Histopathology Inventory For IND 49204

Study No.	90-808	90-803	90-806
Description	Acute	14 day	6 day
Species	Dog	rat	dog
Adrenals	x	x	
Aorta	x	x	
Bladder			
Bone marrow smear			
Bone (femur)	x	x	
Brain	x	x	
Cecum	x	x	
Cervix		x	
Colon (large intestine)	x	x	
Diaphragm			
Duodenum	x	x	
Epididymis	x	x	
Esophagus	x	x	
Eye	x	x	
Fallobian tube			
Gall bladder	x		
Gross lesions		x	
Harderian gland			
Heart	x	x	
Hypophysis			
Ileum (small intestine)	x	x	
Injection site			
Jejunum	x	x	
Kidneys	x	x	
Laryngeal gland			
Larynx			
Liver	x	x	
Lungs		x	
Lymph nodes, cervical			
, mandicular	x		
, mesenteric	x	x	
, submaxillary			
Mammary gland	x	x	
Nasal cavity			
Optic nerves	x	x	
Ovaries	x	x	
Pancreas	x	x	
Parathyroid			
Peripheral nerve		x	
Pharync			
Pituitary	x	x	
Prostate	x	x	
Rectum		x	
Salivary gland	x	x	
Sciatic nerve	x		
Seminal vesicles			
Skeletal muscle	x	x	
Skin	x	x	
Spinal cord	x	x	
Spleen	x	x	
Sternum	x	x	
Stomach	x	x	
Testes	x	x	
Thymus	x	x	
Thyroid	x	x	
Tongue		x	
Trachea	x	x	

Urinary bladder	x	x
Uterus	x	x
Vagina	x	x
Zymbal gland		
Others		
Paranasal sinus		
Oral cavity		
Middle ear		
Teeth		
Nasal pharynx		
Abdominal tissue		
Incisor		
molar		
Sublingual gland		
Haw gland		
Bronchia		
Vertebra		
Coagulating gland		

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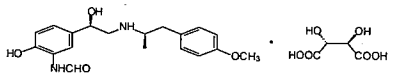
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Division of Pulmonary and Allergy Drug Products (HFD-570)

REVIEW INFORMATION:

Review No.:	4
Reviewer Name:	Luqi Pei, D.V.M., Ph.D.
Key Words:	RR-formoterol, developmental toxicity
Review Completion Date:	December 20, 1999
Information to be Conveyed to Sponsor:	Yes (x) No ( )

APPLICATION INFORMATION:

IND or NDA Application No.:	IND 55,302
Serial No., Content and Date of Submission:	005, toxicology reports, Sept. 28, 1998 010, toxicology data, August 11, 1999 011, a 28-day inhalation toxicity study in dogs, August 17, 1999.
Sponsor:	Sepracor Inc., Marlborough, MA
Drug: *Generic Name:*	(R,R)-Formoterol-L-tartrate
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:	
Class:	β_2-agonist
Indication:	[redacted] b(4)

Summary: This review evaluates general and genetic toxicology of RR-formoterol. General toxicology section includes 4-week inhalation toxicity studies in rats and dogs, and a proposed protocol for a 6-month inhalation toxicity study in rats. Genetic toxicology section includes two short-term genetic toxicity assays: *in vivo* micronucleus induction in mouse and *in vitro* chromosomal aberration in CHO cells. The review finds that RR-formoterol is non-genotoxic and exhibits a toxicity profile typical of beta agonists. The heart was identified as a target organ of toxicity. In juvenile animals the lung and testes were potential target organs of toxicity. RR-formoterol-treated juvenile female dogs showed statistically significant decreases in lung weight. Juvenile male rats and dogs showed degeneration or vacuolation of the germinal epithelium. These effects, which have not been known with beta agonists previously, suggest that the drug might be a developmental toxicant. The review discusses the relevance of these findings and their implications in the safety evaluation of future clinical pediatric protocols. The review recommends the sponsor further evaluate the lung and testicular findings. It also recommends the sponsor slightly modify their protocol for the 6-month inhalation study in rats.

TABLE OF CONTENT

Documents Reviewed:

Report #	Study #	Description	Sub. date	Vol.	Page
090-809	312010	Comparative acute IH tolerance study of RR, SS, and racemic formoterol in dogs	9/28/98	1	1
N/A	312021	28-day inhalation toxicity study in rats	8/11/99	1	App. 2
090-816	312022	28-day inhalation toxicity study in dogs	8/17/99	1	App. 2
090-465	312022	Toxicokinetics of RR-formoterol in a 28-day inhalation toxicity study in dogs	8/17/99	1	App. 1
090-811		In vivo mouse micronucleus assay	9/28/98	1	B-20
090-810		CHO chromosomal aberration assay	9/28/98	1	B-54
N/A	312050	Proposed 6-month inhalation toxicity study protocol	8/11/99	1	1

b(4)

Document Submitted but Not Included in This Review:

Salmonella typhimurium Reverse Mutation Assay, Sepracor Report No. 090-807, Vol. 1, page B-1. This study report was previously submitted (February 20, 1998) and reviewed by Luqi Pei on April 16, 1999.

Previous Pharmacology and Toxicology Reviews:

Review #	Reviewer	Review Date	Review Description
1	Dr. Luqi Pei	April 16, 1999	Review of the original submission
2	Dr. Luqi Pei	Sept. 2, 1999	Review of a 2-year carcinogenicity study protocol in rats
3	Dr. Luqi Pei	Sept. 7, 1999	Executive CAC meeting minutes

INTRODUCTION:

RR-formoterol is an enantiomer of a selective long-acting beta 2 agonist, racemic formoterol. Racemic formoterol is a mixture of RR- and SS-formoterol with an RR/SS ratio of 1. Racemic formoterol has been approved and marketed as a bronchodilator in several foreign countries including Japan (1986) and the United Kingdom (1996). In the US, development of formoterol is rather competitive and rapidly progressing. Norvatis' NDA (NDA No. 20-831) is currently under review by the Agency and [redacted] of clinical development. Sepracor is the [redacted] sponsor attempting to develop formoterol, specifically RR-enantiomer, in the US. b(4)

Sepracor has elected to develop RR-formoterol independently. What sets the Sepracor application apart from the [redacted] is that Sepracor is developing the RR-enantiomer only. [redacted] In a similar situation, the Agency recently approved Xopenex (levalbuterol), an enantiomer of albuterol. b(4)

Sepracor is actively pursuing this IND. Sepracor filed its original IND application with the Agency on February 20, 1998. This IND was allowed to proceed based on the availability of clinical data, although appropriate preclinical data were lacking (Pharmacology and Toxicology Review by Dr. Luqi Pei dated March 24, 1998). This IND is now in phase 2A of clinical development.

Preclinically, Sepracor's IND has a unique development history. Sepracor intended to initiate a carcinogenicity study before completing appropriate dose-ranging toxicity studies. On July 22, 1999, Sepracor submitted a protocol for a 2-year inhalation carcinogenicity study in rats for the Agency's concurrence. Supporting data for the carcinogenicity protocol were a draft summary and unaudited data of a 28-day inhalation toxicity study in rats. The CDER Executive Carcinogenicity Assessment Committee reviewed the protocol on September 7, 1999. The Committee rejected the protocol because of a lack of relevant supporting data (See Exec. CAC Minutes dated September 7, 1999 and Pharmacology and Toxicology Review by Dr. Luqi Pei dated September 3, 1999). Following the submission of the carcinogenicity study protocol, Sepracor submitted inhalation toxicology study reports with treatment durations of up to 4-weeks in rats and dogs and a proposed protocol for a 6-month inhalation study in rats. This review evaluates these toxicology studies and protocols.

REVIEW

Safety Pharmacology:

1. Comparative Acute Inhalation Tolerance Study of RR-, SS- and Racemic Formoterol and RR-desformoterol in Dogs, Submitted on September 28, 1998. Vol. 1, page A-1.

b(4)

Testing lab:	[redacted]
Study number:	[redacted] -312010, Sepracor Report # 090-809
Study Dates:	10/14/97-7/2/98
GLP Statement:	Yes
Dose:	RR: 5, 20, 40 µg/kg; SS and RR-desformoterol: 40 µg/kg, RR/SS: 80 µg/kg
Batch No.	Lot RH924-96, XL-18P9F, RH924-97, 956-40

Beagle dogs (2/sex/group) were exposed to RR-, SS-, racemic formoterol, or RR-desformoterol aerosol for 15 minutes to compare the animals' tolerance to the drugs. Table 1 presents the overall study design. The parameters of evaluation included clinical signs, body weight, EKG and plasma drug levels. The observation period lasted for 14 days after the drug exposure.

Table 1. Study Design of the Single Dose Tolerability Study in Dogs

		Dose (µg/kg)		Animal#
Group	Test Article	Targeted	Delivered	/sex
1	RR-formoterol	40	36	2
2	"	20	22	2
3	"	5	4.4	2
4	SS-formoterol	40	45	2
5	RR-desformoterol	40	46	2
6	Racemic formoterol	80	84	2

RR-formoterol caused dose-related increases in heart rate and increases in incidences of arrythmia, agitation, trembling, emesis and rapid respiration. The mean increases in heart rate were 50, 58 and 73 % for the low, mid and high dose groups, respectively. Heart rate returned to baseline 24 hours after the treatment. The incidences of arrhythmia at 24 hours after treatment were 1/4, 2/4 and 3/4 for the low, mid and high dose groups, respectively. The arrhythmia lasted more than 48 hours post dosing (3/6), but returned to normal by 96 hours post dosing.

Racemic formoterol (80 µg/kg) produced effects comparable to those seen at the high dose of RR-formoterol (40 µg/kg). RR-desformoterol produced mild increases in heart rate only. SS-formoterol produced only a minor and transient decrease in body weight gain.

Toxicology:

General Toxicology:

1. A 28-Day Inhalation Toxicity Study of RR-Formoterol in Rats. WIL Research Laboratories Study No. WIL-312021. Submitted on 8/11/99. Vol. 1. Appendix 2. b(4)

Testing lab:	C ⊐
Study number:	C ⊐-312021, Sepracor Report # not available
Study Dates:	6/1/98 – 8/4/99
GLP Statement:	Yes
Dose:	RR: 104, 424, 784 µg/kg; and RR/SS: 376 µg/kg
Batch No.	Lot 121697A

Inhalation toxicity of RR-formoterol was evaluated in a 28-day toxicity study in rats. Sprague-Dawley rats [C ⊐:CD®(SD)IGS BR, 10/sex/dose, aged 6-7 weeks at the start of exposure] were exposed, nose-only, to the formoterol aerosol for 30 minutes a day for 28 consecutive days. Formoterol dose levels were 104, 424 and 784 µg/kg/day for the low, mid and high dose groups, respectively. Table 2 lists the dose estimation. Vehicle was saline (0.9% sodium chloride). b(4) Additional animals (3/sex/time point) were used for the toxicokinetic study.

Table 2. Dose Estimates of the 28-Day Rat Inhalation Toxicity Study of RR-Formoterol

Groups	Drug	Mean Drug Conc. (µg/L air)	MMAD[1] (µm)	GSD	Inhaled Dose[2] (µg/kg)
2	RR-Formoterol	3.9	0.80	1.75	104
3	RR-Formoterol	16.1	0.61	1.44	424
4	RR-Formoterol	29.8	0.86	1.70	784
5	RR/SS-Formot.	14.2	0.86	1.73	376

1. MMAD = mass median aerodynamic diameter, and GSD = geometric standard deviation.
2. Inhaled dose = [Exposure concentration (µg/L) x Mean Minute Volume (LPM) x Exposure Duration] ÷ Mean Body Weight (kg), where body weight and minute volume were 0.21 kg and 0.2 LPM for days 1 – 14, and 0.28 kg and 0.23 LPM for days 15 – 30, respectively.

The following parameters were monitored during the study.

Parameters	Time of Measurements
Clinical signs:	Daily
Body weight:	Weekly
Food consumption:	Weekly
Heart rate:	During and following exposure in Weeks 0 and 3
Ophthalmology:	Weeks –1 and 3
Clinical pathology:	Weeks 1 and 4
Plasma drug level:	Days 0 and 26 at hours 0.5, 1, 2, 6, and 24 after exposure
Pathology:	
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, testes, prostate, spleen, thymus

Necropsy:	Sacrifice time
Histopathology:	Adrenals, aorta, bone and marrow, brain (fore, mid and hind), eyes (w/ optic nerve), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum), heart, kidneys, larynx, liver, lungs (including bronchi), lymph nodes (bronchial, mediastinal, and mesenteric), mammary glands, nasal cavity and sinuses, ovaries, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skeletal muscle, spinal cord, spleen, testes w/ epididymides, thymus, thyroid and parathyroids, trachea, urinary bladder, uterus, vagina, all gross lesions

Results:

Mortality: No treatment-related mortality was observed. A total of six animals died of accidental asphyxiation during the study. The incidence of the deaths was two (female), one (male), three (female), and 2 (female) for the 0, 100, and 400 µg/kg/day of RR-formoterol and 400 µg/kg/day of RR/SS-formoterol groups, respectively.

Clinical Observations: No treatment-related effects were observed.

Body Weight: Mean cumulative body weight gains (from week 0) in the test article groups were increased throughout the study period when compared to the control group. As a result, mean body weights were increased by 6-10%, 5-6%, and 7-8% for the low, mid and high dose groups, respectively, at week 4. The RR/SS-formoterol group also showed 3-9% increases in mean body weight. These increases, however, did not reach a statistically significant level ($P > 0.05$).

Food Consumption: No treatment-related effects were observed.

Clinical Pathology: Noticeable changes in clinical pathology parameters are listed in Table 3. Changes included dose-related decreases in platelet numbers, plasma albumin, serum glucose, and calcium. The decrease in glucose levels was transient as levels returned to normal by week 4 of treatment.

Table 3. Clinical Pathology Changes in the 28-day Rat Inhalation Toxicity Study

	Dose (µg/kg/day)			
	0	104	424	784
Platelet (thous/µl), wk 4	1107	956	916[a]	906[a]
Albumin (g/dl), week 4	4.3	4.0[a]	4.0[a]	4.1
Glucose (mg/dl), week 1	116	97[a]	86[a]	86[a]
, week 4	151	152	141	150
Calcium (dl), week 4	10.4	10.0[a]	9.8[a]	9.8[a]

a. Significantly different from control ($P < 0.05$)

Ophthalmology: No treatment-related effects were observed.

Heart Rate: A general drug-related increase in mean heart rate was observed during exposure in all dosed groups (Table 4). The mean (male and female at weeks 0 and 3 combined) percent

increases relative to the concurrent control were 8, 9 and 13% for the low, mid and high dose RR-formoterol, respectively. The RR/SS-formoterol group showed an 11% increase in heart rate.

Table 4. Heart Rate in the 28-day Rat Inhalation Toxicity Study (Day 0)

		Male					Female				
Dose (μg/kg/day)		0	104	424	784	376[1]	0	104	424	784	376[1]
Pre-exposure	Mean	486	525	493	506	528	506	517	500	508	514
	SD	37	62	41	45	35	52	48	47	22	52
During exposure	Mean	470	563*	564*	586*	562*	511	555*	565*	577*	558*
	SD	27	25	17	25	23	32	18	19	17	12
2-hr post exposure	Mean		511	489	524	474		448	506	506	480
	SD		35	44	37	76		45	23	31	41
4-hr post exposure	Mean		472		440	509			510	537	513
	SD		28		74	19			23	42	

1. RR/SS-formoterol.
* $P < 0.01$.

Pathology:

Macroscopic examination: Some treated animals showed small testes. The incidences of small testes were 0/10, 1/10, 0/10, 3/10 for the control, low, mid and high dose groups, respectively.

Organ weight: Changes in organ weights were limited to the males only. Changes of interest were trends of decreases in lung and testes weights and increases in heart weights, but they usually did not reach statistically significant levels (Table 5). Table 5 also shows statistically significant changes in organ weight analysis in several organs: liver, lung, spleen and adrenals. However, they were mostly limited to organ weights relative to body weight, and had little toxicological significance because the drug-treated animals had increased body mass that is a typical effect of beta agonists. These differences disappeared when organs weights relative to brain weight, which was relatively constant, were compared.

Table 5. Organ Weights in the 28-Day Rat Inhalation Toxicity Study

	Male				
Dose (μg/kg/day)	0	104	424	784	376[1]
Final body weight (g)	323	335	340	343	331
Brain weight (g)	1.87	1.90	1.86	1.85	1.86
, % of bdwt	0.58	0.57	0.55	0.54	0.56
Liver, (g)	9.64	9.38	8.98	9.39	8.45
, % to bdwt	2.98	2.81	2.64*	2.74	2.56**
, % to brain wt	515	492	482	510	454
Kidney (g)	2.44	2.31	2.29	2.34	2.15
, % to bdwt	0.76	0.69	0.67	0.38	0.65*
, % to brain wt	130	122	123	127	115
Lungs (g)	1.44	1.40	1.36	1.36	1.34
, % to bdwt	0.448	0.42	0.399*	0.395*	0.406
, % to brain wt	77.2	73.7	72.9	73.3	71.9
Heart (g)	1.20	1.26	1.25	1.32	1.20
, % to bdwt	0.369	0.377	0.370	0.382	0.363
, % to brain wt	64.0	66.3	67.5	71.2	64.3
Spleen (g)	0.55	0.50	0.49	0.50	0.45
, % to bdwt	0.172	0.15*	0.14*	0.15*	0.14**
, % to brain wt	29.5	26.0	26.5	27.0	24.0**
Testes/Epidid. (g)	1.31	1.12	1.67	1.12	1.21
, % to bdwt	1.31	1.12	1.12	1.12	1.21
, % to brain wt	225	198	213	208	215
Adrenals (g)	0.063	0.061	0.057	0.059	0.061
, % to bdwt	0.021	0.018*	0.017*	0.017*	0.019
, % to brain wt	3.35	3.22	3.06	3.22	3.27

1. RR/SS-formoterol.

* $p < 0.05$, ** $p < 0.01$.

Note: The organ weight relative to body weight was not determined in the females. This was because the sponsor failed to record the final body weight before necropsy in the females. This shortcoming, however, does not seem to affect overall data interpretation of the study.

Microscopic examination: Most noticeable changes occurred in the high dose males (Table 6). These changes were minimal in the degree of severity and present in the heart, testes/epididymides, lung, kidney, and nasal cavity. The change in the heart was cardiomyopathy that was characterized by multifocal myofiber degeneration accompanied by mononuclear inflammation cells. The change in testes was the degeneration of seminiferous epithelium. A few animals showed lymphoid infiltration in the kidney and mineralization in the lung. The change in the nasal cavity was non-suppurative inflammation of the submucosa; however, this change was limited to the Level 2 section of the nose that is known to be more susceptible to injuries in this species.

Table 6. Microscopic Findings in the 28-Day Rat Inhalation Toxicity Study

	Male					Female				
Dose (µg/kg/day)	0	104	424	784	376[1]	0	104	424	784	376[1]
Heart, cardiomyopathy	2/10	3/10	3/10	6/10	4/10	1/10	0/10	1/10	1/10	1/10
Kidney, lymphoid filtr.	0/10	0/10	2/10	2/10	0/10	3/10	3/10	2/10	5/10	4/8
Lung, mineralization, vascular	1/10	1/10	2/10	3/10	7/10	2/10	3/10	3/10	3/10	0/8
Nasal (level 2), inflammation, nonsuppurat.	0/10	1/10	3/10	5/10	2/10	0/10	1/10	2/10	3/10	2/8
Testes, degeneration	1/10	0/10	1/10	3/10	0/10					
Epididymides, Intratubular cell debris	0/10	0/10	1/10	3/10	0/10					

1. RR/SS-formoterol.

Plasma drug levels: Table 7 presents plasma formoterol levels during the study. A dose-related increase in drug levels was evident.

Table 7. RR-Formoterol PK Parameters in a 28-day Rat Inhalation Toxicity Study

Sex	Dose (µg/kg/day)	AUC 0-24 hr (pg/h/ml)		Cmax (pg/ml)	
		Day 1	Day 26	Day 1	Day 26
Male	104	3,509	6,993	1,487	1,060
	424	17,613	18,768	8,010	5,067
	784	24,477	33,509	8,843	9,733
Female	104	3,428	3,423	1,257	1,040
	424	5,395	17,366	2,777	5,147
	784	32,173	26,074	17,000	9,850

Conclusion: RR-formoterol exhibited a typical toxicity profile for a beta 2 agonist. The heart and testes are the target organs of toxicity. The high dose males showed cardiomyopathy and testicular atrophy. [redacted] The NOAEL may be considered as 424 µg/kg/day in the males and 784 µg/kg/day in the females. b(4)

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2. A 28-Day Inhalation Toxicity Study of RR-Formoterol in Dogs. Sepracor Document No. 090-816, [redacted] -312022. Submitted on 8/17/99. Vol. 1. Appendix 2.

