Mating and Fertility Indexes for male and female rats that (R,R)-formoterol at doses of 0, 1, 5, and 10 mg/kg/day.

Parameter	Sex	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Mating Index, %	M	96.0% (24/25)	100% (24/24)	96.0% (24/25)	96.0% (24/25)
	F	100% (25/25)	100% (25/25)	100% (25/25)	100% (25/25)
Fertility Index, %	M	88.0% (22/25)	95.8% (23/24)	92.0% (23/25)	96.0% (24/25)
	F	92.0% (23/25)	96.0% (24/25)	96.0% (24/25)	100% (25/25)
Pre-Coital Interval, days	M/F	3.6	2.8	3.4	3.2

Terminal and necroscopic evaluations:

Male Rats:

Spermatogenic Endpoint Evaluations: There were no treatment-related effects on testicular and epididymal sperm number (e.g., the left testis and left epididymis were used to determine the number of sperm in millions/g tissue), sperm production rate (e.g., the left testis was used to determine the sperm in millions/g tissue), sperm motility, and sperm morphological differential count.

Gross pathological findings: Absolute and relative weights for the right epididymis, left epididymis, right cauda epididymis, and left cauda epididymis for male treatment groups were slightly decreased; however, these changes appeared to have no toxicological significance as there were no functional consequences on fertility or reproductive performance. Absolute and relative weights for the right epididymis for male treatment groups were decreased to 87.1-91.4% and 84-88.6% of control values (0.70 g and 0.131%), respectively. Absolute and relative weights for the left epididymis for male treatment groups were decreased to 87-91.3% and 81.5-87.7% of control values (0.69 g and 0.130%), respectively. Absolute and relative weights for the right cauda epididymis for male treatment groups were decreased to 86.1-89.3% and 81.4-86.4% of control values (0.3121 g and 0.059%), respectively. Absolute and relative weights for the left cauda epididymis were decreased to 87.7-91.9% and 82.8-87.9% of control values (0.3084 g and 0.058%), respectively. There were no treatment-related gross pathological findings.

Female Rats:

Embryonic data: Mated female rats were sacrificed on day 15 of gestation. 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.

Embryonic data at scheduled necropsy.

Parameter	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Gravid females	23	23	24	25
Corpora lutea/dam	17.2 (396/23)	17.2 (396/23)	18.2 (436/24)	18.6 (465/25)
Pre-implantation loss/dam	2.0 (46/23)	0.9 (20/23)	1.8 (43/24)	1.4 (36/25)
Implantation sites/dam	15.2 (350/23)	16.3 (376/23)	16.4 (393/24)	17.2 (429/24)
Post-implantation loss/dam	1.0 (22/23)	1.2 (27/23)	0.8 (18/24)	1.2 (30/25)
Resorptions/dam -early -late	 1.0 (22/23) 0	 1.2 (27/23) 0	 0.8 (18/24) 0	 1.2 (30/25) 0
Viable embryos	14.3 (328/23)	15.2 (349/23)	15.6 (375/24)	16.0 (399/25)
Dead embryos	0	0	0	0

Maternal gross pathological findings: There were no treatment-related gross pathological findings in F_0 dams. There were no treatment-related changes in absolute and relative weights for the brain, ovaries, and the pituitary gland.

Summary of individual 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. 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. (R,R)-formoterol at oral doses ≤10 mg/kg/day had no effects on fertility or mating indexes in male and female rats. Spermatogenic endpoints (i.e., number of sperm, sperm production, motility, and morphology) 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. There were no treatment-related gross pathological findings in male or female F_0 rats. Absolute and relative weights for the right epididymis, left epididymis, right cauda epididymis, and left cauda epididymis for male treatment groups were slightly decreased; however, these changes appeared to have no toxicological significance as there were no functional consequences on fertility or reproductive performance.

Study Title: A Dose Range-Finding Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rats.

Key study findings:

♦ In a dose range finding (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 0, 10, 20, 40, 80, and 160 mg/kg/day.

♦ Maternal toxicity was evident at 160 mg/kg/day as mortality occurred at this dose.

♦ (R,R)-formoterol was teratogenic as external malformations were evident with doses of 80 and 160 mg/kg/day. Anasarca was observed for 1 fetus at 80 mg/kg/day and 2 fetuses at 160 mg/kg/day. One of these fetuses at 160 mg/kg/day was also observed with ablepharia (bilateral) and mandibular and maxillary micrognathia. Localized fetal

edema (neck and thorax) was observed for 1 fetus at 160 mg/kg/day. The role of maternal toxicity for these observed effects is unknown. Many of these findings (i.e., anasarca and localized fetal edema) were reproduced in the definitive embryofetal development study.

Study no.: Sepracor Document number 090-813, 1998
Volume #, and page #: Amendment #014, Volume 1, Pages 1 to 257
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: November 20, 1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-(L)-tartrate, Lot number RH924-96 (Purity, 99.3%). The active component, (R,R)-Formoterol free base, represented 70% of the test article.
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Female Sprague-Dawley rats [redacted] CD®(SD)IGS BR rats] were obtained from [redacted] Following an acclimation period, each female rat was placed with a resident male rat of the same strain and source for breeding. Selected female rats were approximately 12 weeks old when paired for breeding. Positive evidence of mating was confirmed by presence of a copulatory plug or the presence of sperm in a vaginal smear. The body weight range for mated female rats on day 0 of gestation was 214 to 306 g. b(4)
Doses employed: (R,R)-Formoterol was administered at oral doses of 0, 10, 20, 40, 80, and 160 mg/kg/day.
Route of administration: (R,R)-Formoterol doses of 0, 10, 20, 40, 80, and 160 mg/kg/day were administered by oral gavage using dose volumes of 10, 0.625, 1.25, 2.5, 5, and 10 mL/kg, respectively.
Study design: Mated female rats received (R,R)-formoterol at doses of 10, 20, 40, 80, or 160 mg/kg/day from days 6 to 17 of gestation. Animals were checked twice per day for moribundity and mortality. Clinical observations were conducted prior to dosing during the treatment period. Animals were observed for clinical signs of toxicity at 1 hr after dosing. Female rats that died were necropsied the same day. The number and location of implantation sites and corpora lutea and the number of fetuses were recorded. Tissues were preserved for possible future examination. Body weights and food consumption were measured on days 0, 6 through 18, and 20. All surviving mated female rats were sacrificed on day 20 and submitted to necropsy examination. The uterus and ovaries were removed. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and later resorptions, and the total number of implantation sites were determined. Maternal tissues were preserved for possible future examination. A detailed external examination of each fetus was conducted and included the eyes, palate, and external orifices. Each was then sexed, weighed, euthanized, and discarded.
Number/sex/group: 8 mated female rats/group

Parameters and endpoints evaluated: The potential maternal toxicity and developmental toxicity of (R,R)-formoterol-(L)-tartrate were evaluated.

Results:

Mortality: There were two treatment-related deaths at 160 mg/kg/day. Clinical signs for both animals that preceded death included labored respiration and extremities that appeared pale in color. Female #82933 died on day 9 of gestation. Female #82933 was also observed with prostration, lacrimation (bilateral), and eyes and ears that appeared pale in color. Necropsy examination found that this female had dark red lungs (all lobes) and 15 normally developing implantations in utero. Female #82954 died on day 15 of gestation. Necropsy examination found that this female had 17 normally developing implantations in utero.

Clinical signs: Clinical signs of toxicity were evident at 1 hr after dosing, primarily with doses of 80 and 160 mg/kg/day. At 160 mg/kg/day from days 14-17 of gestation, 6 animals were observed with limbs extended and in a flattened stance. Tan staining around the mouth and/or nose was observed in all (R,R)-formoterol treatment groups. Salivation was observed for rats at 80 and 160 mg/kg/day.

Clinical signs (total occurrence/# animals) at 1 hr after dosing with (R,R)-formoterol at 0, 10, 20, 40, 80, and 160 mg/kg/day.

Clinical Sign	0	10	20	40	80	160
Animals appear flattened, limbs extended	0	0	0	0	0	7/6
Dried tan staining around nose	1/1	0	0	0	5/2	7/5
Dried tan staining around mouth	0	4/3	1/1	13/6	20/7	27/8
Salivation	0	0	0	0	5/3	5/3
Red discharge material at vaginal opening	0	0	0	0	1/1	3/2

General clinical signs (total occurrence/# animals) from days 0 to 20 of gestation. During the treatment period, animals were observed prior to dosing with (R,R)-formoterol at 0, 10, 20, 40, 80, and 160 mg/kg/day.

Clinical Sign	0	10	20	40	80	160
Dried tan staining around nose	0	0	1/1	0	0	5/2
Dried tan staining around mouth	0	0	0	0	6/4	7/3
Dried red material around mouth	0	0	0	0	0	7/3

Body weight: Body weight gain from days 6 to 18 of gestation was unaffected by treatment with (R,R)-formoterol. Small body weight losses were observed for female rats at 40 and 80 mg/kg/day on day 7 and for female rats at 160 mg/kg/day on days 7 and 8. However, body weight gains for these (R,R)-formoterol groups exceeded the control group over the remaining period of treatment. Body weights for vehicle-control rats on days 6 and 18 were 283 and 358 g, respectively. Body weight gains for female

rats at 10, 20, 40, 80, and 160 mg/kg/day from days 6 to 18 were 134.4, 135.8, 142.2, 112.3, and 121.4% of the control, respectively.

Food consumption: No treatment-related effects on food consumption (g/animal/day or g/kg/day) were evident for the overall treatment period (i.e., days 6 to 18). However, food consumption was decreased from days 6-9 for rats at 10, 20, 40, 80, and 160 mg/kg/day and increased from days 9-12 and 12-18 for rats at 40, 80, and 160 mg/kg/day.

Food consumption from days 6-9 for rats at 10, 20, 40, 80, and 160 mg/kg/day was decreased to 85, 75, 80, 50, and 35% of the control (20 g/animal/day), respectively. Food consumption from days 9-12 for rats at 20, 40, 80, and 160 mg/kg/day were all increased to 114.3% of the control (21 g/animal/day), respectively. Food consumption from days 12-18 for rats at 20, 40, 80, and 160 mg/kg/day was increased to 113.6, 122.7, 118.2, and 131.8% of the control (22 g/animal/day), respectively.

Terminal and necroscopic evaluations:

Dams: Fetal body weight was slightly decreased to 92-94% of controls at doses of 80 and 160 mg/kg/day. There were no treatment-related effects on intrauterine growth and survival. Corpora lutea/dam, implantation sites/dam, pre-implantation loss/dam, viable fetuses/dam, dead fetuses/dam, post-implantation loss, and sex ratios were comparable between control and treatment groups.

Fetal data at scheduled necropsy for female rats that received (R,R)-formoterol at doses of 0, 10, 20, 40, 80, and 160 mg/kg/day.

Parameters	0	10	20	40	80	160
Number of gravid females	8	7	8	5	7	6
Corpora lutea/dam	17.5 (140/8)	16.7 (117/7)	17.6 (141/8)	18.0 (90/5)	18.7 (131/7)	17.5 (105/6)
Implantation sites/dam	16.4 (131/8)	16.0 (112/7)	14.9 (119/8)	16.0 (80/5)	15.3 (107/7)	16.3 (98/6)
Pre-implantation loss/dam	1.1 (9/8)	0.7 (5/7)	2.8 (22/8)	2.0 (10/5)	3.4 (24/7)	1.2 (7/6)
Viable fetuses/dam	16.1 (129/8)	15.0 (105/7)	14.4 (115/8)	15.4 (77/5)	14.3 (100/7)	15.5 (93/6)
Dead fetuses/dam	0	0	0	0	0	0
Resorptions						
-Early	0.3 (2/8)	1.0 (7/7)	0.5 (4/8)	0.6 (3/5)	0.9 (6/7)	0.8 (5/6)
-Late	0	0	0	0	0.1 (1/7)	0
-Total	0.3 (2/8)	1.0 (7/7)	0.5 (4/8)	0.6 (3/5)	1.0 (7/7)	0.8 (5/6)
Post-implantation loss/dam	0.3 (2/8)	1.0 (7/7)	0.5 (4/8)	0.6 (3/5)	1.0 (7/7)	0.8 (5/6)
Fetal body weight, g						
-Males	3.6	3.7	3.5	3.6	3.3	3.4
-Females	3.5	3.6	3.3	3.4	3.3	3.2
Sex						
-Males	8.9 (71/8)	8.1 (57/7)	6.5 (52/8)	7.0 (35/5)	6.1 (43/7)	8.2 (49/6)
-Females	7.3 (58/8)	6.9 (48/7)	7.9 (63/8)	8.4 (42/7)	8.1 (57/7)	7.3 (44/6)

Offspring: External malformations were evident at doses of 80 and 160 mg/kg/day. Anasarca was observed for 1 fetus (#82938-16) at 80 mg/kg/day and 2 fetuses (#82936-09 and -10) at 160 mg/kg/day. Localized fetal edema (neck and thorax) was observed for 1 fetus (#82921-02) at 160 mg/kg/day. Fetus #82936-09 at 160 mg/kg/day was also observed with ablepharia (bilateral) and mandibular and maxillary micrognathia. Localized fetal edema and fetal anasarca were observed in a second (Segment II) teratology study (Sepracor Document number 090-820) in which mated female rats received (R,R)-formoterol at doses of 10, 60, and 120 mg/kg/day. The role of maternal toxicity for these observed effects is unknown, although, many of these finding (i.e., anasarca and localized fetal edema) occurred in a reproducible manner in two independent studies. There were no findings of external variations.

Fetuses/litters with external malformations from dams treated with (R,R)-formoterol at doses of 0, 10, 20, 40, 80, and 160 mg/kg/day.

Parameter	0	10	20	40	80	160	Historical Control
Fetuses/Litters	129/8	105/7	115/8	77/5	100/7	93/6	50858/ 3574
Total external malformations	0	0	0	0	1/1	3/2	
Localized fetal edema	0	0	0	0	0	1 (1.1%)/ 1 (16.7%)	0.006%/ (0.0-0.3%)
Fetal anasarca	0	0	0	0	1 (1%)/ 1 (14.3%)	2 (2.2%)/ 1 (16.7%)	0.02%/ (0.0-0.4%)
Maxillary micrognathia	0	0	0	0	0	1 (1.1%)/ 1 (16.7%)	0.008%/ (0.0-1.8%)
Mandibular micrognathia	0	0	0	0	0	1 (1.1%)/ 1 (16.7%)	0.02%/ (0.0-0.4%)
Ablepharia	0	0	0	0	0	1 (1.1%)/ 1 (16.7%)	-

Summary of individual study findings: In a dose range finding (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 0, 10, 20, 40, 80, and 160 mg/kg/day. Maternal toxicity was evident at 160 mg/kg/day as mortality occurred at this dose. (R,R)-formoterol was teratogenic as external malformations were evident with doses of 80 and 160 mg/kg/day. Anasarca was observed for 1 fetus at 80 mg/kg/day and 2 fetuses at 160 mg/kg/day. One of these fetuses at 160 mg/kg/day was also observed with ablepharia (bilateral) and mandibular and maxillary micrognathia. Localized fetal edema (neck and thorax) was observed for 1 fetus at 160 mg/kg/day. The role of maternal toxicity for these observed effects is unknown. Many of these findings (i.e., anasarca and localized fetal edema) were reproduced in the definitive embryofetal development study.

Study Title: A Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rats.

Key study findings:

♦ In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 10, 60, or 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation.

♦ Maternal toxicity (i.e., death and clinical signs) was evident with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day.

♦ Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥10 mg/kg/day and racemic formoterol at 120 mg/kg/day. External malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related external malformations included omphalocele, localized fetal edema of the neck, anasarca, microphthalmia and/or anophthalmia, and micromelia. Omphalocele and microphthalmia and/or anophthalmia were not observed with racemic formoterol. The one incidence of malformation, omphalocele, at 10 mg/kg/day occurred independently of maternal toxicity. Skeletal malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related skeletal malformations that included bent limb bones and vertebral anomaly with or without associated rib anomaly were observed with both compounds. A finding of 1 fetus with 12 pairs of ribs was observed with only (R,R)-formoterol.

♦ Post-implantation loss, consisting primarily of early resorption, was increased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Number of viable fetuses and fetal body weight were decreased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day.

♦ An increased incidence of skeletal variations, consisting primarily of reductions in ossification, was evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Several variations at 10 mg/kg/day occurred independently of maternal toxicity.

Study no.: Sepracor Document number 090-820
Volume #, and page #: Amendment #030, Volumes 3 and 4, Pages 1 to 471
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: November 17, 1998
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity:
(R,R)-Formoterol-L-tartrate, Lot number 1193-15 [Isomeric Purity by HPLC: [redacted] (R,R)-isomer, [redacted] (S,R)-isomer, [redacted] (R,S)-isomer, and [redacted] (S,S)-Isomer; Impurities by HPLC: [redacted] Total]. The active component of (R,R)-formoterol-L-tartrate, (R,R)-formoterol free base, represents 70% of the test article.

Formoterol 0.5 Fumarate monohydrate [Impurities by HPLC: ⊏ ⊐ total]. The components of racemic formoterol fumarate, (R,R)-formoterol (active component) and (S,S)-formoterol free bases, represent ⊏ ⊐ of the test article. b(4)
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Female Sprague-Dawley rats — CD®(SD)IGS BR rats] were obtained from ⊏ ⊐ Following an acclimation period, each female rat was placed with a resident male rat of the same strain and source for breeding. Selected female rats were approximately 12 weeks old when paired for breeding. Positive evidence of mating was confirmed by presence of a copulatory plug or the presence of sperm in a vaginal smear. The body weight range for mated female rats on day 0 of gestation was 200 to 294 g. b(4)
Doses employed: (R,R)-formoterol was administered at oral doses of 10, 60, and 120 mg/kg/day. Racemic formoterol was administered at an oral dose of 120 mg/kg/day. Doses for each test article were expressed as Formoterol free base equivalents.
Route of administration: The vehicle or drug solution was administered by oral gavage. The vehicle was administered at a dose volume of 7.5 mL/kg. For (R,R)-formoterol administered at doses of 10, 60, and 120 mg/kg/day, dose volumes were 0.625, 3.75, and 7.5 mL/kg, respectively. For racemic formoterol administered at a dose of 120 mg/kg/day, the dose volume was 7.5 mL/kg.
Study design: Mated female rats received (R,R)-formoterol at doses of 10, 60, or 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day from days 6 to 17 of gestation. Animals were checked twice per day for moribundity and mortality. Animals were monitored for clinical signs of toxicity at 1 and 2 hr after dosing. Body weights and food consumption were measured on days 0, 6 through 18, and 20. Mated female rats were sacrificed on day 20 and submitted to necropsy examination. The uterus and ovaries were removed. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and later resorptions, and the total number of implantation sites were determined. Maternal tissues were preserved for possible future examination. A detailed external examination of each fetus was conducted and included the eyes, palate, and external orifices. The sex of each fetus was determined internally. A visceral examination of each fetus was conducted using a fresh dissection technique that included the heart and major vessels. The heads from approximately one-half of the fetuses in each litter were placed in Bouin's solution for subsequent processing and soft-tissue examination using Wilson's technique. The heads from remaining fetuses in each litter were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethanol. Following fixation in alcohol, each fetus was macerated in KOH and stained with Alizarin Red S using a modification of Dawson's method for subsequent skeletal examination.
Number/sex/group: 25 mated female rats/group
Parameters and endpoints evaluated: The potential maternal and developmental toxicity of (R,R)-formoterol and racemic formoterol were evaluated in the rat.

Results:

Mortality: Treatment-related mortality was observed with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. The majority of deaths

occurred on day 6 of gestation, the first day of treatment. One female in the 60 mg/kg/day (R,R)-formoterol group (#11971) and seven female rats in the 120 mg/kg/day (R,R)-formoterol group (#11903, 11919, 11948, 11965, 11980, 11881, and 12015) died on day 6. Two females in the 120 mg/kg/day racemic formoterol group died, one each on days 6 (#11926) and 9 (#11992). Clinical signs prior to death, consisting of prostration and gasping, were only observed for one female rat in the 120 mg/kg/day (R,R)-formoterol group. All animals were pregnant except for one in the 120 mg/kg/day (R,R)-formoterol group.

Clinical signs: Clinical signs of toxicity were observed for female rats that received (R,R)-formoterol at doses of 60 and 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day.

Altered posture (i.e., lying on their back, side, and/or abdomen with extended or splayed hindlimbs and/or forelimbs) was observed for 18 animals in the 60 mg/kg/day (R,R)-formoterol group, 16 animals in the 120 mg/kg/day (R,R)-formoterol group, and 21 animals in the 120 mg/kg/day racemic formoterol group. These observations occurred from days 13 to 17 of gestation at 1 and/or 2 hr after dosing.

Lacrimation was observed for 4 animals in the 60 mg/kg/day (R,R)-formoterol group, 6 animals in the 120 mg/kg/day (R,R)-formoterol group, and 7 animals in the 120 mg/kg/day racemic formoterol group. These observations occurred from days 6 to 8 of gestation at 1 hr after dosing.

Clinical signs at 1 hr after dosing (total occurrence/number of animals).

Clinical signs	(R,R)-Formoterol				Racemic Formoterol
	0 mg/kg/day	10 mg/kg/day	60 mg/kg/day	120 mg/kg/day	120 mg/kg/day
Lying on back; forelimbs/hindlimbs extended	0/0	0/0	3/3	15/10	10/8
Abdomen contacts surface, hindlimbs splayed	0/0	0/0	10/10	14/9	16/11
Lying on side; hindlimbs extended	0/0	0/0	10/9	6/6	12/9
Lacrimation, right eye	0/0	0/0	3/3	6/6	8/6
Lacrimation, left eye	0/0	0/0	4/3	7/6	8/6

Clinical signs at 2 hr after dosing (total occurrence/number of animals).

