agonists are known to cause cardiac lesions in animals. These points argue unfavorably against using such a high dose (three times of the established safe dose) in the clinical trials. (The concern about the cardiac toxicity of the drug was raised in the division safe meeting. It was concluded that close EKG monitoring in the trial will be conducted to check its cardiac effects to ensure patient safety.)

Table 10. Previous Human Experience

Report#	Population	Route/ duration	Dose (μg)	n	Observation
91-001	Healthy subjects	IH, 1x, cross over	6, 12, 24, 48, 72	16	VHD: headache, nausea, abdominal pain, ↑ QTc
91-002	Asthmatic patients	IH, 1x	48	6	Incomplete study

The proposed clinical trial is not supported by preclinical data, but may proceed based on the available clinical experience if found acceptable by the medical officer.

Recommendations:

1. The proposed protocol of R,R-formoterol was not supported by preclinical data, nor was the dose selection. However, R,R-formoterol had been given to human subjects previously and the proposed doses appeared to be tolerated in humans. Based on the available human experience, the trial may proceed at doses determined by the medical officer upon the review of clinical data

2. The sponsor is reminded that inhalation studies in 2 species with sufficient testing duration prior to the multidose trials in humans are generally required.

3. The sponsor is reminded to comply with the ICH guidelines for the full battery of genotoxicity testing prior to the repeated dose exposure in humans.

Luqi Pei, Ph.D.
Pharmacologist/Toxicologist

Ori: IND HFD-570/Division File
HFD-570/Dr. Pei/ Dr. Sheevers/ Ms. Toyer