Testing lab:	[redacted]
Study number:	[redacted] -312022, Sepracor Report # 090-816
Study Dates:	6/18/98 – 8/9/99, Report Date: 8/8/99
GLP Statement:	Yes
Dose:	RR: 6, 22, 41 µg/kg; SS: 45 µg/kg/day
Batch No.	Lot 121697A

b(4)

Inhalation toxicity of RR-formoterol was evaluated in a 28-day toxicity study in dogs. Beagle dogs [4/sex/dose, approximately 6 months old) were exposed, nose-only, to the formoterol aerosol for 15 minutes daily for 28 consecutive days. Mean RR-formoterol dose levels were 6, 22, and 41 µg/kg/day for the low, mid and high dose groups, respectively. Table 8 lists the dose estimation. Vehicle was saline (0.9% sodium chloride).

Table 8. Dose Estimates of the 28-Day Dog Inhalation Toxicity Study of RR-Formoterol

Groups	Drug	Mean Drug Conc. (µg/L air)	MMAD[1] (µm)	GSD	Inhaled Dose[2] (µg/kg)
1	Saline	0			0
2	RR-Formoterol	0.83	0.9	1.96	5.8
3	RR-Formoterol	3.2			21.8
4	RR-Formoterol	7.8	1.0	2.09	41.4
5	SS-Formoterol	5.8	1.0	1.84	45.0

1. MMAD = mass median aerodynamic diameter, GSD = geometric standard deviation.
2. The mean RR-formoterol dose level was 5.8, 22.5, and 40.8 µg/kg/day for the males and 5.8, 21.0, and 42.0 µg/kg/day for the females, respectively. The mean SS-formoterol dose level was 65.3 and 36.3 µg/kg/day for the males and females, respectively.

The following parameters were monitored during the study.

Parameters	Time of Measurements
Clinical signs:	Daily
Body weight:	Weekly, the first week of exposure is designated as week 0.
Food consumption:	Weekly
Heart rate:	Weeks 0, 1, and 3 during and 2, 4, 24 hours after exposure
Ophthalmology:	Weeks –3 and 3
Clinical pathology:	Weeks –2, 0, 3
Plasma drug level:	Days 0 and 23 at hours 0.5, 1, 2, 6, and 24 after exposure using LC/MS/MS
Particle sizing:	Week 1 and 3 for the high dose group and week 3 for the low dose group
Pathology:	
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, testes, thyroid
Necropsy:	Sacrifice time
Histopathology:	Adrenals, aorta, bone and marrow, brain (fore, mid and hind), eyes (w/

optic nerve), femur, gall bladder, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum), heart, kidneys, larynx, liver, lungs (including bronchi), lymph nodes (bronchial, mediastinal, and mesenteric), nasal cavity and sinuses, ovaries, pancreas, peripheral nerve (sciatic), pituitary, prostate, salivary glands, skeletal muscle, skin with mammary glands, spinal cord, spleen, testes w/ epididymides, thymus, thyroid and parathyroids, trachea, urinary bladder, uterus, vagina, gross lesions

Results:

Mortality: No treatment-related mortality was observed.

Clinical Observations: The report indicated that technicians noticed increased flushing of the body surface in RR-formoterol treated dogs at 1 hour post exposure, but it did not give the incidence of occurrence.

Body Weight: No treatment-related effects were observed.

Food Consumption: No treatment-related effects were observed.

Clinical Pathology: Slight changes in clinical pathology were noticed, mostly in the males. Table 9 lists the noticeable changes. The high dose males showed changes in hematology and clinical chemistry. Hematology changes included decreases in hemoglobin concentration and hematocrit, and an increase in APTT. Clinical chemistry changes included decreases in albumin, total protein, glucose, and creatine kinase activity.

Table 9. Clinical Pathology Changes in the 28-day Dog Inhalation Toxicity Study (Week 3)

	Male					Female				
Dose (µg/kg/day)	0	6	23	41	65[1]	0	6	21	42	36[1]
Hematology										
Hemoglobin (g/dl)	15.2	15.3	14.4	13.0*	15.5	15.5	15.6	14.1*	14.4	15.1
Hematocrit (%)	43.5	43.1	40.7	37.1*	43.8	43.6	45.1	40.0*	40.7	42.8
APTT (seconds)	13.6	15.1	14.2	15.5*	13.6	12.5	12.8	13.3	13.1	12.2
Clinical Chemistry										
Albumin (g/dl)	3.9	3.6	3.6	3.5	3.7	3.8	3.7	3.6	3.8	3.8
Protein $_{total}$ (g/dl)	6.0	5.7	5.6*	5.6*	5.8	5.8	5.8	5.6	5.9	5.7
Glucose (mg/dl)	107	100	95**	92**	104	98	89	100	95	100
Creatine kinase (u/l)	272	206	211	163**	224	293	539	279	322	199

1. SS-formoterol.
* $P < 0.01$.

Ophthalmology: No treatment-related effects were observed.

Heart Rate: RR-Formoterol treated dogs showed increases in heart rate (Table 10); however, a clear dose-response relationship was lacking. All dosed groups showed approximately an 80% increase in heart rate, relative to pre-exposure. The SS-formoterol treated dogs did not

experience an increase in heart rate.

Table 10. Heart Rate in the 28-day Formoterol Dog Inhalation Toxicity Study (Week 0, p122)

		Male (bpm)					Female (bpm)				
Dose (µg/kg/day)		0	6	23	41	65[1]	0	6	21	42	36[1]
Pre-exposure	Mean	N/A	129	160	133	148	N/A	141	138	131	133
	SD		35.5	14.7	15.2	29.7		42.3	6.5	46.6	34.6
During exposure	Mean		192	235	208	149		214	234	233	130
	SD		35.9	40.9	19.4	42.1		29.2	23.4	43.7	29.9
2-hr post exposure	Mean		226	213	208	147		234	205	210	129
	SD		6.6	34.3	9.0	41.1		13.3	28.4	32.6	7.6
4-hr post exposure	Mean		203	229	222			210	232	213	
	SD		42.4	4.8	11.8			21.6	9.0	25.8	
24-hr post exposure	Mean		115	140	113			122	121	118	
	SD		28	9.0	4.5			11.0	7.0	36.6	

1. SS-formoterol.
* $P < 0.01$.

Pathology:

Macroscopic examination: Two male dogs in the low and high dose RR-formoterol groups (one each) showed small testes. One female dog in the high dose RR-formoterol group showed a firm area in the lung.

Organ weight: Changes in organ weights were limited to testes in the males and lung weight in the females (Table 11). In the males, both absolute and relative testes weights were decreased in the treated groups. The decrease in testes weight relative to brain weight was approximately 35%. In the females, both absolute and relative lung weights were decreased in all treated groups. These decreases, however, lacked an apparent dose-response relationship.

Table 11. Organ Weight in the 28-Day Dog Inhalation Toxicity Study

	Male					Female				
Dose (µg/kg/day)	0	6	23	41	65[1]	0	6	21	42	36[1]
Final body weight (kg)	9.6	10.1	9.7	9.9	9.9	7.8	8.2	8.0	7.9	7.7
Brain (g)	77.8	79.1	77.3	78	77	71.2	74.8	71.5	74.1	73.9
Heart, g	64.3	74.3	70.8	71.4	77.2	62.9	61.6	57.6	64.2	60.2
, relative to bdwt (%)	0.67	0.74	0.73	0.72	0.78	0.81	0.76	0.72	0.81	0.78
Lung (g)	95.9	93.3	89.3	96.0	93.4	98.9	79.5*	76.3*	78.3*	76.2*
, relative to bdwt (%)	0.99	0.93	0.92	0.98	0.94	1.29	0.99*	0.96*	0.99*	0.99*
, relative to brain wt (%)	123	118	116	123	121	139	106	107	106	103
Testes (g)	16.4	12.2	13.4	10.6	13.2					
, relative to bdwt (%)	0.17	0.12*	0.13	0.11*	0.13					
, relative to brain wt (%)	21	15	17	14	17					

1. SS-formoterol
* $p < 0.05$.

Microscopic examination: Microscopic changes were generally unremarkable. Table 12 lists findings that were possibly treatment-related. Changes were seen in the kidney, liver, lung and testes of the high dose RR-formoterol males. The change in the kidney was dilatation of the medullary renal tubules. Cytoplasmic vacuolation of the periportal region was noticed in the liver.

In the lung, granulomatous inflammation was noticed in all formoterol treated groups, but an apparent dose-response relationship was lacking. Minimal vacuolation of the germinal epithelium was noticed in the testes. All the changes were minimal in the degree of severity. Two dogs showed non-suppurative inflammation in the nasal cavity; however, this change was limited to the Level 2 section of the nose that is known to be more susceptible to injuries in this species.

Table 12. Microscopic Findings in the 28-Day Dog Inhalation Toxicity Study

	Male				
Dose (μg/kg/day)	0	6	23	42	65[1]
Kidney, tubular dilation/medullary	0/4	0/4	0/4	2/4	2/4
Liver, vacuolation/cytoplasmic	0/4	0/4	0/4	2/4	0/4
Lung, inflammation/granulomatous	0/4	2/4	1/4	2/4	1/4
Nasal (level 2), inflammation, Non-suppurative	0/4	0/4	0/4	2/4	1/4
Testes, vacuolation/germinal epithelium	0/4	0/4	0/4	1/4	0/4

1. RR/SS-formoterol.

In the female dogs, histologic changes were limited to the lung. Macrophages (minimal) were present in the alveoli (incidence: 0/4-C, 0/4-LD, 0/4-MD, 1/4-HD, and 2/4-SS).

Plasma drug levels: Table 13 presents plasma formoterol levels during the study. Plasma RR-formoterol levels increased in a dose-related fashion. Some accumulation may have occurred, as indicated by an approximately 50-100% increase in AUC values between day 0 and day 23. For example, AUC's in the high dose male dogs were approximately 11 and 28 μg.h/ml for days 0 and 23, respectively. The report indicated that these animals received a slightly higher inhaled RR-formoterol dose (about 20%, p824) on day 23, but this minor increase in inhaled dose is not large enough to account for the magnitude of increase in plasma levels.

Table 13. RR-Formoterol PK Parameters in the 28-day Dog Inhalation Toxicity Study

Sex	Dose	AUC 0-24 hr (pg/h/ml)		Cmax (pg/ml)	
	(µg/kg/day)	Day 0	Day 23	Day 1	Day 23
Male[a]	0	115	269[b]	9.65	23.0
	6	1480	1930	194	239
	22	5480	7360	900	1140
	42	10900	27700	2130	5150
	65 (SS)	11700	12500	2500	2230
Female	0	57.1	115[c]	6.92	5.90
	6	1880	2960	185	357
	22	3890	9630	705	1060
	42	9610	16100	1750	2480
	37 (SS)	8670	4720	1190	1090

a. N = 4 unless otherwise specified; b, n = 3; and c, n = 2.

Conclusion:

This study reveals that the heart, lung, and testes may be the target organs of RR-formoterol. This is illustrated by increases in heart rate, a statistically significant decrease in lung weight in the treated females compared to controls, and degeneration of seminiferous epithelium in the testis of one high dose male. RR-formoterol also produced mild anemia and decreases in glucose. If the increase in heart rate and decrease in lung weight are considered, no NOAEL was established. However, the increase in heart rate was transient and it is not clear whether the effect on lung weight was a treatment-related effect (see Summary and Evaluation Section). If the heart rate and lung effects are not considered, the NOAEL is 22 µg/kg/day.

Genetic Toxicology:

1. *In vivo* Mouse Micronucleus Assay of Formoterol and RR-desformoterol. Sepracor Document No. 090-811. Submitted on September 28, 1998. Vol.1, page B-20.

Testing lab: [redacted]
Study number: [redacted] No. 18969-0-455, Sepracor Report # 090-811 b(4)
Study Dates: 12/09/97 – 1/23/98
GLP Statement: Yes
Batch No. Lot RH924-96

The clastogenicity of the formoterol enantiomers was tested *in vivo* in a micronuclei induction assay in mice. Male mice [redacted] CD-1®(ICR)BR] were dosed intravenously with the test substance as illustrated in Table 14. The vehicle control was saline (0.9% sodium chloride). The positive control was Endoxan® (cyclophosphamide, 80 mg/kg). Animals (5/sex) were scheduled to be b(4) sacrificed at 24 or 48 hours after a single dose. The positive controls were evaluated at the 24-hour sacrifice time point only. Bone marrow was collected at the time of sacrifice and slides were made. The frequency of micronucleated polychromatic erythrocytes (MPCE) was evaluated

microscopically. Two thousand polychromatic and normal erythrocytes were counted in each animal. The frequency of MPCE and the ratio of PCE to normal erythrocytes were compared. The criterion for a positive result was a statistically significant and dose-related increase in the frequency of micronucleated PCE for at least one of the time points.

Table 14. Design of the Formoterol Mouse Micronucleus Assay

Group	1	2	3	4	5	6	7	8
Treatment	RR-formoterol			SS-formoterol	RR/SS-formoterol	Desformoterol	Vehicle	Pos. Control
Dose (mg/kg)	19	37.5	75	19	19	75	-	80
Sacrifice (hr post dosing)	24	24	24, 48	24, 48	24, 48	24, 48	24, 48	24

No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes were noted in any of the treated groups over the concurrent controls. The positive control induced a typical positive response. Dose selection for RR-formoterol was acceptable as significant and dose-related increases in mortality occurred in the mid (37.5 mg/kg) and high dose (75 mg/kg) RR-formoterol group. The incidence of death was 0/5, 2/5, and 6/12 for the low, mid and high dose groups, respectively. Deaths usually occurred within 30 second after dosing. Mortality also occurred in the RR-desformoterol (3/18) and RR/SS-formoterol (3/16) groups.

The lack of increase in the frequency of chromosomal aberration suggests that RR-formoterol be non-clastogenic under the experimental conditions, despite the deficiency of small sample size at the 48-hour time point (below).

Comment: This study had an n of two for the high dose at the 48-hr time point. The small sample size is a shortcoming of the study and it raises doubts about the validity of the test. The small sample size was apparently due to the excessive mortality (4/6) in this group. Nonetheless, the study had three dose groups and all three doses yielded negative results at 24-hour post dosing, including the mid and high doses which were lethal to some animals. The two surviving high dose animals also produced negative results at the 48-hour time point. Given that RR-formoterol tested negative in *in vitro* bacterial gene mutation and mammalian chromosomal aberration assays, it seems reasonable to rely on results from a reduced number of animals in the micronucleus test. Thus, this assay may be considered as acceptable.

2. CHO Cell Chromosomal Aberration Tests of Formoterol Enantiomers and RR-desformoterol. Sepracor Document No. 090-810. Submitted on September 28, 1998. Vol.1, page B-54. b(4)

Testing lab: [redacted]
Study number: [redacted] No. 18969-0-437, Sepracor Report # 090-810
Study Dates: 12/09/97 –3/19/98
GLP Statement: Yes
Batch No. Lot RH924-96

The ability of formoterol enantiomers and RR-desformoterol to induce chromosomal aberrations was tested in a Chinese Hamster Ovary (CHO) cell assay. Formoterol enantiomers were RR-, SS- and RR/SS-formoterol tartrate. Concentrations of each test substance are listed in Table 15. Selections of test substance concentrations were acceptable because they were based on cytotoxicity (i.e., ≥50% reduction in mitotic index) in the dose-ranging studies. Negative controls were culture medium and DMSO (up to 10 µl/ml) in the absence and presence of S9 fraction, respectively. Positive controls were mitomycin (0.75 – 1.5 µg/ml) and cyclophosphamide (5.0 – 10 µg/ml) in the absence and presence of S9 fraction, respectively. The S9-fraction was prepared from rats treated with Aroclor 1254.

Table 15. Test Article Concentrations of the CHO Genotoxicity Assay

Testing Substance	Concentrations (µg/ml)		Results
	-S9 Fraction	+ S9 Fraction	
RR-Formoterol	200, 300, 400, 600, 800	400, 600, 800, 1000	Negative
SS-Formoterol	284, 405, 578, 826	199, 284, 405, 578	Negative
RR/SS -Formoterol	100, 200, 300, 400	500, 600, 700, 800	Negative
RR-Desformoterol	200, 300, 450	200, 450, 925, 1100	Negative

Criteria for an acceptable assay were: 1) the negative and vehicle control cultures must contain less than approximately 5% cells with aberrations, and 2) the positive control result must be significantly higher ($p < 0.01$) than the vehicle controls at one or more dose levels. Statistical analysis was a Cochran-Armitage test for linear trend and Fisher's Exact test. Percentage of cells with aberrations was the major parameter of evaluation. Consideration was also given to other factors such as percentage of cells with more than one aberration and dose-relatedness of any increase in aberrations.

No significant increases in chromosomal aberrations were observed at any concentration of the formoterol enantiomers or RR-desformoterol. Thus, formoterol and RR-desformoterol are considered non-clastogenic under the testing conditions.

Protocol review:

A Proposed Study Protocol for a 6-Month Inhalation Toxicity Study in Rats. Submitted on August 11, 1999, Vol. 1, page 1.

Testing lab to conduct the study:	[redacted]
Study Number:	[redacted] 312050 (assigned)
Study Dates:	N/A
GLP Statement:	Will comply with GLP

b(4)

The sponsor proposes to conduct a 6-month inhalation study in rats to evaluate general toxicity of RR-formoterol. Table 16 presents the overall design of the proposed study. Sprague-Dawley Rats [redacted] :CD®9(SD)IGS BR, 15-20/sex/dose] will be exposed to formoterol via nose-only exposure tubes for 30 minutes/day for six months. Additional animals (15/dose/sex) will be used for toxicokinetic studies. Rats will be 7 – 8 weeks old at the beginning of the treatment and weigh 180 – 280 g and 150 – 250 g for the males and females, respectively.

b(4)

Table 16. Study Design of the 6-Month RR-Formoterol Study in Rats

Group	Treatment	Dose (µg/kg/day)[1]	Animals (No./sex)
1	Saline	0	20[2]
2	RR-Formoterol (LD)	100	15
3	" (MD)	200	15
4	" (HD)	400	20

1. Inhaled dose (ID). ID = (C x T x MV)/BW, where C = aerosol drug concentration (µg/L), T = daily exposure duration (min), MV = minute volume (L), and BW = body weight (kg).
2. Five animals per sex in the control and high dose groups will undergo a 1-month recovery period.

Dose selection is based on a 28-day inhalation toxicity study (WIL-312021) from which toxicokinetic data are available (Table 7). Table 17 further summarizes the sponsor's basis for dose selection. RR-formoterol aerosol will be generated from 0.9% saline solution using a Collison jet nebulizer. Actual drug concentrations will be determined by chemical analysis of aerosol samples collected on filters. Particle sizes will be determined (monthly) with a cascade impactor. Actual doses will be estimated according to the experimental conditions (See Table 16 for the calculation).

Table 17. Rationale for the 6-month Rat Inhalation Toxicity Study

Dose (μg/kg/day)	Sex	Rationale	Expected AUC Ratio*
100	Male	Expected pharmacologically active dose	50
	Female	"	24
200	Male	Expected to produce tolerable AE	> 50
	Female	Expected to produce an AUC ratio of 50	50
400	Male	Expected MTD	134
	Female	Expected to produce a large AUC ratio	124

* Based on plasma drug levels on day 26 in rats and after a single dose in humans.

The following parameters will be observed during the study: viability (twice a day), clinical observations (daily), physical examination (weekly), body weight (weekly), food consumption (weekly), ophthalmic examinations (pre-study and week 26), clinical pathology (weeks 1 and 26), and gross and microscopic examinations (time of sacrifice). Plasma drug levels (months 3 and 6) will be checked from the satellite animals. Blood samples will be collected from 3 rats/sex/time point (0.5, 1, 2, 6 and 24 hr post dosing).

Microscopic examinations will be done for all animals in the control and high dose groups, and animals that are euthanized *in extremis*. The heart, lung, liver, kidney, nasal cavity, and all lesions will be examined from animals in the low and mid dose groups. If a potential target organ is noted in the high dose group, that tissue will also be examined in the low and mid dose groups. All tissues showing treatment-related lesions at the 6-month interval will be examined in recovery animals in the control and high dose group.