Clinical signs	(R,R)-Formoterol				Racemic Formoterol
	0 mg/kg/day	10 mg/kg/day	60 mg/kg/day	120 mg/kg/day	120 mg/kg/day
Lying on back; forelimbs/hindlimbs extended	0/0	0/0	0/0	7/7	2/2
Abdomen contacts surface, hindlimbs splayed	0/0	0/0	6/5	4/4	6/6
Lying on side;	0/0	0/0	1/1	12/8	8/7

hindlimbs extended					

Body weight: There were no treatment-related effects on body weight gains, although, body weights from days 6 to 7 of gestation were reduced for rats that received (R,R)-formoterol at 60 and 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Body weights for female controls on days 6, 7, and 18 were 284, 288, and 369 g, respectively. Body weights for female rats that received (R,R)-formoterol at doses of 60, and 120 mg/kg/day from days 6 to 7 were decreased by 3.8 and 6.25% of initial weights, respectively. Body weights for female rats that received racemic formoterol at a dose of 120 mg/kg/day from days 6 to 7 were decreased by 3.8% of initial weights, respectively. Body weight gains for female rats that received (R,R)-formoterol at doses of 10, 60, and 120 mg/kg/day from days 6 to 18 were increased to 131.6, 115.2, and 105.6% of the control, respectively. Body weight gains for female rats that received racemic formoterol at a dose of 120 mg/kg/day from days 6 to 18 were increased to 120.7% of the control.

Food consumption: Overall food consumption from days 6 to 18 of gestation was unaffected by treatment with either (R,R)-formoterol or racemic formoterol. However, food consumption for all treatment groups was decreased from days 6 to 9 and increased from days 9 to 12 and 12 to 18. Results were essentially the same, whether food consumption was expressed as g/animal/day or g/kg/day. From days 6 to 9, food consumption for female rats that received (R,R)-formoterol at doses of 10, 60, and 120 mg/kg/day was decreased to 85, 62.5, and 52.5% of the control (80 g/kg/day), respectively. From days 6 to 9, food consumption for female rats that received racemic formoterol at a dose of 120 mg/kg/day was decreased to 57.5% of the control. From days 9 to 12, food consumption for female rats that received (R,R)-formoterol at doses of 60 and 120 mg/kg/day was increased to 107.9 and 109.2% of the control (76 g/kg/day), respectively. From days 9 to 12, food consumption for female rats that received racemic formoterol at a dose of 120 mg/kg/day was increased to 107.9% of the control. From days 12 to 18, food consumption for female rats that received (R,R)-formoterol at doses of 10, 60, and 120 mg/kg/day was increased to 110.8, 114.9, and 118.9% of the control (74 g/kg/day), respectively. From days 12 to 18, food consumption for female rats that received racemic formoterol at a dose of 120 mg/kg/day was decreased to 116.2% of the control.

Terminal and necroscopic evaluations:

Dams: For dams sacrificed at scheduled termination on day 20 of gestation, post-implantation losses, consisting primarily of early resorption, were increased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Number of viable fetuses and fetal body weight were decreased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. (R,R)-formoterol at 10 mg/kg/day had no effects on post-implantation loss, viable litter size, or mean fetal body weight. Neither (R,R)-formoterol nor racemic formoterol had any effects on sex ratio. Gravid uterine weights for dams treated with (R,R)-formoterol at 120 mg/kg/day were decreased to 88.4% of the control (82.1 g).

Fetal Data at Scheduled Necropsy

Parameter	0 mg/kg/day	10 mg/kg/day	60 mg/kg/day	120 mg/kg/day	120 mg/kg/day (Racemic)
Number of gravid females	25	23	22	16	21
Corpora lutea/dam	17.3 (433/25)	17.7 (406/23)	17.3 (381/22)	18.6 (297/16)	18.2 (382/21)
Implantation sites/dam	15.4 (384/25)	15.8 (364/23)	15.5 (341/22)	15.6 (249/16)	15.6 (328/21)
Pre-implantation loss/dam	2.0 (49/25)	1.8 (42/23)	2.0 (45/22)	3.0 (48/16)	2.6 (54/21)
Viable fetuses/dam	14.8 (369/25)	15.4 (355/23)	13.6* (300/22)	13.1* (209/16)	14.3* (301/21)
Dead fetuses/dam	0	0	0	0	0
Resorptions -Early -Late -Total	 0.6 (15/25) 0 0.6 (15/25)	 0.4 (9/23) 0 0.4 (9/23)	 1.8 (39/22)* 0.1 (2/22) 1.9 (41/22)*	 2.4 (38/16)* 0.1 (2/16) 2.5 (40/16)*	 1.2 (25/21) 0.1 (2/21) 1.3 (27/21)*
Post-implantation loss/dam	0.6 (15/25)	0.4 (9/23)	1.9 (41/22)*	2.5 (40/16)*	1.3 (27/21)*
Fetal body weight, g -Males -Females	 3.7 3.5	 3.5 3.4	 3.3* 3.2*	 3.1* 2.9*	 3.3* 3.1*
Sex -Males -Females	 7.6 (191/25) 7.1 (178/25)	 7.5 (173/23) 7.9 (182/23)	 7.0 (155/22) 6.6 (145/22)	 6.3 (101/16) 6.8 (108/16)	 7.8 (163/21) 6.6 (138/21)

* $p < 0.05$

Offspring: External and skeletal malformation were evident for fetuses obtained from dams treated with (R,R)-formoterol or racemic formoterol.

External malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Omphalocele (i.e., several loops of the intestine protruded through an opening in the umbilicus, and remnants of a membranous sac were discernable) was evident at incidences that exceeded the historical controls for (R,R)-formoterol at 10, 60, and 120 mg/kg/day; however, this finding was not observed with racemic formoterol. Omphalocele observed at 10 mg/kg/day occurred independently of maternal toxicity. Localized fetal edema of the neck was evident at incidences that exceeded the historical controls for (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Microphthalmia and/or anophthalmia (i.e., moderate or severe reduction in size of the eye and/or complete absence of eyes or presence of vestigial eyes) was evident at incidences that exceeded the historical controls for (R,R)-formoterol at 60 and 120 mg/kg/day; however, there were no findings with racemic formoterol. Anasarca (i.e., generalized massive edema) was evident at incidences that exceeded the historical controls for (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Micromelia (i.e., shorter than normal femora, tibiae, and/or fibulae and bent scapule, radii, femora, and fibulae and/or ulnae) was

evident at incidences that exceeded the historical controls for (R,R)-formoterol at 120 mg/kg/day and racemic formoterol at 120 mg/kg/day.

Skeletal malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Bent limb bones were evident at incidences that exceeded the historical controls for (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Vertebral anomaly with or without associated rib anomaly was evident at incidences that exceeded the historical controls for (R,R)-formoterol at 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. The finding of only 12 pairs of ribs present was evident for one fetus in the 120 mg/kg/day (R,R)-formoterol group.

A visceral malformation, consisting of heart and/or great vessel anomaly (i.e., accessory aorta arose from ascending aorta, coursed retroesophageal, and rejoined the descending aorta; the right carotid and right subclavian arteries arose independently from the aortic arch; no brachiocephalic trunk) was observed for one fetus in the 120 mg/kg/day (R,R)-formoterol group.

External, visceral, and skeletal malformations for fetuses from dams that received (R,R)-formoterol at doses of 10, 60, and 200 mg/kg/day or racemic formoterol at a dose of 200 mg/kg/day from days 6 through 17 of gestation. Data is expressed as number of fetuses (%)/number of litters (%).

Parameter	(R,R)-Formoterol				Racemic Formoterol	Historical Control[a], % Fetuses % Litters
	0	10	60	120	120 (Racemic)	
Fetuses/litters	369/25	355/23	300/22	209/16	301/21	
External (Total Effected)	1/1	1/1	7/7	9/6	3/3	
Localized fetal edema	0	0	3 (1%)/ 3 (13.6%)	2 (1%)/ 2 (12.5%)	1 (0.3%)/ 1 (4.8%)	0.01%/ (0-0.3%)
Micromelia	0	0	0	2 (1%)/ 2 (12.5%)	1 (0.3%)/ 1 (4.8%)	0.002%/ (0-0.3%)
Microphthalmia and/or anophthalmia	0	0	1 (0.3%)/ 1 (4.6%)	1 (0.5%)/ 1 (6.3%)	0	0.06%/ (0-1.3%)
Anasarca	0	0	4 (1.3%)/ 4 (18.2%)	6 (2.9%)/ 4 (25%)	2 (0.7%)/ 2 (9.5%)	0.02%/ (0-0.4%)
Omphalocele	0	1 (0.3%)/ 1 (4.6%)	1 (0.3%)/ 1 (4.6%)	2 (1%)/ 1 (6.3%)	0	0.03%/ (0-1.7%)
Visceral (Total Effected)	0	0	0	1/1	0	
Heart and/or great vessel anomaly	0	0	0	1 (0.5%)/ 1 (6.3%)	0	0.04%/ (0-1.9%)
Skeletal (Total Effected)	0	0	18/8	12/7	8/4	
Bent limb bones	0	0	18 (6%)/ 8 (36.4%)	10 (4.8%)/ 6 (37.5%)	7 (2.3%)/ 3 (14.3%)	0.007%/ (0-0.5%)
Vertebral anomaly with or without associated rib anomaly	0	0	0	1 (0.5%)/ 1 (6.3%)	1 (0.3%)/ 1 (4.8%)	0.06%/ (0-0.7%)
Only 12 pairs of ribs	0	0	0	1 (0.5%)/	0	0.0025%/

present				1 (6.3%)		(0-0.5%)

a. The historical control consists of the mean percent of fetuses affected over the range of percent litter effects.

An increased incidence of skeletal variations, consisting primarily of reductions in ossification, were evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Variations at 10 mg/kg/day occurred independently of maternal toxicity. Increased incidences of sternebra(e) #5 and/or # 6 unossified, sternebra(e) #1, #2, #3, and/or #4 unossified, and bent ribs, that exceeded historical controls, were observed for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. The proportion of cervical centrum #1 ossified was decreased for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. An increased incidence of reduced ossification of the vertebral arches, that exceeded historical controls, was observed for (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Increased incidences of entire sternum unossified and pubis unossified, that exceeded historical controls, were observed for (R,R)-formoterol at 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. An increased incidence of reduced ossification of the 13th rib(s), that slightly exceeded historical controls, was observed for (R,R)-formoterol at 60 and 120 mg/kg/day. An increased incidence of hyoid unossified, that exceeded historical controls, was observed for (R,R)-formoterol at 10 mg/kg/day or racemic formoterol at 120 mg/kg/day, although, the treatment relationship of the finding for (R,R)-formoterol at 10 mg/kg/day was questionable. Increased incidences of 7th cervical ribs, 14th rudimentary ribs, 27 presacral vertebrae, 14th full ribs, sternebra(e) misaligned (slight or moderate), and reduced ossification of the skull were observed for (R,R)-formoterol and/or racemic formoterol treatment group(s) as compared to the concurrent control; however, incidences were generally within the historical control range and there treatment-relationship was somewhat questionable.

Skeletal variations for fetuses from dams that received (R,R)-formoterol at doses of 10, 60, and 200 mg/kg/day or racemic formoterol at a dose of 200 mg/kg/day from days 6 through 17 of gestation.

Parameter	(R,R)-Formoterol				Racemic Formoterol	Historical Control[a], % Fetuses % Litters
	0	10	60	120	120 (Racemic)	
Fetuses/litters	369/25	355/23	300/22	209/16	301/21	40676/ 3574
Sternebra(e) #5 and/or #6 unossified	12 (3.3%)/ 7 (28%)	83 (23.4%)/ 13 (56.5%)	59 (19.7%)/ 14 (63.6%)	78 (37.3%)/ 13 (81.3%)	123 (40.9%)/ 14 (66.7%)	10.45%/ (0.6-37.5%)
Sternebra(e) #1, #2, #3, and/or #4 unossified	0	1 (0.3%)/ 1 (4.4%)	3 (1%)/ 3 (13.6%)	18 (8.6%)/ 9 (56.3%)	18 (6%)/ 7 (33.3%)	3.5%/ (0-3.5%)
Bent ribs	0	12 (3.4%)/ 5 (21.7%)	64 (21.3%)/ 15 (68.2%)	36 (17.2%)/ 9 (56.3%)	37 (12.3%)/ 14 (66.7%)	0.8%/ (0-4.6%)
Cervical centrum #1 ossified	60(16.3%)/ 16 (64%)	13 (3.7%)/ 7 (30.4%)	8 (2.7%)/ 4 (18.2%)	2 (0.96%)/ 2 (12.5%)	6 (2%)/ 4 (19%)	8.8%/ (0-31.6%)
Entire sternum unossified	0	0	0	1 (0.5%)/ 1 (6.3%)	4 (1.3%)/ 2 (9.5%)	0.03%/ (0-1%)
Reduced ossification of the 13th rib(s)	1 (0.3%)/ 1 (4%)	4 (1.1%)/ 2 (8.7%)	5 (1.7%)/ 4 (18.2%)	9 (4.3%)/ 2 (12.5%)	1 (0.3%)/ 1 (4.8%)	1.63%/ (0-11.5%)
7th cervical rib(s)	0	2 (0.6%)/ 1 (4.4%)	1 (0.3%)/ 1 (4.6%)	4 (1.9%)/ 2 (12.5%)	1 (0.3%)/ 1 (4.8%)	0.6%/ (0-5.6%)
Pubis unossified	0	0	0	1 (0.5%)/ 1 (6.3%)	1 (0.3%)/ 1 (4.8%)	0.05%/ (0-2%)
Reduced ossification of the vertebral arches	0	0	3 (1%)/ 3 (13.6%)	4 (1.9%)/ 4 (25%)	3 (1%)/ 3 (14.3%)	0.09%/ (0-1.7%)
27 presacral vertebrae	0	0	0	0	1 (0.3%)/ 1 (4.8%)	0.22%/ (0-4.2%)
14th full ribs	0	0	0	1 (0.5%)/ 1 (6.3%)	0	0.7%/ (0-1.3%)
Hyoid unossified	3 (0.8%)/ 2 (8%)	22 (6.2%)/ 9 (39.1%)	3 (1%)/ 2 (9.1%)	2 (1%)/ 2 (12.5%)	13 (4.3%)/ 6 (28.6%)	0.8%/ (0-14.3%)
Sternebra(e) misaligned (slight or moderate)	0	0	0	3 (1.4%)/ 2 (12.5%)	0	1.22%/ (0-14.1%)
14th rudimentary ribs	10 (2.7%)/ 6 (24%)	11 (3.1%)/ 6 (26.1%)	14 (4.7%)/ 5 (22.7%)	7 (3.4%)/ 2 (12.5%)	18 (6%)/ 4 (19%)	4.6%/ (0-39.3%)
Reduced ossification of the skull	0	0	0	1 (0.5%)/ 1 (6.3%)	1 (0.3%)/ 1 (4.8%)	0.09%/ (0-6.1%)

a. The historical control consists of the mean percent of fetuses effected over the range of percent litter effects.

Summary of individual study findings:

In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 10, 60, or 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation.

Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥10 mg/kg/day and racemic formoterol at 120 mg/kg/day.

Maternal toxicity was evident with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Treatment-related mortality was observed with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. The majority of deaths occurred on day 6 of gestation, the first day of treatment. Clinical signs of toxicity were observed for female rats that received (R,R)-formoterol at doses of 60 and 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day. Clinical signs consisted of altered posture (i.e., lying on their back, side, and/or abdomen with extended or splayed hindlimbs and/or forelimbs) and lacrimation.

For dams sacrificed at scheduled termination on day 20 of gestation, post-implantation loss, consisting primarily of early resorption, was increased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Number of viable fetuses and fetal body weight were decreased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day.

External malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Omphalocele was evident for (R,R)-formoterol at 10, 60, and 120 mg/kg/day; however, this finding was not observed with racemic formoterol. The single occurrence of omphalocele at 10 mg/kg/day occurred independently of maternal toxicity. Localized fetal edema of the neck and anasarca was evident for (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Microphthalmia and/or anophthalmia were evident at incidences that exceeded historical controls for (R,R)-formoterol at 60 and 120 mg/kg/day; however, there were no findings with racemic formoterol. Micromelia was evident at incidences that exceeded the historical controls for (R,R)-formoterol at 120 mg/kg/day and racemic formoterol at 120 mg/kg/day.

Skeletal malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Bent limb bones were evident for (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Vertebral anomaly with or without associated rib anomaly was evident for (R,R)-formoterol at 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. The finding of only 12 pairs of ribs present was evident for one fetus in the 120 mg/kg/day (R,R)-formoterol group.

A visceral malformation, consisting of heart and/or great vessel anomaly was observed for one fetus in the 120 mg/kg/day (R,R)-formoterol group.

An increased incidence of skeletal variations, consisting primarily of reductions in ossification, was evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Skeletal variations at 10 mg/kg/day occurred independently of maternal toxicity. Increased incidences of sternebra(e) #5 and/or # 6 unossified, sternebra(e) #1, #2, #3, and/or #4 unossified, and bent ribs were observed for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. The proportion of cervical centrum #1 ossified was decreased for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. An increased incidence of reduced ossification of the vertebral

arches was observed for (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Increased incidences of entire sternum unossified and pubis unossified were observed for (R,R)-formoterol at 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. An increased incidence of reduced ossification of the 13th rib(s) was observed for (R,R)-formoterol at 60 and 120 mg/kg/day. Increased incidences of 7th cervical ribs, 14th rudimentary ribs, 27 presacral vertebrae, 14th full ribs, sternebra(e) misaligned (slight or moderate), and reduced ossification of the skull were observed for (R,R)-formoterol and/or racemic formoterol treatment group(s) as compared to the concurrent control; however, incidences were generally within the historical control range and their treatment-relationship was somewhat questionable.

Study Title: A Study of the Effects of (R,R)-Formoterol and Racemic Formoterol on Embryo/Fetal Development in Rats.

Key study findings:

♦ In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at doses of 1, 5, or 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation.

♦ There was no evidence of maternal toxicity with (R,R)-formoterol at ≤10 mg/kg/day.

♦ Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥1 mg/kg/day and racemic formoterol at 10 mg/kg/day. An external malformation, omphalocele, was observed with fetuses in the 1, 5, and 10 mg/kg/day (R,R)-formoterol groups, although, there was not a dose-response relationship. Omphalocele was reported in the previous teratology study (Sepracor Document number 090-820) with (R,R)-formoterol doses of 10, 60, and 120 mg/kg/day. The NOAEL for omphalocele appears to be <1 mg/kg/day. Omphalocele occurred independently of maternal toxicity. Umbilical herniation of the intestine (i.e., several loops of the intestine protruded through an opening in the umbilicus) was observed for 1 fetus in the 10 mg/kg/day racemic formoterol group.

♦ Skeletal variations consisting of decreased ossification were increased for all (R,R)-formoterol groups as well as the racemic formoterol group.

♦ On gestation days 6 or 17, the dose of 1 mg/kg/day produced an AUC that was approximately 750 to 900 times the AUC observed with a human inhalation dose of 48 µg/day; however, it should be noted that 1 mg/kg/day is not a NOAEL. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Study no.: Sepracor Document number 090-825, 2001
Volume #, and page #: Amendment #030, Volumes 5 and 6, Pages 1 through 511
Conducting laboratory and location:

Date of study initiation: July 8, 1999

b(4)

GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity:
(R,R)-Formoterol-L-tartrate, Lot number 010799A
The active component of (R,R)-formoterol-L-tartrate, (R,R)-formoterol free base, represents 70% of the test article.
Formoterol 0.5 Fumarate monohydrate, Lot number BX9041
The components of racemic formoterol fumarate, (R,R)-formoterol (active component) and (S,S)-formoterol free bases, represent [redacted] of the test article. b(4)
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Female Sprague-Dawley rats [—:CD®(SD)IGS BR rats] were obtained from [redacted] Following an acclimation period, each female rat was placed with a resident male rat of the same strain and source for breeding. b(4) Selected female rats were approximately 12 weeks old when paired for breeding. Positive evidence of mating was confirmed by presence of a copulatory plug or the presence of sperm in a vaginal smear. The body weight range for mated female rats on day 0 of gestation was 232 to 308 g.
Doses employed: (R,R)-formoterol was administered at oral doses of 1, 5, and 10 mg/kg/day. Racemic formoterol was administered at an oral dose of 10 mg/kg/day. Doses for each test article were expressed as Formoterol free base equivalents.
Route of administration: The vehicle or drug solutions were administered by oral gavage using a dose volume of 5 mL/kg.
Study design: Mated female rats received (R,R)-formoterol at doses of 1, 5, or 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 to 17 of gestation. Animals were checked twice per day for moribundity and mortality. Female rats that died were necropsied the same day. The number and location of implantation sites and corpora lutea and the number of pups were recorded. Pups and maternal gross lesions were preserved for possible future examination. Animals were monitored for clinical signs of toxicity at 1 and 2 hr after dosing. Body weights and food consumption were measured on days 0, 6 through 18, and 20. All surviving mated female rats were sacrificed on day 20 and submitted to necropsy examination. The uterus and ovaries were removed. The number of corpora lutea on each ovary was determined. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and later resorptions, and the total number of implantation sites were determined. Maternal gross lesions were preserved for possible future examination. A detailed external examination of each fetus was conducted and included the eyes, palate, and external orifices. The sex of each fetus was determined internally. A visceral examination of each fetus was conducted using a fresh dissection technique that included the heart and major vessels. The heads from approximately one-half of the fetuses in each litter were placed in Bouin's solution for subsequent processing and soft-tissue examination using Wilson's technique. The heads from remaining fetuses in each litter were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethanol. Following fixation in alcohol, each fetus was macerated in KOH and stained with Alizarin Red S using a modification of Dawson's method for subsequent skeletal examination.
Number/sex/group: 25 mated female rats/group

Parameters and endpoints evaluated: The potential maternal and developmental toxicity of (R,R)-formoterol and racemic formoterol were evaluated in the rat.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs. One dam (#28876) that received (R,R)-formoterol at 5 mg/kg/day delivered on gestation day 20 due to an error in the detection of mating. Seven pups were delivered and no external malformations were evident for any of the pups.

Body weight: There were no treatment-related effects on body weight gain. Body weights for female control dams on days 6 and 18 were 294 and 375 g, respectively. Body weight gains for female dams that received (R,R)-formoterol at 1, 5, or 10 mg/kg/day were 126.4, 128.5, and 131.8% of the control, respectively. The body weight gain for female dams that received racemic formoterol at 10 mg/kg/day was 130.9% of the control.