OVERALL SUMMARY AND EVALUATION:

This section summarizes and evaluates safety pharmacology and general and genetic toxicology studies of RR-formoterol. The safety pharmacology section includes an acute inhalation study to compare the tolerability of formoterol enantiomers in dogs. The general toxicology section includes a review of two study reports of 4-week inhalation toxicity testing in rats and dogs, and a proposed protocol for a 6-month inhalation toxicity study in rats. The genetic toxicology section includes an assessment of an *in vivo* mouse micronucleus assay and a CHO cell chromosomal aberration assay.

Summary:

Summary of Safety Pharmacology:

The sponsor compared the tolerability of formoterol enantiomers in dogs. Beagle dogs (2/sex/dose) received a single inhalation dose of RR-formoterol (5, 20, and 40 μg/kg), SS-formoterol (40 μg/kg), RR/SS-formoterol (80 μg/kg), or RR-desformoterol (40 μg/kg). Cardiovascular effects of the test articles were evaluated via EKG. RR-Formoterol caused dose-related increases in heart rate (50%-LD, 58%-MD and 73%-HD) and increases in incidences of

arrhythmia, agitation, trembling, emesis and rapid respiration. Heart rate returned to baseline 24 hours after the treatment. The incidence of arrhythmia 24 hours after treatment was 1/4, 2/4 and 3/4 for the low, mid and high dose groups, respectively. The arrhythmia lasted more than 48 hours post dosing (3/6), but returned to normal by 96 hours post dosing. Racemic formoterol (80 μg/kg) produced effects comparable to those seen with the high dose RR-formoterol (40 μg/kg). RR-desformoterol produced mild increases in heart rate only. SS-formoterol caused a minor and transient decrease in body weight gain.

Summary of General Toxicology:

General toxicity of RR-formoterol was evaluated in two 4-week inhalation toxicity studies in rats and dogs. In the rat study, Sprague-Dawley rats (10/sex/dose) were exposed, nose-only, to RR-formoterol at dose levels of 104, 424 and 784 μg/kg/day (total inhaled dose) for 28 days. The study revealed minimal toxicity of the drug. Changes in clinical observations were slight increases in mean body weight and heart rates in all treated groups. The increases in mean body weights (8%-LD, 6%-MD, and 7%-HD over controls) were not statistically significant, nor were they dose-related. The increases in heart rate were statistically significant (8%-LD, 9%-MD, and 13%-HD over control) and seemed to be dose-related. Clinical pathology changes included a slight (up to 18%) decrease in platelet numbers, slight (less than 7%) and apparently dose-unrelated, but statistically significant decreases in serum albumin and calcium levels, and a transient decrease (17% - 26%) in serum glucose levels. The noticeable microscopic findings were limited to males only. These animals showed increases in incidences of minimal cardiomyopathy (2/10-C vs 6/10-HD) and degeneration of seminiferous epithelium in the testes (1/10-C vs 3/10-HD). Histological changes in testes were correlated with small testes noted during the macroscopic examination. The NOAEL value was 424 μg/kg/day in the males and at least 784 μg/kg/day in the females.

In the dog study, beagle dogs (4/sex/dose) were exposed, nose-only, to RR-formoterol at dose levels of 6, 22 and 41 μg/kg/day (total inhaled dose) for 28 days. Changes in clinical observations were non-dose-related increases in heart rates (maximum increase of 72-82%) in all treated groups. Also observed was the flushing of body surface. Clinical pathology changes were mostly limited to the high dose males. These changes were hematological and biochemical. Hematology changes included decreases in hemoglobin concentration (15%) and hematocrit (15%), and an increase in APTT (14%). Clinical chemistry changes included decreases in albumin (10%), total protein (7%), glucose (14%), and creatine kinase activity (40%). All changes, except for the decrease in albumin, were statistically significant.

Statistically significant decreases in lung weight were seen in all treated female dogs. Noticeable microscopic findings were limited to high dose males only (Table 12). These animals showed increases in incidences of vacuolation of germinal epithelium in testes (0/4-C vs 1/4-HD), minimal non-suppurative inflammation of the nasal cavity (0/4-C vs 2/4-HD), and minimal liver vacuolation (0/4-C vs 2/4-HD). The effect on testes seemed to correlate with the decrease in testicular weight (Table 11). In the lung minimal alveolar granulomatous inflammation was observed, but the incidence and severity lacked a dose-response relationship (incidences: 0/4-C, 2/4-LD, 1/4-MD, and 2/4-HD). Granulomatous inflammation is usually not chemical specific and is readily reversible once the exposure is withdrawn. If the increase in heart rate and decrease in

lung weight are considered, no NOAEL was established. However, the increase in heart rate was transient and it is not clear whether the effect on lung weight was a treatment-related effect (see Evaluation, below). If the heart rate and lung effects are not considered, the NOAEL is 22 µg/kg/day.

Summary of Genetic Toxicology:

Genotoxicity of RR-formoterol was evaluated in an *in vivo* mouse micronucleus assay, a CHO cell chromosomal aberration assay and a bacterial mutation assay (the latter assay was previously reviewed, see Pharmacology and Toxicology Review by Dr. Luqi Pei dated April 16, 1999). RR-formoterol tested negative in all three assays and, therefore, is considered non-genotoxic.

Evaluation:

This section discusses and evaluates previously unknown effects of RR-formoterol on the lung and testes in animals. A 4-week inhalation toxicity study showed that RR-formoterol-treated juvenile female dogs developed small lungs. In the same study, one high dose male dog showed testicular vacuolation. A 4-week inhalation study in rats revealed that RR-formoterol treated males had an increased incidence of testicular lesions. These findings suggest that the lung and testes might be the potential target organs of RR-formoterol.

Evaluation of the Effect of Inhaled RR-Formoterol on the Developing Lung in Juvenile Dogs

Juvenile (6-month old) female dogs treated with inhaled RR-formoterol for 4-weeks showed a statistically significant decrease in lung weight (Sepracor Document No. 090-816). This decrease was apparent in all three parameters that are generally used to compare organ weight: the absolute lung weight, lung weight relative to body weight, and lung weight relative to brain weight (Table 11). This decrease in lung weight, however, was not accompanied by histologic changes. Significance of the decreased lung weight is unknown at present because of the lack of histologic changes, but the possibility of it being a treatment-related effect cannot be excluded based on the following rationale.

RR-formoterol might be a pulmonary developmental toxicant in juvenile dogs. Dogs in this study aged 5-6 and 6-7 months at the beginning and end of the exposure, respectively. They are considered juveniles from the developmental viewpoint. Literature data indicates that lung development in beagle dogs continues beyond one year of age. For example, Mauderly[1] reported that tidal volumes in beagle dogs were 69, 178, and 191 ml for the age of 3 months, 1 and 5 years, respectively. Unfortunately, lung development in juvenile dogs has not been extensively studied. Despite the lack of detailed understanding of lung development in these animals, many agents (e.g., inhaled corticosteroids) are known to disrupt their lung development. Thus, it is possible that RR-formoterol may adversely affect lung development, resulting in smaller lungs in dogs.

1. Mauderly, JL: Effect of age on pulmonary structure and function of immature and adult animals and man. *Fed Proc*, 1979;38:173-177.

However, the following three points may argue against such an interpretation:

1. RR-Formoterol did not cause similar effects in the males.
2. The effect of RR-formoterol on lung weight lacks a clear dose-response relationship.
3. The decrease in lung weight, since observed in the females only, may be a spurious finding, possibly due to the control females having unusually large lungs.

These arguments appear to be reasonable; however, they may be flawed as discussed below:

1. *RR-Formoterol did not cause similar effects in the males.* Smaller lungs were observed in the females only, not in the males. As previously discussed, lung development in juvenile beagle dogs has not been extensively studied. Despite the lack of knowledge about lung development in beagle dogs, it is well known that children (both males and females) exhibit different critical growth and developmental windows with regard to age. Generally, girls develop ahead of boys by approximately two years. A possible critical development window may also be present in dogs. It is unclear that lung development correlates well with growth in children, and much less is known in dogs. Nonetheless, the theory of a critical development window may explain the sex difference in RR-formoterol's effect on lung development. Because the duration of formoterol exposure was fairly short, the exposure might have hit the critical window of lung development in the females, but missed it in the males. Thus, the lack of similar findings in the males does not preclude the possibility that the decreased lung size in the females is a treatment-related effect.

2. *The effect of RR-formoterol on lung weight lacks a clear dose-response relationship.* However, it is possible that even the lowest dose may have produced the maximum effect of the drug in inhibiting lung development. This hypothesis is consistent with the observation that the treated animals showed a non-dose-related increase in heart rate, the most reliable and sensitive index of beta agonist activity *in vivo*. On the other hand, it is also possible that the exposure levels may have been so low that they did not reach the steep portion of the dose-response curve. The doses in this study ranged from 6 to 42 μg/kg/day only, so it is possible that a full dose-response curve was not obtained. Thus, it is apparent that the argument that a lack of dose-response relationship renders the finding irrelevant may be flawed.

3. *The decrease in lung weight, since observed in the females only, may be a spurious finding.* This argument is based on a theory that the control females may have had unusually large lungs. The following two observations seem to support this theory: 1) the control female dogs had approximately 30% higher lung weight relative to body weight than the control males (1.29-F vs 0.99-M), and 2) this relative lung weight was similar between the treated females (0.96 – 0.99) and control males (0.99). However, the following observations run counter to the above argument. The lung weight relative to brain weight, a more stable index, is similar between the male and female control animals (123%-M vs 139%-F), while the treated females showed lower lung weight relative to the brain weight than the control (139%-C vs 103-106%-T).

Overall, Argument 3 is plausible, but data are insufficient to determine whether it is correct. Additional data are needed for an appropriate evaluation of this argument. Therefore, we should look into the historical background of this parameter in the test laboratory to determine the validity of Argument 3. At present, unless additional data indicate otherwise, the argument of spurious finding is not be acceptable.

The above discussions suggest that RR-formoterol might adversely affect lung development in female dogs.

Most laboratories do not conduct appropriate assessments of drug effects on lung development in their general toxicology studies. Both morphologic and morphometric examinations of the lung structure are essential to assess possible detrimental effects of drugs on lung development, but most testing laboratories rarely conduct morphometric studies. Furthermore, when morphologic examinations are conducted, they often do not include an evaluation of alveolar formation, the most critical parameter in lung development.

Morphometric assessment of chemical effects on lung development in animals is well established in the literature[2]. It has also been used in assessing drug effects on lung development in dogs[3]. The morphometric assessment of lung development often includes the determination of the size of the lung and respiratory tract structure, alveolar numbers and size, and lung weight. The lung weight is an easy and sensitive, but crude parameter to determine effect of drugs on the lung. Other parameters should also be examined for a proper assessment of drugs on the developing lung. Pulmonary functional tests are also useful in determining effects of drugs on lung development. Unfortunately, they are only occasionally used.

The finding of small lungs in the female juvenile dogs treated with RR-formoterol suggests that RR-formoterol may adversely affect lung development. This finding may have significant implications in the safety evaluation of any pediatric clinical development plans. The sponsor should further evaluate this observation to either confirm or refute the possibility that RR-formoterol disrupts lung development in juvenile dogs. The Division should take this finding into consideration in the safety evaluation of future pediatric protocols of the drug.

2. Hyde, DM. et al: Morphometric approaches for evaluating pulmonary toxicity in mammals: implication in risk assessment. Risk Anal, 1994;14:293-302
3. Mehkerjee, A. Pharmacologic and Toxicologic Review dated January 19, 1993 in NDA 18-153.

Evaluation of the Testicular Effect of RR-Formoterol

As discussed previously, RR-formoterol treated animals showed decreases in testicular weight and histological changes in the testes. A 4-week inhalation study in rats [redacted] Study No. [redacted] 312021) revealed that RR-formoterol treated males had an increase in the incidence of degeneration of testicular epithelium (Table 6). In a 4-week inhalation study in dogs (Sepracor Document No. 090-816), one of the 4 high dose male dogs showed testicular vacuolation (Table 12). None of the 15 other dogs in the control and lower doses showed such a lesion. Also, the mean testicular weight of the RR-formoterol-treated dogs was decreased (Table 11). The occurrence of testicular lesions across species suggests that the testes may be a target organ for RR-formoterol. b(4)

[redacted] b(4)

The effect of RR-formoterol on the development of the male reproductive system should be taken into consideration in the safety evaluation of clinical pediatric protocols in the future.

Evaluation of the Proposed 6-month Toxicity Study Protocol

This section evaluates a proposed protocol for a 6-month inhalation study of RR-formoterol in rats. The sponsor proposes total inhaled doses of at 100, 200, and 400 μg/kg/day for the low, mid and high dose groups, respectively. Parameters to be monitored are those generally expected for inhalation toxicology studies. Thus, the study protocol is generally acceptable. However, the following modifications are strongly recommended:

1. The high dose in females should be increased. The sponsor argues that their proposed dose (400 μg/kg/day) is acceptable because this dose is anticipated to produce a plasma exposure 124 times the expected human plasma exposure in clinical trials. However, exposure ratios are not a basis for dose selection in general toxicology studies, and the dose of 400 μg/kg/day in a 28-day study did not show any detectable toxicity in this sex. The sponsor should raise this dose to identify potential target organs of the drug in this sex. Also, the anticipated AUC ratio may not be reliable because it is based on human plasma levels from a single dose study. It is unknown whether accumulation will occur in clinical trials. Should drug accumulation occur, the exposure ratio may be significantly reduced. It is recommended that the sponsor, if possible, raise the high dose and adjust the low and mid dose levels accordingly.

2. The acceptable range of particle size should be defined. Particle size is a predominant factor in determining the level of exposure (systemic and pulmonary) during an inhalation toxicity

study. For particles with an aerodynamic diameter of 3 – 5 μm, only less than 10% of an inhaled dose usually deposits in the lung in rats. This number decreases rapidly as the particle size increases. A predefined range of particle size would ensure sufficient pulmonary and systemic exposure.

3. The uterus in females should be weighed because it is a target organ of beta agonists in rats.

Conclusion:

This review finds that RR-formoterol exhibits a toxicity profile typical of beta agonists and is non-genotoxic. Target organs of toxicity of the drug may include the heart, lungs, testes, liver and kidney. In addition, the review identifies that the drug may potentially interfere with lung development and cause testicular damage in juvenile dogs. RR-formoterol-treated female dogs had statistically smaller lungs than controls. This observation has not been reported previously. Both high-dose rats and dogs showed testicular abnormalities. The review finds these observations may be of safety concern in the evaluation of future pediatric protocols of the drug. The review finds the proposed protocol for a 6-month inhalation study to be generally acceptable, but modifications are suggested.

Recommendations:

The following recommendation relates to the evaluation of the effect of RR-formoterol on lung development in juvenile dogs.

1. To better evaluate the decrease in lung weight observed in the female dogs, (Report No. 090-816), the sponsor should submit historical control data on lung weights and other information relevant to assessment of the effect of drugs and chemicals on lung development in juvenile beagle dogs.

For the 6-month inhalation toxicity study in rats, the sponsor should:

1. Given that no toxicity was observed in females dosed at 400 μg/kg/day in the 28-day study, increase the high dose in females and adjust the low and mid doses accordingly.

2. Define the acceptable range of particle size.

3. Obtain uterus weight at time of sacrifice in females.

Luqi Pei, D.V.M., Ph.D.
Pharmacologist/Toxicologist

Robin Huff, Ph.D.
Pharm/Tox Team Leader

Cc: IND 55,302/HFD-570 Division File
Drs. Pei/Huff/Anthracite/Shah/Jani

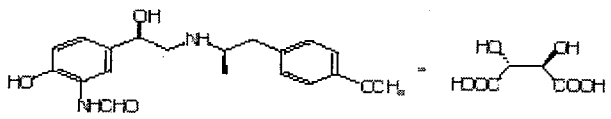
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

IND number: 55,302
Review number: #07
Sequence number/date/type of submission: Amendment #031 dated May 4, 2001
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Sepracor Inc.
111 Locke Drive
Marlborough, MA 01752
Manufacturer for drug substance : Same

Reviewer name: Timothy W. Robison, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: June 13, 2001

Drug:

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



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

Drug class: β_2-Adrenergic Agonist

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

Clinical formulation: (R,R)-Formoterol will be provided in 1 mL unit dose vials (UDVs) for the 21-day safety study with doses of 90 and 120 µg/day. The doses for the manufactured 1.0 mL UDVs are 15, 30, and 60 µg. Actual drug solution formulations were not provided.

Route of administration: Oral Inhalation

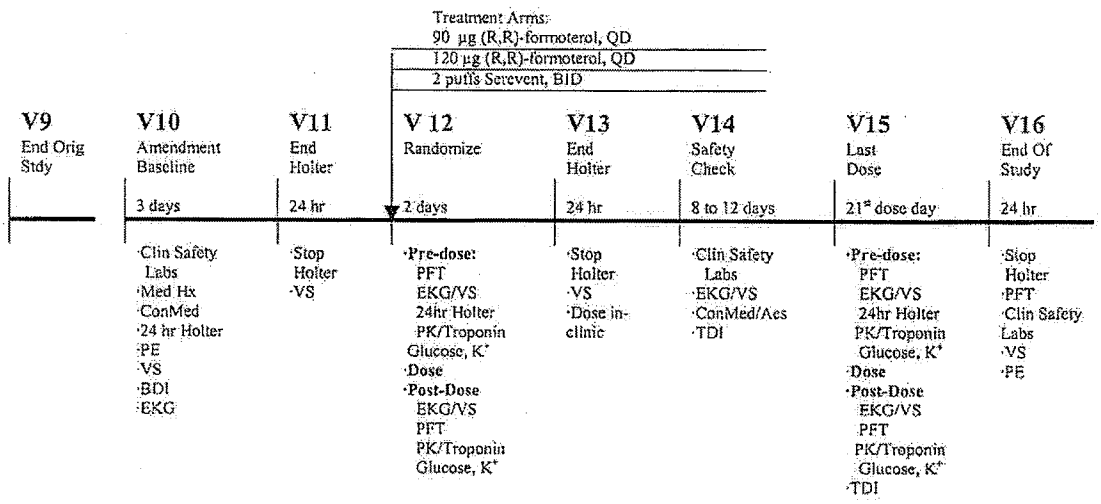
Proposed clinical protocol:

The sponsor proposed a randomized, placebo- and active-controlled, five-way, crossover study of (R,R)-formoterol tartrate inhalation solution and salmeterol in subjects with chronic obstructive pulmonary disease (COPD) designated as Protocol #091-021. It was originally proposed that (R,R)-formoterol would be administered as single inhaled doses at 0 µg/day (placebo), 9.6 µg/day, 48 µg/day, 96 µg/day, and 24 µg BID with a 6-day washout period between treatments (these doses will be delivered with PARI LC Plus nebulizers, which have a residual volume of approximately [redacted] UDVs will apparently not be used for administration of these doses). In the present amendment, the sponsor has added a 21-day parallel group treatment in which study subjects (≥35 years old with COPD) will be randomized in a 1 to 1 to 1 format to receive either 90 µg (R,R)-formoterol (1.8 µg/kg for a 50-kg person), 120 µg (R,R)-formoterol (2.4 µg/kg for a 50-kg person), or 42 µg B.I.D. salmeterol (Servent®). The study duration will be 21 days. There will be approximately 20 subjects per treatment arm for a total of 60 subjects. b(4)

(R,R)-formoterol will be administered using a PARI LC Plus nebulizer and Dura-Neb 3000 compressor at home by study subjects except during visits 12, 13, and 15 when drug will be administered in the clinic. Servent® will be administered using the marketed, metered dose inhaler (MDI). (R,R)-Formoterol at 90 µg will be administered once per day using 1 x 30 µg/mL and 1 x 60 µg/mL (total volume = 2 mL). (R,R)-Formoterol at 120 µg will be administered once per day using 2 x 60 µg/mL (total volume = 2 mL). Salmeterol at 42 µg (2 puffs, 21 µg delivered per actuation) will be administered twice per day. The nebulizer used to administer (R,R)-formoterol has a residual volume of approximately [redacted] of the (R,R)-formoterol dose will be actually delivered. Therefore, total therapeutic daily doses of (R,R)-formoterol will be [redacted] of 90 µg) and [redacted] of 120 µg). b(4)

Electrocardiograms will be recorded during visits 10, 12, 14, and 15 as illustrated in the diagram below. Holter monitoring will occur from visits 10 to 11, 12 to 13, and 15 to 16.

2. Study Schematic



Previous clinical experience:

The sponsor has administered (R,R)-formoterol to human subjects at inhaled doses up to 96 µg in single dose clinical trials. No data from repeat dose clinical trials are available at present.

Single dose studies with (R,R)-formoterol have been conducted in healthy and asthmatic human subjects. Clinical Protocol #091-001 found that approximately half of healthy human subjects receiving (R,R)-formoterol at single doses of 48 or 72 µg experienced tremors. Clinical Protocol #091-002 found that the incidence of tremor in asthmatic patients was higher with (R,R)-formoterol at 48 µg as compared to Foradil (racemic mixture) at 12 µg. These clinical findings of tremor parallel findings of tremor in acute toxicity studies with animals. Further, large doses of formoterol and other β-adrenergic agonists are known to cause cardiac lesions in animals.

In Amendment #012 dated December 13, 1999, the sponsor submitted a clinical protocol (#091-004) for administration of (R,R)-formoterol to asthmatic patients at inhaled doses of 24, 48, or 72 µg/day for 3 weeks. The status (i.e., not started, in progress, or completed) of this study is not known.

All clinical trials conducted to date have delivered a 3.0 mL nebulization solution with the exception of Protocol #091-021, which used both 2.0 and 3.0 mL doses. The nebulizers used in these clinical trials have a residual volume of approximately [redacted] Therefore, the nebulized dose is just [redacted] of the 3.0 mL dose or [redacted] of the 2.0 mL dose as shown in the table below.

b(4)

Total Dose	3.0 mL Nebulized Dose (2/3 of dose)	2.0 mL Nebulized Dose (1/2 of dose)
96 µg	66 µg	48 µg
72 µg	48 µg	36 µg
48 µg	32 µg	24 µg
24 µg	16 µg	12 µg
9.6 µg	6.6 µg	4.8 µg

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

OVERALL SUMMARY AND EVALUATION:

Introduction:

(R,R)-formoterol is a β_2-adrenergic agonist under development for the treatment of [redacted] COPD. In the present submission, the sponsor has submitted an amendment to protocol #091-021 in which (R,R)-formoterol will be administered to patients with COPD at inhaled doses of 90 and 120 µg/day for 3 weeks. b(4)

The sponsor proposed a randomized, placebo- and active-controlled, five-way, crossover study of (R,R)-formoterol tartrate inhalation solution and salmeterol in subjects with chronic obstructive pulmonary disease (COPD) designated as Protocol #091-021. It was originally proposed that formoterol would be administered as single inhaled doses at 0 µg/day (placebo), 9.6 µg/day, 48 µg/day, 96 µg/day, and 24 µg BID with a 6-day washout period between treatments. In the present amendment, the sponsor has added a 21-day parallel group treatment in which study subjects (≥35 years old with COPD) will be randomized in a 1 to 1 to 1 format to receive either 90 µg (R,R)-formoterol (1.8 µg/kg for a 50-kg person), 120 µg (R,R)-formoterol (2.4 µg/kg for a 50-kg person), or 42 µg B.I.D. salmeterol (Servent®). The study duration will be 21 days. There will be approximately 20 subjects per treatment arm for a total of 60 subjects. (R,R)-Formoterol at 90 µg will be administered once per day using 1 x 30 µg/mL and 1 x 60 µg/mL (total volume = 2 mL). (R,R)-Formoterol at 120 µg will be administered once per day using 2 x 60 µg/mL (total volume = 2 mL). Salmeterol at 42 µg (2 puffs, 21 µg delivered per actuation) will be administered twice per day. The nebulizer used to administer (R,R)-formoterol has a residual volume of approximately of [redacted] of the (R,R)-formoterol dose will be actually delivered. Therefore, total therapeutic daily doses of (R,R)-formoterol will be [redacted] of 90 µg) and [redacted] of 120 µg). b(4)

From a preclinical standpoint, safety of these proposed clinical doses will be evaluated using NOAELs identified in 28-day inhalation toxicity studies with rats and dogs. It will be assumed that total doses of 90 and 120 µg are delivered (i.e., 100% of dose was deposited in the lungs). Safety margins will be determined in terms of deposited dose.

In the 28-Day inhalation toxicity study with (R,R)-formoterol in rats, total doses were 104, 424, and 784 µg/kg/day (See Pharmacology Review dated December 20, 1999). Using a deposition factor of 0.09, deposited doses were calculated to be 9.4, 38.2, and 70.6 µg/kg/day, respectively. The NOAEL was identified as 38.2 µg/kg/day. Increases in heart rate were evident for all treatment groups during the daily exposure period; however, there was no evidence of a dose-response relationship. The incidence of cardiomyopathy, characterized by multifocal myofiber degeneration and infiltration of mononuclear cells, was increased for male rats at 70.6 µg/kg/day. Histopathological changes also occurred in the testes, epididymides, nasal cavity, lungs, and kidneys that appeared to be treatment-related.

Estimated Doses for the 28-Day Inhalation Toxicity Study with (R,R)-Formoterol in Rats.

Group	Drug	Mean Drug Conc. (µg/L air)	MMAD[1] µm	GSD	Inhaled dose[2] µg/kg/day	Deposited dose[3] µg/kg/day
2	R,R-Formoterol	3.9	0.80	1.75	104	9.4
3	R,R-Formoterol	16.1	0.61	1.44	424	38.2
4	R,R-Formoterol	29.8	0.86	1.70	784	70.6
5	R,R/S,S-Formoterol	14.2	0.86	1.73	376	33.8

1. MMAD = mass median aerodynamic diameter and GSD = geometric standard deviation.
2. Inhaled dose = [Exposure concentration (µg/L) x Mean Minute Volume (L/min) x Exposure Duration] ÷ Mean Body Weight (kg), where body weight and minute volume were 0.21 kg and 0.2 L/min for days 1-14, and 0.28 kg and 0.23 L/min for days 15-30, respectively.
3. Deposited dose calculated using a 0.09 deposition factor.

In the 28-day inhalation toxicity study with (R,R)-formoterol in dogs, total doses were 5.8, 21.8, and 41.4 µg/kg/day (See Pharmacology Review dated December 20, 1999). Using a deposition factor of 0.17, deposited doses were calculated to be 1, 3.7, and 7 µg/kg/day, respectively. The NOAEL was identified as 3.7 µg/kg/day. Heart rate was increased in male and female treatment groups during the exposure period and at 2- and 4-hr post exposure, although, there was no evidence of dose-response relationships. 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.

Estimated Doses for the 28-Day Inhalation Toxicity Study with (R,R)-Formoterol in Dogs.

Group	Drug	Mean Drug Conc. (µg/L air)	MMAD[1] µm	GSD	Inhaled dose[2] µg/kg/day	Deposited dose[3] µg/kg/day
1	Saline	0			0	0
2	R,R-Formoterol	0.83	0.9	1.96	5.8	1
3	R,R-Formoterol	3.2			21.8	3.7
4	R,R-Formoterol	7.8	1.0	2.09	41.4	7
5	S,S-Formoterol	5.8	1.0	1.84	45.0	7.65

1. MMAD = mass median aerodynamic diameter and GSD = geometric standard deviation.
2. The mean (R,R)-formoterol dose levels were 5.8, 22.5, and 40.8 µg/kg/day for male dogs and 5.8, 21.0, and 42.0 µg/kg/day for female dogs. The mean (S,S)-formoterol dose levels for male and female dogs were 65.3 and 36.3 µg/kg/day, respectively.
3. Deposited dose calculated using a 0.17 deposition factor.

In Amendment #012 dated December 13, 1999, the sponsor submitted clinical protocol (#091-004) for administering (R,R)-formoterol to asthmatic patients at inhaled doses of 24, 48, or 72 µg/day for 3 weeks (See Pharmacology Review dated February

14, 2000). This status of this study is unknown at present. Thus, no results from repeat dose clinical trials are available at present.

Safety evaluation: The sponsor has proposed to administer (R,R)-formoterol to asthmatic patients at doses of 90 and 120 µg/day for 3 weeks. The NOAEL of 3.7 µg/kg/day (deposited dose) identified in the 28-day inhalation toxicity study with (R,R)-formoterol in dogs provides 2- and 1.5-fold margins of safety for proposed clinical doses of 90 and 120 µg/day, respectively. This NOAEL was selected on the basis of histopathology in the kidney, liver, nasal cavity, and testes. No cardiac pathology was observed in the dog. Cardiac pathology was identified in the rat, but safety margins of 21 and 16 exist for the proposed clinical doses of 90 and 120 µg/day, respectively.

Exposure Ratios for Clinical Doses of 90 µg/day and 120 µg/day

Species	Exposure Ratios based upon Deposited Dose			Exposure Ratios based upon Body Surface Area		
	NOAEL	90 µg	120 µg	NOAEL	90 µg	120 µg
		1.8 µg/kg	2.4 µg/kg		66.6 µg/m^2	88.8 µg/m^2
Rat	38.2 µg/kg	21.2	15.9	2544 µg/m^2	38.2	28.6
Dog	3.7 µg/kg	2	1.5	440 µg/m^2	6.6	4.95

Safety issues relevant to clinical use: Tremors have been observed in healthy human subjects that received (R,R)-formoterol at single doses of 48 or 72 µg. The incidence of tremor in asthmatic patients was higher with (R,R)-formoterol at 48 µg as compared to Foradil® (racemic mixture) at 12 µg. These clinical findings of tremor parallel findings of tremor in acute toxicity studies with animals. Further, large doses of formoterol and other β-adrenergic agonists are known to cause cardiac lesions in animals. Cardiac lesions could potentially occur in human subjects receiving repeated high doses of (R,R)-formoterol. No results from repeat dose clinical trials are available at present.

Other clinically relevant issues: None

Conclusions: The NOAELs, expressed in terms of deposited dose, in the 28-day inhalation toxicity studies with (R,R)-formoterol in rats and dogs provide sufficient margins of safety for proposed clinical doses of 90 and 120 µg/day. There is concern that cardiac lesions could occur in human patients that receive repeated high doses of (R,R)-formoterol. No results from repeat dose clinical trials are available at present.

Communication review:

Investigator's brochure/informed consent review: None.

RECOMMENDATIONS: None.

Internal comments: None.

External recommendations (to sponsor): None.

Draft letter content for sponsor (if not same as above):

Future development or issues: The sponsor's use of repeated high doses of (R,R)-formoterol in clinical protocols needs to be carefully monitored.

Reviewer signature:

Timothy W. Robison, Ph.D. Date
Pharmacologist, HFD-570

Team leader signature [concurrence/non-concurrence]:

Robin Huff, Ph.D. Date
Supervisory Pharmacologist, HFD-570

cc: list:
IND 55,302, Division File, HFD-570
OstroffC, HFD-570
HuffR, HFD-570
RobisonT, HFD-570

Addendum to review (if necessary): The 21-day clinical safety study with doses of 90 and 120 µg/day proposed in Amendment 2 to Protocol 091-021 was withdrawn by the sponsor in a Teleconference with the Division on June 4, 2001.

Studies reviewed within this submission:

1. In vitro blood-to-plasma partitioning and the plasma protein binding of [3H]-(R)-albuterol in human, and the effect of (S)-albuterol, (R,R/S,S)-formoterol and (R,R)-formoterol on the protein binding of (R)-albuterol.

[

b(4)

]

Introduction and drug history:

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

b(4)

The sponsor has apparently conducted single dose clinical trials with inhaled doses of (R,R)-formoterol up to 96 µg. At present, there are no results from repeat dose clinical trials. The sponsor has conducted 28-day inhalation toxicity studies in rats and dogs in support of clinical trials. Six-month inhalation toxicity studies are apparently in progress.

A pharmacology review dated February 14, 2000 evaluated inhaled doses of (R,R)-formoterol at 24, 48, or 72 µg/day in a proposed 3-week clinical trial (Protocol #091-004) that was submitted in Amendment #012 dated December 13, 1999. It was concluded that a sufficient safety margin (i.e., approximately ≥8 with doses expressed in terms of body surface area) existed between the NOAEL identified in the 28-day inhalation toxicity study with dogs and the proposed doses in humans. The status of this clinical study in unknown at present.

TABLE OF CONTENTS

PHARMACOKINETICS/TOXICOKINETICS:

Distribution:

In Vitro Blood-to-Plasma Partitioning and the Plasma Protein Binding of [^{3}H]-(R)-Albuterol in Human, and the Effect of (S)-Albuterol, (R,R/S,S)-Formoterol and (R,R)-Formoterol on the Protein Binding of (R)-Albuterol.

b(4)

Study no: ⊏ ⊐ Number 99-7137, Sepracor Document No. 090-505
Volume #, and page #: Volume 1, Pages 1-58 of Sepracor Document No. 090-505
Conducting laboratory and location: ⌐

b(4)

⌟

Date of study initiation: January 10, 2000
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: See table

Test Article	Supplier	Lot Number	Purity, %
[^{3}H]-(R)-Albuterol	⊏ ⊐	324-142-014	98.8%
(S)-Albuterol [(S)-Albuterol HCl]	Sepracor Inc.	022/176	98.49
(R,R/S,S)-Formoterol [(R,R/S,S)-Formoterol 0.5 fumarate hydrate]	Sepracor Inc.	XL018p9F	99.22
(R,R)-Formoterol [(R,R)-Formoterol-L-tartrate]	Sepracor Inc.	121697A	99.2

Dosing:

Species/strain: Blood was obtained from human male donors.

Methods: The in vitro blood-to-plasma partitioning and the plasma protein binding of [^{3}H]-(R)-albuterol were examined and the effects of (S)-albuterol, (R,R/S,S)-formoterol, and (R,R)-formoterol on the protein binding of (R)-albuterol were assessed. [^{3}H]-(R)-albuterol concentrations were 0.1, 0.5, 1, 10, and 25 ng/mL. Blood was obtained from human male donors. Whole blood was used for the blood-to-plasma partition study. Plasma protein binding of [^{3}H]-(R)-Albuterol was determined using an ultrafiltration method following a 2 hr incubation at 37°C. The effects of (S)-albuterol, (R,R/S,S)-formoterol, and (R,R)-formoterol on plasma protein binding of [^{3}H]-(R)-albuterol at 1.0 ng/mL were assessed at final concentrations of 10 ng/mL, 20 pg/mL, and 10 pg/mL, respectively, with a 1 hr incubation at 37°C. All concentrations listed are nominal concentrations.

Results: The fraction of [^{3}H]-(R)-albuterol, at concentrations of 0.1 to 25 ng/mL, distributed to red blood cells was 0.46 to 0.50. Plasma protein binding of [^{3}H]-(R)-albuterol at concentrations of 0.1 to 25 ng/mL ranged from 5.4 to 13.5% and was independent of concentration. Plasma protein binding of [^{3}H]-(R)-albuterol at 1.0 ng/mL was approximately 7.3 ± 4.5%. Plasma protein binding of [^{3}H]-(R)-albuterol at 1.0 ng/mL

in the presence of (S)-albuterol (10 ng/mL), (R,R/S,S)-formoterol (20 pg/mL), and (R,R)-formoterol (10 pg/mL) was 6.8 ± 2.2, 3.0 ± 1.5, and 12.6 ± 0.9%, respectively. Thus, these agents at indicated concentrations had no effects on plasma protein binding of [^{3}H]-(R)-albuterol at 1.0 ng/mL. Plasma concentrations of formoterol in clinical subjects are not known at present. (R,R)-Formoterol concentrations used in this experiment appear to be extremely low as compared to albuterol concentrations, assuming comparable molecular weights.

PK/TK summary: The fraction of [^{3}H]-(R)-albuterol, at concentrations of 0.1 to 25 ng/mL, distributed to red blood cells was 0.46 to 0.50. Plasma protein binding of [^{3}H]-(R)-albuterol at concentrations of 0.1 to 25 ng/mL ranged from 5.4 to 13.5% and was independent of concentration. Plasma protein binding of [^{3}H]-(R)-albuterol at 1.0 ng/mL was unaffected by (S)-albuterol (10 ng/mL), (R,R/S,S)-formoterol (20 pg/mL), and (R,R)-formoterol (10 pg/mL).

PK/TK conclusions: Plasma protein binding of [^{3}H]-(R)-albuterol at 1.0 ng/mL was unaffected by (R,R)-formoterol (10 pg/mL).

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/s/

Timothy Robison
6/13/01 01:21:53 PM
PHARMACOLOGIST

Robin Huff
6/13/01 03:36:13 PM
PHARMACOLOGIST
I concur.

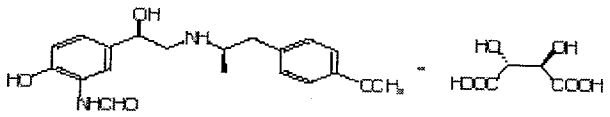
PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 55,302
Review number: #10
Sequence number/date/type of submission:
#014/February 4, 2000/Amendment
#029/February 1, 2001/Amendment
#030/March 16, 2001/Amendment
#038/June 12, 2001/Amendment
#041/July 16, 2001/ Amendment
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Sepracor Inc.
111 Locke Drive
Marlborough, MA 01752
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: November 26, 2001

Drug:

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



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

Drug class: • ₂-Adrenergic Agonist

Indication: ⊏ ⊐ Chronic Obstructive Pulmonary Disease (COPD)

b(4)

Clinical formulation: Not provided.

Route of administration: Oral Inhalation

Proposed clinical protocol: Clinical protocols were not provided in the Amendments reviewed.

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

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

Introduction and drug history:

(R,R)-Formoterol, a •$_2$-adrenergic agonist, is under development for treatment of [redacted] COPD. The formoterol molecule contains two chiral centers and therefore, two pairs of enantiomers (i.e., (R,R)-, (S,S)-, (R,S)-, and (S,R)-). It has been suggested that the (S,S)-formoterol enantiomer lacks therapeutic effects and contributes only to adverse reactions. The (R,R)-formoterol enantiomer is apparently twice as potent as racemic formoterol based on unit weight, [redacted]

b(4)

[redacted]

An End of Phase II meeting was held with the Sponsor on September 5, 2001. The sponsor's proposed Phase III studies consist of two pivotal studies in subjects (≥35 years old) with COPD. (R,R)-Formoterol will be administered at inhaled doses of 15 µg BID, 25 µg BID, and 50 µg QD using a nebulizer. The proposed treatment period is 12 weeks. Subjects that successfully complete the clinical trial with (R,R)-formoterol at 25 µg BID or 50 µg QD will be offered the opportunity to enroll in a chronic safety study. The proposed treatment period is 9 months.

Studies reviewed within this submission:

Study Title	Amendment / Sepracor Document Number
PHARMACOLOGY	
SAFETY PHARMACOLOGY	
Cardiovascular	
Addendum: Comparative acute inhalation tolerance study of (R,R)-, (S,S)-, and racemic formoterol and (R,R)-desformoterol in dogs.	Amendment #014/090-809
PHARMACOKINETICS/TOXICOKINETICS	
PK/TK Parameters	
Mice	
Toxicokinetics of formoterol during an acute inhalation tolerance study of (R,R)-formoterol in mice.	Amendment #014/090-460
Toxicokinetics of formoterol during a 28-day oral tolerance study of (R,R)-formoterol in mice in support of — -312042.	Amendment #029/090-470
Toxicokinetics of formoterol during a 28-day inhalation tolerance study of (R,R)-formoterol in mice in support of — 312026.	Amendment #029/090-461
Rats	
Toxicokinetics of formoterol during a 28-day inhalation toxicity study of (R,R)- and (R,R/S,S)-formoterol in rats in support of — 312021.	Amendment #029/090-463
Dogs	
Toxicokinetics of formoterol and [redacted] ratios	Amendment #014/090-464

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during an acute inhalation tolerance study of (R,R)-, (S,S)-, and (R,R/S,S)-formoterol and (R,R)-desformoterol in dogs.	
Distribution	
[^{3}H]-(R,R)-Formoterol: In vitro blood to plasma partitioning and plasma protein binding in rat, dog, human, and mouse.	Amendment #041/090-417, 090-418, 090-419, and 090-452
TOXICOLOGY	
Acute Toxicity	
Mice	
Acute inhalation tolerance study of (R,R)-formoterol in mice.	Amendment #029/090-818
Rats	
Comparative Acute Inhalation Tolerance Study of (RR)-, (SS)-, (R,R/S,S)-Formoterol and (R,R)-Desformoterol in Rats.	Amendment #038/090-815
Subacute/Subchronic Toxicity	
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Amendment: A 28-day inhalation toxicity study of (R,R)-formoterol in rats.	Amendment #029/090-817
REPRODUCTIVE TOXICOLOGY	
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A study of the effects of (R,R)-formoterol on fertility and early embryonic development to implantation in rats.	Amendment #030/090-831
A dose range-finding study of the effects of (R,R)-formoterol on embryo/fetal development in rats.	Amendment #014/090-813
A study of the effects of (R,R)-formoterol on embryo/fetal development in rats.	Amendment #030/090-820
A study of the effects of (R,R)-formoterol and racemic formoterol on embryo/fetal development in rats.	Amendment #030/090-825
Rabbits	
A dose range-finding study of the effects of (R,R)-formoterol on embryo/fetal development in rabbits.	Amendment #029/090-812
A study of the effects of (R,R)-formoterol on embryo/fetal development in rabbits.	Amendment #030/090-819
A study of the effects of (R,R)-formoterol and racemic formoterol on embryo/fetal development in rabbits. Toxicokinetics of Formoterol During an Embryo/Fetal Development Study in Rabbits.	Amendment #030/090-826 Amendment #029/090-468

9. Method validation for the determination of formoterol isomers in dosing formulations and inhalation chamber samples (Sepracor Document number 090-814, 1999).