Food consumption: Overall food consumption from days 6 to 18 of gestation was unaffected by treatment with either (R,R)-formoterol or racemic formoterol. However, food consumption was slightly decreased from days 6 to 9 for (R,R)-formoterol groups at 5 and 10 mg/kg/day and slightly increased from days 12 to 18 for all treatment groups. Results were essentially similar, whether food consumption was expressed as g/animal/day or g/kg/day. Food consumption from days 6 to 9 for (R,R)-formoterol groups at 5 and 10 mg/kg/day were both decreased to 87% of the control (23 g/animal/day). Food consumption from days 12 to 18 for (R,R)-formoterol and racemic formoterol treatment groups were all increased to 108% of the control (25 g/animal/day).

Toxicokinetics: AUC values for (R,R)-formoterol increased with elevating dose. AUC values for (R,R)-formoterol at 5 and 10 mg/kg/day were less than dose proportional as compared to 1 mg/kg/day. AUC values for (R,R)-formoterol at 5 and 10 mg/kg/day were approximately dose proportional. The dose of 1 mg/kg/day administered to mated female rats on gestation days 6 or 17 produced an AUC that was approximately 750 to 900 times the AUC observed with a human dose of 48 µg/day; however, it should be noted that 1 mg/kg/day is not a NOAEL.

Formoterol toxicokinetic parameters following daily oral gavage dosing of 1, 5, and 10 mg/kg/day (R,R)-formoterol and 10 mg/kg/day racemic formoterol for 12 days in female rats.

Formoterol Oral Dose, mg/kg/day	$AUC_{0\text{-}24hr}$, ng.hr/mL		Exposure Ratio for Human AUC = 0.0338 ng·hr/mL[a]		C_{max}, ng/mL		T_{max}, hr	
	GD 6	GD17	GD 6	GD 17	GD 6	GD17	GD 6	GD17
(R,R)-Formoterol								
1	30.9	25.4	914	751	1.92	1.52	6.0	0.5
5	69.9	75.8	2068	2243	6.79	9.84	0.5	0.5
10	120	165	3550	4882	7.96	17.6	6.0	0.5
Racemic Formoterol								
10	72.5	130	-	-	6.18	13.1	0.5	0.5

a. (R,R)-Formoterol administered to human volunteers by inhalation at a dose of 48 µg/day produced an AUC of 0.0338 ng·hr/mL (Study No. 091-004). This dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Terminal and necroscopic evaluations:

Dams: Gravid uterine weights were unaffected by treatment. There were no treatment-related effects on numbers of corpora lutea/dam, implantation sites/dam, viable fetuses/dam, dead fetuses/dam, pre-implantation loss/dam, post-implantation loss/dam, and resorptions/dam. There were also no effects on sex ratios or fetal body weights.

Fetal data at scheduled necropsy.

Parameter	Control	(R,R)-Formoterol			Racemic Formoterol
	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	10 mg/kg/day	10 mg/kg/day
Number of gravid female rats	25	24	24	24	24
Corpora lutea/dam	18.5 (462/25)	18.8 (451/24)	18.8 (450/24)	18.3 (440/24)	18.0 (433/24)
Implantation sites/dam	16.2 (406/25)	16.3 (391/24)	16.3 (391/24)	16.3 (390/24)	16.3 (392/24)
Pre-implantation loss/dam	2.2 (56/25)	2.5 (60/24)	2.5 (59/24)	2.1 (50/24)	1.7 (41/24)
Viable fetuses/dam	15.3 (383/25)	15.5 (373/24)	15.5 (372/24)	15.8 (378/24)	15.7 (376/24)
Dead fetuses/dam	0	0	0	0	0
Resorptions/dam -early -late -total	 0.9 (23/25) 0 0.9	 0.7 (17/24) 0 0.7	 0.8 (19/24) 0 0.8	 0.5 (12/24) 0 0.5	 0.7 (16/24) 0 0.7
Post-implantation loss/dam	0.9 (23/25)	0.7 (17/24)	0.8 (19/24)	0.5 (12/24)	0.7 (16/24)
Sex -males -females	 7.2 (180/25) 8.1 (203/25)	 7.5 (179/24) 8.1 (194/24)	 7.9 (189/24) 7.6 (183/24)	 7.3 (176/24) 8.4 (202/24)	 7.8 (186/24) 7.9 (202/24)
Fetal body weight, g -males -females	 3.8 3.6	 3.8 3.6	 3.7 3.6	 3.7 3.6	 3.8 3.6

Offspring:

Omphalocele (i.e., several loops of the intestine protruded through an opening in the umbilicus and remnants of a membranous sac were discernible) was observed with fetuses in the 1, 5, and 10 mg/kg/day (R,R)-formoterol groups, although, there was not a dose-response relationship. Omphalocele was reported in the previous teratology study (Sepracor Document number 090-820) with (R,R)-formoterol doses of 10, 60, and 120 mg/kg/day. The NOAEL for omphalocele appears to be <1 mg/kg/day. Omphalocele occurred independently of maternal toxicity. Umbilical herniation of the intestine (i.e., several loops of the intestine protruded through an opening in the umbilicus) was observed for 1 fetus in the 10 mg/kg/day racemic formoterol group. The sponsor has contended that umbilical herniation of the intestine and omphalocele are generally considered within a similar classification, the former is the result of incomplete retraction of the intestine and the latter is an abdominal closure defect involving protrusion of the abdominal viscera. There were no treatment-related visceral or skeletal malformations.

External, visceral, and skeletal malformations for fetuses from dams that received (R,R)-formoterol at doses of 1, 5, and 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 through 17 of gestation. Data is expressed as number of fetuses (%)/number of litters (%).

Parameter	(R,R)-Formoterol				Racemic Formoterol	Historical Control[a], % Fetuses % Litters
	0	1	5	10	10 (Racemic)	
Fetuses/litters	383/25	373/24	372/24	378/24	376/24	
External (Total Effected)	0	1/1	3/2	1/1	1/1	
Omphalocele	0	1 (0.3%)/ 1 (4.2%)	3 (0.8%)/ 2 (8.3%)	1 (0.3%)/ 1 (4.2%)	0	0.03%/ (0-1.7%)
Umbilical herniation of intestine	0	0	0	0	1 (0.3%)/ 1 (4.2%)	0.01%/ (0-0.4%)
Anury	0	0	1 (0.3%)/ 1 (4.2%)	0	0	
Skeletal (Total Effected)	0	0	2/2	0	0	
Vertebral anomaly with or without associated rib anomaly	0	0	2 (0.5%)/ 2 (8.3%)	0	0	0.06%/ (0-0.7%)

a. The historical control consists of the mean percent of fetuses effected over the range of percent litter effects.

Incidences of unossified sternebrae #5 and/or #6 were increased for all (R,R)-formoterol groups as well as the racemic formoterol group. Incidences of cervical centrum #1 ossified were decreased for all (R,R)-formoterol groups as well as the racemic formoterol group. The incidence of 14th rudimentary rib was increased for fetuses in the 10 mg/kg/day (R,R)-formoterol group. Bent ribs and 27 presacral vertebrae were each observed for 1 fetus in the 10 mg/kg/day (R,R)-formoterol group. Fourteen (14) full ribs was observed for 1 fetus in each of the 1 and 10 mg/kg/day (R,R)-formoterol groups, although, a dose-response relationship was not evident. In the

previous teratology study (Sepracor Document number 090-820), increased incidences of sternebra(e) #5 and/or # 6 unossified and bent ribs were observed for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. In addition, the proportion of cervical centrum #1 ossified was decreased for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day.

A visceral variation, major blood vessel variation, was observed for 1 and 2 fetuses in the 1 and 10 mg/kg/day (R,R)-formoterol groups, respectively, although, there was no dose-response relationship. There were no treatment-related external variations. In previous teratology study (Sepracor Document number 090-820), no treatment-related external or visceral variations were observed with (R,R)-formoterol at doses of 10, 60, and 120 mg/kg/day.

These skeletal and visceral variations occurred independently of maternal toxicity.

Visceral and skeletal variations for fetuses from dams that received (R,R)-formoterol at doses of 1, 5, and 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 through 17 of gestation.

Parameter	(R,R)-Formoterol				Racemic Formoterol	Historical Control[a], % Fetuses % Litters
	0	1	5	10	10 (Racemic)	
Fetuses/litters	383/25	373/24	372/24	378/24	376/24	40676/ 3574
Visceral						
Major blood vessel variation	0	1 (0.3%)/ 1 (4.2%)	0	2 (0.5%)/ 1 (4.2%)	0	0.05%/ (0.0-1.5%)
Skeletal						
Sternebra(e) #5 and/or #6 unossified	33 (8.6%)/ 13 (52%)	55 (14.8%)/ 11 (45.8%)	59 (15.9%)/ 13 (54.2%)	45 (11.9%)/ 13 (54.2%)	106 (28.2%)/ 18 (75%)	10.45%/ (0.6-37.5%)
Cervical centrum #1 ossified	51 (13.3%)/ 15 (60%)	27 (7.2%)/ 13 (54.2%)	23 (6.2%)/ 11 (45.8%)	25 (6.6%)/ 11 (45.8%)	15 (4%)/ 9 (37.5%)	8.8%/ (0-31.6%)
14th rudimentary ribs	18 (4.7%)/ 12 (48%)	29 (7.8%)/ 11 (45.8%)	18 (4.8%)/ 8 (33.3%)	51 (13.5%)/ 15 (62.5%)	19 (5%)/ 9 (37.5%)	4.6%/ (0-39.3%)
Bent ribs	0	0	0	1 (0.3%)/ 1 (4.2%)	1 (0.3%)/ 1 (4.2%)	0.8%/ (0-4.6%)
27 presacral vertebrae	0	0	0	1 (0.3%)/ 1 (4.2%)	0	0.22%/ (0-4.2%)
14th full ribs	0	1 (0.3%)/ 1 (4.2%)	0	1 (0.3%)/ 1 (4.2%)	0	0.7%/ (0-1.3%)
25 presacral vertebrae	0	0	0	0	1 (0.3%)/ 1 (4.2%)	0.16%/ (0-3.8%)

a. The historical control consists of the mean percent of fetuses effected over the range of percent litter effects.

Summary of individual study findings:

In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at doses of 1, 5, or 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation.

There was no evidence of maternal toxicity with (R,R)-formoterol at doses ≤10 mg/kg/day.

Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥1 mg/kg/day and racemic formoterol at 10 mg/kg/day.

An external malformation, omphalocele, was observed with fetuses in the 1, 5, and 10 mg/kg/day (R,R)-formoterol groups, although, there was not a dose-response relationship. Omphalocele was reported in the previous teratology study (Sepracor Document number 090-820) with (R,R)-formoterol doses of 10, 60, and 120 mg/kg/day. The NOAEL for omphalocele appears to be <1 mg/kg/day. Omphalocele occurred independently of maternal toxicity. Umbilical herniation of the intestine (i.e., several loops of the intestine protruded through an opening in the umbilicus) was observed for 1 fetus in the 10 mg/kg/day racemic formoterol group. There were no treatment-related visceral or skeletal malformations.

Incidences of unossified sternebrae #5 and/or #6 were increased for all (R,R)-formoterol groups as well as the racemic formoterol group. Incidences of cervical centrum #1 ossified were decreased for all (R,R)-formoterol groups as well as the racemic formoterol group. These variations were independent of maternal toxicity. In the previous teratology study (Sepracor Document number 090-820), increased incidences of sternebra(e) #5 and/or # 6 unossified and bent ribs were observed for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. In addition, the proportion of cervical centrum #1 ossified was decreased for (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. In the present study, there were no apparent treatment-related external or visceral variations.

On gestation days 6 or 17, the dose of 1 mg/kg/day produced an AUC that was approximately 750 to 900 times the AUC observed with a human inhalation dose of 48 µg/day; however, it should be noted that 1 mg/kg/day is not a NOAEL. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Rabbits

Study Title: A Dose Range-Finding Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rabbits.

Key study findings:

♦ In a dose range finding (Segment II) teratology study, artificially inseminated rabbits received (R,R)-formoterol at oral doses of 0, 20, 40, 80, 160, and 320 mg/kg/day from days 7 to 20 of gestation.

♦ Maternal toxicity was evident at doses of 40, 80, 160, and 320 mg/kg/day. Mortality occurred at doses of 40, 80, 160, and 320 mg/kg/day. All rabbits in the 160 and 320 mg/kg/day were euthanized on day 13 due to excessive toxicity. One female at 80 mg/kg/day aborted on day 28.

♦ (R,R)-formoterol appeared to be teratogenic as a treatment-related external malformation was observed at 80 mg/kg/day. Adactyly was observed at an incidence that exceeded concurrent and historical controls. The role of maternal toxicity in this finding is unknown. Adactyly was reproduced in the embryofetal development study with oral doses of 20, 40, and 80 mg/kg/day.

♦ Post-implantation loss was increased at 80 mg/kg/day.

Study no.: Sepracor Document number 090-812, 2000
Volume #, and page #: Amendment #029, Volume 2, Pages 1 to 261
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: November 20, 1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-(L)-tartrate, Lot number 121697A
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Sexually mature, virgin female New Zealand White rabbits were obtained from [redacted] b(4) Female rabbits were artificially inseminated using sperm collected from 6 resident male rabbits of the same strain and supplier. Immediately following insemination, each female rabbit received human chorionic gonadotropin by intravenous administration. Selected female rabbits were approximately 6.5 months old at initiation of insemination. The body weight range for selected female rabbits on day 0 of gestation was 3390 to 3920 g.
Doses employed: (R,R)-formoterol was administered at oral doses of 0, 20, 40, 80, 160, and 320 mg/kg/day from days 7 to 20 of gestation.
Route of administration: Vehicle and drug solutions were administered by oral gavage using a dose volume of 4 mL/kg.
Study design:

Potential maternal toxicity and developmental toxicity of (R,R)-formoterol (L)-tartrate were evaluated in this study. (R,R)-formoterol was administered by oral gavage at doses of 0, 40, 80, 160, and 320 mg/kg/day to 6 artificially inseminated female rabbits per group from days 7 to 20 of gestation. Dose selection was based upon a preliminary toxicity study in which (R,R)-formoterol was administered to non-mated female rabbits at oral doses of 80, 160, 320, and 640 mg/kg/day for 3 days. Mortality occurred for 1 of 3 rabbits at 160 mg/kg/day and 1 of 3 rabbits at 320 mg/kg/day. Body weight losses were evident at 160, 320, and 640 mg/kg/day. At 640 mg/kg/day, decreased defecation as well as rapid respiration 1-hr after dosing were observed for all animals. The 1 animal that died at 640 mg/kg/day was observed with dark red lungs, foamy contents in the trachea, and red fluid contents in the thoracic cavity.

Basal diet and reverse osmosis-treated drinking water were provided *ad libitum* during the study period. Rabbits were monitored twice per day for moribundity and mortality. Animals were observed for clinical signs of toxicity at 1 hr after dosing. Gross examinations were conducted on female rabbits that died during the study period. Maternal tissues were preserved for possible future histopathological examination. Body weights were measured on days 0, 7 to 21, 24, and 29 of gestation. Food consumption was measured daily from days 0 to 29 of gestation. All surviving female rabbits were sacrificed on day 29 of gestation. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and total number of implantation sites were recorded. Maternal tissues were preserved for possible future histopathological examination. Each fetus was weighed and an external examination was conducted that included the eyes, palate, and external orifices. The sex of each fetus was determined internally. Each fetus was euthanized and discarded.
Number/sex/group: 6 artificially inseminated female rabbits/group
Parameters and endpoints evaluated: Potential maternal toxicity and developmental toxicity of (R,R)-formoterol (L)-tartrate.

Results:

Mortality: Between days 7 to 11 of gestation, death occurred for 1 of 6 rabbits at 40 mg/kg/day (day 11), 1 of 6 rabbits at 80 mg/kg/day (day 7), 3 of 6 rabbits at 160 mg/kg/day (all died on day 7), and 4 of 6 rabbits at 320 mg/kg/day (2 died on day 7, 1 died on day 9, and 1 died on day 11). Gross necropsy examinations were conducted all rabbits that died. At 40 mg/kg/day, the 1 female that died had a reddened trachea, dark red areas in the lungs, a distended, gas-filled colon, and normally developing implantations in utero. At 80 mg/kg/day, the 1 rabbit that died had a reddened trachea, dark red lobes of the lungs, and normally developing implantations in utero. At 160 mg/kg/day, the 3 rabbits that died were found to have a reddened trachea, dark red lobes of the lungs, and normally developing implantations in utero. At 320 mg/kg/day, 3 of the 4 rabbits that died were found to have a reddened trachea, dark red areas in the lungs, and/or red lungs. One rabbit at 320 mg/kg/day had foamy contents in the trachea. On day 13 of gestation, the sponsor elected to sacrifice all remaining rabbits at 160 and 320 mg/kg/day due to excessive toxicity (i.e., death, body weight loss, decreased food consumption), and all animals were discarded without further examination.

Clinical signs: One female (#26629) at 80 mg/kg/day aborted on day 28 of gestation. It was unclear if this abortion was treatment-related, although, abortions were observed with a dose of 80 mg/kg/day in the second teratology study with rabbits (Sepracor Document number 090-819).

Body weight: Body weight losses were evident for rabbits at 160 and 320 mg/kg/day and remaining animals in these groups were sacrificed on day 13. There were no treatment-related effects on body weight gains for rabbits at doses of 20, 40, and 80 mg/kg/day.

On day 13 of gestation, body weights for rabbits at 160 and 320 mg/kg/day had decreased by 1.5 and 5.8%, respectively, of body weights on day 7.

Body weights for vehicle-control rabbits on days 7 and 21 were 3931 and 4157 g, respectively, yielding a 5.75% increase of body weight. Body weights on day 21 for rabbits at 20, 40, and 80 mg/kg/day were increased by 6.2, 8.1, and 8.6%, respectively, as compared to body weights on day 7. From days 7 to 9 of gestation, body weight was decreased by 0.7% for rabbits at 80 mg/kg/day as compared to 0.08% increase for vehicle-controls.

Food consumption: Food consumption (g/animal/day or g/kg/day) for rabbits at 80, 160, and 320 mg/kg/day was decreased from days 7-10 and 10-13. Examination of food consumption for the entire treatment period of days 7 to 21 found no treatment-related effects for rabbits at 20, 40, and 80 mg/kg/day. For days 7 to 10, food consumption for rabbits at 20, 40, 80, 160, and 320 mg/kg/day was decreased to 81.5, 87.2, 67.8, 50.7, and 21.3% of the control (211 g/animal/day), respectively. For days 10 to 13, food consumption for rabbits at 80, 160, and 320 mg/kg/day was decreased to 87.9, 71.4, and 58.8% of the control (182 g/animal/day), respectively. For days 7 to 21, food consumption for rabbits at 20, 40, and 80 mg/kg/day were 84.2, 94.2, and 83.7% of the control (190 g/animal/day), respectively; however, no treatment-related effect could be inferred given the lack of a dose-response relationship.

Terminal and necroscopic evaluations:

Dams: Post-implantation loss was increased for rabbits at 80 mg/kg/day as compared to vehicle-controls. Fetal body weight was reduced at 40 and 80 mg/kg/day. At scheduled necropsy on day 29 of gestation, 1 female at 80 mg/kg/day was found to have dark red areas on all lobes of the lungs.

Fetal Data at Scheduled Necropsy

Parameter	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day
Number of gravid females	6	6	4	3
Corpora lutea/dam	12.3 (74/6)	12.2 (73/6)	14.8 (59/4)	13.0 (39/3)
Implantation sites/dam	7.5 (45/6)	5.3 (32/6)	8.8 (35/4)	8.7 (26/3)
Pre-implantation loss/dam	4.8 (29/6)	6.8 (41/6)	6.0 (24/4)	4.3 (13/3)
Viable fetuses/dam	6.3 (38/6)	5.0 (30/6)	8.8 (35/4)	6.0 (18/3)
Dead fetuses/dam	0	0	0	0
Resorptions -Early -Late -Total	 1.0 (6/6) 0.2 (1/6) 1.2 (7/6)	 0.3 (2/6) 0 0.3 (2/6)	 0 0 0	 1.7 (5/3) 1.0 (3/3) 2.7 (8/3)
Post-implantation loss/dam	1.2 (7/6)	0.3 (2/6)	0	2.7 (8/3)
Fetal body weight, g (M + F)	46.2	41.7	38.5	38.1

Offspring: A treatment-related external malformation was observed with 80 mg/kg/day (R,R)-formoterol at an incidence that exceeded concurrent and historical controls. Adactyly was observed at 80 mg/kg/day. From the second teratology study in rabbits (Sepracor Document number 090-819), this finding was described as the absence or reduced ossification of metatarsals, phalanges, and/or claws, and/or fused metatarsals. The role of maternal toxicity in this finding is unknown, although, it occurred in a reproducible manner in two independent studies. No external variations were observed in the present study.

Malformations: Fetuses/Litters in the 0, 20, 40, and 80 mg/kg/day.

Parameters	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day	Historical Control
Fetuses/Litters	38/5	30/6	35/4	18/3	10873/1588
External					
Adactyly	0	0	0	2 (11.1%)/ 1 (33.3%)	0.02%/ (0.0-0.8%)

Summary of individual study findings: In a dose range finding (Segment II) teratology study, artificially inseminated rabbits received (R,R)-formoterol at oral doses of 0, 20, 40, 80, 160, and 320 mg/kg/day from days 7 to 20 of gestation. (R,R)-formoterol appeared to be teratogenic as a treatment-related external malformation was observed at 80 mg/kg/day. Maternal toxicity was evident at doses of 40, 80, 160, and 320 mg/kg/day. Mortality occurred at doses of 40, 80, 160, and 320 mg/kg/day. All rabbits in the 160 and 320 mg/kg/day were euthanized on day 13 due to excessive toxicity (i.e., mortality, body weight loss, and decreased food consumption). One female at 80 mg/kg/day aborted on day 28. Post-implantation loss was increased at 80 mg/kg/day. An external malformation, adactyly, was observed with 80 mg/kg/day (R,R)-formoterol at an incidence that exceeded concurrent and historical controls. The role of maternal toxicity in this finding is unknown. Adactyly was reproduced in an embryofetal study with oral doses of 20, 40, and 80 mg/kg/day.

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

Key study findings:

♦ In a (Segment II) teratology study, (R,R)-formoterol was administered by oral gavage at doses of 0, 20, 40, and 80 mg/kg/day to artificially inseminated female rabbits from days 7 to 20 of gestation.

♦ Maternal toxicity was evident at 40 and 80 mg/kg/day. One rabbit at 40 mg/kg/day and three rabbits at 80 mg/kg/day aborted between days 23 and 29 of gestation. The incidence of decreased defecation was increased in a dose-related manner in all (R,R)-formoterol treatment groups.

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

♦ Post-implantation loss at 80 mg/kg/day was increased when compared to the control. This increase was attributed to higher early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control.

♦ Visceral variations were observed at 40 and 80 mg/kg/day (R,R)-formoterol and skeletal variations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. In addition, there were histopathological findings in fetal tissues at 20, 40, and 80 mg/kg/day that could not be classified as variations. Findings at 20 mg/kg/day appeared to occur independently of maternal toxicity.

Study no.: Sepracor Document number 090-819
Volume #, and page #: Amendment #030, Volumes 6 and 7, Pages 1 to 395
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: November 17, 1998
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol (L)-tartrate, Lot #113098A. The active component, (R,R)-Formoterol free base, represents 70% of the test article.
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Sexually mature virgin female New Zealand White rabbits were obtained from [redacted] Female rabbits were artificially inseminated using sperm collected from 11 resident male rabbits of the same strain and supplier. b(4)
Immediately following insemination, each female rabbit received human chorionic gonadotropin by intravenous administration. Selected female rabbits were approximately 6 months old at initiation of insemination. The body weight range for selected female rabbits on day 0 of gestation was 3063 to 4286 g.
Doses employed: (R,R)-formoterol was administered at doses of 0, 20, 40, and 80 mg/kg/day. Doses are expressed as (R,R)-formoterol free base.
Route of administration: The vehicle and dosing solutions were administered by oral gavage using a dose volume of 4 mL/kg.

Study design: Potential maternal toxicity and developmental toxicity of (R,R)-formoterol (L)-tartrate were evaluated in this study. (R,R)-formoterol was administered by oral gavage at doses of 0, 20, 40, and 80 mg/kg/day to 22 artificially inseminated female rabbits per group from days 7 to 20 of gestation. Basal diet and reverse osmosis-treated drinking water were provided *ad libitum* during the study period. Rabbits were monitored twice per day for moribundity and mortality. Clinical examinations of rabbits were conducted prior to dosing during the treatment period. Animals were observed for clinical signs of toxicity at 1 hr after dosing. Gross examinations were conducted on female rabbits that died during the study period. Maternal tissues and recognizable fetuses were preserved for possible future histopathological examination. Body weights were measured on days 0, 7 to 21, 24, and 29 of gestation. Food consumption was measured daily from days 0 to 29 of gestation. All surviving female rabbits were sacrificed on day 29 of gestation. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and total number of implantation sites were recorded. Maternal tissues were preserved for possible future histopathological examination. Each fetus was weighed and an external examination was conducted that included the eyes, palate, and external orifices. The sex of each fetus was determined internally. Each fetus was submitted to a visceral examination using a fresh dissection technique that included the heart and major vessels. Fetal kidneys were examined and graded for renal papillae development. The brain from each fetus was examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation in alcohol, each fetus was macerated in KOH, stained with Alizarin Red S using Dawson's method, and submitted to skeletal examination.
Number/sex/group: 22 female rabbits per group
Parameters and endpoints evaluated: Potential maternal toxicity and developmental toxicity of (R,R)-formoterol (L)-tartrate.

Results:

Mortality: The sponsor contended that there were no treatment-related deaths. Two female rabbits at 80 mg/kg/day died during the treatment period, although, both deaths were attributed to gavage errors. Two female rabbit at 40 mg/kg/day were found dead during the treatment period. These two deaths at 40 mg/kg./day were not considered to be related to the test article due to an apparent lack of treatment-related death at 80 mg/kg/day. Treatment-related death was observed at 40 and 80 mg/kg/day in the dose range finding study.

Two female rabbits at 80 mg/kg/day died immediately after dosing due to aspiration of the test article. One female (#27748) died on day 9. Necropsy examination of this animal found foamy contents in the trachea, mottled lobes of the lung, not all lobes of the lungs fully collapsed, an accessory spleen, and three normally developing implantations in utero. One female (#27807) died on day 12. Necropsy examination found that this animal was internally normal and had 9 normally developing implantations in utero.

Two female rabbits at 40 mg/kg/day were found dead during the treatment period. One female (#27753) was found dead on day 20 of gestation. Clinical signs for this animal prior to death included vocalization, head held high, labored respiration, red nasal discharge, and red material around the mouth 1 hr following dosing. Necropsy examination of animal #27753 found foamy contents in the trachea and in all lobes of the lungs, and was gravid (6 normally developing implantations, 1 late resorption, and 1 early resorption). One female (#27787) died on gestation day 7. Necropsy examination found that this animal was internally normal and had nine normally developing implantations in utero.

Clinical signs: Three female rabbits at 80 mg/kg/day and one female rabbit at 40 mg/kg/day aborted. The incidence of decreased defecation in treatment groups was observed to increase in a dose-related manner.

Three female rabbits at 80 mg/kg/day aborted between days 23 and 29 of gestation. Female #27773 aborted on day 23 of gestation. Female #27821 aborted on day 25 of gestation and was observed with decreased defecation during days 9-13 and 15-23 of gestation. Female #27800 aborted on day 29 of gestation.

Female #27792 at 40 mg/kg/day aborted on day 26 of gestation and was observed with decreased defecation from days 17 to 25 of gestation.

The incidence of decreased defecation in treatment groups was increased in a dose-related manner.

Clinical signs from days 0 to 29 of gestation (total number of observations/number of animals).

Clinical sign	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day
Decreased defecation	12/4	31/9	48/8	56/8
Wet red material on cage bedding	0	2/2	1/1	4/4
Urine appears thick and white in color	0	0	0	1/1

Body weight: There were no treatment-related effects on body weight gain from days 7 to 21 of gestation. Body weights for female controls on days 7 and 21 were 3971 and 4249 g, respectively, yielding a 7.0% increase. Body weights for female rabbits at 20, 40, and 80 µg/kg/day on day 21 of gestation were increased by 9.3, 9.7, and 7.5%, respectively, as compared to day 7.

Food consumption: Food consumption (g/animal/day or g/kg/day) for female rabbits at 40 and 80 mg/kg/day from days 7 to 21 of gestation were decreased to 86.4 and 79.6% of the control (191 g/animal/day), respectively. These results appear somewhat contradictory to observed increases in body weight gains.

From days 7 to 10, food consumption for female rabbits at 20, 40, and 80 mg/kg/day was decreased to 82.1, 83.7, and 69.9% of the control (196 g/animal/day), respectively. From days 10 to 13, food consumption for female rabbits at 80 mg/kg/day

was decreased to 89.7% of the control (185 g/animal/day). From days 13 to 21, food consumption for female rabbits at 40 and 80 mg/kg/day was decreased to 86 and 82.8% of the control (186 g/animal/day), respectively.

Terminal and necroscopic evaluations:

Dams: There were no treatment-related gross necropsy findings in maternal tissues. Gravid uterine weight for female rabbits at 80 mg/kg/day was decreased to 75% of the control (433.4 g). Post-implantation loss at 80 mg/kg/day was increased when compared to the control. This increase was attributed to higher early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control. There were no effects on intrauterine parameters (i.e., corpora lutea/dam, implantation sites/dam, pre-implantation loss/dam, viable fetuses/dam, dead fetuses/dam, resorptions, post-implantation loss/dam, fetal body weight, and sex ratios) at 20 mg/kg/day. Pre-implantation losses for dams at 0, 20, 40, and 80 mg/kg/day (R,R)-formoterol were high at 30.2, 38.7, 31.2, and 34.1%, respectively, although, implantation occurred prior to the start of treatment and there was no dose response relationship.

Fetal Data at Scheduled Necropsy

Parameter	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day
Number of gravid females	20	21	19	16
Corpora lutea/dam	10.9 (217/20)	10.7 (224/21)	11.1 (221/19)	9.6 (154/16)
Implantation sites/dam	7.4 (147/20)	6.3 (133/21)	7.7 (146/19)	6.3 (101/16)
Pre-implantation loss/dam	3.5 (70/20)	4.3 (91/21)	3.4 (65/19)	3.3 (53/16)
Viable fetuses/dam	6.7 (134/20)	5.5 (116/21)	6.8 (130/19)	4.8* (76/16)
Dead fetuses/dam	0.1 (1/20)	0	0	0
Resorptions				
-Early	0.4 (8/20)	0.7 (14/21)	0.5 (10/19)	0.9 (14/16)
-Late	0.2 (4/20)	0.1 (3/21)	0.3 (6/19)	0.7 (11/16)*
-Total	0.6 (12/20)	0.8 (17/21)	0.8 (16/19)	1.6 (25/16)*
Post-implantation loss/dam	0.7 (13/20)	0.8 (17/21)	0.8 (16/19)	1.6 (25/16)*
Fetal body weight, g				
-Males	46.9	46.3	43.0	40.9*
-Females	45.2	44.6	41.4	40.7*
Sex				
-Males	3.4 (67/20)	3.0 (63/21)	3.2 (60/19)	2.3 (36/16)
-Females	3.4 (67/20)	2.5 (53/21)	3.7 (70/19)	2.5 (40/16)

* $p < 0.05$

Offspring: Total malformations (i.e., external, visceral, and skeletal) at 20, 40, and 80 mg/kg/day were increased in a dose-related manner to 10(5) 11(9), and 26(12), respectively, as compared to 3 fetuses (2 litters) for the control. The role of maternal toxicity for effects observed at 40 and 80 mg/kg/day is unclear.

Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol at incidences that exceeded concurrent and historical controls. Adactyly, involving 1 or 2 digits, was observed at 80 mg/kg/day. Skeletally, this finding consisted of absence or reduced ossification of metatarsals, phalanges, and/or claws, and/or fused metatarsals. Adactyly was also observed in the dose range finding study. Syndactyly was observed for 1 fetus (#27777-06) at 80 mg/kg/day. There was no apparent skeletal origin noted for this finding. Syndactyly has not been previously observed in the testing laboratory. Brachydactyly was observed at 40 and 80 mg/kg/day. Skeletally, brachydactyly consisted of absent phalanges and claws, and/or reduced ossification of phalanges. Short tails were observed for 1 fetus each at 40 and 80 mg/kg/day. One fetus (#27827-01) at 80 mg/kg/day had an umbilical herniation of the intestine with several loops of the intestine protruding through an opening in the umbilicus.

Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol at incidences that exceeded concurrent and historical controls. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Malpositioned kidney at 20 mg/kg/day appeared to occur independently of maternal toxicity. In each instance, the right kidney was noted immediately anterior to the urinary bladder. Heart and/or great vessel anomalies were observed at 40 and 80 mg/kg/day. A bulbous aorta was observed for 1 fetus at 40 mg/kg/day and 5 fetuses at 80 mg/kg/day. For these 5 fetuses at 80 mg/kg/day, 2 had interventricular septal defects (i.e., opening in the interventricular septum) and 1 had cardiomegaly (enlarged ventricles and right atrium). Two other fetuses at 80 mg/kg/day had interventricular septal defects (i.e., opening in the interventricular septum) without abnormalities of the aorta. One fetus in this group had a common truncus arteriosus. Lobular dysgenesis of the lungs, consisting of the right accessory lobe being absent, was observed for 4 fetuses in 1 litter (#27726) at 80 mg/kg/day.

Skeletal malformations (i.e., costal cartilidge anomaly, metatarsals fused, skull bone(s) fused) were observed with 80 mg/kg/day (R,R)-formoterol; however, the treatment relationship of these findings appear to be questionable. Other malformations were reported, although, incidences were not dose-related (i.e., observed at low and/or mid doses but not at the high dose).

Total malformations: number of affected fetuses/litters.

Malformations	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day
External	0	1/1	3/2	10/8
Soft tissue	0	3/2	4/4	18/10
Skeletal	3/2	6/3	5/5	3/3

Malformations: Fetuses/Litters in the 0, 20, 40, and 80 mg/kg/day.

Parameters	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day	Historical Control
Fetuses/Litters	134/20	116/20	130/18	76/15	10873/1588
External					
Adactyly	0	0	0	4(5.3%)/ 3 (20%)	0.02%/ (0.0-0.8%)
Short tail	0	0	1 (0.8%)/ 1 (5.6%)	1 (1.3%)/ 1 (6.7%)	-
Syndactyly	0	0	0	1 (1.3%)/ 1 (6.7%)	-
Brachydactyly	0	0	2 (1.5%)/ 1 (5.6%)	7 (9.2%)*/ 6 (40%)	-
Umbilical herniation of the intestine	0	0	0	1 (1.3%)/ 1 (6.7%)	0.01%/ (0.0-0.7%)
Visceral					
Malpositioned kidney(s)	0	3 (2.6%)/ 2 (10%)	3 (2.3%)/ 3 (16.7%)	8 (10.5%)*/ 6 (40%)	-
Bulbous aorta	0	0	1 (0.8%)/ 1 (5.6%)	5 (6.6%)*/ 4 (26.7%)	-
Lungs-lobular dysgenesis	0	0	0	4 (5.3%)/ 1 (6.7%)	-
Interventricular septal defect	0	0	0	4 (5.3%)*/ 4 (26.7%)	-
Cardiomegaly	0	0	0	1 (1.3%)/ 1 (6.7%)	-
Common truncus arteriosus	0	0	0	1 (1.3%)/ 1 (6.7%)	-
Skeletal					
Costal cartilidge anomaly	0	0	0	1 (1.3%)/ 1 (6.7%)	0.07%/ (0.0-1.3%)
Metatarsals fused	0	0	0	1 (1.3%)/ 1 (6.7%)	-
Skull bone(s) fused	0	0	0	1 (1.3%)/ 1 (6.7%)	-

Note: Only malformations with a possible treatment relationship have been listed.

Visceral and skeletal variations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. There were no treatment-related external variations. In addition, there were histopathological findings in fetal tissues at 20, 40, and 80 mg/kg/day that could not be classified as variations. Findings at 20 mg/kg/day appeared to occur independently of maternal toxicity.

Visceral variations were evident at 40 and 80 mg/kg/day. The incidences of major blood vessel variations were slightly increased at 40 and 80 mg/kg/day as compared to the concurrent and historical controls, although, the treatment relationship of these findings appears questionable.

Skeletal variations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. The incidences of sternebrae with thread-like attachment and 27 presacral vertebrae were increased at 20, 40, and 80 mg/kg/day as compared to concurrent and historical controls. The incidence of accessory skull bones was increased at 80 mg/kg/day as compared to concurrent and historical controls.

There were several histopathological findings for fetal tissues at 20, 40, and 80 mg/kg/day. White areas in the liver, oviduct cysts, and dark red areas on the liver or mesenteric cyst(s) were observed in fetuses from all (R,R)-formoterol treatment groups with no corresponding findings in the control group. Cysts in the liver were observed in fetuses at 40 and 80 mg/kg/day with no corresponding findings in the control group.

Variations: Fetuses/Litters in the 0, 20, 40, and 80 mg/kg/day.

Parameters	0 mg/kg/day	20 mg/kg/day	40 mg/kg/day	80 mg/kg/day	Historical Control
Fetuses/Litters	134/20	116/20	130/18	76/15	10873/1588
Visceral					
Major blood vessel variation	7 (5.2%)/ 4 (20%)	5 (4.3%)/ 4 (20%)	13 (10%)/ 6 (33.3%)	12 (15.8%)/ 5 (33.3%)	5.4%/ (0.0-31.5%)
Renal papilla(e) not developed and/or distended ureters	0	0	0	1 (1.3%)/ 1 (6.7%)	0.1%/ (0.0-2.9%)
No classification					
Cysts in the liver	0	0	10 (7.7%)/ 4 (22.2%)	20 (26.3%)/ 6 (40%)	
White areas in the liver	0	1 (0.9%)/ 1 (5%)	7 (5.4%)/ 2 (11.1%)	15 (19.7%)/ 5 (33.3%)	
Oviduct cysts	0	1 (0.9%)/ 1 (5%)	7 (5.4%)/ 3 (16.7%)	3 (4%)/ 2 (13.3%)	
Dark red areas on the liver or mesenteric cyst(s)	0	3 (2.6%)/ 1 (5%)	2 (1.5%)/ 2 (11.1%)	1 (1.3%)/ 1 (6.7%)	
Skeletal					
27 presacral vertebrae	5 (3.7%)/ 3 (15%)	20 (17.2%)*/ 10 (50%)	18 (13.85%)/ 8 (44.4%)	13 (17.1%)*/ 10 (66.7%)	18.4%/ (0.0-45.8%)
Sternebrae with thread-like attachment	0	5 (4.3%)/ 2 (10%)	9 (6.9%)*/ 6 (33.3%)	5 (6.6%)*/ 3 (20%)	1.1%/ (0.0-9.1%)
Accessory skull bones	0	0	0	4 (5.3%)*/ 4 (26%)	0.8%/ (0.0-5.0%)
Extra site of ossification anterior to sternebra #1	0	0	2 (1.5%)/ 2 (11.1%)	1 (1.3%)/ 1 (6.7%)	0.8%/ (0.0-6.7%)

Sternebra(e) malaligned (slight or moderate)	0	0	2 (1.5%)/ 2 (11.1%)	1 (1.3%)/ 1 (6.7%)	2%/ (0.0-17.1%)

Summary of individual study findings:

In a (Segment II) teratology study, (R,R)-formoterol was administered by oral gavage at doses of 0, 20, 40, and 80 mg/kg/day to 22 artificially inseminated female rabbits per group from days 7 to 20 of gestation. Surviving animals were sacrificed on day 29 of gestation. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥20 mg/kg/day.

Maternal toxicity was evident at 40 and 80 mg/kg/day and also possibly at 20 mg/kg/day. One rabbit at 40 mg/kg/day and three rabbits at 80 mg/kg/day aborted between days 23 and 29 of gestation. The incidence of decreased defecation was increased in a dose-related manner in all (R,R)-formoterol treatment groups.

Post-implantation loss at 80 mg/kg/day was increased when compared to the control. This increase was attributed to higher early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control.

Total malformations (i.e., external, visceral, and skeletal) at 20, 40, and 80 mg/kg/day were increased in a dose-related manner to 10(5) 11(9), and 26(12), respectively, as compared to 3 fetuses (2 litters) for the control. Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol. Adactyly, syndactyly, and umbilical herniation of the intestine were observed at 80 mg/kg/day. Brachydactyly and short tails were observed at 40 and 80 mg/kg/day. Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Malpositioned kidney at 20 mg/kg/day occurred independently of maternal toxicity except decreased defecation. Heart and/or great vessel anomalies were observed at 40 and 80 mg/kg/day. Lobular dysgenesis of the lungs was observed at 80 mg/kg/day. The role of maternal toxicity in these findings at 40 and 80 mg/kg/day is not clear. Adactyly was reproduced in two studies.

Visceral variations were observed at 40 and 80 mg/kg/day (R,R)-formoterol and skeletal variations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. In addition, there were histopathological findings in fetal tissues at 20, 40, and 80 mg/kg/day that could not be classified as variations. Findings at 20 mg/kg/day appeared to occur independently of maternal toxicity. The incidences of major blood vessel variations were slightly increased at 40 and 80 mg/kg/day, although, the treatment relationship of these findings appears questionable. The incidences of sternebrae with thread-like attachment and 27 presacral vertebrae were increased at 20, 40, and 80 mg/kg/day. The incidence of accessory skull bones was increased at 80 mg/kg/day. There were several histopathological findings for fetal tissues at 20, 40, and 80 mg/kg/day. White areas in the liver, oviduct cysts, and dark red areas on the liver or mesenteric cyst(s) were observed in fetuses from all (R,R)-formoterol treatment groups. Cysts in the liver were observed in fetuses at 40 and 80 mg/kg/day.

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

Key study findings:

♦ In a (Segment II) teratology study, artificially inseminated female rabbits received (R,R)-formoterol at oral doses of 2, 10, and 20 mg/kg/day from days 7 to 20 of gestation. Racemic formoterol at a dose of 20 mg/kg/day was included as a comparator.

♦ In the present study, (R,R)-formoterol at doses ≤20 mg/kg/day was not teratogenic in contrast to the earlier study (Sepracor document number 090-819) where malformations were evident at doses ≥20 mg/kg/day.

♦ The incidences of major blood vessel variations were increased for (R,R)-formoterol and racemic formoterol treatment groups as compared to the concurrent control, although, there was no evidence of a dose response relationship.

♦ The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical dose of 48 µg/day. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Study no.: Sepracor Document number 09-826, 2001 and 090-468, 2000
Volume #, and page #: Amendment #030, Volumes 8 and 9, Pages 1 to 501
Amendment #029, Volumes 2, Pages 1 to 20
Conducting laboratory and location: ┌

b(4)

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Date of study initiation: July 8, 1999 and September 20, 1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity:

(R,R)-Formoterol (L)-tartrate, Lot #010799A. The active component, (R,R)-Formoterol free base, represents 70% of the test article.
Racemic Formoterol ½ fumarate monohydrate, Lot #BX9041. The components of racemic formoterol fumarate, (R,R)-formoterol (active component) and (S,S)-formoterol free bases, represented [redacted] of the test material. b(4)
Formulation/vehicle: 0.5% carboxymethylcellulose

Methods:
Species/strain: Sexually mature, virgin female New Zealand White rabbits were obtained from [redacted] Female rabbits were b(4)
artificially inseminated using sperm collected from 22 resident male rabbits of the same strain and supplier. Immediately following insemination, each female rabbit received human chorionic gonadotropin by intravenous administration. Rabbits for the teratology study were 6½ months old at the initiation of insemination and had a body weight range of 2935 to 4849 g. Rabbits for the toxicokinetic study were 5 to 9 months old at the start of the in-life phase and had a body weight range of 3.0 to 4.5 kg.
Doses employed: (R,R)-formoterol was administered at oral doses of 0, 2, 10, or 20 mg/kg/day. Racemic formoterol was administered at an oral dose of 20 mg/kg/day.
Route of administration: The vehicle and dosing solutions were administered by oral gavage using a dose volume of 4 mL/kg.
Study design:

Potential maternal toxicity and developmental toxicity of (R,R)-formoterol (L)-tartrate were evaluated in this study. (R,R)-formoterol was administered by oral gavage at doses of 0, 2, 10, and 20 mg/kg/day to 22 artificially inseminated female rabbits per group from days 7 to 20 of gestation. The vehicle-control group received 0.5% carboxymethylcellulose. Racemic formoterol at a dose of 20 mg/kg/day was used as a comparator. Basal diet and reverse osmosis-treated drinking water were provided *ad libitum* during the study period. Rabbits were monitored twice per day for moribundity and mortality. Clinical examinations of rabbits were conducted prior to dosing during the treatment period. Animals were observed for clinical signs of toxicity at 1 hr after dosing. A gross necropsy examination was performed on one female that aborted during the study. The number and location of implantation sites and corpora lutea and the number of viable fetuses were recorded. No maternal tissues were retained for this animal and products of conception were discarded. Body weights were measured on days 0, 7 to 21, 24, and 29 of gestation. Food consumption was measured daily from days 0 to 29 of gestation. All surviving female rabbits were sacrificed on day 29 of gestation. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and total number of implantation sites were recorded. Maternal tissues were preserved for possible future histopathological examination. Each fetus was weighed and an external examination was conducted that included the eyes, palate, and external orifices. The sex of each fetus was determined internally. Each fetus was submitted to a visceral examination using a fresh dissection technique that included the heart and major vessels. The brain from each fetus was examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation in alcohol, each fetus was macerated in KOH, stained with Alizarin Red S using Dawson's method, and submitted to skeletal examination.