TABLE OF CONTENTS – PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics:

Mechanism of action:

Study of IN-0712 in the Human β_1- and β_2-Adrenergic Receptor Binding Assays (Amendment #041; Sepracor Document number 090-484, 2001).

The affinity of (R,R)-desmethyl formoterol (IN-0712) for human recombinant β_1- and β_2-adrenergic receptors obtained from Sf9 cells was assessed. IN-0712 was tested in β_1- and β_2-adrenergic receptor radioligand binding assays at concentrations of 0.001, 0.1, and 10 µM. IN-0712 was further tested in the β_2-adrenergic receptor assay at ten concentrations in duplicate to obtain a full competition curve. IN-0712 displayed no affinity for the β_1-adrenergic receptor. IN-0712 at 10 µM displayed an affinity for the β_2-adrenergic receptor at 61%. The IC_{50} of IN-0712 for the β_2-adrenergic receptor was determined to be 3270 nM.

Drug activity related to proposed indication:

Effect of (RS)-, (R)- and (S)-Albuterol and (R,R/S,S)-, (R,R)-, and (S,S)-Formoterol on Tumor Necrosis Factor-α (TNFα) Induced Hyperreactivity in Tracheal Smooth Muscle of the Guinea Pig In Vitro (Amendment #30; Sepracor Document No. 090-480, 2000).

The effects of (R,S)-, R-, and S-albuterol and (R,R/S,S)-, (R,R)-, and (S,S)-formoterol on tumor necrosis factorα (TNFα)-induced hyperreactivity in guinea pig tracheal smooth muscle in vitro were assessed. It was previously reported that recombinant human TNFα increased maximal isotonic contraction of guinea pig tracheal smooth muscle to methacholine. Tracheal strips (each containing 3-4 cartilage rings), obtained from male Hartley guinea pigs, were attached to force-displacement transducers in order to measure isometric contractions and submersed in a tissue bath. In the present experiments, pre-incubation of tracheal preparations with TNFα caused an average 19.9% increase in the maximum contraction inducible by methacholine. Effects of (R,S)-, R-, and S-albuterol and (R,R/S,S)-, (R,R)-, and (S,S)-formoterol at concentrations of 10^{-5}, 10^{-7}, and 10^{-9} M on methacholine-induced contraction of tracheal smooth muscle were tested in the presence and absence of TNFα pretreatment. (R,S)-Albuterol, R-albuterol, (R,R/S,S)-formoterol, and (R,R)-formoterol decreased the responsiveness of smooth muscle preparations to methacholine as determined by a dose-dependent increase in the apparent EC_{50} of methacholine (with or without TNFα pretreatment). S-Albuterol and (S,S)-formoterol had no significant effects on the responsiveness of tracheal smooth muscle preparations to methacholine, having no effect on the apparent EC_{50} (with or without TNFα pretreatment). (R,S)-Albuterol, R-albuterol, S-albuterol, (R,R)-formoterol, and (S,S)-formoterol decreased TNFα-induced hyperreactivity. (R,R/S,S)-formoterol had a smaller effect on TNFα-induced hyperreactivity as compared to other compounds.

Effect of R-albuterol, (R,S)-albuterol, and S-albuterol on TNFα-induced hyperreactivity of guinea pig tracheal smooth muscle.

Pretreatment	B_2-Agonist M	R-Albuterol treatment		R,S,-Albuterol treatment		S-Albuterol treatment	
		Maximum Contraction [1]	EC_{50}, M (2)	Maximum Contraction [1]	EC_{50}, M (2)	Maximum Contraction [1]	EC_{50}, M (2)
Control	-	97.1%	8.42×10^{-7}	97.2%	1.22×10^{-6}	95.4%	7.32×10^{-7}
TNFα	-	119.8%	6.05×10^{-7}	116.2%	9.70×10^{-7}	113.6%	5.96×10^{-7}
Control	10^{-9}	122.5%	1.47×10^{-6}	111.6%	6.32×10^{-7}	93.2%	4.17×10^{-7}
TNFα	10^{-9}	99.9%	5.45×10^{-7}	108.4%	8.40×10^{-7}	97.5%	5.28×10^{-7}
Control	10^{-8}	117.7%	5.49×10^{-6}	109.4%	9.23×10^{-6}	94.0%	8.12×10^{-7}
TNFα	10^{-8}	116.5%	7.55×10^{-6}	107.8%	8.05×10^{-6}	98.4%	8.60×10^{-7}
Control	10^{-7}	98.1%	3.47×10^{-5}	103.1%	1.38×10^{-5}	111.0%	8.27×10^{-7}
TNFα	10^{-7}	106.6%	1.38×10^{-5}	108.4%	1.09×10^{-5}	110.0%	7.85×10^{-7}

1. Maximum contraction inducible by methacholine.
2. EC_{50} for methacholine for methacholine.

Effect of (R,R)-formoterol, (R,R/S,S)-Formoterol, and (S,S)-Formoterol on TNFα-induced hyperreactivity of guinea pig tracheal smooth muscle.

Pretreatment	B_2-Agonist M	R,R-Formoterol treatment		RR,SS-Formoterol treatment		S,S-Formoterol treatment	
		Maximum Contraction [1]	EC_{50}, M (2)	Maximum Contraction [1]	EC_{50}, M (2)	Maximum Contraction [1]	EC_{50}, M (2)
Control	-	94.8%	7.06×10^{-7}	96.9%	9.67×10^{-7}	95.1%	7.53×10^{-7}
TNFα	-	116.9%	7.50×10^{-7}	120.6%	1.31×10^{-6}	113.7%	3.04×10^{-7}
Control	10^{-9}	99.5%	1.25×10^{-5}	94.6%	2.38×10^{-6}	100.2%	4.57×10^{-7}
TNFα	10^{-9}	98.9%	9.15×10^{-6}	106.0%	6.90×10^{-6}	97.8%	3.15×10^{-7}
Control	10^{-8}	97.6%	1.07×10^{-5}	90.1%	5.91×10^{-6}	108.3%	7.14×10^{-7}
TNFα	10^{-8}	97.9%	1.52×10^{-5}	112.6%	8.00×10^{-6}	96.3%	8.05×10^{-7}
Control	10^{-7}	95.6%	2.04×10^{-5}	94.5%	1.09×10^{-5}	108.9%	1.37×10^{-5}
TNFα	10^{-7}	100.2%	2.98×10^{-5}	105.7%	1.08×10^{-5}	90.7%	5.76×10^{-6}

1. Maximum contraction inducible by methacholine.
2. EC_{50} for methacholine for methacholine.

Effect of Methacholine, (R,S)-, (R)-, and (S)-Albuterol, and (R,R)-, (S,S)- and (R,R/S,S)-Formoterol on Mucociliary Transport in Calf Trachea (Sepracor Document number 090-479, 2000).

Effects of methacholine, (R,S)-, R-, and S-albuterol, and (R,R)-, (S,S)- and (R,R/S,S)-formoterol on mucociliary transport were evaluated in vitro using the calf trachea. Tracheae were obtained from healthy male calves. Six to seven ring lengths of trachea were removed and bisected. Each specimen was pinned down on a tissue holder and floated in a dish to perfuse the serosal side of the tissue. On the mucosal surface at the caudal end of the tracheal specimen, 10 µL of carbon emulsion was deposited at two sites, approximately 3 mm distant from each other. Movement of this carbon emulsion was measured by use of a metric ruler in the ocular part of the microscope. Movement was recorded at both sites and velocity was determined by measuring distance transported during a 30-sec observation period. Dose-effect curves were developed for methacholine, (R,S)-, R-, and S-albuterol, and (R,R)-, (S,S)- and (R,R/S,S)-formoterol. All compounds were found to enhance mucociliary transport

velocity, although, the test compounds were more potent than methacholine. R-Albuterol and (R,S)-albuterol were not significantly different from one another; however, they were both more potent than S-albuterol. (R,R)-Formoterol and (R,R/S,S)-formoterol were not significantly different from one another; however, they were both more potent than (S,S)-formoterol. R-Albuterol and (R,R)-formoterol were more potent than their corresponding S-enantiomers. R-Albuterol was the most potent compound tested.

Relative Efficacy and Potency of Methacholine, (R,S)-, R-, and S-Albuterol and (R,R/S,S)-, (R,R)-, and (S,S)-Formoterol in Enhancing Mucociliary Transport in Calf Trachea.

Compound	Peak Effect (Maximum % Increase in Transport Velocity ± SEM)	EC_{50} (M) (log EC_{50} ± SEM)
Methacholine	68.3 ± 9.8	6.07×10^{-5} (-4.22 ± 0.41)
(R,S)-Albuterol	101.6 ± 10.8	5.97×10^{-8} (-6.81 ± 0.28)
R-Albuterol	110.6 ± 10.7	4.09×10^{-9} (-8.39 ± 0.31)
S-Albuterol	55.9 ± 7.6	1.03×10^{-7} (-6.99 ± 0.26)
(R,R/S,S)-Formoterol	96.8 ± 18.0	5.28×10^{-8} (-7.39 ± 0.30)
(R,R)-Formoterol	109.3 ± 20.6	1.78×10^{-8} (-7.75 ± 0.34)
(S,S)-Formoterol	68.2 ± 4.5	1.40×10^{-7} (-6.85 ± 0.21)

Study of Four Compounds on Various Cytokine Secretions Using Basal Models (Sepracor Document number 090-481, 2000).

Stimulatory effects of (R,R)-formoterol (IN-0343), (S,S)-formoterol (IN-0230), R-albuterol (IN-0466), and S-albuterol (IN-0479) on secretion of interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin-6 (IL-6) and tumor necrosis factor$_\alpha$ (TNF_α) were assessed with human peripheral blood mononuclear cells (PBMC) or U-937 (human macrophage-like) cells. Concanavalin A (Con A) and phorbol 12-myristate 13-acetate (PMA) were used included as positive controls. Test compounds at a concentration of 1 µM were incubated with cells for 48 hr at 37°C. IL-4 and IL-5 secretions were assessed with PBMC. IL-6 and TNF_α secretions were assessed with U-937 cells. Cytokines were measured with enzyme-immunoassays. (S,S)-Formoterol had stimulatory effects on secretion of IL-5 and TNF_α that were approximately 4 times greater than that observed for (R,R)-formoterol. (S)-Albuterol was approximately 3 times more potent than R-albuterol in stimulating secretion of TNF_α. (R)-Albuterol was approximately 5 times more than (S)-albuterol in stimulating secretion of IL-6.

Stimulatory effects of (R,R)-formoterol, (S,S)-formoterol, R-albuterol, and S-albuterol on secretion of IL-4, IL-5, IL-6)and TNF_α from human peripheral blood mononuclear cells or U-937 cells.

Compound (1 μM)	IL-4 pg/mL	IL-5 pg/mL	IL-6 pg/mL	TNF_α pg/mL
(R,R)-Formoterol	0	1.85	0	1.04
(S,S)-Formoterol	0.23	7.13	0.645	4.185
(R)-Albuterol	0	2.93	2.47	1.22
(S)-Albuterol	0	3.36	0.555	3.01
Con A (20 μg/mL)	15.42	78.54	-	-
PMA (10 nM)	-	-	93.26	140.99

Study of Four Compounds in Various Inflammation Assays (Sepracor Document number 090-478, 2000).

The inhibitory activity of (R,R)-formoterol-L-tartrate (IN-0343), (S,S)-formoterol-D-tartrate (IN-0230), R-albuterol (IN-0466; aldehyde), and S-albuterol (IN-0479; aldehyde) toward stimulus-provoked secretion of the following inflammatory mediators was assessed: prostaglandin D_2 (A23187-stimulated secretion of PGD_2 from rat mast cells), prostaglandin I_2 (basal secretion of PGI_2 from human umbilical vein endothelial cells), leukotriene C_4 (A23187-stimulated secretion of LTC_4 from human HL-60 cells), interleukin 4 (concanavalin A (Con A)-stimulated secretion of IL-4 from human peripheral blood mononuclear cells), interleukin 5 (Con A-stimulated secretion of IL-5 from human PBMC), tumor necrosis factorα (phorbol 12-myristate 13-acetate (PMA)-stimulated secretion of TNFα from human macrophage-like U-937 cells) and interleukin-6 (PMA-stimulated secretion of IL-6 from human macrophage-like U-937 cells). Compounds were tested in each assay at a concentration of 10 μM. (R,R)-formoterol, (S,S)-formoterol, (R)-albuterol, or S-albuterol at a concentration of 10 μM had minimal inhibitory effects on various inflammation assays.

Inhibitory activity (%) of (R,R)-formoterol, (S,S)-formoterol, (R)-albuterol, and S-albuterol in various inflammation assays.

Assays	(R,R)-Formoterol	S-Albuterol	R-Albuterol	(S,S)-Formoterol
PGD_2 secretion	46	53	46	43
PGI_2 secretion	-	-	-	-
LTC_4 secretion	27	-	-	-
IL-4 secretion	-	-	24	-
IL-5 secretion	27	26	36	12
TNFα secretion	47	43	56	34
IL-6 secretion	-	24	29	-

Study of Compounds, Racemic Formoterol (IN-0259), (R,R)-Formoterol (IN-0291), and (S,S)-Formoterol (IN-0293), in Various Binding, Enzyme, and Ion Transport Assays (Sepracor Document number 090-482, 2000).

Racemic formoterol, (R,R)-formoterol, and (S,S)-formoterol were assessed for their effects on various receptors, enzymes, and ion transport assays. Compounds were tested in each assay at 10 μM. Racemic formoterol (10 μM) produced an

increased binding of [^{125}I]endothelin-1 to the human endothelin$_A$ (ETA) receptor >50%. There were no other significant interactions with receptors, enzymes, or ion transport.

Pharmacology summary:

(R,R)-desmethyl formoterol (IN-0712) displayed no affinity for the β_1-adrenergic receptor. The IC_{50} of IN-0712 for the β_2-adrenergic receptor was determined to be 3270 nM. (R,S)-Albuterol, R-albuterol, (R,R/S,S)-formoterol, and (R,R)-formoterol decreased the responsiveness of guinea pig tracheal smooth muscle preparations to methacholine as determined by a dose-dependent increase in the apparent EC_{50} of methacholine (with or without TNFα pretreatment). S-Albuterol and (S,S)-formoterol had no significant effects on the responsiveness of tracheal smooth muscle preparations to methacholine, having no effect on the apparent EC_{50} (with or without TNFα pretreatment). (R,S)-Albuterol, R-albuterol, S-albuterol, (R,R)-formoterol, and (S,S)-formoterol decreased TNFα-induced hyperreactivity in guinea pig tracheal smooth muscle in vitro. (R,R/S,S)-formoterol had a smaller effect on TNFα-induced hyperreactivity as compared to other compounds. Methacholine, (R,S)-, R-, and S-albuterol, and (R,R)-, (S,S)- and (R,R/S,S)-formoterol were found to enhance mucociliary transport velocity in the calf trachea in vitro, although, the test compounds were more potent than methacholine. R-Albuterol and (R,R)-formoterol were found to be more potent than their corresponding S-enantiomers. R-Albuterol was the most potent compound tested. (S,S)-Formoterol had stimulatory effects on secretion of IL-5 and TNF_α from human peripheral blood mononuclear cells and U-937 (human macrophage-like) cells, respectively, that were approximately 4 times greater than that observed for (R,R)-formoterol. (R,R)-formoterol, (S,S)-formoterol, R-albuterol, or S-albuterol at a concentration of 10 µM had minimal inhibitory effects toward stimulus-provoked secretion of the inflammatory mediators, prostaglandin D_2, prostaglandin I_2, leukotriene C_4, interleukin 4, interleukin 5, tumor necrosis factorα, and interleukin-6. (R,R)-formoterol and (S,S)-formoterol at 10 µM had no significant effects on various receptors, enzymes, and ion transport assays. Racemic formoterol (10 µM) produced an increased binding of [^{125}I]endothelin-1 to the human endothelin$_A$ (ETA) receptor >50%.

Pharmacology conclusions: (R,R)-formoterol decreased the responsiveness of guinea pig tracheal smooth muscle preparations to methacholine as determined by a dose-dependent increase in the apparent EC_{50} of methacholine (with or without TNFα pretreatment). (R,R)-formoterol decreased TNFα-induced hyperreactivity in guinea pig tracheal smooth muscle in vitro. (R,S)-, R-, and S-albuterol, and (R,R)-, (S,S)- and (R,R/S,S)-formoterol were found to enhance mucociliary transport velocity in the calf trachea in vitro. R-Albuterol and (R,R)-formoterol were found to be more potent than their corresponding S-enantiomers. (R,R)-formoterol at a concentration of 10 µM had minimal inhibitory effects toward stimulus-provoked secretion of the inflammatory mediators, prostaglandin D_2, prostaglandin I_2, leukotriene C_4, interleukin 4, interleukin 5, tumor necrosis factorα, and interleukin-6. (R,R)-formoterol at 10 µM had no significant effects on various receptors, enzymes, and ion transport assays.

II. SAFETY PHARMACOLOGY:

Cardiovascular effects:

Study title: **Amendment to the Final Report entitled "Comparative Acute Inhalation Tolerance Study of (R,R), (S,S)-, and Racemic Formoterol and (R,R)-Desformoterol in Dogs."**

Study no: Sepracor Document number 09-809, Addendum 1999
Volume #, and page #: Amendment #014, Volume 2, Pages 1-13
Conducting laboratory and location: [redacted] b(4)

GLP compliance: Yes
QA report: yes (X) no ( )

The original report was evaluated in the review dated December 20, 1999. After the release of the final report, the sponsor requested that all ECG recordings from all animals be reevaluated. Cardiac response was evaluated in terms of probable diagnosis (i.e., tachycardia or the presence of arrhythmia) and recovery during the post-exposure period. In addition, cardiac effects between groups were qualitatively compared. No remarkable additional evidence of abnormality in the cardiac patterns was observed. Five of six animals that exhibited abnormal cardiac patterns during the previous evaluation were confirmed upon reevaluation. One female (#5321) in the 80 µg/kg racemic formoterol group that had been reported as exhibiting abnormal cardiac patterns was no longer included following the reevaluation. However, one male (#5297) in the 20 µg/kg (R,R)-formoterol group that was not previously reported was included.

The reevaluation would appear to have no impact on the original review of this study. It is unclear why the sponsor is excluding some study animals from the analysis.

Safety pharmacology conclusions: An amendment to the Final Report entitled "Comparative Acute Inhalation Tolerance Study of (R,R), (S,S)-, and Racemic Formoterol and (R,R)-Desformoterol in Dogs" has no impact on the evaluation of the study in the review dated December 20, 1999.

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III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Mice

Study title: **Toxicokinetics of Formoterol During A 28-Day Oral Toxicity Study of (R,R)-Formoterol in Mice.**

Study no: Sepracor Document number 090-470
Volume #, and page #: Amendment #029, Volume2, Pages 1 to 22
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: June 21, 1999
GLP compliance: There was no statement of compliance with GLP regulations.
QA report: yes (X) no ( ); A quality assurance audit of this toxicokinetic report was conducted by [redacted] b(4)
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-L-tartrate, Lot number 010799A
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods (unique aspects): Toxicokinetic parameters of plasma formoterol were assessed in mice that received (R,R)-formoterol at oral doses of 5, 15, 50, and 150 mg/kg/day for 28 days. There were several errors in the summary that referred to inhalation exposure; however, drug was administered by oral gavage in the present study as reflected by text in the body of the report.
Dosing:
Species/strain: Male and female Charles River [redacted] CD-1®(ICR)BR mice
#/sex/group or time point (main study): 30 mice/sex/group b(4)
Age: Animals were approximately 8 weeks old at the initiation of dosing.
Weight: The body weight range for male and female mice combined was 18-36 g at the initiation of dosing.
Doses in administered units: Oral doses of 5, 15, 50, and 150 mg/kg/day (R,R)-formoterol.
Route, form, volume, and infusion rate: Vehicle or drug solution was administered by the oral gavage using a dose volume of 10 mL/kg.