Toxicokinetic parameters for plasma formoterol were evaluated in artificially inseminated female rabbits that received (R,R)-formoterol at doses of 2, 10, or 20 mg/kg/day or racemic formoterol at a dose of 20 mg/kg/day. Blood samples for measurement of plasma formoterol levels were collected on gestation days 7 and 20 at 0.5, 1, 2, 6, and 24 hr after dosing. Plasma concentrations of formoterol 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 method lacked chiral specificity and concentration data were expressed as formoterol. Although, the method was not validated to measure desformoterol, the MRM mass channel for desformoterol was acquired and presented as a ratio with respect to the MRM signal of formoterol for qualitative purposes. The MRM signal for an equal quantity of desformoterol was assumed to be approximately equal to that for formoterol.
Number/sex/group: 22 artificially inseminated female rabbits per group for the teratology study and 3 artificially inseminated female rabbits per group for the toxicokinetic study.
Parameters and endpoints evaluated: Potential maternal and developmental toxicity of (R,R)-formoterol-L-tartrate and racemic formoterol were evaluated in the rabbit.

Results:

Mortality: None.

Clinical signs:

Female #28518 that received (R,R)-formoterol at 10 mg/kg/day aborted on gestation day 26. Given the lack of dose-response relationship, this abortion was not considered treatment-related. This female was lethargic on gestation day 24 and had decreased defecation and dried brown material at the base of the tail for 2 days (GD 24 and 25) prior to abortion. This female aborted 3 late resorbing fetuses with no apparent malformations and had 3 former implantation sites in utero. At necropsy, this female had cystic oviducts.

The incidence of decreased defecation was increased for treatment groups, although, a majority of occurrences were observed between days 21 and 29 of gestation.

Clinical signs (total occurrence/# of animals).

Clinical Sign	(R,R)-Formoterol				Racemic Formoterol
	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	20 mg/kg/day	20 mg/kg/day
Decreased defecation	5/2	8/6	9/3	9/5	22/6

Body weight: There were no treatment-related effects on body weight gains from days 7 to 21 of gestation. Mean body weights for vehicle-control animals on days 7 and 21 of gestation were 3918 and 4211 g, respectively, yielding a 7.5% increase of initial body weight. Body weights on day 21 for female rabbits that received (R,R)-formoterol at doses of 2, 10, and 20 mg/kg/day were increased by 10.2, 8.6, and 10.8%, respectively,

of body weights on day 7. Body weight on day 21 for female rabbits that received racemic formoterol at 20 mg/kg/day was increased by 10.6% of body weight on day 7.

Food consumption: There were no treatment-related effects on food consumption (g/animal/day or g/kg/day) from days 7 to 21 of gestation. Food consumption (g/animal/day or g/kg/day) was transiently decreased from days 7 to 8 of gestation. Food consumption on days 7 to 8 for female rabbits that received (R,R)-formoterol at doses of 2, 10, and 20 mg/kg/day were decreased to 86.3, 83.3, and 72.1% of the control (233 g/animal/day), respectively. Food consumption on days 7 to 8 for female rabbits that received racemic formoterol at 20 mg/kg/day was decreased to 82.8% of the control.

Toxicokinetics: AUC values for formoterol on days 7 and 20 of gestation in female rabbits that received (R,R)-formoterol at doses of 2, 10, and 20 mg/kg/day increased in an approximate dose-related manner. Plasma levels of the metabolite, desformoterol, generally ranged from 3 to 20% of plasma levels of formoterol for female rabbits that received (R,R)-formoterol at 2, 10, and 20 mg/kg/day. A similar relationship for plasma levels of desformoterol was observed for female rabbits that received racemic formoterol at 20 mg/kg/day. The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical dose of 48 µg/day. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Toxicokinetic parameters for plasma formoterol on gestation day (GD) 7 and 20 in pregnant female rabbits that received (R,R)-formoterol at doses of 2, 10, or 20 mg/kg/day or racemic formoterol at a dose of 20 mg/kg/day.

Group	$AUC_{0\text{-}24hr}$, ng·hr/mL		Exposure Ratio for Human AUC = 0.0338 ng·hr/mL[a]		C_{max}, ng/mL		T_{max}, hr	
	GD 7	GD 20	GD 7	GD 20	GD 7	GD 20	GD 7	GD 20
2 mg/kg/day (R,R)-Formoterol	70.5	63.7	2086	1885	15.1	463	0.83	0.67
10 mg/kg/day (R,R)-Formoterol	429	340	12692	10059	123	157	1.67	0.83
20 mg/kg/day (R,R)-Formoterol	807	582	23876	17219	162	331	2.00	0.67
20 mg/kg/day Racemic Formoterol	822	360	-	-	194	154	1.33	0.83

a. (R,R)-Formoterol administered to human volunteers by inhalation at a dose of 48 µg/day produced an AUC of 0.0338 ng·hr/mL (Study No. 091-004). This dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Terminal and necroscopic evaluations:

Dams:

At scheduled necropsy, female #29603 that received racemic formoterol at 20 mg/kg/day was found to have a pale liver (all lobes), pale lungs (all lobes), and dark red contents in the stomach and cecum.

There were no treatment-related effects on intrauterine growth and survival (i.e., no effects on corpora lutea/dam, implantation sites/dam, pre-implantation loss/dam, viable fetuses/dam, post-implantation loss/dam, fetal body weight, and sex ratios). Pre-implantation losses for dams at 0, 2, 10, and 20 mg/kg/day (R,R)-formoterol and dams at 20 mg/kg/day racemic formoterol were high at 39.3, 31.5, 43.4, 32.2, and 38.4%, respectively, although, implantation occurred prior to the start of treatment and there were no treatment-related effects.

Female #29632 that received (R,R)-formoterol at 20 mg/kg/day was found to have congenital segmental aplasia of the right uterine horn and agenesis of the right uterine horn. This female was non-gravid.

Fetal Data at Scheduled Necropsy

Parameter	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	20 mg/kg/day	20 mg/kg/day Racemic
Number of gravid females	21	18	17	19	18
Corpora lutea/dam	10.9 (229/21)	13.4 (242/18)	11.2 (190/17)	11.1 (211/19)	13.2 (238/18)
Implantation sites/dam	6.4 (135/21)	8.3 (150/18)	6.2 (106/17)	7.2 (137/19)	7.6 (136/18)
Pre-implantation loss/dam	4.5 (94/21)	5.1 (92/18)	4.9 (84/17)	3.9 (74/19)	5.7 (102/18)
Viable fetuses/dam	6.0 (125/21)	8.1 (146/18)	5.9 (100/17)	6.4 (121/19)	6.9 (125/18)
Dead fetuses/dam	0	0	0	0	0
Resorptions					
-Early	0.4 (8/21)	0.1 (1/18)	0.4 (6/17)	0.7 (14/19)	0.3 (5/18)
-Late	0.1 (2/21)	0.2 (3/18)	0	0.1 (2/19)	0.3 (6/18)
-Total	0.5 (10/21)	0.2 (4/18)	0.4 (6/17)	0.8 (16/19)	0.6 (11/18)
Post-implantation loss/dam	0.5 (10/21)	0.2 (4/18)	0.4 (6/17)	0.8 (16/19)	0.6 (11/18)
Fetal body weight, g					
-Males	49.0	47.7	50.2	45.0	46.0
-Females	45.8	45.7	48.2	44.2	46.9
Sex					
-Males	3.1 (66/21)	3.9 (70/18)	2.7 (46/17)	3.3 (62/19)	3.3 (60/18)
-Females	2.8 (59/21)	4.2 (76/18)	3.2 (54/17)	3.1 (59/19)	3.6 (65/18)

* $p < 0.05$

Offspring:

There appeared to be no treatment-related external, visceral, or skeletal malformations with (R,R)-formoterol doses ≤20 mg/kg/day in contrast to the earlier study (Sepracor document number 090-819) where malformations were evident at ≥20 mg/kg/day. Malformations consisting of cleft lip, spleen absent, and lobular dysgenesis of the lung(s) were each observed for only 1 fetus and do not appear to be treatment-related, although, lobular dysgenesis of the lung(s) was observed at an increased incidence with a dose of 80 mg/kg/day in the earlier study.

The incidences of major blood vessel variations were increased for (R,R)-formoterol and racemic formoterol treatment groups as compared to the concurrent control, although, there was no evidence of a dose response relationship. The incidence of extra site of ossification anterior to sternebra #1 was increased for female rabbits that received (R,R)-formoterol at 20 mg/kg/day or formoterol at 20 mg/kg/day. Incidences of skeletal variations, consisting of sternebrae with thread-like attachment and 7th sternebra, were increased for (R,R)-formoterol groups, although, there treatment relationships were unclear given low incidences within the historical control and lack of dose response relationships.

Fetuses and litters with malformations

Parameters	(R,R)-Formoterol				Racemic Formoterol	Historical Controls
	0	2	10	20	20	
Fetuses/Litters	125/20	146/18	100/17	121/18	125/18	10873/ 1588
External						
Cleft lip	0	0	0	1 (0.8%)/ 1 (5.6%)	0	0.3%/ (0.0-1.0%)
Visceral						
Spleen-absent	0	0	0	0	1 (0.8%)/ 1 (5.6%)	0.009%/ (0.0-1.0%)
Lung(s), lobular dysgenesis	0	0	0	1 (0.8%)/ 1 (5.6%)	0	-
Skeletal						
Vertebral anomaly with or without associated rib anomaly	0	1 (0.7%)/ 1 (5.6%)	2 (2%)/ 2 (11.8%)	0	0	1.2%/ (0.0-9.8%)
Vertebral centra anomaly	0	0	0	1 (0.8%)/ 1 (5.6%)	0	0.04%/ (0.0-0.7%)

Fetuses and litters with variations

Parameters	(R,R)-Formoterol				Racemic Formoterol	Historical Controls
	0	2	10	20	20	
Fetuses/Litters	125/20	146/18	100/17	121/18	125/18	10873/ 1588
Visceral						
Major blood vessel variation	2 (1.6%)/ 2 (10%)	11 (7.5%)/ 7 (38.9%)	10 (10%)/ 7 (41.2%)	8 (6.6%)/ 4 (22.2%)	6 (4.8%)/ 6 (33.3%)	5.4%/ (0.0-31.5%)
Gallbladder absent or small	0	0	0	0	2 (1.6%)/ 2 (11.1%)	1.9%/ (0.0-12.2%)

Skeletal						
Sternebrae with thread-like attachment	0	6 (4.1%)/ 1 (5.6%)	1 (1%)/ 1 (5.9%)	1 (0.8%)/ 1 (5.6%)	2 (1.6%)/ 1 (5.6%)	1.1%/ (0.0-9.1%)
7th Sternebra	0	2 (1.4%)/ 1 (5.6%)	1 (1%)/ 1 (5.9%)	2 (1.6%)/ 1 (5.6%)	2 (1.6%)/ 2 (11.1%)	0.26%/ (0.0-3.8%)
Extra site of ossification anterior to sternebra #1	0	1 (0.7%)/ 1 (5.6%)	0	4 (3.3%)/ 2 (11.1%)	3 (2.4%)/ 3 (16.7%)	0.8%/ (0.0-6.7%)

Summary of individual study findings: In a (Segment II) teratology study, artificially inseminated female rabbits received (R,R)-formoterol at doses of 2, 10, and 20 mg/kg/day from days 7 to 20 of gestation. Racemic formoterol at a dose of 20 mg/kg/day was included as a comparator. In the present study, (R,R)-formoterol at doses ≤20 mg/kg/day was not teratogenic in contrast to the earlier study (Sepracor document number 090-819) where malformations were evident at doses ≥20 mg/kg/day. The incidences of major blood vessel variations were increased for (R,R)-formoterol and racemic formoterol treatment groups as compared to the concurrent control, although, there was no evidence of a dose response relationship. The incidence of extra site of ossification anterior to sternebra #1 was increased for female rabbits that received (R,R)-formoterol at 20 mg/kg/day or formoterol at 20 mg/kg/day. The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical inhalation dose of 48 µg/day. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Reproductive and developmental toxicology summary:

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. 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. (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.

In a dose range finding (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 0, 10, 20, 40, 80, and 160 mg/kg/day. Maternal toxicity was evident at 160 mg/kg/day as mortality occurred at this dose. (R,R)-formoterol was teratogenic as external malformations were evident with doses of 80 and 160 mg/kg/day. Anasarca was observed for 1 fetus at 80 mg/kg/day and 2 fetuses at 160 mg/kg/day. One of these fetuses at 160 mg/kg/day was also observed with ablepharia (bilateral) and mandibular and maxillary micrognathia. Localized fetal edema (neck and thorax) was observed for 1 fetus at 160 mg/kg/day. The role of maternal toxicity for these effects is not known. Anasarca and localized fetal edema were reproduced in the definitive embryofetal development study.

In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 10, 60, or 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥10 mg/kg/day and racemic formoterol at 120 mg/kg/day. Maternal toxicity (i.e., death and clinical signs) was evident with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Post-implantation loss, consisting primarily of early resorption, was increased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Number of viable fetuses and fetal body weight were decreased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. External malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related external malformations included omphalocele, localized fetal edema of the neck, anasarca, microphthalmia and/or anophthalmia, and micromelia. Omphalocele and microphthalmia and/or anophthalmia were not observed with racemic formoterol. Omphalocele occurred independently of maternal toxicity. Skeletal malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related skeletal malformations included bent limb bones, vertebral anomaly with or without associated rib anomaly and 12 pairs of ribs. An increased incidence of skeletal variations, consisting primarily of reductions in ossification, was evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related variations consisted of sternebra(e) #5 and/or # 6 unossified, sternebra(e) #1, #2, #3, and/or #4 unossified, bent ribs, decreased proportion of cervical centrum #1 ossified, reduced ossification of the vertebral arches, entire sternum unossified, pubis unossified, reduced ossification of the 13th rib(s). Several variations occurred independently of maternal toxicity.

In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 1, 5, or 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation. There was no evidence of maternal toxicity with (R,R)-formoterol at ≤10 mg/kg/day. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥1 mg/kg/day and racemic formoterol at 10 mg/kg/day. An external malformation, omphalocele, was observed with fetuses in the 1, 5, and 10 mg/kg/day (R,R)-formoterol groups, although, there was not a dose-response relationship. Omphalocele was reported in the previous teratology study (Sepracor Document number 090-820) with (R,R)-formoterol doses of 10, 60, and 120 mg/kg/day. Omphalocele occurred independently of maternal toxicity. The NOAEL for omphalocele appears to be <1 mg/kg/day. Umbilical herniation of the intestine (i.e., several loops of the intestine protruded through an opening in the umbilicus) was observed for 1 fetus in the 10 mg/kg/day racemic formoterol group. Incidences of unossified sternebrae #5 and/or #6 were increased for all (R,R)-formoterol groups as well as the racemic formoterol group. Incidences of cervical centrum #1 ossified were decreased for all (R,R)-formoterol groups as well as the racemic formoterol group. The dose of 1 mg/kg/day administered to mated female rats on gestation days 6 or 17 produced an

AUC that was approximately 750 to 900 times the AUC observed with a clinical dose of 48 µg/day; however, it should be noted that 1 mg/kg/day is not a NOAEL. This dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

In a dose range finding (Segment II) teratology study, artificially inseminated rabbits received (R,R)-formoterol at oral doses of 0, 20, 40, 80, 160, and 320 mg/kg/day from days 7 to 20 of gestation. (R,R)-formoterol appeared to be teratogenic as a treatment-related external malformation was observed at 80 mg/kg/day. Maternal toxicity was evident at doses of 40, 80, 160, and 320 mg/kg/day. Mortality occurred at doses of 40, 80, 160, and 320 mg/kg/day. All rabbits in the 160 and 320 mg/kg/day were euthanized on day 13 due to excessive toxicity. One female at 80 mg/kg/day aborted on day 28. Post-implantation loss was increased at 80 mg/kg/day. An external malformation, adactyly, was observed with 80 mg/kg/day (R,R)-formoterol at an incidence that exceeded concurrent and historical controls. The role of maternal toxicity in this finding is unknown. Adactyly was also observed in the (Segment II) teratology study with rabbits in which (R,R)-formoterol was administered at oral doses of 20, 40, and 80 mg/kg/day.

In a (Segment II) teratology study, (R,R)-formoterol was administered by oral gavage at doses of 0, 20, 40, and 80 mg/kg/day to artificially inseminated female rabbits. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥20 mg/kg/day. Maternal toxicity was evident at 40 and 80 mg/kg/day and also possibly at 20 mg/kg/day. One rabbit at 40 mg/kg/day and three rabbits at 80 mg/kg/day aborted between days 23 and 29 of gestation. The incidence of decreased defecation was increased in a dose-related manner in all (R,R)-formoterol treatment groups. Post-implantation loss at 80 mg/kg/day was increased when compared to the control. This increase was attributed to higher early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control. Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol. Adactyly, syndactyly, and umbilical herniation of the intestine were observed at 80 mg/kg/day. Brachydactyly and short tails were observed at 40 and 80 mg/kg/day. Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Malpositioned kidney occurred independently of maternal toxicity except for decreased defecation. Heart and/or great vessel anomalies were observed at 40 and 80 mg/kg/day. Lobular dysgenesis of the lungs was observed at 80 mg/kg/day. Visceral variations were observed at 40 and 80 mg/kg/day (R,R)-formoterol and skeletal variations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. In addition, there were histopathological findings in fetal tissues at 20, 40, and 80 mg/kg/day that could not be classified as variations. These effects at 20 mg/kg/day appeared to occur independently of maternal toxicity.

In a (Segment II) teratology study, artificially inseminated female rabbits received (R,R)-formoterol at oral doses of 2, 10, and 20 mg/kg/day from days 7 to 20 of gestation. Racemic formoterol at a dose of 20 mg/kg/day was included as a comparator. In the present study, (R,R)-formoterol at doses ≤20 mg/kg/day was not

teratogenic in contrast to the earlier study (Sepracor document number 090-819) where malformations were evident at doses ≥20 mg/kg/day. The incidences of major blood vessel variations were increased for (R,R)-formoterol and racemic formoterol treatment groups as compared to the concurrent control, although, there was no evidence of a dose response relationship. The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical inhalation dose of 48 µg/day. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Reproductive and developmental toxicology conclusions: (R,R)-formoterol was teratogenic in studies with both rats and rabbits.

External and skeletal malformations were evident in studies conducted with rats. Omphalocele (i.e., several loops of the intestine protruded through an opening in the umbilicus, and remnants of a membranous sac were discernable) was observed in two studies with combined doses of 1, 5, 10, 60, and 120 mg/kg/day. Omphalocele occurred independently of maternal toxicity. A NOAEL was not established for this finding (i.e., there was no threshold dose).

External and visceral malformations were evident in rabbits in one study with doses of 20, 40, and 80 mg/kg/day. A malpositioned right kidney was observed at doses ≥20 mg/kg/day. In a second study with doses of 2, 10, and 20 mg/kg/day, there were no teratogenic findings. Malpositioned kidney appeared to occur independently of maternal toxicity. The NOAEL could be considered 10 mg/kg/day. A threshold dose appears to exist with rabbits. The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical inhalation dose of 48 µg/day. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

Labeling recommendations: Teratogenic findings with (R,R)-formoterol in rats and rabbits should be conveyed in the labeling.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

(R,R)-Formoterol, a β_2-adrenergic agonist, is under development for treatment of [redacted] COPD. In the present amendments in support of the continued development of (R,R)-formoterol, the sponsor has provided preclinical pharmacology and toxicology studies as follows: pharmacology; safety pharmacology; toxicokinetic studies with mice (inhalation and oral exposure), rats (inhalation exposure), and dogs (inhalation exposure); in vitro blood to plasma partitioning and plasma protein binding in rat, dog, human, and mouse; acute inhalation toxicity studies with mice and rats; an amendment to the 28-day inhalation toxicity study with rats; and reproductive toxicology studies consisting of a fertility and reproductive performance study in rats, a dose range finding embryo/fetal development study in rats, two embryo/fetal development studies in rats, a dose range finding embryo/fetal development study in rabbits, and two embryo/fetal development studies in rabbits. b(4)

The pharmacological activity of (R,R)-formoterol was assessed with several in vitro assays. (R,R)-Formoterol and (R,R/S,S)-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 tumor necrosis factorα (TNFα) pretreatment). (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,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. (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. (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 or (S,S)-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, TNFα, 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%.

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 study in the review dated December 20, 1999.

Toxicokinetic parameters of formoterol were assessed in mice, rats, and dogs following exposure to (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.

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 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.

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. 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. (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.

In a dose range finding (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 0, 10, 20, 40, 80, and 160 mg/kg/day. Maternal toxicity was evident at 160 mg/kg/day as mortality occurred at this dose. (R,R)-formoterol was teratogenic as external malformations were evident with doses of 80 and 160 mg/kg/day. Anasarca was observed for 1 fetus at 80 mg/kg/day and 2 fetuses at 160 mg/kg/day. One of these fetuses at 160 mg/kg/day was also observed with ablepharia (bilateral) and mandibular and maxillary micrognathia. Localized fetal edema (neck and thorax) was observed for 1 fetus at 160 mg/kg/day. The role of maternal toxicity for these effects is not known. Anasarca and localized fetal edema were reproduced in the definitive embryofetal development study.

In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 10, 60, or 120 mg/kg/day or racemic formoterol at a dose of 120 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥10 mg/kg/day and racemic formoterol at 120 mg/kg/day. Maternal toxicity (i.e., death and clinical signs) was evident with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Post-implantation loss, consisting primarily of early resorption, was increased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. Number of viable fetuses and fetal body weight were decreased with (R,R)-formoterol at 60 and 120 mg/kg/day and racemic formoterol at 120 mg/kg/day. External malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related external malformations included omphalocele, localized fetal edema of the neck, anasarca, microphthalmia and/or anophthalmia, and micromelia. Omphalocele and microphthalmia and/or anophthalmia were not observed with racemic formoterol. Omphalocele at 10 mg/kg/day occurred independently of maternal toxicity. Skeletal malformations were evident for fetuses obtained from dams treated with (R,R)-formoterol at 60 or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Treatment-related skeletal malformations included bent limb bones and vertebral anomaly with or without associated rib anomaly. A finding of 1 fetus with 12 pairs of ribs was observed with only (R,R)-formoterol at 120 mg/kg/day. An increased incidence of skeletal variations, consisting primarily of reductions in ossification, was evident for fetuses obtained from dams treated with (R,R)-formoterol at 10, 60, or 120 mg/kg/day or racemic formoterol at 120 mg/kg/day. Several variations occurred at 10 mg/kg/day, independent of maternal toxicity. Treatment-related variations consisted of sternebra(e) #5 and/or # 6 unossified, sternebra(e) #1, #2, #3, and/or #4 unossified, bent ribs, decreased proportion of cervical centrum #1 ossified, reduced ossification of the vertebral arches, entire sternum unossified, pubis unossified, reduced ossification of the 13^{th} rib(s).

In a (Segment II) teratology study, mated female rats received (R,R)-formoterol at oral doses of 1, 5, or 10 mg/kg/day or racemic formoterol at a dose of 10 mg/kg/day from days 6 to 17 of gestation. Sacrifice was scheduled for day 20 of gestation. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥1 mg/kg/day and racemic formoterol at 10 mg/kg/day. There was no evidence of maternal toxicity with (R,R)-formoterol at ≤10 mg/kg/day. An external malformation, omphalocele, was observed with fetuses in the 1, 5, and 10 mg/kg/day (R,R)-formoterol groups, although, there was not a dose-response relationship. Omphalocele was reported in the previous teratology study (Sepracor Document number 090-820) with (R,R)-formoterol doses of 10, 60, and 120 mg/kg/day. Occurrence of omphalocele was clearly independent of maternal toxicity. The NOAEL for omphalocele appears to be <1 mg/kg/day. Umbilical herniation of the intestine (i.e., several loops of the intestine protruded through an opening in the umbilicus) was observed for 1 fetus in the 10 mg/kg/day racemic formoterol group. Incidences of unossified sternebrae #5 and/or #6 were increased for all (R,R)-formoterol groups as well as the racemic formoterol group. Incidences of cervical centrum #1 ossified were decreased for all (R,R)-formoterol groups as well as the racemic formoterol group. These variations occurred independently of maternal toxicity. On gestation days 6 or

17, the dose of 1 mg/kg/day produced an AUC that was approximately 750 to 900 times the AUC observed with a clinical inhalation dose of 48 µg/day; however, it should be noted that 1 mg/kg/day is not a NOAEL. This dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

In a dose range finding (Segment II) teratology study, artificially inseminated rabbits received (R,R)-formoterol at oral doses of 0, 20, 40, 80, 160, and 320 mg/kg/day from days 7 to 20 of gestation. (R,R)-formoterol appeared to be teratogenic as a treatment-related external malformation was observed at 80 mg/kg/day. Maternal toxicity was evident at doses of 40, 80, 160, and 320 mg/kg/day. Mortality occurred at doses of 40, 80, 160, and 320 mg/kg/day. All rabbits in the 160 and 320 mg/kg/day were euthanized on day 13 due to excessive toxicity. One female at 80 mg/kg/day aborted on day 28. Post-implantation loss was increased at 80 mg/kg/day. An external malformation, adactyly, was observed with 80 mg/kg/day (R,R)-formoterol at an incidence that exceeded concurrent and historical controls. The role of maternal toxicity for this finding is not known. Adactyly was also observed in the (Segment II) teratology study with rabbits in which (R,R)-formoterol was administered at oral doses of 20, 40, and 80 mg/kg/day.

In a (Segment II) teratology study, (R,R)-formoterol was administered by oral gavage at doses of 0, 20, 40, and 80 mg/kg/day to artificially inseminated female rabbits from days 7 to 20 of gestation. Teratogenic effects were evident in fetuses obtained from dams treated with (R,R)-formoterol at doses ≥20 mg/kg/day. Maternal toxicity was evident at 40 and 80 mg/kg/day and also possibly at 20 mg/kg/day. One rabbit at 40 mg/kg/day and three rabbits at 80 mg/kg/day aborted between days 23 and 29 of gestation. The incidence of decreased defecation was increased in a dose-related manner in all (R,R)-formoterol treatment groups. Post-implantation loss at 80 mg/kg/day was increased when compared to the control. This increase was attributed to higher incidences of early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control. Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol. Adactyly, syndactyly, and umbilical herniation of the intestine were observed at 80 mg/kg/day. Brachydactyly and short tails were observed at 40 and 80 mg/kg/day. Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Malpositioned kidney at 20 mg/kg/day occurred independently of maternal toxicity except for decreased defecation. Heart and/or great vessel anomalies were observed at 40 and 80 mg/kg/day. Lobular dysgenesis of the lungs was observed at 80 mg/kg/day. Visceral variations were observed at 40 and 80 mg/kg/day (R,R)-formoterol and skeletal variations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. In addition, there were histopathological findings in fetal tissues at 20, 40, and 80 mg/kg/day that could not be classified as variations. These effects at 20 mg/kg/day appeared to occur independently of maternal toxicity.

In a (Segment II) teratology study, artificially inseminated female rabbits received (R,R)-formoterol at oral doses of 2, 10, and 20 mg/kg/day from days 7 to 20 of gestation. Racemic formoterol at a dose of 20 mg/kg/day was included as a

comparator. In the present study, (R,R)-formoterol at doses ≤20 mg/kg/day was not teratogenic in contrast to the earlier study (Sepracor document number 090-819) where malformations were evident at doses ≥20 mg/kg/day. The incidences of major blood vessel variations were increased for (R,R)-formoterol and racemic formoterol treatment groups as compared to the concurrent control, although, there was no evidence of a dose response relationship. The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical dose of 48 µg/day. This clinical dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

General Toxicology Issues:

(R,R)-formoterol was teratogenic in studies with both rats and rabbits. Racemic formoterol, included as a comparator in these studies, was also teratogenic in rats; however, in a study in rabbits, the dose of the (R,R/S,S)-racemate (i.e., 20 mg/kg/day) was too low to allow teratogenicity determination, as comparable doses of the (R,R)-enantiomer (i.e., 2, 10, and 20 mg/kg/day) produced negative findings, in contrast to an earlier study with higher doses. The profile of teratogenic findings with the (R,R)- and (R,R/S,S)-formoterol in rats were generally similar given that the racemate contains the (R,R)-enantiomer. [redacted] b(4)

[redacted] The lack of findings with racemic formoterol may be considered somewhat ambiguous given that the racemic mixture contains the (R,R)-isomer, and that the (R,R/S,S)-racemate was found to be teratogenic in this IND.

External and skeletal malformations (i.e., localized fetal edema, micromelia, microphthalmia and/or anophthalmia, anasarca, omphalocele, bent limb bones, vertebral anomaly with or without associated rib anomaly, only 12 pairs of ribs) were evident in studies conducted with (R,R)-formoterol in rats. Omphalocele (i.e., several loops of the intestine protruded through an opening in the umbilicus, and remnants of a membranous sac were discernable) was observed in two studies with combined doses of 1, 5, 10, 60, and 120 mg/kg/day. The occurrence of omphalocele was clearly independent of maternal toxicity at doses ≤10 mg/kg/day. A NOAEL was not established for this finding (i.e., there was no threshold dose). The dose of 1 mg/kg/day produced an AUC on gestation days 6 or 17 that was approximately 750 to 900 times the AUC observed with a clinical inhalation dose of 48 µg/day; however, it should be noted that 1 mg/kg/day is not a NOAEL. This dose is comparable to doses proposed for Phase 3 trials (i.e., 50 mg QD and 25 mg BID).

External and visceral malformations (i.e., adactyly, short tail, syndactyly, brachydactyly, malpositioned kidney(s), bulbous aorta, lungs-lobular dysgenesis, interventricular septal defect) were evident in rabbits in one study with (R,R)-formoterol at doses of 20, 40, and 80 mg/kg/day. Malpositioned kidney(s) were observed at doses ≥20 mg/kg/day. This malformation at 20 mg/kg/day appeared to occur independently of maternal toxicity. In a second study with doses of 2, 10, and 20 mg/kg/day, there were

no teratogenic findings. The NOAEL could be considered 10 mg/kg/day. A threshold dose appeared to exist in studies with rabbits. The threshold dose of (R,R)-formoterol at 10 mg/kg/day, for both embryo/fetal toxicity studies in rabbits, produced an AUC that was 10059-12692 times the AUC observed with a clinical inhalation dose of 48 µg/day.

Teratogenic findings have been identified in studies with the β_2-agonists, albuterol sulfate, salmeterol, and metaproterenol. A study in CD-1 mice with albuterol sulfate at subcutaneous doses of 0.25 and 2.5 mg/kg/day induced cleft palate formation in 5 of 111 (4.5%) and 10 of 108 (9.3%) fetuses, respectively. The drug did not induce cleft palate formation when administered at a subcutaneous dose of 0.025 mg/kg. The positive control, isoproterenol at a subcutaneous dose of 2.5 mg/kg/day, induced cleft palate in 22 of 72 (30.5%) fetuses. In a reproduction study with Stride Dutch rabbits, albuterol at an oral dose of 50 mg/kg induced cranioschisis in 7 of 19 (37%) fetuses. Salmeterol was not teratogenic in rats at oral doses up to 2 mg/kg. However, in pregnant Dutch rabbits, salmeterol at oral doses ≥1 mg/kg exhibited fetal toxic effects characteristically resulting from beta-adrenoceptor stimulation that included precocious eyelid openings, cleft palate, sternebral fusion, limb and paw flexures, and delayed ossification of the frontal cranial bones. No effects were observed with an oral dose of 0.6 mg/kg. New Zealand White rabbits were less sensitive since only delayed ossification of the frontal cranial bones was observed at an oral dose of 10 mg/kg. Metaproterenol was found to be teratogenic in rats and embryotoxic in rabbits. Teratogenic effects included skeletal abnormalities, hydrocephalus, and skull bone separation

Malformations and variations observed with beta agonists may be class effects of these agents. $Beta_2$ agonists may induce several changes that include increased heart rate, hemodynamic perturbations (i.e., alterations of blood pressure and flow), hyperglycemia, hypokalemia, cardiac arrhythmias, pulmonary edema, myocardial ischemia, and alterations of uterine contractility that could result in adverse effects for fetuses. Treatment of chick embryos with cathecholamines, including epinephrine, can produce a variety of predictable aortic arch and intracardiac malformations (Teratology 12: 33-46, 1975; Teratology 21: 299-307, 1980; Teratology 28: 9-14, 1983; Teratology 38: 291-296, 1988). The pregnancy labeling for salmeterol includes the statement, "Extensive use of other beta-agonists has provided no evidence that these class effects in animals are relevant to use in humans." Possibly, malformations and variations produced by (R,R)-formoterol in rats and rabbits may be class effects of beta-adrenergic agonists, although, the benefits and risks of use of this agent in pregnant women need to be carefully considered. In studies conducted under this IND, there were no significant differences in the profile of teratogenic findings in rats between (R,R)-formoterol and racemic formoterol. In a study in rabbits, the dose of racemic formoterol (i.e., 20 mg/kg/day) was too low to allow teratogenicity determination, as comparable doses of the (R,R)-enantiomer (i.e., 2, 10, and 20 mg/kg/day) produced negative findings, in contrast to an earlier study with higher doses.

It is unclear if these findings are class effects of beta agonists and their relevance to humans in unknown. The Investigator's Brochure and Informed Consent should be modified to appropriately warn physician and patients, respectively, of the teratogenic potential of (R,R)-formoterol.

Recommendations:

Internal comments:

(R,R)-formoterol induced teratogenic effects in both rats and rabbits. It is unclear if these findings were class effects of beta agonists and their relevance to humans is not known. A threshold dose for teratogenic findings in rats was not established. However, with regard to findings in rabbits, a threshold dose was established at 10 mg/kg/day and the AUC at this dose was 10059-12692 times the AUC observed with a clinical inhalation dose of 48 µg/day. The Investigator's Brochure and Informed Consent need to be modified to inform physician and patients, respectively, of the teratogenic potential of (R,R)-formoterol.

Appropriate birth control measures should be included in all clinical trials with (R,R)-formoterol, including Phase III clinical trials. For the NDA, if submitted, product labeling should convey teratogenic findings with rats and rabbits.

External recommendations (to sponsor):

1. Teratogenic effects were evident in reproductive toxicology studies with rats and rabbits. Modify the Investigator's Brochure and Informed Consent to inform physician and patients, respectively, of the teratogenic potential of (R,R)-formoterol. Submit the modified text to the Division for our review and evaluation.

2. Clarify that the study entitled "Toxicokinetics of Formoterol During A 28-Day Oral Toxicity Study of (R,R)-Formoterol in Mice" (Sepracor Document number 090-470) was conducted using the oral route of administration. The summary provided with the study report makes references to the inhalation route.

3. Provide the spontaneous incidence of cardiomyopathy in — CD®(SD)IGS BR rats, 8-10 weeks of age. b(4)

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

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

/s/

Timothy Robison
11/26/01 03:07:35 PM
PHARMACOLOGIST

Robin Huff
11/27/01 05:11:07 PM
PHARMACOLOGIST

Appendix 8 IND 55,302 Review #11 dated February 6, 2002

PHARMACOLOGY/TOXICOLOGY COVER SHEET

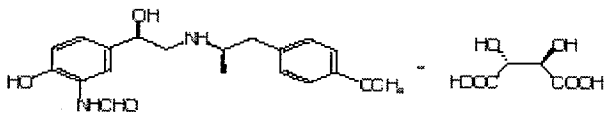
IND number: 55,302
Review number: #11
Sequence number/date/type of submission: #045/September 18, 2001/Amendment
#046/October 1, 2001/Amendment
#050/November 2, 2001/Amendment
#052/December 18, 2001/Amendment

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

Reviewer name: Timothy W. Robison, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: February 6, 2002

Drug:

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



b(4)

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

Drug class: β_2-Adrenergic Agonist

Indication: ⊏ ⊐ Chronic Obstructive Pulmonary Disease (COPD)

b(4)

Clinical formulation: Not provided.

Route of administration: Oral Inhalation

Proposed clinical protocol:

The studies described below comprise the sponsor's Phase III program for (R,R)-Formoterol. There are two pivotal studies, one non-pivotal comparison study, and one chronic safety study. Females of childbearing potential enrolled into these studies must be using an acceptable method of birth control. All females of childbearing potential must have a serum pregnancy test conducted at visit 1 and confirmed negative prior to randomization. Females who are considered not of childbearing potential must be either surgically sterile (defined as status posthysterectomy or bilateral tubal ligation) or postmenopausal (defined as documented cessation of menstruation for ≥12 consecutive months and a serum FSH in the postmenopausal range [>40 IU/L]).

Pivotal Studies:

Protocol Title: A double-blind, double-dummy, randomized, placebo- and active-controlled, multicenter, parallel-group study of (R,R)-Formoterol in the treatment of subjects with chronic obstructive pulmonary disease.
Protocol number 091-050
This study is designed to assess the bronchodilator effect and safety of multiple daily doses of (R,R)-formoterol administered for 12 weeks as maintenance treatment in patients with chronic obstructive pulmonary disease. This study is similar in design to the 091-051 protocol and will serve as a confirmatory study. The sponsor has proposed a randomized, double-blind, placebo- and active-controlled, parallel-group study in subjects (≥35 years old) with chronic obstructive pulmonary disease (COPD). (R,R)-Formoterol will be administered at inhaled doses of 15 µg B.I.D., 25 µg B.I.D., and 50 µg Q.D. Formoterol will be administered using a nebulizer. As an active comparator, salmeterol will be administered at an inhaled dose of 42 µg B.I.D. using a metered dose inhaler. A placebo-control group will also be included. The treatment period is 12 weeks. Approximately 800 subjects will be enrolled in the study and it is expected that approximately 625 will complete the study. There will be approximately 125 subjects per treatment group.

Protocol Title: A double-blind, double-dummy, randomized, placebo- and active-controlled, multicenter, parallel-group study of (R,R)Formoterol in the treatment of subjects with chronic obstructive pulmonary disease (Amendment #050).
Protocol number: 091-051
The present study is designed to assess the bronchodilator effect and safety of multiple daily doses of (R,R)-formoterol administered for 12 weeks as maintenance treatment in patients with COPD. The sponsor has proposed a randomized, double-blind, placebo- and active-controlled, parallel-group study in subjects (≥35 years old) with chronic obstructive pulmonary disease (COPD). (R,R)-Formoterol will be administered at inhaled doses of 15 µg B.I.D., 25 µg B.I.D., and 50 µg Q.D. Formoterol will be administered using a nebulizer. As an active comparator, salmeterol will be administered at an inhaled dose of 42 µg B.I.D. using a metered dose inhaler. A placebo-control group will also be included. The treatment period is 12 weeks. Approximately 800 subjects will be enrolled in the study and it is expected that

approximately 575 will complete the study. There will be approximately 115 subjects per treatment group.

Other studies described in the EOP2 meeting package are listed below.

Non-pivotal Comparison Study:

The sponsor has proposed a randomized, double-blind, placebo- and active-controlled, parallel-group study in subjects (≥35 years old) with COPD. (R,R)-Formoterol will be administered at inhaled doses of 25 µg BID and 50 µg QD using a nebulizer. As an active comparator, salmeterol will be administered at an inhaled dose of 42 µg B.I.D. using a metered dose inhaler. A placebo-control group will also be included. The treatment period is 4 weeks. Approximately 140 subjects will be enrolled in the study and it is expected that approximately 100 will complete the study.

Chronic Safety Study:

The sponsor has proposed a randomized, open-label, placebo- and active-controlled, parallel-group study using subjects from the two pivotal studies that received (R,R)-formoterol at 25 µg BID or 50 µg QD, salmeterol at 42 µg BID, or placebo. The treatment period is 9 months. Approximately 600 subjects will be enrolled in the study and it is expected that 300 subjects will complete 6 months of treatment and 100 subjects will complete 12 months (i.e., 3 + 9 months) of treatment.

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

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

Introduction and drug history:

(R,R)-Formoterol, a β_2-adrenergic agonist, is under development for treatment of 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)

An EOP2 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 for that study is 9 months.

Preclinical questions submitted by the sponsor at the EOP2 meeting and Division responses are listed below.

Human Pharmacokinetics and Bioavailability

2. Desformoterol is a degradant and an expected metabolites of (R,R)-formoterol. At a concentration much greater than would be clinically or toxicologically relevant (150 µg/mL), approximately 25% of (R,R)-formoterol added to human plasma held at 37°C is selectively transformed to desformoterol within 12 hr. Based on in vitro receptor binding studies, (R,R)-desformoterol possesses affinity for the B_2-receptor but is comparatively weak relative to the parent (R,R)-formoterol, suggesting that most of the pharmacological activity resides with (R,R)-formoterol. Rat and dog single-dose inhalation toxicology studies have shown that (R,R)-desformoterol administration results in the same or fewer effects than those observed for comparable doses of (R,R)-formoterol. Also, two genotoxicity studies, an in vitro chromosome aberration study with Chinese hamster ovary (CHO) cells and an in vivo mouse micronucleus test, were each negative with this material.

Several attempts to quantitate desformoterol exposure in animals and humans have been unsuccessful. Sepracor therefore proposes to complete the qualification of desformoterol at the levels expected in the drug product in accord with ICH guidelines by conducting a 28-day dog inhalation study.

Does the Division agree?

♦ From a preclinical standpoint, identification of metabolites and measurement of metabolite exposure levels in both animal species (i.e., mice, rats, and dogs) and humans is essential for the evaluation of several nonclinical studies (i.e., general toxicity, carcinogenicity).

♦ With regard to the presence of (R,R)-desformoterol, qualification might be achieved by measurement of metabolite exposure; however, this might not apply to other isomers of desformoterol. Qualification of degradants and impurities can be achieved with a 90-day inhalation study using the most appropriate species.

Nonclinical Drug Metabolism and Pharmacokinetics

1. A series of oral ADME studies with (R,R)-formoterol in the species used for the nonclinical safety, drug metabolism, and pharmacokinetic programs (rats, mice, and dogs) is in progress. It is proposed that results form the completed studies to date, in combination with the results from the ongoing and planned nonclinical studies (see Table 10.3.1-1), will be sufficient to support the proposed Phase III clinical program and the NDA for (R,R)-formoterol in COPD.

Does the Division agree that the proposed program is sufficient to support the NDA?

♦ Toxicokinetic data obtained primarily from inhalation studies with rats and dogs are essential to allow comparisons with human exposure. Toxicokinetic data from oral

studies may be provided, and would appear to be particularly important in the evaluation of the oral (gavage) carcinogenicity study with mice.

♦ Metabolism of (R,R)-formoterol should also be thoroughly characterized in mice, rats, and dogs to allow comparisons with human metabolism. This will be particularly important in the evaluation of carcinogenicity studies conducted with mice and rats.

Toxicology

1. Does the FDA concur that the animal safety data available to date adequately support the initiation of Phase III and exposure of the planned number of subjects?

♦ The longest toxicology studies submitted to the Division are the 28-day inhalation toxicology studies in rats and dogs. Thus, animal safety data available to date are <u>not</u> adequate to support the initiation of the proposed Phase III clinical trials.