Toxicokinetics: Mice received (R,R)-formoterol at oral doses of 5, 15, 50, and 150 mg/kg/day for 28 days. Blood samples for measurement of plasma formoterol levels were collected on days 0 and 27 at 0.5, 1, 2, 6, and 24 hr after dosing. Three mice/sex/group were used for each time point. Plasma was pooled from 3 mice per time point. Plasma concentrations of formoterol and the desformoterol/formoterol ratios were

measured using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The validated quantitation range of formoterol was 2.50 to 200 pg/mL using a 1-mL sample volume. The assay method lacked chiral specificity and concentration data was expressed as formoterol. The method was not validated for desformoterol; however, the MRM mass channel for this compound was acquired and presented as a ratio with respect to the MRM signal of formoterol. The MRM signal for an equal quantity of desformoterol was assumed to be approximately the same as that for formoterol.

Results: AUC and C_{max} values for formoterol in male and female mice increased with elevating dose, although, dose proportionality was not evident. AUC and C_{max} values for formoterol in male mice at 50 and 150 mg/kg/day and female mice at 150 mg/kg/day were greater on day 27 as compared to day 0 suggesting some drug accumulation. A sex-dependent effect was evident as AUC and C_{max} values for formoterol were generally greater in males as compared to females. Pooled desformoterol/formoterol MRM ratios in male and female mice were only detectable at doses of 50 and 150 mg/kg/day for a few time points. The ratios were <0.08 suggesting little exposure to desformoterol following administration of (R,R)-formoterol.

Toxicokinetic parameters for formoterol on days 0 and 27 in mice that received (R,R)-formoterol at oral doses of 5, 15, 50, and 150 mg/kg/day.

Dose mg/kg/day	Sex	$AUC_{0.5\text{-}24hr}$, ng·hr/mL		C_{max}, ng/mL		T_{max}, hr	
		Day 0	Day 27	Day 0	Day 27	Day 0	Day 27
5	Male	67.8	22.2	19.5	15.2	2.0	1.0
	Female	19.5	11.9	14.4	9.20	0.50	0.50
15	Male	106	77.7	31.2	84.5	0.50	0.50
	Female	121	40.9	45.4	33.8	1.0	0.50
50	Male	527	1370	142	497	1.0	0.50
	Female	278	254	120	144	0.50	0.50
150	Male	1990	50900	735	4430	1.0	6.0
	Female	1390	2010	662	931	0.50	0.50

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Toxicokinetics of Formoterol During a 28-Day Inhalation Tolerance Study of (R,R)-Formoterol in Mice in Support of ⊏ ⊐ 312026.

Study no: Sepracor Document number 090-461, 2000
Volume #, and page #: Amendment #029, Volume2, Pages 1 to 22
Conducting laboratory and location: ⌐

b(4)

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Date of study initiation: February 15, 1999
GLP compliance: There was no statement of compliance with GLP regulations.
QA report: yes (X) no ( ); A quality assurance audit of this toxicokinetic report was conducted by ⊏ ⊐

b(4)

Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-L-tartrate, Lot number 121697A

Methods (unique aspects)**:** Toxicokinetic parameters of plasma formoterol were assessed in mice that received (R,R)-formoterol by nose-only inhalation at doses of 0, 100, 400, and 800 µg/kg/day for 28 days. The vehicle-control group was exposed to an aerosol of the vehicle, 0.9% sodium chloride.

Dosing:

Species/strain: Male and female ⊏ ⊐ CD-1®(ICR)BR mice

#/sex/group or time point (main study): 30 mice/sex/group b(4)

Age: Animals were approximately 8-9 weeks old at the initiation of dosing.

Weight: The body weight range for male and female mice combined was 18-37 g at the initiation of dosing.

Doses in administered units: (R,R)-formoterol was administered by nose-only inhalation at doses of 100, 400, and 800 µg/kg/day

Route, form, volume, and infusion rate: A liquid droplet aerosol of the vehicle or (R,R)-formoterol was generated from saline solutions using a Collision jet nebulizer and was administered by nose-only inhalation.

Toxicokinetics: Mice received (R,R)-formoterol by nose-only inhalation at doses of 100, 400, and 800 µg/kg/day for 28 days. Blood samples for measurement of plasma formoterol levels were collected on days 0 and 26 at 0.5, 1, 2, 6, and 24 hr after dosing. Three-mice/sex/group were used for each time point. Plasma was pooled from 3 mice per time point. Plasma concentrations of formoterol and the desformoterol/formoterol ratios were measured using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The validated quantitation range of formoterol was 2.50 to 200 pg/mL using a 1-mL sample volume. The assay method lacked chiral specificity and concentration data was expressed as formoterol. The method was not validated for desformoterol; however, the MRM mass channel for this compound was acquired and presented as a ratio with respect to the MRM signal of formoterol. The MRM signal for an equal quantity of desformoterol was assumed to be approximately the same as that for formoterol.

Results: AUC and C_{max} values for formoterol in male and female mice increased with elevating dose and were approximately dose proportional. A sex-dependent effect was evident as AUC and C_{max} values for formoterol were generally greater in males as compared to females. Pooled desformoterol/formoterol MRM ratios in male and female mice were detectable at all doses for a few time points. The ratios were <0.1 suggesting low exposure to desformoterol following administration of (R,R)-formoterol.

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Toxicokinetic parameters for formoterol on days 0 and 26 in mice that received (R,R)-formoterol by nose-only inhalation at doses of 100, 400 and 800 µg/kg/day. Deposited doses, based upon a 0.07 deposition factor, are estimated to be 7, 28, and 56 µg/kg/day, respectively.

Nominal Dose µg/kg/day	Sex	$AUC_{0.5\text{-}24hr}$, ng·hr/mL		C_{max}, ng/mL		T_{max}, Hr	
		Day 0	Day 26	Day 0	Day 26	Day 0	Day 26
100	Male	6240	7300	2550	2340	0.5	1.0
	Female	5720	6910	2000	1680	0.5	0.5
400	Male	20500	24600	7960	7730	0.5	0.5
	Female	15300	19700	9410	7800	0.5	0.5
800	Male	41500	34500	33800	29700	0.5	0.5
	Female	37100	42000	27800	38600	0.5	0.5

Rats

Toxicokinetics of Formoterol During a 28-Day Inhalation Toxicity Study of (R,R)-Formoterol and (R,R/S,S)-Formoterol in Rats in Support of — 312021.

Study no: Sepracor Document number 090-463, 1999
Volume #, and page #: Amendment #029, Volume2, Pages 1 to 22
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: October 6, 1998
GLP compliance: There was no statement of compliance with GLP regulations.
QA report: yes (X) no ( ); A quality assurance audit of this toxicokinetic report was conducted by [redacted]
Drug, lot #, radiolabel, and % purity:
(R,R)-Formoterol-L-tartrate, Lot number 121697A (Purity, 99.8%)
(R,R/S,S)-Formoterol, Lot number XL018P9F (Purity, 99.4%)
Formulation/vehicle:

Methods (unique aspects): Toxicokinetic parameters of plasma formoterol were assessed in rats that received (R,R)-formoterol by nose-only inhalation at doses of 0, 100, 400, and 800 µg/kg/day for 28 days. The positive-control group was exposed to (R,R/S,S)-Formoterol at 400 µg/kg/day.
Dosing:
Species/strain: Male and female — :CD®(SD)IGS BR rats
#/sex/group or time point (main study): 30 rats/sex/group
Age: Animals were approximately 7-8 weeks old at the initiation of dosing.

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Weight: Body weight ranges were 180-240 g for male rats and 150-180 g for female rats. *Doses in administered units:* (R,R)-formoterol was administered by nose-only inhalation at doses of 100, 400, and 800 µg/kg/day. (R,R/S,S)-formoterol was administered by nose-only inhalation at a dose of 400 µg/kg/day.
Route, form, volume, and infusion rate: An aerosol was generated from a solution of (R,R)- or (R,R/S,S)-formoterol in saline. Rats were exposed to the aerosol by nose-only inhalation for 30 minutes daily.

Toxicokinetics: Rats received (R,R)-formoterol by nose-only inhalation at doses of 100, 400, and 800 µg/kg/day for 28 days. The positive-control group was exposed to (R,R/S,S)-Formoterol at 400 µg/kg/day. Blood samples for measurement of plasma formoterol levels were collected on days 1 and 26 at 0.5, 1, 2, 6, and 24 hr after dosing. Three-rats/sex/group were used for each time point. Plasma concentrations of formoterol and the desformoterol/formoterol ratios were measured using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The validated quantitation range of formoterol was 2.50 to 200 pg/mL using a 1-mL sample volume. The assay method lacked chiral specificity and concentration data was expressed as formoterol. The method was not validated for desformoterol; however, the MRM mass channel for this compound was acquired and presented as a ratio with respect to the MRM signal of formoterol. The MRM signal for an equal quantity of desformoterol was assumed to be approximately the same as that for formoterol.

Results: AUC and C_{max} values following administration of (R,R)-formoterol to male and female rats at doses of 100, 400, or 800 µg/kg/day increased with elevating dose, although, dose proportionality was not evident. AUC values on day 26 for formoterol in male rats exposed to (R,R)-formoterol were greater than values on day 1 suggesting drug accumulation. AUC values for formoterol following exposure to (R,R)-formoterol at 400 µg/kg/day or (R,R/S,S)-formoterol at 400 µg/kg/day were comparable between male rats; however, values were higher in female rats exposed to (R,R/S,S)-formoterol. AUC values for formoterol following exposure to (R,R/S,S)-formoterol were higher in female rats as compared to male rats. Desformoterol/formoterol ratios in male rats were only detectable with (R,R)-formoterol at a dose of 400 µg/kg/day and (R,R/S,S)-formoterol at a dose of 400 µg/kg/day for a few erratic time points. Ratios were ≤0.22 suggesting low exposure to desformoterol. Desformoterol/formoterol ratios in female rats were only detectable with (R,R)-formoterol at doses of 100, 400, and 800 µg/kg/day for a few erratic time points. Ratios were ≤0.49 suggesting minimal exposure to desformoterol.

Toxicokinetic parameters for formoterol on days 0 and 26 in rats that received (R,R)-formoterol by nose-only inhalation at doses of 100, 400, or 800 µg/kg/day or (R,R/S,S)-formoterol by nose-only inhalation at a dose of 400 µg/kg/day. Deposited doses, based upon a 0.10 deposition factor, are estimated to be 10, 40, 80, and 40 µg/kg/day, respectively.

Nominal Dose µg/kg/day	Sex	$AUC_{0.5\text{-}24hr}$, ng·hr/mL		C_{max}, ng/mL		T_{max}, hr	
		Day 1	Day 26	Day 1	Day 26	Day 1	Day 26
100 (R,R)	Male	3140	6730	1490	1060	0.5	0.5
	Female	3060	3160	1460	1040	0.5	0.5
400 (R,R)	Male	15600	17500	8010	5070	0.5	0.5
	Female	4700	16100	2780	5150	0.5	0.5
800 (R,R)	Male	22300	31100	8840	9730	0.5	0.5
	Female	27900	23600	17000	9850	0.5	0.5
400 (R,R/S,S)	Male	14700	17400	6000	6690	0.5	0.5
	Female	18100	20500	9060	9560	0.5	0.5

Dogs

Toxicokinetics of Formoterol and Desformoterol and [redacted] Ratios During an Acute Inhalation Tolerance Study of (R,R)-, (S,S)-, and (R,R/S,S)-Formoterol and (R,R)-Desformoterol in Dogs in Support of [redacted] 312010. b(4)

Study no: Sepracor Document number 090-464, 1999
Volume #, and page #: Amendment #014, Volume2, Pages 1 to 22
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: October 24, 1997
GLP compliance: There was no statement of compliance with GLP regulations.
QA report: yes (X) no ( ); A quality assurance audit of this toxicokinetic report was conducted by [redacted]
Drug, lot #, radiolabel, and % purity:
(R,R)-Formoterol tartrate, Lot number RH-924-96 (Purity, 99.49%)
(S,S)-Formoterol tartrate, Lot number RH-924-97 (Purity, 99.51%) b(4)
(R,R/S,S)-Formoterol fumarate monohydrate, Lot number XL018P9F (Purity, 99.22%)
(R,R)-Desformoterol tartrate, Lot number 956-40 (Purity, 96.29%)
Formulation/vehicle: Saline

Methods (unique aspects): Toxicokinetic parameters of plasma formoterol were assessed in dogs that received (R,R)-formoterol at 5, 20, or 40 µg/kg, (S,S)-formoterol at 40 µg/kg, (R,R/S,S)-formoterol at 80 µg/kg, or (R,R)-desformoterol at 40 µg/kg by inhalation exposure.
Dosing:
Species/strain: Beagle dogs

#/sex/group or time point (main study): 2 dogs/sex/group
Age: Animals were approximately 21-23 weeks old at the initiation of dosing.
Weight: The body weight range was 7-10 kg.
Doses in administered units: (R,R)-formoterol at 5, 20, or 40 µg/kg, (S,S)-formoterol at 40 µg/kg, (R,R/S,S)-formoterol at 80 µg/kg, or (R,R)-desformoterol at 40 µg/kg were administered by nose-only inhalation exposure.
Route, form, volume, and infusion rate: Nose-only inhalation exposure for 15 min. An aerosol was generated from a solution of the test article in saline.

Toxicokinetics: Toxicokinetic parameters of plasma formoterol were assessed in dogs that received (R,R)-formoterol at 5, 20, or 40 µg/kg, (S,S)-formoterol at 40 µg/kg, (R,R/S,S)-formoterol at 80 µg/kg, or (R,R)-desformoterol at 40 µg/kg by inhalation exposure. Blood samples for measurement of plasma formoterol levels were collected at 0.5, 1, 2, 6, and 24 hr after dosing. Two dogs/sex/group were used for each time point. Plasma concentrations of formoterol and the desformoterol/formoterol ratios were measured using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The validated quantitation range of formoterol was 2.50 to 200 pg/mL using a 1-mL sample volume. The assay method lacked chiral specificity and concentration data was expressed as formoterol. The method was not validated for desformoterol; however, the MRM mass channel for this compound was acquired and presented as a ratio with respect to the MRM signal of formoterol. The MRM signal for an equal quantity of desformoterol was assumed to be approximately the same as that for formoterol.

Results: AUC and C_{max} values for formoterol following exposure to 5, 20, or 40 µg/kg (R,R)-formoterol increased with elevating dose and increases were approximately dose proportional. C_{max} values following exposure to (R,R)-formoterol, (S,S)-formoterol, (R,R/S,S)-formoterol, or (R,R)-desformoterol were greater in male dogs as compared to female dogs. Following exposure to 40 µg/kg (R,R)-formoterol, 40 µg/kg (S,S)-formoterol, 40 µg/kg (R,R)-desformoterol, or 80 µg/kg (R,R/S,S)-formoterol, AUC values were observed to be in the following order: (R,R)-desformoterol<(R,R)-formoterol<(S,S)-formoterol<(R,R/S,S)-formoterol. Plasma desformoterol/formoterol MRM ratios were undetectable following inhalation of any dose of formoterol in male or female dogs. Following inhalation of 40 µg/kg (R,R)-desformoterol, plasma [redacted] MRM ratios were generally greater in females than in males. b(4)

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Formoterol (and Desformoterol*) toxicokinetic parameters following inhalation of 5, 20, and 40 µg/kg (R,R)-formoterol, 40 µg/kg (S,S)-formoterol, 80 µg/kg (R,R/S,S)-formoterol, and 40 µg/kg (R,R)-desformoterol in male and female dogs. Deposited doses, based upon a 0.25 deposition factor, are estimated to be 1.25, 5, 10, 10, 16, and 5 µg/kg, respectively.

Dose µg/kg	$AUC_{0.5\text{-}24hr}$, pg·hr/mL		C_{max}, pg/mL		T_{max}, hr	
	Male	Female	Male	Female	Male	Female
5 µg/kg (R,R)-Formoterol	2020	1210	203	185	2.0	0.5
20 µg/kg (R,R)-Formoterol	6950	4830	1340	873	0.75	0.5
40 µg/kg (R,R)-Formoterol	10500	10700	1960	1650	0.5	0.5
40 µg/kg (S,S)-Formoterol	19900	22400	3370	2870	1.0	1.0
80 µg/kg (R,R/S,S)-Formoterol	39700	34300	7990	6980	0.75	0.75
40 µg/kg (R,R)-Desformoterol*	49.4	73.4	12.6	11.8	0.5	0.5

Distribution:

[^{3}H]-(R,R)-Formoterol: In Vitro Blood to Plasma Partitioning and Plasma Protein Binding in the Rat, Dog, Human, and Mouse.

Study no: Sepracor Document numbers 090-417, 2000; 090-418, 2000; 090-419, 2000; and 090-452, 2000.

Volume #, and page #: Amendment #041, Volume 1

Conducting laboratory and location: [b(4)

Date of study initiation: June 15, 1999-July 1, 1999

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: [^{3}H]-(R,R)-formoterol, Lot number 140-098-0080 (Purity 99.9%)

Methods: In vitro blood-to-plasma partitioning and plasma protein binding of [^{3}H]-(R,R)-formoterol was assessed with male rats, female dogs, male humans, and mice. Plasma protein binding was assessed using an ultrafiltration method. Concentrations of [^{3}H]-(R,R)-formoterol in studies with rat whole blood and plasma were 1.0, 10.0, 20.0, and 100 ng/mL. Concentrations of [^{3}H]-(R,R)-formoterol in studies with dog and mouse whole blood and plasma were 1.0, 2.5, 5.0, and 100 ng/mL. Concentrations of [^{3}H]-(R,R)-formoterol in studies with human whole blood and plasma were 0.25, 0.50, and 1.0 ng/mL. Partitioning and binding parameters were measured at 0.5, 1.0, 2.0, and 3.0 hr after incubation at 37°C.

Results: The majority of the (R,R)-formoterol content in EDTA-preserved whole blood from rat, dog, human, and mouse was associated with red blood cells and concentration-independent. The percent of drug bound to rat, dog, human, and mouse

plasma protein was weak and concentration-independent. Freeze-thawing of plasma had minimal effects on protein binding of [^{3}H]-(R,R)-formoterol.

In vitro blood-to-plasma partitioning and plasma protein binding of [^{3}H]-(R,R)-formoterol

Species and Concentration	Fraction distributed to red blood cells, FBC	% Plasma protein binding
Rat, 1 to 100 ng/mL	0.68-0.71	37.2-48.9%
Dog, 1 to 100 ng/mL	0.68-0.73	35.9-47.8%
Human, 0.25 to 1 ng/mL	0.68-0.71	52.1-64.8%
Mouse, 1 to 100 ng/mL	0.48-0.58	28.2-33.7%

PK/TK summary:

Toxicokinetic parameters were determined in mice, rats, and dogs following treatment with (R,R)-formoterol. AUC and C_{max} values for formoterol in male and female mice increased with elevating dose following oral administration of (R,R)-formoterol at doses of 5 to 150 mg/kg/day for 28 days. Drug accumulation was evident at higher doses. AUC and C_{max} values for formoterol in male and female mice increased in a dose proportional manner following nose-only inhalation of (R,R)-formoterol at doses of 100 to 800 µg/kg/day for 28 days. For these oral and inhalation studies, a sex-dependent effect was evident as AUC and C_{max} values for formoterol were generally greater in males as compared to females. AUC and C_{max} values for formoterol increased with elevating dose in rats that received (R,R)-formoterol by nose-only inhalation at doses of 100 to 800 µg/kg/day for 28 days. Drug accumulation was evident on day 26. AUC and C_{max} values for formoterol increased in a dose proportional manner for dogs that received single doses of (R,R)-formoterol at 5, 20, or 40 µg/kg by inhalation exposure. C_{max} values following exposure to (R,R)-formoterol were greater in male dogs as compared to female dogs.

In vitro blood-to-plasma partitioning and plasma protein binding of [^{3}H]-(R,R)-formoterol was assessed with male rats, female dogs, male humans, and mice. The majority of the (R,R)-formoterol content in EDTA-preserved whole blood from rat, dog, human, and mouse was associated with red blood cells (i.e., the fraction distributed to red blood cells ranged from 0.48-0.73) and concentration-independent. The percent of drug bound to rat, dog, human, and mouse plasma protein was weak (i.e., % plasma protein binding ranged from 28.2 to 64.8%) and concentration-independent.