> The 6-month inhalation study in rats and the 13-week inhalation study in dogs should be submitted to the Division prior to initiation of proposed trials (unaudited reports are acceptable). Assuming adequate safety margins are established in both studies, which are review issues, there may be adequate support for the initiation of the proposed clinical trial.
>
> Prior to extension of the proposed clinical trials beyond 12 weeks of treatment, the 9-month inhalation study in dogs should be submitted to the Division.

2. Does the Division agree with the adequacy of the nonclinical safety evaluation program that is proposed to support the NDA?

♦ Completion of the preclinical development program as outlined in Table 10.2-1 would appear adequate to support an NDA submission. These studies should be submitted to the Division, in an appropriate time frame as described in the Guidance for Industry: M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (July 1997, ICH). Adequacy of individual studies is a review issue.

Studies reviewed within this submission:

STUDY	Sepracor Document #	Amendment
TOXICOLOGY:		
Subchronic/Chronic Toxicity		
Rat		
6-month inhalation toxicology study with rats.	090-827	#046
Dog		
13-week inhalation toxicology study with dogs.	090-830	#046
9-month inhalation toxicology study with dogs.	090-829	#052

b(4)

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PHARMACOLOGY/TOXICOLOGY REVIEW

IV. GENERAL TOXICOLOGY:

Subchronic/Chronic Toxicity

Rats

Study title: A 6-Month Inhalation Toxicity Study of (R,R)—Formoterol in Rats with a 1-Month Recovery Period.

Key study findings:

♦ In a 6-month inhalation toxicology study, rats were exposed to (R,R)-formoterol at target doses of 100, 400, and 800 µg/kg/day. Deposited doses were 10, 40, and 77 µg/kg/day, respectively.

♦ The NOAEL was identified as 10 µg/kg/day due to findings of treatment-related mortality with doses of 40 and 77 µg/kg/day.

♦ There was no apparent target organ of toxicity. However, an increased incidence of thymic hemorrhage was observed at 77 µg/kg/day.

♦ AUC values for (R,R)-formoterol at the NOAEL for male and female rats were 2920 and 4010 pg·hr/mL, respectively.

Study no: Sepracor Document number 090-827
Volume #, and page #: Amendment #046, Volumes 1-4, Pages 1 to 1211
Conducting laboratory and location: ⌐ ⌋ b(4

Date of study initiation: June 18, 1999
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-formoterol tartrate, lot number 113098A (Purity, 99.6%)
Formulation/vehicle: 0.9% sodium chloride for injection

Methods (unique aspects)**:** Toxic effects of (R,R)-formoterol were evaluated in rats exposed by nose-only inhalation to doses of 0, 100, 400, and 800 µg/kg/day for 6 months (182 consecutive days). Recovery was evaluated during and following a 1-month recovery period.
Dosing:
Species/strain — CD®(SD)IGS BR rats were obtained from ⌐ ⌋ b(4)
⌐ ⌋ on August 3, 1999.
#/sex/group or time point (main study): For the toxicology study groups, the air-control and high dose groups of 20 rats/sex/group. The low and mid dose groups consisted of

15 rats/sex/day. Following 26-weeks of exposure, 15 rats/sex/group were assigned to the primary necropsy. The remaining 5 rats/sex/group in the air-control and high dose groups were assigned to a 1-month recovery period.
Satellite groups used for toxicokinetics or recovery: For the toxicokinetic study, there were 3 groups of 15 rats/sex/group. In addition, there was a sentinel group composed of 15 rats/sex/group.
Age: Animals were approximately 9.5 weeks old at the start of treatment.
Weight: For toxicology groups, body weight ranges were 295-375 g for male rats and 186-247 g for female rats. For toxicokinetic groups, body weight ranges were 297-376 g for male rats and 170-249 g for female rats.
Doses in administered units: Target doses were 100, 400, and 800 µg/kg/day (all doses are expressed as free base equivalents). Deposited doses were 10, 40, and 77 µg/kg/day, respectively.

Inhaled and deposited doses of (R,R)-formoterol in rats.

Group[1]	Target Inhaled Dose, µg/kg/day	Actual Inhaled Dose, µg/kg/day	Deposited Dose[2], µg/kg/day
1	0	0	-
2 and 5	100	99	10
3 and 6	400	396	40
4 and 7	800	773	77

1. Toxicology groups were Groups 1-4 and Toxicokinetic groups were Groups 5-7.
2. The deposition factor was estimated to be 0.10. MMAD ± GSD determined for the 100 and 800 µg/kg/day dose levels were 0.8 ± 1.70 and 0.9 ± 1.82 µm, respectively.

Estimated dose levels were calculated as follows:

$$\text{Inhaled dose} = \frac{\text{Exposure concentration (µg/L) x minute volume (L/min) x Duration (min)}}{\text{mean body weight (kg)}}$$

Exposure concentrations were determined by chemical analysis of test exposure atmosphere samples that were collected on glass-fiber filters during the 30-min exposure periods. Material deposited on filter discs was analyzed by HPLC to determine the amount of (R,R)-formoterol present.

Route, form, volume, and infusion rate: Inhalation

Exposures were conducted using ⊏ ⊐ (directed flow) nose-only exposure systems. Saline and test aerosols were delivered from the generation apparatus to a modified glass and Plexiglass chromatography jar and then to each nose-only system. For the test article exposures of each group, liquid droplet aerosol atmospheres were generated by jet nebulization from saline solutions of the test article. The modified ⊏ ⊐ Collision nebulizer ⊏ ⊐ was selected for use based on production of aerosols with particle sizes (MMAD) less than 2 µm. b(4)

For each exposure, the animals were placed in nose-only restraint tubes. The actual exposure concentration for each group was used with the exposure duration, group mean body weight, and minute volume to calculate an estimated inhaled dose. For each treatment level, all toxicology group and toxicokinetic group male and female

rats were exposed together. Target concentrations were based on combined male and female groups. The exposure period was 30 min for all groups. Due to changes in mean body weights and estimated minute volumes during the phase, calculated target exposure concentrations varied.

		Target Dosage Level (µg/kg/day)								
		100			400			800		
Date Implemented	No. of Days	MV (L/min)	Mean BW (kg)	Target Conc. (µg/L)	MV (L/min)	Mean BW (kg)	Target Conc. (µg/L)	MV (L/min)	Mean BW (kg)	Target Conc. (µg/L)
08/31/99	17	0.20	0.270	4.5	0.20	0.268	18	0.19	0.266	37
09/17/99	13	0.23	0.326	4.7	0.23	0.326	19	0.23	0.323	37
09/30/99	27	0.24	0.355	4.9	0.24	0.355	20	0.24	0.350	39
10/27/99	27	0.26	0.382	4.9	0.26	0.384	20	0.25	0.373	40
11/23/99	29	0.27	0.398	4.9	0.27	0.396	20	0.26	0.378	39
12/22/99	35	0.28	0.415	4.9	0.28	0.411	20	0.26	0.390	40
01/26/00	36	0.28	0.422	5.0	0.28	0.412	20	0.27	0.394	39

Dosing solutions were prepared at least weekly and stored refrigerated. Based upon the observation of particulate matter in the test article solution for the 800 µg/kg/day groups (Groups 4 and 7) prepared on study day 148 (which did not have the particulate matter when reformulated), the test article solution for the 800 µg/kg/day groups was filtered through a 0.2 µm filter beginning on day 155. Beginning on day 163, the test article solution for all groups (Groups 2 through 7) was filtered through a 0.2 µm filter.

Observations and times:

Clinical signs: Animals were observed twice daily for moribundity/mortality. Clinical examinations were conducted on all animals immediately following exposure (after unloading from restraint tubes). Clinical examinations were conducted once daily during the recovery period. Physical examinations (toxicology groups only) were conducted weekly.

Body weights: Body weights were measured weekly.

Food consumption: Food consumption was measured weekly.

Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of exposures and during week 25. Examinations were not conducted at the end of recovery period due to an apparent lack of findings at the end of treatment period.

EKG: Not performed.

Hematology: For the treatment period (10 rats/sex/group), hematology parameters were determined during weeks 1 and 26. For the recovery period (5 rats/sex/group), hematology parameters were determined for the control and high dose groups during week 30.

Clinical chemistry: For the treatment period (10 rats/sex/group), serum chemistry parameters were determined during weeks 1 and 26. For the recovery period (5 rats/sex/group), serum chemistry parameters were determined for the control and high dose groups during week 30.

Urinalysis: For the treatment period (10 rats/sex/group), urinalysis parameters were determined during weeks 1 and 26. For the recovery period (5 rats/sex/group), urinalysis parameters were determined for the control and high dose groups during week 30.
Gross pathology: Necropsy examinations were conducted on all toxicology group animals at the end of the treatment and recovery periods.
Organs weighed: Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes with epididymides, thymus gland, thyroid (with parathyroids), and uterus.
Histopathology: Following collection of protocol-specified tissues (see histopathology inventory table), the entire head was removed and preserved. Following decalcification, six cross-sections of the nasal cavities were prepared for microscopic examination. After fixation, protocol-specified tissues were trimmed, processed into paraffin blocks, sectioned at 4-8 µm, mounted on glass slides, stained with hematoxylin and eosin, and submitted to microscopic examination. The sponsor reported that tissues were examined for the rat at 800 µg/kg/day that was sacrificed in a moribund condition all animals at the primary necropsy (i.e., 26 weeks). However, only results from analysis of the heart, kidneys, liver, lungs, and nasal cavity were provided for the control, low dose, mid dose, and high dose groups, while results from analysis of all other tissues were limited to control and high dose groups. Microscopic examination of tissues was not performed at the recovery necropsy (i.e., 30 weeks) since no test article-related microscopic changes were observed at the primary necropsy.
Toxicokinetics: Toxicokinetic groups consisting of 15 rats/sex/group were exposed to (R,R)-formoterol at doses of 100, 400, and 800 µg/kg/day for 182 consecutive days. Blood samples for measurement of (R,R)-formoterol levels were collected on day 0 and during weeks 13 and 26 at 0.167, 0.5, 1, 2, 6, and 24 hr following exposure. For each blood collection interval, 3 rats/sex/group/time point were used. Animals used for the 10 min time point were also used for the 24 hr time point with the following exception. Due to accidental deaths of one male and one female in the 100-µg/kg/day group prior to the final blood collection interval, only two animals/sex from that group were used for the 24-hr time point. The same two animals/sex and one animal/sex from those assigned to the 6-hr time point were used for the 10-min 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 multiple reaction monitoring (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. Animals were euthanized following the final blood collection and discarded without examination.

Results: Deposited doses for low, mid, and high dose (R,R)-formoterol groups were 10, 40, and 77 µg/kg/day, respectively.

Mortality: Treatment-related deaths were observed in the 40- and 77-µg/kg/day toxicology groups. There were no deaths in the 10-µg/kg/day toxicology group.

However, two deaths occurred in the 10-µg/kg/day toxicokinetic group, but the treatment relationship of these deaths was uncertain (e.g., possibly accidental).

In the 40-µg/kg/day toxicology group, 3 male rats (numbers 27774, 27779, and 27704) died on day 1. These animals were replaced with 3 male rats (numbers 27739, 27681, and 27736) on day 2. No necropsy examinations were conduced on animals that died. There was no evidence of accidental trauma due to restraint tubes in any of these animals that died.

In the 77-µg/kg/day group, 1 male rat (number 27671) died on day 1 and was replaced with 1 male rat (number 27783) on day 2. This male rat (number 27783) died on day 2 and was replaced with another male rat (number 27706) on day 3. In the 77-µg/kg/day toxicokinetic group, 1 male rat (number 27791) died on day 1 before its scheduled 24-hr blood collection. This animal was replaced with another male rat (number 27765), which was exposed on day 1 and used for blood collection on days 1 and 2. Blood collected from animal #27791 was not used for toxicokinetic evaluation. A necropsy examination was conducted on #27783; however, no examinations were conducted on #27671 and #27791. There was no evidence of accidental trauma due to restraint tubes in any of these animals that died.

One female rat (#27809) in the 77-µg/kg/day group was sacrificed in a moribund condition on day 180. Clinical signs for this animal prior to sacrifice consisted of lethargy, emaciation, labored respiration, decreased defecation, wet brown material on the trunk, and wet red material on the urogenital and anogenital areas. Tissues from this animal were submitted to microscopic examination. Chronic active inflammation (severe) and ulceration (severe) were observed in the rectum. Acute inflammation (moderate) was observed in the uterus. Ulceration (minimal) and chronic active inflammation were observed in the urinary bladder. Myeloid hyperplasia was observed in the sternum bone marrow and spleen. Hepatocellular necrosis (minimal) was observed in the liver.

In the control group, 1 male rat (number 27675) died during blood collection on day 8 and was replaced with 1 male rat (number 27777) on the same day).

One male rat (number 27679) and one female rat (number 27845) from the 10-µg/kg/day toxicokinetic group died during weeks 22 and 17, respectively. These animals were discarded without further evaluation.

Accidental deaths of one male rat and one female rat in the 10-µg/kg/day toxicokinetic group occurred prior to the final blood collection interval. Animal numbers were not reported and it is not clear if these are numbers 27679 and 27845.

Note: All replacement animals were selected from the same original shipment of animals received on August 3, 1999.

Clinical signs: Lethargy was observed at 77 µg/kg/day for a total of 19 males and 14 females. Lethargy was also observed at a low incidence for 4 males at 40 µg/kg/day. Lethargy for male and female rats at 77 µg/kg/day had an onset at study days 26 and

91, respectively, and continued sporadically throughout the remainder of the treatment period. Lethargy did not persist for 24-hr post-exposure, and it did not persist into the recovery period.

Clinical signs immediately following exposure (number of occurrences/number of animals).

Clinical Sign	Sex	Deposited doses, µg/kg/day			
		0	10	40	77
Lethargy	Male Female	0 0	0 0	4/4 0	105/19 49/14

Body weights: During the treatment period, absolute body weight and body weight gain for male rats at 77 µg/kg/day were suppressed during the treatment period. During the recovery period, body weight gains for male and female rats from the 77-µg/kg/day group were suppressed.

Body weights relative to the control and body weight gains for deposited doses of 10, 40, and 77 µg/kg/day at week 26 (end of the treatment period).

Body weight (BW) measurements	Control		10 µg/kg/day		40 µg/kg/day		77 µg/kg/day	
	M	F	M	F	M	F	M	F
BW, g at Week 0	341	211	341	221	341	213	334	218
BW, g at Week 26	538	292	536	334	534	328	497	322
% of Control BW at Week 26	100	100	99.6	114.4	99.3	112.3	**92.4**	110.3
BW change, g	197	81	195	113	193	115	163	104
BW gain relative to control, %	100	100	98.9	133.2	98	140.6	**84.5**	124.3

Body weight gain for a deposited dose of 77 µg/kg/day at week 30 (end of the recovery period).

Body weight (BW) measurements	Control		77 µg/kg/day	
	Male	Female	Male	Female
BW, g at Week 27	529	314	495	309
BW, g at Week 30	570	330	518	310
BW change	41	16	23	1
BW gain relative to control, %	100	100	60	6.3

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

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

Hematology: Platelet counts during week 26 were decreased for all female treatment groups and the male high dose group.

Week 1: Platelet counts for male and female rats at 77 µg/kg/day were decreased to 92.1 and 92.6% of controls (1124 x 10^3/µL and 1069 x 10^3/µL), respectively.

Week 26 (End of Treatment Period): Platelet counts for male rats at 77 µg/kg/day were decreased to 82.6% of the control (1046 x 10^3/µL). Platelet counts for female rats at 10, 40, and 77 µg/kg/day were decreased to 78.2, 80.25, and 74.6% of the control (1099 x 10^3/µL), respectively. There were slight changes of activated partial thromboplastin time (APTT), although, they appeared to have no biological significance. There were no changes of prothrombin time.

Week 30 (End of Recovery Period): Platelet counts for male and female rats at 77 µg/kg/day were decreased to 90.5 and 86.8% of controls (1119 x 10^3/µL and 973 x 10^3/µL), respectively.

Clinical chemistry: Decreased levels of glucose and amylase activity in male and female treatment groups appeared to be related to the pharmacological activity of (R,R)-formoterol. Blood urea nitrogen levels were elevated in male and female treatment group; however, no treatment-related histopathological changes were evident in the kidneys. Slight changes of albumin levels, globulin levels and albumin to globulin ratios were evident in male and female treatment groups, although, the toxicological significance of these changes appeared to be questionable.

Week 1:

Glucose levels for male rats at 10, 40, and 77 µg/kg/day were decreased to 74.8, 69.1, and 71.5% of the control (123 mg/dL), respectively. Glucose levels for female rats at 10, 40, and 77 µg/kg/day were decreased to 79.5, 76.1, and 74.4% of the control (117 mg/dL), respectively. Amylase levels for male rats at 10, 40, and 77 µg/kg/day were decreased to 84.2, 75.6, and 81% of the control (1387 U/L), respectively. Amylase

levels for female rats at 10, 40, and 77 µg/kg/day were decreased to 86.5, 88.8, and 82.75% of the control (858 U/L), respectively.

Blood urea nitrogen levels for male rats at 40 and 77 µg/kg/day were increased to 121.15 and 123.1% of the control (15.6 mg/dL), respectively. The blood urea nitrogen level for female rats at 77 µg/kg/day was increased to 116% of the control (17.5 mg/dL).

Albumin levels for female treatment groups were all decreased to 93.8% of the control (4.8 g/dL). The albumin to globulin (A/G) ratios for female rats at 10, 40, and 77 µg/kg/day were decreased to 92.7, 88, and 85.4% of the control (2.74), respectively.

Cholesterol levels for female rats at 10, 40, and 77 µg/kg/day were decreased to 89.2, 78.5, and 78.5% of the control (65 mg/dL), respectively.

Week 26 (End of Treatment Period):
Glucose levels for male rats at 10, 40, and 77 µg/kg/day were decreased to 80.2, 64.3, and 62.7% of the control (126 mg/dL), respectively. Glucose levels for female rats at 10, 40, and 77 µg/kg/day were decreased to 69.6, 60.8, and 57.6% of the control (125 mg/dL), respectively. Amylase levels for male rats at 10, 40, and 77 µg/kg/day were decreased to 93.6, 73, and 79.7% of the control (1383 U/L), respectively. Amylase levels for female rats at 10, 40, and 77 µg/kg/day were decreased to 76.2, 77.1, and 71.5% of the control (1230 U/L), respectively.

Blood urea nitrogen levels for male rats at 10, 40, and 77 µg/kg/day were decreased to 125.5, 134.2, and 131.7% of the control (16.1 mg/dL), respectively. Blood urea nitrogen levels for female rats at 10, 40, and 77 µg/kg/day were increased to 117.2, 112.6, and 123% of the control (17.4 mg/dL), respectively.

Albumin levels for male treatment groups were decreased to 91.3-95.7% of the control (4.6 g/dL). Globulin levels for male treatment groups were slightly increased to 108.3-116.7% of the control (2.4 g/dL). Globulin levels for female rats at 40 and 77 µg/kg/day were 113.6 and 118.2% of the control (2.2 g/dL), respectively. The A/G ratios for male treatment groups were decreased to 79.3-89.1% of the control (1.93), respectively. The A/G ratios for female rats at 10, 40, and 77 µg/kg/day were decreased to 85.8, 79.6, and 75.8% of the control (2.60), respectively.

Aspartate transferase levels for male rats at 40 and 77 µg/kg/day were slightly increased to 131.25 and 126% of the control (96 U/L), respectively. Alkaline phosphatase levels for female rats at 77 µg/kg/day were increased to 140.5% of the control (37 U/L).

Potassium levels for female rats at 40 and 77 µg/kg/day were increased to 109.3 and 111.9% of the control (5.03 mEq/L).

Week 30 (End of Recovery Period): Glucose levels for male rats at 77 µg/kg/day were decreased to 89.8% of the control (128 mg/dL), respectively. Blood urea nitrogen levels for male and female rats at 77 µg/kg/day were increased to 119.4 and 122% of controls (15.5 and 15.9 mg/dL). Globulin levels for female rats at 77

µg/kg/day were increased to 113% of the control (2.3 g/dL). The A/G ratio for female rats at 77 µg/kg/day was decreased to 90% of the control (2.31). Alkaline phosphatase levels for female rats at 77 µg/kg/day were increased to 126.7% of the control (30 U/L).

Urinalysis: No treatment-related changes of urinalysis parameters were evident at weeks 1, 26, and 30.

Organ weights: Changes of organ weights were evident; however, there were no apparent corresponding histopathological findings.

Week 26 (End of Treatment Period):

Heart: Relative heart weights for male rats at 10, 40, and 77 µg/kg/day were increased to 108.1, 114.6, and 119.4% of the control (0.309%), respectively. Absolute heart weights for female rats at 10, 40, and 77 µg/kg/day were increased to 118.3-125% of the control (1.04 g). No changes of heart weight relative to body weight were evident for female treatment groups. However, heart weight relative to brain weight values for female rats at 10, 40, and 77 µg/kg/day were increased to 120.7-124.8% of the control (55.057%). Heart weight changes for female treatment groups may have been due to increased body weight.

Thymus: Absolute thymus weight for male rats at 77 µg/kg/day was decreased 73.1% of the control (0.1586 g). Thymus weight relative to brain weight for male rats at 10, 40, and 77 µg/kg/day were decreased to 82, 84.6, and 77.3% of the control (7.719%), respectively.

Thyroid/Parathyroid: Absolute thyroid/parathyroid weights for female rats at 10, 40, and 77 µg/kg/day were increased to 122.7, 122.7, and 134.3% of the control (0.0181 g), respectively. Thyroid/parathyroid to brain weight values for female rats at 10, 40, and 77 µg/kg/day were increased to 123.1, 126.3, and 137.8% of the control (0.953%), respectively. No changes in thyroid/parathyroid to body weight values were evident. Changes in thyroid/parathyroid weights for female treatment groups may have been due to increased body weight.

Week 30 (End of Recovery Period):

Thymus: Thymus weight relative to brain weight for male rats at 77 µg/kg/day was decreased to 79% of the control (7.334%).

Gross pathology:

There were no treatment-related gross pathological findings at the end of the treatment or recovery periods.

The sentinel group was sacrificed and necropsied at the week 30 evaluation. There were apparently no findings of macroscopic changes suggestive of an infectious or contagious process.