PK/TK conclusions: The majority of the (R,R)-formoterol content in EDTA-preserved whole blood from rat, dog, human, and mouse was associated with red blood cells (i.e., the fraction distributed to red blood cells ranged from 0.48-0.73) and concentration-independent. The percent of drug bound to rat, dog, human, and mouse plasma protein was weak (i.e., % plasma protein binding ranged from 28.2 to 64.8%) and concentration-independent.

IV. TOXICOLOGY:

Acute Toxicity

Mice

Study title: Acute Inhalation Tolerance Study of (R,R)-Formoterol in Mice.

Key study findings:

♦ Mice were exposed by acute nose-only inhalation to (R,R)-formoterol at nominal doses of 400, 800, and 1600 µg/kg (deposited doses of 29.7-31.3, 50-54, and 102-125 µg/kg, respectively).

♦ There was no treatment-related mortality.

♦ Microscopic examination was limited to the heart from animals in the 102 µg/kg (R,R)-formoterol and unexposed control groups that were sacrificed two days after exposure. Cardiomyopathy, characterized by small aggregates of mononuclear inflammatory cells in foci of myofiber degeneration (vacuolated and/or hyalinized sarcoplasm) in the inner third of the left ventricle was observed for mice treated with (R,R)-formoterol at a deposited dose of 102 µg/kg.

Study no: Sepracor Document number 090-818, 1999 and 090-460, 1999
Volume #, and page #: Amendment #029, Volume 1, Pages 1 of 218 and Amendment #014, Volume 1, Pages 1 to 19
Conducting laboratory and location: [redacted]

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Date of study initiation: October 12, 1998
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-L-tartrate, Lot number 121697A (Purity 99.8%)
Formulation/vehicle: Saline aerosol exposure (0.9% NaCl for Injection USP)

Methods (unique aspects): Inhalation tolerance and potential adverse effects of (R,R)-formoterol were evaluated in an acute inhalation study with CD®-1(ICR)BR mice.
Dosing:
Species/strain: CD-1®(ICR)BR mice were obtained from [redacted]

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#/sex/group or time point (main study): 6 mice/sex/group in treatment groups and 3 mice/sex/group in the unexposed control group.
Satellite groups used for toxicokinetics or recovery: 9 mice/sex/group
Age: Male and female mice were approximately 9 and 12 weeks old, respectively, at the initiation of exposure.
Weight: Body weight ranges at initiation of exposure were 28.0 to 38.9 g for male mice and 25.5 to 35.7 g for female mice.
Doses in administered units: (R,R)-formoterol was administered by nose-only inhalation at doses of 400, 800, and 1600 µg/kg. All doses were expressed as (R,R)-formoterol free base equivalents. Animals in Group 7 were randomized separately from other animals in toxicology and toxicokinetic groups. Animals in Group 8 were selected without randomization and used as an unexposed control group. For each (R,R)-formoterol dose, animals in the toxicology group were exposed first. On the following day, the animals assigned to the respective toxicokinetic group were exposed. Inhaled doses were calculated as follows:

$$\text{Inhaled Dose (µg/kg)} = \frac{\text{Exposure conc. (µg/L) x Min. volume (L/min) x Duration (min)}}{\text{Mean Body Weight (kg)}}$$

Toxicology Groups

Group[b]	Target Dose µg/kg	# mice/sex/ group	Recovery Period, Days	Actual Dose µg/kg	Deposited Dose[a], µg/kg
1	400	6	14	424	29.7
2	1600	6	14	1780	125
3	800	6	14	772	54
7	1600	6	2	1455	102
8	0 (Control)	3	2	0	0

a. The deposited dose was calculated using a deposition factor of 0.07 based upon a MMAD ± GSD for (R,R)-formoterol of approximately of 1.00 ± 1.79 µm.
b. Groups 7 and 8 were sacrificed following a 2-day observation period and the heart was processed for microscopic evaluation. All other toxicology groups were sacrificed following a 14-day observation period.

Toxicokinetic Groups

Group	Target Dose µg/kg	# mice/sex/ group	Recovery Period, Days	Actual Dose µg/kg	Deposited Dose, µg/kg
4	400	9	0	447	31.3
5	1600	9	0	1621	113.5
6	800	9	0	715	50

a. The deposited dose was calculated using a deposition factor of 0.07 based upon a MMAD ± GSD for (R,R)-formoterol of approximately of 1.00 ± 1.79 µm.

Actual exposure concentrations for toxicology and toxicokinetic groups.

Group	Target Dose µg/kg	Minute Volume L/min	Mean BW kg	Exposure Conc. µg/L	Target Exposure Conc. µg/L	% Target Conc.	Exposure Time (min)	Inhaled Dose µg/kg	% of Target Dose
1	400	0.04	0.0283	20.0	20.0	100	15	424	106
2	1600	0.04	0.0306	22.7	20.0	114	60	1780	111
3	800	0.04	0.0311	20.0	20.0	100	30	772	97
4	400	0.04	0.0295	22.0	20.0	110	15	447	112
5	1600	0.04	0.0311	21.0	20.0	105	60	1621	101
6	800	0.04	0.0319	19.0	20.0	95	30	715	89
7	1600	0.04	0.0330	20.0	20.0	100	60	1455	91

Route, form, volume, and infusion rate: A nose-only inhalation exposure system was used for these studies.

Mice were restrained in nose-only tubes during exposures. Immediately prior to test article exposure, animals were exposed to a saline aerosol for 15 min to allow acclimation to restraint. A single target exposure concentration of 20 µg/L and three exposure durations of 15, 30, and 60 min were used to target (R,R)-formoterol (free base) doses of 400, 800, and 1600 µg/kg, respectively. Male and female mice within each group were exposed simultaneously. Liquid aerosol atmospheres were generated by jet nebulization (using a modified —— type Collision nebulizer) from saline solutions of the test article. Saline or test aerosols were delivered from the nebulizer to a modified glass and plexiglass chromatography jar. The chromatography jar was placed in the system to act as a mixing plenum, if addition of dilution air was required. Vacuum, provided by the facility exhaust system, captured and removed aerosol from the nose-only system. The nose-only system was operated under negative pressure at 0.4 inches of water.

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Actual exposure concentrations of (R,R)-formoterol free base were determined by chemical analysis of aerosol samples collected on filters. For each 15, 30, or 60 min exposure, one, two, and three aerosol samples, respectively, of the exposure atmosphere were collected. Each sample was collected on a 25-mm glass-fiber filter held in an in-line filter holder. Total sample volumes were measured by recording the sampling airflow rate (0.9 LPM for all samples) and the sample collection time (10-15 min for all samples). The mass of formoterol free base on each filter was determined using an HPLC method. Actual exposure concentrations (as free base) were calculated by dividing the analytically determined mass of free base by the sample volume.

Observations and times:

Clinical signs: Animals were observed twice daily for moribundity/mortality. Animals were observed for clinical signs of toxicity immediately following exposure. Toxicology animals were monitored once daily for clinical signs. Toxicology animals received physical examinations on the day prior to exposure, the day of exposure, and the day after exposure. Physical examinations were also performed on post-exposure days 7 and 14.

Body weights: Body weights for animals in Group 1-6 were measured on the day prior to exposure, day 0 (prior to test article exposure), and on post-exposure days 7 and 14.

Animals in Group 7 were weighed on the day of exposure and prior to necropsy on post-exposure day 2. It should be noted there was no corresponding control group to assess changes in body weight gain.
Food consumption: Not measured.
Ophthalmoscopy: Not performed.
EKG: Not performed.
Hematology: Not performed.
Clinical chemistry: Not performed.
Urinalysis: Not performed.
Gross pathology: Necropsy examinations were conducted on toxicology animals. Groups 7 and 8 were sacrificed following a 2-day observation period and the heart was processed for microscopic evaluation. All other toxicology groups were sacrificed following a 14-day observation period. Animals in Group 8 (unexposed controls) were euthanized and the hearts were collected for examination.
Organs weighed: Not performed.
Histopathology: Hearts from animals in Group 7 (1600 µg/kg) and Group 8 (unexposed control) were processed, embedded in paraffin blocks, sectioned at 5 to 8 µM, and stained with hematoxylin and eosin. A pathologist conducted microscopic examination of hearts.
Toxicokinetics: Blood samples for measurement of plasma formoterol levels were collected from 3 rats/sex/group at 0.5, 1, and 2 hr following exposure. Plasma samples were shipped to [redacted] for analysis. Results of toxicokinetic analysis will apparently be reported at a later time. b(4)
Other: None.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs.

Gross pathology: There were no treatment-related gross pathological findings.

Histopathology: Microscopic examination was limited to the heart from animals in Group 7 (deposited dose of 102 µg/kg) and Group 8 (unexposed control) that were sacrificed two days after exposure. Cardiomyopathy, characterized by small aggregates of mononuclear inflammatory cells in foci of myofiber degeneration (vacuolated and/or hyalinized sarcoplasm) in the inner third of the left ventricle was observed for 4 of 6 males and 2 of 6 females treated with (R,R)-formoterol at a deposited dose of 102 µg/kg. Cardiomyopathy was graded as minimal. For 1 female at 102 µg/kg, macrophage infiltrate without myofiber degeneration was observed. There were no findings of cardiomyopathy in hearts from the unexposed control group.

Histomorphological diagnosis of the heart from mice in Group 7 (deposited dose of 102 µg/kg) and Group 8 (unexposed control).

Heart	102 µg/kg (R,R)-Formoterol		Unexposed Control	
	Male	Female	Male	Female
Number examined	6	6	3	3
-cardiomyopathy (minimal)	4	2	0	0
-hypertrophy of epicardium (minimal)	3	1	0	1
-macrophage infiltrate (minimal)	0	1	0	0

Toxicokinetics: AUC and C_{max} values for formoterol increased with elevating dose; however, increases were less than dose proportional. Mean plasma desformoterol/formoterol ratios in male and female mice that received (R,R)-formoterol at inhaled doses ≤1600 µg/kg were not measurable at any time point.

Formoterol toxicokinetic parameters following administration of (R,R)-formoterol to mice at inhaled doses of 400, 800, and 1600 µg/kg.

Dose, µg/kg	AUC0.5-2hr, pg·hr/mL		C_{max}, pg/mL		T_{max}, hr	
	Male	Female	Male	Female	Male	Female
400	6670	7420	9720	12000	0.5	0.5
800	14100	9410	18500	14600	0.5	0.5
1600	19100	16600	26100	27400	0.5	0.5

Summary of individual study findings: Mice were exposed by acute nose-only inhalation to (R,R)-formoterol at nominal doses of 400, 800, and 1600 µg/kg (deposited doses of 29.7-31.3, 50-54, and 102-125 µg/kg, respectively). There was no treatment-related mortality. Microscopic examination was limited to the heart from animals in the 102 µg/kg (R,R)-formoterol and unexposed control groups that were sacrificed two days after exposure. Cardiomyopathy, characterized by small aggregates of mononuclear inflammatory cells in foci of myofiber degeneration (vacuolated and/or hyalinized sarcoplasm) in the inner third of the left ventricle was observed for 4 of 6 males and 2 of 6 females treated with (R,R)-formoterol at a deposited dose of 102 µg/kg. Cardiomyopathy was graded as minimal. For an additional female at 102 µg/kg, macrophage infiltrate without myofiber degeneration was observed. There were no findings of cardiomyopathy in hearts from the unexposed control group.

Rats

Study title: Comparative Acute Inhalation Tolerance Study of (RR)-, (SS)-, (R,R/S,S)-Formoterol and (R,R)-Desformoterol in Rats.

Key study findings:

♦ Rats were exposed by acute inhalation to (R,R)-formoterol at nominal doses ranging from 40 to 1600 µg/kg (deposited doses ranging from 3 to 155 µg/kg), (S,S)-formoterol at a nominal dose of 1600 µg/kg (deposited dose of 199 µg/kg), (R,R/S,S)-formoterol at

a nominal dose of 1600 µg/kg (deposited dose of 76.9 µg/kg), or (R,R)-desformoterol at a nominal dose of 1600 µg/kg (deposited dose of 118 µg/kg). Observation periods following exposure were 2 or 14 days.

♦ One male rat exposed to (R,R)-formoterol at a deposited dose of 148 µg/kg died during the test article exposure period.

♦ (R,R)-formoterol at deposited doses of 132 to 155 µg/kg increased heart rates in male and female rats to 131.7 and 118.7% of baseline rates, respectively. (R,R/S,S)-Formoterol at 76.9 µg/kg and (R,R)-desformoterol at 118 µg/kg increased heart rates in male rats; however, these compounds had minimal effects on heart rates in female rats. (S,S)-Formoterol at 199 µg/kg had no effect on heart rate.

♦ Microscopic examination was limited to the heart collected from the unexposed control and 148 µg/kg (R,R)-formoterol groups at 2 days after treatment. Cardiomyopathy (minimal to mild) was observed for 2 of 6 rats in the unexposed control group and 5 of 5 animals in the 148 µg/kg (R,R)-formoterol group.

Study no: Sepracor Document number 090-815, 2001
Volume #, and page #: Amendment #038, Volume 1, Pages 1 to 359
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: June 18, 1998
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity:
(R,R)-Formoterol-(L)-tartrate, Lot number 121697A (Purity, 99.8%)
(S,S)-Formoterol Fumarate, Lot number GY-880-13 (Purity, 100%)
(R,R/S,S)-Formoterol Fumarate Hydrate, Lot number XL018P9F (Purity, 99.4%)
(R,R)-Desformoterol-(L)-Tartrate (Purity, 100%)
Formulation/vehicle: 0.9% Sodium Chloride for Injection USP

Methods (unique aspects): The objective of this study was to determine the acute inhalation tolerance and potential adverse effects of (R,R)-formoterol, (S,S)-formoterol, (R,R/S,S)-formoterol, and (R,R)-desformoterol when administered as single inhalation exposures to rats.
Dosing:
Species/strain — CD®(SD)IGS BR rats were obtained from [redacted] Animals for studies with (R,R)-formoterol were obtained on July 21, 1998. Animals for studies with (S,S)-formoterol, (R,R/S,S)-formoterol, and (R,R)-desformoterol were obtained on December 15, 1998. b(4)
#/sex/group or time point (main study): 3 rats/sex/group
Satellite groups used for toxicokinetics or recovery: 9 rats/sex/group for toxicokinetic measurements of plasma formoterol levels.
Age: Animals were approximately 8 to 10 weeks old at the initiation of exposures.

Weight: Body weight ranges at the initiation of exposure were 208 to 310 g for male rats and 188 to 242 g for female rats.
Doses in administered units: All dose levels are expressed as formoterol free base equivalents. The maximum concentration for racemic (R,R/S,S)-formoterol was limited by the relatively poor solubility of this test article. Inhaled doses were calculated as follows:

$$\text{Inhaled Dose (µg/kg)} = \frac{\text{Exposure conc. (µg/L) x Min. volume (L/min) x Duration (min)}}{\text{Mean Body Weight (kg)}}$$

Toxicology Groups (3 rats/sex/group)

Group	Test Article	Target Dose (µg/kg)	Inhaled Dose (µg/kg)	Deposited Dose[a] (µg/kg)
1	(R,R)-Formoterol	40	33	3
2	(R,R)-Formoterol	200	165	15
3	(R,R)-Formoterol	400	435	39
4[b]	(R,R)-Formoterol	1600	1649	148
5	(R,R)-Formoterol	1600	1719	155
6[b]	Unexposed Control	0	0	0
11	(R,R)-Formoterol	1600	1463	132
12	(S,S)-Formoterol	1600	2208	199
13	(R,R/S,S)-Formoterol	1600	854	77
14	(R,R)-Desformoterol	1600	1314	118

a. The deposited dose was calculated using a 0.09 deposition factor based upon a MMAD ± GSD for (R,R)-formoterol of approximately of 1.02 ± 2.08 µm (Gravimetric) and 0.93 ± 2.52 µm (Analytical). Particle sizes were not determined for (S,S)-formoterol, racemic (R,R/S,S)-formoterol, or (R,R)-desformoterol, although, they were considered to be similar to (R,R)-formoterol.
b. Groups 4 and 6 were sacrificed following a 2-day observation period and the heart was processed for microscopic evaluation. All other toxicology groups were sacrificed following a 14-day observation period.

Toxicokinetic Groups (9 rats/sex/group)

Group	Test Article	Target Dose (µg/kg)	Inhaled Dose (µg/kg)	Deposited Dose[a] (µg/kg)
7	(R,R)-Formoterol	40	26	2.3
8	(R,R)-Formoterol	200	154	14
9	(R,R)-Formoterol	400	365	33
10	(R,R)-Formoterol	1600	1927	173
15	(S,S)-Formoterol	1600	1976	178
16	(R,R/S,S)-Formoterol	1600	986	89
17	(R,R)-Desformoterol	1600	1580	142

a. The deposited dose was calculated using a 0.09 deposition factor based upon a MMAD ± GSD for (R,R)-formoterol of approximately of 1.02 ± 2.08 µm (Gravimetric) and 0.93 ± 2.52 µm (Analytical). Particle sizes were not determined for (S,S)-formoterol, racemic (R,R/S,S)-formoterol, or (R,R)-desformoterol.

b(4)

INHALATION TABLE 2

PROJECT NO. — -312020 COMPARATIVE ACUTE INH. STUDY OF FORMOTEROL IN RATS PAGE 1

SPONSOR:SEPRACOR INC. EXPOSURE CONCENTRATIONS AND INHALED DOSE LEVELS FOR INDIVIDUAL EXPOSURES

DATE	GROUP	SEX	MINUTE VOLUME	MEAN BODY WEIGHT	EXPOSURE DURATION	EXPOSURE CONC.	TARGET EXPOSURE CONC.	% OF TARGET CONC.	INHALED DOSE	TARGET INHALED DOSE	% OF TARGET DOSE
			L/MIN	KG	MIN	ug/L	ug/L		ug/KG	ug/KG	
08/03/98	1	M/F	0.2	0.232	15	2.6	3.1	83	33	40	83
		M		0.239		2.2	3.1	71			
		F		0.225		3.0	3.1	96			
08/10/98	2	M/F	0.2	0.256	15	14	17	82	165	200	82
		M		0.282		14	19	74			
		F		0.229		14	15	93			
08/13/98	3	M/F	0.2	0.273	15	40	36	109	435	400	109
		M		0.299		43	40	107			
		F		0.247		37	33	111			
08/26/98	4	M/F	0.2	0.321	60	44	43	103	1649	1600	103
		M		0.370		41	49	83			
		F		0.271		47	36	131			
08/26/98	5	M/F	0.2	0.308	60	44	41	107	1719	1600	107
		M		0.347		41	46	88			
		F		0.268		47	36	131			
08/04/98	7	M/F	0.2	0.250	15	2.2	3.3	65	26	40	65
08/11/98	8	M/F	0.2	0.284	15	15	19	77	154	200	77
08/14/98	9	M/F	0.2	0.294	15	36	39	91	365	400	91
08/28/98	10	M/F	0.2	0.332	60	53	44	120	1927	1600	120
01/07/99	11	M/F	0.2	0.283	60	35	38	92	1463	1600	91
12/30/98	12	M/F	0.2	0.250	60	46	33	138	2208	1600	138
01/12/99	13	M/F	0.2	0.288	60	21	38	53	854	1600	53
01/05/99	14	M/F	0.2	0.274	60	30	37	82	1314	1600	82
12/31/98	15	M/F	0.2	0.255	60	42	34	124	1976	1600	124
01/08/99	16	M/F	0.2	0.286	60	24	38	62	986	1600	62
01/06/99	17	M/F	0.2	0.281	60	37	37	100	1580	1600	99

M = MALE, F = FEMALE, L/MIN = LITERS/MINUTE, ug/L = MICROGRAMS/LITER, ug/KG = MICROGRAMS/KILOGRAM BODY WEIGHT

GROUP 6 = UNEXPOSED CONTROL GROUP

Route, form, volume, and infusion rate: Inhalation (nose only) exposure

Immediately prior to exposure to the designated test article, each group was placed into exposure tubes for at least 15 min before exposure to an aerosol of saline (0.9% NaCl) for 15 min. Animals were exposed to a test atmosphere containing an aerosol of the designated test article for a single 15 or 60 min period. Exposure duration for Groups 1-3 and 7-9 was 15 min. Groups 4, 5, and 10-17 were exposed to the appropriate test article for 60 min. For each dose level, animals in toxicology groups were generally exposed first and animals in toxicokinetic groups were exposed on the following day.

A nose-only exposure system was used for this study. Filtered air was supplied to the generation apparatus. Saline or test aerosols were delivered from a nebulizer (Collision nebulizer manufactured by [redacted] to a modified glass and plexiglass chromatography jar used as a mixing plenum. The test atmosphere was delivered to the nose-only system through respiratory tubing. A vacuum was used to capture and remove aerosol from the nose-only system. The nose-only system was operated under approximately 0.4-0.5 inches of water of negative pressure. b(4)

Actual exposure concentrations of free base were determined by chemical analysis of aerosol samples collected on filters. For each 15 min exposure, one aerosol sample of the exposure atmosphere was collected on a 25-mm glass-fiber filter. For each 60 min exposure, two samples were collected. Total sample volumes were determined by recording the sampling airflow rate (1 LPM for all samples) and the sample collection time. The mass of formoterol free base on each filter was determined using an HPLC method. The actual exposure concentration (as free base) was calculated by dividing the analytically determined mass of free base by the sample volume.

Observations and times:

Clinical signs: Rats were monitored twice daily for moribundity/mortality. Animals were observed for clinical signs of toxicity prior to exposure and immediately following exposure. Physical examinations were conducted on all toxicology animals on the day prior to exposure and on the day following exposure. Physical examinations were also conducted on animals in Group 4 on post-exposure day 2 and on all animals in other toxicology groups on post-exposure days 7 and 14. Groups 4 and 6 were observed daily for a 2-day period following exposure. All other toxicology groups were observed daily for a 14-day period following exposure.

Body weights: Body weights were measured on the day prior to exposure and on post-exposure days 7 and 14. Body weights for animals in Group 4 were conducted on the day prior to exposure and prior to necropsy on post-exposure day 2.

Food consumption: Not performed.

Ophthalmoscopy: Not performed.

EKG: Electrocardiograms (EKG) for determination of heart rate were conducted for each rat. Animals were restrained in nose-only exposure holding tubes that were modified to allow EKG electrode leads to be attached to the chest and to each hindlimb. During 15 min exposures for Groups 1, 2, and 3, EKGs were recorded for 3-5 min near the end of the saline aerosol exposure, for 3-5 min near the end of the test article exposure, and at 2 hr post-exposure. During 60-min exposures for Groups 4, 5, 11, 12,

13, and 14, EKGs were recorded during the first and last 15 min of the exposure period. The heart rate determined during the last 15 min of the exposure period was reported and used for comparison to heart rate during saline pre-exposure. If test article-related heart rate changes were observed for any group, EKGs were recorded at 4 hr following completion of exposure.
Hematology: Not performed.
Clinical chemistry: Not performed.
Urinalysis: Not performed.
Gross pathology: Necropsy examinations were conducted on all toxicology animals. Groups 4 and 6 were sacrificed following a 2-day observation period. All other toxicology groups were sacrificed following a 14-day observation period. The heart was collected from each animal and placed in 10% neutral buffered formalin.
Organs weighed: Not performed.
Histopathology: Hearts from Groups 4 and 6 were processed, embedded in paraffin blocks, sectioned at 5 to 8 µm, and stained with hematoxylin and eosin. Microscopic examinations of heart tissue sections were conducted.
Toxicokinetics: Blood samples were collected from 3 rats/sex/group at 0.5, 1, and 2 hr after dosing. Plasma concentrations of formoterol were measured using a validated LC/MS/MS method. The lower limit of quantitation was 2.50 pg/mL. The validated quantitation range was 2.50 to 200 pg/mL using a 1.0-mL sample volume. The assay method lacked chiral specificity and concentration data were consequently expressed as formoterol. The method was not validated to quantify desformoterol, although, its MRM mass channel was determined and acquired for qualitative purposes. The signal for the desformoterol MRM mass channel was assumed to be approximately the same as that for formoterol. Ratios of the MRM signal for ⊏ ⊐ internal standard were used for the semiquantitative assessment of desformoterol plasma exposure following inhalation of (R,R)-desformoterol. b(4)

Results:

Mortality: One male rat in Group 4 that was exposed to (R,R)-formoterol at a deposited dose of 148 µg/kg died during the test article exposure period. Gross necropsy findings for this animal included a pale spleen and red matting on the external surface.

Clinical signs: There were no treatment-related clinical signs.

Electrocardiography: (R,R)-formoterol at deposited doses of 132 to 155 µg/kg increased heart rate in male and female rats by 132 and 119% of baseline rates, respectively. Heart rate returned to the baseline rate by 2 hr after exposure in female rats, while heart rate was still elevated in male rats at 4 hr after exposure. (R,R/S,S)-Formoterol at 77 µg/kg and (R,R)-desformoterol at 118 µg/kg increased heart rate in male rats by 135.6 and 128.5% of baseline rates, respectively; however, these compounds had minimal effects on heart rate in female rats. (S,S)-Formoterol at 199 µg/kg had no effect on heart rate in male or female rats.

Heart rate (bpm) changes following exposure to (R,R)-formoterol at doses of 132 to 155 µg/kg, (S,S)-Formoterol at 199 µg/kg, (R,R/S,S)-formoterol at 77 µg/kg, and (R,R)-desformoterol at 118 µg/kg. The nominal dose for all compounds was 1600 µg/kg. The values in parentheses represent the percent of the saline pre-exposure.

Period	(R,R)-Formoterol 132-155 µg/kg		(S,S)-Formoterol 199 µg/kg		(R,R/S,S)-Formoterol 77 µg/kg		(R,R)-Desformoterol 118 µg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Saline pre-exposure	432	481	450	503	388	397	425	541
During exposure	569 (132%)	571 (118.7)	509 (113%)	507 (101%)	526 (136%)	456 (115%)	546 (128%)	547 (101%)
2 hr post-exposure	497 (115%)	487 (101%)	393 (87%)	476 (95%)	447 (115%)	448 (113%)	488 (115%)	539 (99.6%)
4 hr post-exposure	479 (111%)	498 (103%)	-	-	453 (117%)	-	496 (117%)	519 (95.9%)

Gross pathology: There were no treatment-related gross pathological effects.

Histopathology: Microscopic examination was limited to the heart collected from the unexposed control (Group 6) and 148 µg/kg (R,R)-formoterol (Group 4) groups at 2 days after exposure. Cardiomyopathy (minimal to mild) was observed for 2 of 6 rats in the unexposed control group and 5 of 5 animals in the 148 µg/kg (R,R)-formoterol group. The occurrence of cardiomyopathy in unexposed control rats at an incidence of 33% would appear to be unusual.

Histopathological examination of the heart from Group 6 (Unexposed control) and Group 4 (Exposed to (R,R)-Formoterol at a deposited dose of 148.4 µg/kg).

Heart	Unexposed control (Group 6)		148 µg/kg (R,R)-Formoterol	
	Male	Female	Male	Female
Number examined	3	3	2	3
Cardiomyopathy				
-minimal	0	2	2	1
-mild	0	0	0	2
-total	0	2	2	3

Toxicokinetics: AUC and C_{max} levels for formoterol following exposure to (R,R)-formoterol at doses ranging from 40 to 1600 µg/kg increased in a dose proportional manner. A sex-related effect was evident as AUC and C_{max} levels were higher in female rats as compared to male rats. AUC and C_{max} levels for formoterol following exposure to 1600 µg/kg (S,S)-formoterol or 1600 µg/kg (R,R/S,S)-formoterol in male and female rats were similar. AUC and C_{max} levels for desformoterol following exposure to (R,R)-desformoterol were higher in female rats as compared to male rats.

Formoterol (Desformoterol*) toxicokinetic parameters following inhalation of 40, 200, 400, and 1600 µg/kg (R,R)-formoterol, 1600 µg/kg (S,S)-formoterol, 1600 µg/kg (R,R/S,S)-formoterol, and 1600 µg/kg desformoterol in male and female rats.

Test Article	$AUC_{0.5\text{-}2hr}$, pg·hr/mL		C_{max}, pg/mL		T_{max}, hr	
	Males	Females	Males	Females	Males	Females
(R,R)-Formoterol, 40 µg/kg (Dep. Dose 2.3 µg/kg)	847	1110	819	1200	0.50	0.50
(R,R)-Formoterol, 200 µg/kg (Dep. Dose 14 µg/kg)	3740	5090	4160	5800	0.50	0.50
(R,R)-Formoterol, 400 µg/kg (Dep. Dose 33 µg/kg)	8570	12700	6510	14700	1.00	0.50
(R,R)-Formoterol, 1600 µg/kg (Dep. Dose 173 µg/kg)	67600	76500	78500	66600	0.50	0.50
(S,S)-Formoterol, 1600 µg/kg (Dep. Dose 178 µg/kg)	18000	16100	19500	20200	0.50	0.50
(R,R/S,S)-Formoterol, 1600 µg/kg (Dep. Dose 89 µg/kg)	26800	27700	24600	23200	0.50	0.50
(R,R)-Desformoterol*, 1600 µg/kg (Dep. Dose 142 µg/kg)	148	624	133	877	2.00	2.00

Summary of individual study findings: Rats were exposed by acute inhalation to (R,R)-formoterol at nominal doses ranging from 40 to 1600 µg/kg (deposited doses ranging from 3 to 155 µg/kg), (S,S)-formoterol at a nominal dose of 1600 µg/kg (deposited dose of 199 µg/kg), (R,R/S,S)-formoterol at a nominal dose of 1600 µg/kg (deposited dose of 76.9 µg/kg), or (R,R)-desformoterol at a nominal dose of 1600 µg/kg (deposited dose of 118 µg/kg). Observation periods following exposure were 2 or 14 days. One male rat exposed to (R,R)-formoterol at a deposited dose of 148 µg/kg died during the test article exposure period. (R,R)-formoterol at deposited doses of 132 to 155 µg/kg increased heart rate in male and female rats to 132 and 119% of baseline rates, respectively. Heart rate returned to the baseline rate by 2 hr after exposure in female rats, although, it was still elevated in male rats at 4 hr after exposure. (R,R/S,S)-Formoterol at 77 µg/kg and (R,R)-desformoterol at 118 µg/kg increased heart rate in male rats to 136 and 128% of baseline rates, respectively; however, these compounds had minimal effects on heart rate in female rats. (S,S)-Formoterol at 199 µg/kg had no effect on heart rate in male or female rats. Microscopic examination was limited to the heart collected from the unexposed control and 148 µg/kg (R,R)-formoterol groups at 2 days after treatment. Cardiomyopathy (minimal to mild) was observed for 2 of 6 rats in the unexposed control group and 5 of 5 animals in the 148 µg/kg (R,R)-formoterol group.

Subacute/Subchronic Toxicity

Rats

Study title: Amendment to the Final Report for the 28-Day Inhalation Toxicity Study of (R,R)-Formoterol in Rats.

Study no: Sepracor Document number 090-817
Volume #, and page #: Amendment #029, Volume 2, Pages 1-5
Conducting laboratory and location: ┌

b(4)

GLP compliance: Yes
QA report: yes (X) no ( ) ┘

The original report was evaluated in the review dated December 20, 1999. The sponsor submitted this amendment to correct a misidentified organ. On Page 13 of the Summary, the first sentence of the concluding paragraph was reworded to indicate that there were testicular, not renal, effects. In Page 48 of the Discussion and Conclusions, the first sentence of the concluding paragraph was reworded to indicate that there were testicular, not renal, effects.

These errors in the report appeared to have no impact on the review of this study, as testicular effects were identified and reported.

Toxicology summary:

Mice were exposed by acute nose-only inhalation to (R,R)-formoterol at nominal doses of 400, 800, and 1600 µg/kg (deposited doses of 29.7-31.3, 50-54, and 102-125 µg/kg, respectively). There was no treatment-related mortality. Microscopic examination was limited to the heart from animals in the 102 µg/kg (R,R)-formoterol and unexposed control groups at 2 days after treatment. Cardiomyopathy, characterized by small aggregates of mononuclear inflammatory cells in foci of myofiber degeneration (vacuolated and/or hyalinized sarcoplasm) in the inner third of the left ventricle, was observed for 4 of 6 males and 2 of 6 females treated with (R,R)-formoterol at a deposited dose of 102 µg/kg. Cardiomyopathy was graded as minimal. For an additional female at 102 µg/kg, macrophage infiltrate without myofiber degeneration was observed. There were no findings of cardiomyopathy in hearts from the unexposed control group.

Rats were exposed by acute inhalation to (R,R)-formoterol at nominal doses ranging from 40 to 1600 µg/kg (deposited doses ranging from 3 to 155 µg/kg), (S,S)-formoterol at a nominal dose of 1600 µg/kg (deposited dose of 199 µg/kg), (R,R/S,S)-formoterol at a nominal dose of 1600 µg/kg (deposited dose of 77 µg/kg), or (R,R)-desformoterol at a nominal dose of 1600 µg/kg (deposited dose of 118 µg/kg). Observation periods following exposure were 2 or 14 days. One male rat exposed to (R,R)-formoterol at a deposited dose of 148 µg/kg died during the test article exposure period. (R,R)-formoterol at deposited doses of 132 to 155 µg/kg increased heart rate in male and female rats to 132 and 119% of baseline rates, respectively. (R,R/S,S)-Formoterol at 77 µg/kg and (R,R)-desformoterol at 118 µg/kg increased heart rate in

male rats; however, these compounds had minimal effects on heart rate in female rats. (S,S)-Formoterol at 199 µg/kg had no effect on heart rate. Microscopic examination was limited to the heart collected from the unexposed control and 148 µg/kg (R,R)-formoterol groups at 2 days after treatment. Cardiomyopathy (minimal to mild) was observed for 2 of 6 rats in the unexposed control group and 5 of 5 animals in the 148 µg/kg (R,R)-formoterol group.

An amendment to the final report for the 28-day inhalation toxicity study of (R,R)-formoterol in rats has no impact on the evaluation of this study in the review dated December 20, 1999.

Toxicology conclusions: Acute inhalation exposure of mice and rats to (R,R)-formoterol at deposited doses of 102 and 148 µg/kg, respectively, produced cardiomyopathy by 2 days after treatment.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Rats

Study title: A Study of the Effects of (R,R)-Formoterol on Fertility and Early Embryonic Development to Implantation in Rats.

Key study findings:

♦ 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 mating indexes in male and female rats.

♦ There were no effects on estrus cyclicity in female rats prior to mating. Spermatogenic endpoints were unaffected. In mated female rats sacrificed on day 15, there were no treatment-related effects on numbers of corpora lutea/dam, implantation sites/dam, viable embryos/dam, or resorptions/dam. There were no effects on pre- and post-implantation loss per dam.

Study no.: Sepracor Document Number 090-831
Volume #, and page #: Amendment #030, Volumes 1 and 2, Pages 1-601
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: October 15, 1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-(L)-Tartrate, Lot number 010799A
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Sprague-Dawley rats — :CD®(SD)IGS BR) were obtained from [redacted] For male rats at the start of treatment, animals were approximately 11 weeks old and had a body weight range of 316 to 460 g. For female rats at the start of treatment, animals were approximately 13 weeks old and had a body weight range of 220 to 323 g.
Doses employed: 0, 1, 5, and 10 mg/kg/day (Doses are expressed in terms of the free base). b(4)
Route of administration: Vehicle or drug solutions were administered by oral gavage using a dose volume of 5 mL/kg.
Study design: Fertility and reproductive parameters were evaluated in male and female rats that received (R,R)-formoterol at doses of 0, 1, 5, and 10 mg/kg/day. For male rats, treatment with (R,R)-formoterol was initiated 30 days prior to mating and dosing continued until female rats reached day 14 of gestation. For female rats, treatment with

(R,R)-formoterol was initiated 14 -15 days prior to mating and dosing continued until day 7 of gestation. Animals were monitored twice daily for mortality and moribundity. Detailed clinical observations were recorded daily for each rat throughout the study period. Animals were also observed for clinical signs of toxicity at 1 hr after dosing. Body weights for male rats were measured twice per week during the treatment period. Body weights for female rats were measured twice per week during the pre-mating and mating periods. Body weights for female rats, with confirmed mating, were measured on days 0, 3, 7, 10, 13, and 15 of gestation. Food consumption during the pre-mating period was measured twice per week. Food consumption was measured on days 0, 3, 7, 10, 13, and 15 of gestation. Vaginal smears were prepared to assess the regularity and duration of the estrous cycle for each female rat for 10 days prior to mating and continuing until evidence of mating was observed or to the end of the breeding period. Male and female rats were paired on a 1 to 1 basis within each group for a maximum of 15 days. If no evidence of mating was obtained after 10 days, the female rat was placed with another male for an additional 5 days. Female rats, with confirmed mating, were sacrificed on day 15 of gestation and submitted to a necropsy examination. The uterus and ovaries were examined. The number of corpora lutea in each ovary was determined. The number and location of all embryos, early resorptions, and total number of implantation sites in the uterus were recorded. Viability of embryos was determined. Male rats were sacrificed and submitted to necropsy examination. The right testis and epididymis were excised and weighed separately. Sperm were obtained from the right cauda epididymis for evaluation of motility and morphology. Abnormal forms of sperm (i.e., double heads, double tails, microcephalic and megacephalic, etc.) from a differential count of 200 spermatozoa per animal were recorded. The left testis and epididymis from all males were weighed, homogenized, and evaluated for determination of homogenization-resistant spermatid count. Organs and tissues were collected from male and female rats and preserved for possible future histopathological examination as follows: cervix, coagulating gland, epididymis (2) ovaries and oviduct (2), pituitary, prostate, seminal vesicles, testes, uterus with vagina, vas deferens, and all gross lesions. The testes, epididymides, ovaries, brain, and pituitary gland were weighed for all F_0 rats that were sacrificed at scheduled termination.
Number/sex/group: 25 rats/sex/group
Parameters and endpoints evaluated: Fertility and reproductive parameters.

Results:

Mortality: There was no treatment-related mortality. Male #36220 in the 1 mg/kg/day group was sacrificed in moribund condition on day 25. Clinical signs prior to sacrifice included hypoactivity, labored respiration, decrease defecation, and a mass on the lateral abdominal area prior to sacrifice. A necropsy examination of this animal found enlarged, pale kidneys with a dark red area in the left cortex and a dilated pelvis. In addition, a distended urinary bladder with calculi and apparent blockage, and distended ureters were also noted for this animal.

Clinical signs: A number of nonspecific clinical signs were increased in incidence for male and female treatment groups. The incidence of dried red material around the eyes, nose, and mouth was increased for male and female treatment groups, although, dose-response relationships were not always present. The incidence of soft stool was

increased for male treatment groups. The incidence of redness of the ears was increased for female treatment groups.

Clinical signs for male and female rats that received (R,R)-formoterol at oral doses of 0, 1, 5, and 10 mg/kg/day (Total occurrence/number of animals).

Clinical Sign	Sex	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Dried red material around right eye	M	0/0	74/2	67/6	17/5
	F	0/0	0/0	4/4	3/3
Dried red material round left eye	M	0/0	79/3	48/8	7/5
	F	0/0	6/1	5/2	3/2
Dried red material around nose	M	25/13	53/17	51/17	76/16
	F	22/9	47/13	27/11	42/16
Dried red material round mouth	M	1/1	1/1	0/0	4/1
	F	-	-	-	-
Soft stool	M	5/4	13/7	27/13	18/9
	F	1/1	-	-	2/2
Right ear appears red in color	M	-	-	-	-
	F	8/2	20/2	32/2	35/1
Left ear appears red in color	M	-	-	-	-
	F	7/2	20/2	30/1	36/1

Body weight: There were no treatment-related effects on body weight gain for either male or female rats. Body weights for male controls on days 0 and 60 were 388 and 530 g, respectively. Body weight gains for male rats at 1, 5, and 10 mg/kg/day were 118, 124.7, and 114.9% of the control, respectively. Body weights for female controls on days 16 (dosing initiated) and 29 were 258 and 271 g, respectively. Body weight gains for female rats at 1, 5, and 10 mg/kg/day from days 16 to 29 were 275.85, 304.7, and 283.5% of the control, respectively. Body weights for female controls on days 0 and 7 of gestation were 275 and 307 g, respectively. Body weight gains for female rats at 1, 5, and 10 mg/kg/day from days 0 to 7 of gestation were 100, 99.7, and 100.6% of the control, respectively.

Food consumption: There were no treatment-related effects on food consumption (expressed as either g/animal/day or g/kg/day) for either male or female rats. This result does not agree with weight gains observed in male and female treatment groups.

In-life observations:

Fertility and Reproductive Performance: Female estrous cyclicity prior to pairing was unaffected by (R,R)-formoterol. There were no treatment-related effects on mating and fertility indexes in male and female rats. The pre-coital interval was unaffected by (R,R)-formoterol. One female (#36310) with no detected evidence of mating, delivered prior to scheduled necropsy.