Histopathology:

Week 26 (End of Treatment Period): An increased incidence of thymic hemorrhage was evident for male and female rats at 77 µg/kg/day. The sponsor considered thymic hemorrhage a sporadic finding related to the euthanasia or tissue collection processes and not a direct test article-related effect. Thymic hemorrhage was not observed in the 28-day inhalation toxicology study with rats. Additional histopathological changes were evident at the high dose; however, any treatment relationships were questionable because findings were generally of low incidence and confined to one sex. These findings in the high dose group were as follows: angiectasis in the adrenal cortex for female rats; cyst in the par distalis of the pituitary gland for female rats; and sinus histiocytosis in the mesenteric lymph node for female rats. Subacute inflammation in the lungs was evident at low incidence in mid and high dose groups. Plasmacytosis in the medullary lymph node was evident at a low incidence in the high dose group. Cardiomyopathy was evident in the control and treatment groups. For 12 to 13 month studies with Sprague-Dawley rats, the incidence of cardiomyopathy was reported to range from 11.1-73.3% in males and 10-40% in females in testing laboratories (Charles River Laboratories; Spontaneous Neoplastic Lesions and Selected Non-neoplastic Lesions in the CRL:CD®BR rats February 1992). Corpora amylacea in the nasal cavity was evident in control and treatment groups.

Week 30 (End of Recovery Period): Histopathological examinations of tissues was not conducted at the end of the recovery period due to a lack of treatment-related findings at the end of the treatment period.

Histopathological findings at the end of the treatment period for rats that received (R,R)-formoterol at deposited doses of 0, 10, 40, and 77.3 µg/kg/day.

Organ/Tissue	Sex	Deposited Dose, µg/kg/day			
		0	100	400	800
Thymus					
-hemorrhage (minimal)	M	2/15	-	-	9/15
	F	2/15	-	-	5/14
-hyperplasia, epithelial (minimal)	M	0/15	-	-	0/15
	F	0/15	-	-	3/14
Adrenal cortex					
-angiectasis (minimal)	M	0/15	-	-	0/15
	F	1/15	-	-	5/14
Pituitary gland					
-cyst, pars distalis (minimal)	M	0/15	-	-	0/15
	F	0/15	-	0/1	3/14
Lungs					
-subacute inflammation (minimal)	M	0/15	0/15	0/15	2/15
	F	0/15	0/15	1/15	0/15
Medullary lymph node					
-plasmacytosis	M	0/15	-	-	3/14
	F	0/15	0/1	0/1	1/14
Mesenteric lymph node					
-sinus histiocytosis (minimal)	M	0/15	-	-	0/15
	F	0/15	-	-	3/14
Heart					
-cardiomyopathy (minimal)	M	4/15	8/15	6/15	4/15
	F	1/15	3/15	3/15	1/14
Nasal Level 4					
-corpora amylacea (minimal)	M	1/15	3/15	3/15	1/15
	F	0/15	1/15	1/15	2/14
Nasal Level 5					
-corpora amylacea (minimal)	M	10/15	11/15	7/15	11/15
	F	0/15	3/15	3/15	5/14

Toxicokinetics:

Systemic exposure to (R,R)-formoterol was significantly higher on day 1 for all dose levels as compared to months 3 and 6. For doses of 40 and 77 µg/kg/day, systemic exposure to (R,R)-formoterol declined as the study progressed (i.e., systemic exposure at day 1 > month 3 > month 6). For the dose of 10 µg/kg/day, AUC values at months 3 and 6 were comparable. On day 1, AUC values increased in a dose proportional manner. At months 3 and 6, AUC values at 40 and 77 µg/kg/day were approximately dose proportion; however, these values were significantly greater than expected based upon values at 10 µg/kg/day. Female rats had higher C_{max} values as compared to male rats.

Mean plasma desformoterol/formoterol ratios in male and female rats following the inhalation of (R,R)-formoterol were measurable in all dose groups on day 1 and during month 3, but undetectable during month 6. Desformoterol/formoterol ratios at collection time points for doses of 10, 40, or 77 µg/kg/day were ≤0.15.

Toxicokinetic parameters for plasma (R,R)-formoterol following inhalation exposure of rats to (R,R)-formoterol at doses of 100, 400, and 800 µg/kg/day.

Dose µg/kg/day	Study Interval	$AUC_{(0.17\text{-}24\ hr)}$, pg·hr/mL		C_{max}, pg/mL		T_{max}, hr	
		Males	Females	Males	Females	Males	Females
10	1 Dose	14500	14800	2010	2690	0.17	0.17
	Month 3	3860	3910	833	1190	0.17	0.17
	Month 6	2920	4010	926	1320	0.17	0.17
40	1 Dose	27800	30900	6870	8520	0.17	0.17
	Month 3	21100	22000	8770	9290	0.17	0.17
	Month 6	13700	19700	4850	6230	0.17	0.17
77	1 Dose	58700	56200	22100	22700	0.17	0.17
	Month 3	38700	38300	15900	19500	0.17	0.17
	Month 6	21000	32900	9170	8690	0.17	0.17

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

Dogs

Study title: A 13-Week Inhalation Toxicity Study (with Recovery) of (R,R)-Formoterol in Dogs.

Key study findings:

♦ In a 13-week nose-only aerosol inhalation toxicology study, beagle dogs received (R,R)-formoterol by nose-only aerosol inhalation at target inhaled doses of 0, 5, 40, and 70/100 µg/kg/day (deposited doses of 0, 1, 8, and 14/20 µg/kg/day, respectively). Due to clinical effects including respiratory depression and cyanosis (during exposure) for two male dogs and morbidity for 1 female dog, the high dose was lowered from 100 to 70 µg/kg/day on day 3 for males and day 22 for females.

♦ Treatment-related deaths occurred at the high dose for one male and one female, which were both euthanized in extremis on days 87 and 21, respectively.

♦ A NOAEL was not established due to electrocardiographic abnormalities (i.e., ventricular ectopic patterns) consisting of premature ventricular beat and/or ventricular escape beat or rhythm, which were observed after the first exposure to R,R-formoterol at all dose levels.

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

♦ There were no target organs of toxicity.

Study no: 090-830
Volume #, and page #: Volumes 5-9, Pages 1-1742
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: October 14, 1999 (Initiation of exposures for male and female dogs were December 7 and 9, 1999, respectively)
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol (L)-Tartrate, Lot number 113098A
Formulation/vehicle: The vehicle used during acclimation and saline aerosol exposures and in the formulation of the test article solutions was 0.9% Sodium Chloride for Injection, USP.

Methods (unique aspects): Beagle dogs received (R,R)-formoterol by nose-only aerosol inhalation at total inhaled doses of 0, 5, 40, and 70/100 µg/kg/day (deposited doses of 0, 1, 8, and 14/20 µg/kg/day, respectively) for 92 consecutive days. Due to clinical effects including respiratory depression and cyanosis (during exposure) for two male dogs and morbidity for 1 female dog, the high dose was lowered from 100 to 70 µg/kg/day on day 3 for males and day 22 for females. It should be noted that the sponsor designated the first week of treatment as week 0. Animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm.
Dosing:

Species/strain: Beagle dogs were obtained from [redacted]

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#/sex/group or time point (main study): The control and high dose groups were composed of 6 dogs/sex/group. The low and mid dose groups were composed of 4 dogs/sex/group.
Satellite groups used for toxicokinetics or recovery: 2 dogs/sex/group from the control and high dose groups were designated for a 1-month recovery period following the treatment period.
Age: Dogs were approximately 6.5 months old at the start of treatment.
Weight: Body weight ranges were 6.9-8.2 kg for male dogs and 5.8-7.6 kg for female dogs.
Doses in administered units: (R,R)-Formoterol was administered at target inhaled doses of 0, 5, 40, and 70/100 µg/kg/day. All doses are expressed as free base equivalents of (R,R)-formoterol. For the high dose group, the dosage level was decreased from 100 to 70 µg/kg/day on day 3 for males and day 22 for females. The control group was exposed to an aerosol of the vehicle, 0.9% sodium chloride (USP), at a level approximately matching the saline concentration in the test atmosphere for the high dose group.

Group	Test Article	Daily Exposure Duration (min)	Target Inhaled Dose µg/kg/day	Number of animals	
				Males	Females
1	Saline	15	0	6	6
2	(R,R)-Formoterol	15	5	4	4
3	(R,R)-Formoterol	15	40	4	4
4	(R,R)-Formoterol	15	70/100	6	6

a. For the high dose group, the dosage level was decreased from 100 to 70 µg/kg/day on day 3 for males and day 22 for females.

The estimated dosage (as inhaled dose in µg/kg) for each male and female group of dogs was calculated from the exposure using the following equation:

Inhaled Dose (µg/kg) = Exposure concentration (µg/L) x mean minute volume to mean body weight ratio (L/min/kg) x Exposure duration (min)

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

Group	Minute volume/BW L/min/kg[a]		Exposure Duration (min)	Mean Exposure concentration[b], µg/L		Target exposure concentration, µg/L (mean)		Mean Estimated Dose µg/kg/day (% of target)	
	Male	Female		Male	Female	Male	Female	Male	Female
Low Dose	0.479 0.425	0.408 0.494	15	0.73	0.74	0.70 0.78 (0.74)	0.82 0.67 (0.74)	4.9 (98.1%)	5.0 (100%)
Mid Dose	0.435 0.459	0.478 0.442	15	5.8	5.4	6.1 5.8 (5.9)	5.6 6.0 (5.8)	39.0 (97.5%)	37.1 (91.8%)
High Dose	0.450 0.432 0.450	0.450 0.472	15	10.3	10.9	14.8 (1d) 15.4 (2d) 10.8 10.4 (10.7)	14.8 10.4 9.9 (11.1)	68.5 (96.7%)	75.6 (99.0%)

a. The mean minute volume to body weight ratio (L/min/kg) for each male and female group was used for these calculations. Two sets of ratios were used for this study. The first set was based on minute volume determined near the initiation of dosing and body weight at randomization and was used for exposures up to and including 1-17-00. Adjusted ratios were implemented on 1-18-00 and were based on the minute volume determinations from January 2000 and most recent body weights.

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

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

Aerosol particle size in test atmosphere for the 70/100 µg/kg (R,R)-Formoterol group.

Date	MMAD	GSD
11-30-1999	0.6	2.52
12-3-1999	0.9	1.84
1-7-2000	0.7	1.71
2-4-2000	0.7	1.54
Mean	0.7	1.90

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

Target Dose µg/kg/day	Estimated Inhaled Dose µg/kg/day		Deposited Dose[a] µg/kg/day	
	Male	Female	Male	Female
5	4.9	5.1	1.0	1.0
40	39.0	37.1	7.8	7.4
70/100	68.5	75.8	13.7	15.2

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

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

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

For test article exposures of treatment groups, liquid droplet aerosol atmospheres were generated by jet nebulization from saline solutions of the test article. A modified [redacted] collision nebulizer was used to produce aerosols with particle sizes <2 µm. b(4)

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Observations and times:

Clinical signs: Animals were observed twice daily for mortality and moribundity. Animals were monitored for clinical signs of toxicity during the time of exposure and approximately 1-2 hr after exposure. Animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm.

Body weights: Body weights were measured weekly.

Food consumption: Individual food consumption was recorded daily and weekly averages were reported.

Ophthalmoscopy: Ophthalmic examinations were conducted on animals prior to the start of treatment (week -1) and during week 12. The sponsor reported that since no treatment-related ocular lesions were observed during week 12, examinations were not performed at the end of the recovery period.

EKG: Modified lead 1, 2, 3 electrocardiograms (ECGs) for heart rate evaluation were recorded for each dog using a , prior to the initiation of exposure (study week –1), at 1 month of exposure (study week 3), during the 13th week of exposure (study week 12), and prior to recovery necropsy (study week 16). During study weeks 3 and 12, these ECGs were recorded approximately 20-26 hr following exposure. In addition, ECGs were also recorded using a . The ECGs evaluations were recorded for 3 to 5 min and were collected near the end of saline exposure period

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and near the end of the 15-min test article exposure period during weeks 0, 3, and 12. Also during study weeks 0, 3, and 12, ECGs were recorded using a three-channel [redacted] ECG evaluations were recorded at 2, 4, and 24 hr after completion of exposure. Of these three time points, only the 2-hr post-exposure time period was collected for the control group (i.e., the 4 and 24 hr time points following exposure were omitted). b(4)

Hematology: Blood samples for determination of hematology parameters were collected prior to test article exposure (week –1), prior to the 7th exposure (week 1), at one month (study week 4), and prior to each scheduled necropsy (study weeks 12 and 17).

Clinical chemistry: Blood samples for determination of serum chemistry parameters were collected prior to test article exposure (week –1), prior to the 7th exposure (week 1), at one month (study week 4), and prior to each scheduled necropsy (study weeks 12 and 17).

Urinalysis: Urine samples for determination of urinalysis parameters were collected prior to test article exposure (week –1), prior to the 7th exposure (week 1), at one month (study week 4), and prior to each scheduled necropsy (study weeks 12 and 17).

Gross pathology: Necropsy examinations were conducted on all animals. Two animals were sacrificed in a moribund condition. Remaining animals were sacrificed at the scheduled terminations (i.e., following the treatment and recovery periods).

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

Histopathology: Tissues were processed into paraffin blocks, sectioned at 4-8 µm, mounted on glass microscope slides, stained with hematoxylin and eosin, and examined by light microscopy. Tissues were examined for animals euthanized in extremis and all animals at the primary necropsy. The sponsor reported that microscopic examination of tissues was not performed for tissues collected at the recovery necropsy since no test article-related microscopic changes were observed at the primary necropsy.

Toxicokinetics: Blood for measurement of plasma drug levels were collected from 4 dogs/sex/group after the first exposure, after approximately 1 month of exposure (week 4), and during the last week of exposure (week 12). Samples were collected at 0.5, 1, 2, 6, and 24 hr after completion of exposure. Samples were analyzed by [redacted] . Plasma concentrations of formoterol and the desformoterol/formoterol ratios were measured using a LC/MS/MS method. The lower limit of quantitation for formoterol was 2.50 pg/mL. The validated quantitation range of formoterol was 2.50 to 200 pg/mL using a 1-mL sample volume. Because the assay method lacks chiral specificity, concentration data were expressed as formoterol. Although, the method was not validated to quantify desformoterol, a metabolite of formoterol, its multiple reaction monitoring (MRM) mass channel was determined and acquired for qualitative purposes only. The signal for the desformoterol MRM mass channel was assumed to be approximately the same as that for formoterol. Toxicokinetic analysis was conducted by [redacted] b(4)

Results:

Mortality: One male (#6852) and one female (#6885) from the high dose group were euthanized in extremis. Clinical signs, noted below, might have resulted from exaggerated pharmacological effects and/or secondary physiological effects on the cardiovascular and respiratory systems. These two deaths at 70/100 µg/kg/day are considered treatment-related.

Male #6852 in the 70/100 µg/kg/day group was euthanized during week 12 (day 87). This animal had a history of intense struggling during exposure periods. On day 2, this animal was observed with severe respiratory depression (i.e., shallow respiration) and became cyanotic. The dog was not exposed on day 3. The animal returned to its regular dosing schedule on day 4. On day 79, exposure was terminated early due to severe respiratory depression. The animal was not exposed on days 80 and 81. The animal was exposed on days 82 through 86. The dog was reported to have a history of intense struggling during exposure and the study director considered this animal to be at high risk of dying prior to scheduled termination on day 92. Animal #6852 was euthanized on day 87 with a total of 84 exposures. The sponsor suggested that this animal received the drug at inhaled doses in excess of the target level due to agitation and hyperventilation during exposure periods. Necropsy findings consisted of reddened medullary lymph nodes. Histopathological findings for this animal were as follows. Bilateral, multifocal medullary tubular mineralization was observed in the kidneys. Hemorrhage was observed in the medullary lymph nodes. Unilateral, intratubular, multinucleated giant cells were observed in one testis. Multifocal cytoplasmic vacuolation was observed in the zona glomerulosa of the adrenal cortex. These histopathological findings appear to be generally spontaneous in nature and would not be expected to contribute substantially to signs of moribundity.

Female #6885 was euthanized during week 3 (day 21) and was exposed at only the 100 µg/kg/day level. Prior to sacrifice, this animal displayed clinical signs that included decreased activity, prostration, lateral recumbency, unresponsiveness, bloody diarrhea, dehydration, and only had a palpebral response. Necropsy findings for this animal consisted on dark red areas in the ileum and colon, white areas in the heart, and red and brown matting of the skin. Histopathological findings for this animal were as follows. Multifocal degeneration of the papillary muscle was observed in the heart. Minimal congestion was observed in the ileum. Bilateral, multifocal medullary tubular mineralization was observed in the kidneys. Minimal hemorrhage was observed in the medullary lymph nodes. Unilateral, focal, minimal corpora amylacea of the nasal turbinate was observed at nasal level 4. Focal glandular dilatation was observed in the rectum. Minimal lymphoid necrosis was observed in the thymus. Multifocal hemorrhage was observed in the adrenal cortex. It is unclear if these histopathological findings contributed to observed clinical signs.

Clinical signs:

Due to clinical effects including respiratory depression and cyanosis (during exposure) for two male dogs (#6851 and 6852) on day 2 and morbidity for 1 female dog on day 21, the high dose was lowered from 100 to 70 µg/kg/day on day 3 for males and day 22 for females.

Treatment-related clinical signs were evident for dogs treated with (R,R)-formoterol at all dose levels. Incidences of flushing of the body surface and facial area (i.e., vasodilation) and reddening of the ears and gums were observed to increase in a dose-related manner at 1 hr after treatment with (R,R)-formoterol. Female dogs at 70/100 µg/kg/day had an increase occurrence of clear discharge from the eyes at 1 hr after exposure. The incidences of injected sclera in the right and left eyes was increased in male and female treatment groups at 1 hr after dosing. Male dogs at 70/100 µg/kg/day had an increased occurrence of wet clear material around the mouth at 1 hr after exposure. During the recovery period, low incidences of flushed facial area, injected sclera in the eyes, clear discharge in the eyes, and reddened gum persisted for dogs from the high dose group, although, it was unclear if these observations had any relationship to earlier drug treatment.

During the time of exposure, the incidence of struggling and vocalization were increased for male dogs at the high dose and all female treatment groups. A small incidence of respiration shallow and animal not breathing, mask removed was observed for male dogs at mid and high doses during the exposure period. At 1hr after exposure, hypoactivity was observed for male and female dogs in mid and high dose groups. The incidence of partial eye closure at 1 hr after dosing was increased for male dogs at the low dose and male and female dogs at mid and high doses. During physical examinations, one female dog at the high dose was observed to be prostrate.

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

Clinical signs	Sex	Control	Low dose	Mid dose	High dose
Behavior/CNS					
-struggled during exposure	M	114/6	90/4	72/4	215/6
	F	49/6	111/4	132/4	89/6
-vocalization during exposure	M	64/5	95/4	25/3	109/6
	F	11/3	29/4	74/4	77/6
Body/Integument					
-body flushed	M	0	0	1/1	8/5
	F	0	0	4/3	7/4
-flushed facial area	M	1/1	0	3/1	9/2
	F	1/1	2/2	2/1	7/5
Cardio-Pulmonary					
-respiration shallow	M	0	0	1/1	8/5
	F	0	0	0	0
-animal not breathing, mask removed	M	1/1	0	3/1	9/2
	F	0	0	0	0
Eyes/Ears/Nose					
-reddened left ear	M	1/1	1/1	5/3	8/5
	F	3/2	1/1	4/2	3/2
-reddened right ear	M	1/1	1/1	5/3	9/5
	F	3/2	1/1	4/2	3/2
-injected sclera, left eye	M	7/3	17/4	16/3	16/6
	F	31/3	21/4	16/4	15/6
-injected sclera, right eye	M	5/2	16/4	13/3	18/6
	F	24/3	23/4	16/4	15/6
-clear discharge, left eye	M	26/1	1/1	14/1	60/3
	F	1/1	75/1	3/3	47/2
-clear discharge, right eye	M	2/1	3/1	7/1	45/3
	F	1/1	73/1	2/2	41/2
Oral/Dental					
-wet clear material round mouth	M	59/6	42/3	13/4	150/6
	F	34/5	85/4	81/4	30/5
-gums reddened	M	0	0	4/2	3/3
	F	0	0	3/3	8/5

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

Clinical signs	Sex	Control	Low dose	Mid dose	High dose
Behavior/CNS					
-hypoactivity	M	0	0	4/2	32/6
	F	0	0	4/2	45/4
-partial closure, left eye	M	0	4/2	10/3	29/6
	F	1/1	0	5/2	45/4
-partial closure, right eye	M	0	4/2	10/3	27/6
	F	2/1	0	5/2	47/4
Body/Integument					
-body flushed	M	0	59/4	296/4	504/6
	F	0	44/4	331/4	454/6
-flushed facial area	M	18/3	35/4	160/4	360/6
	F	15/4	126/4	200/4	318/6
Eyes/Ears/Nose					
-reddened left ear	M	2/1	63/4	190/4	379/6
	F	2/1	58/4	250/4	351/6
-reddened right ear	M	2/1	67/4	190/4	382/6
	F	2/1	62/4	254/4	354/6
-injected sclera, left eye	M	91/5	183/4	122/4	183/6
	F	100/6	195/4	192/4	237/5
-injected sclera, right eye	M	93/5	190/4	120/4	186/6
	F	107/6	193/4	190/4	245/5
-clear discharge, left eye	M	46/1	2/2	15/2	59/2
	F	12/1	82/1	5/1	87/4
-clear discharge, right eye	M	4/1	7/1	6/1	47/2
	F	3/1	83/1	4/1	86/4
Oral/Dental					
-wet clear material round mouth	M	0	0	3/2	13/6
	F	3/2	1/1	2/2	4/3
-gums reddened	M	2/1	42/4	125/4	280/6
	F	26/4	85/4	199/4	277/6

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Daily Examinations – Recovery Period (total occurrence/# of animals).

Clinical Signs	Sex	Control	High Dose

Body/Integument			
-flushed facial area	M	0	3/2
	F	1/1	3/2
Eyes/Ears/Nose			
-reddened left ear	M	0	1/1
	F	0	0
-reddened right ear	M	0	1/1
	F	0	0
-injected sclera, left eye	M	2/2	8/1
	F	4/2	12/2
-injected sclera, right eye	M	2/2	9/1
	F	4/2	14/2
-clear discharge, left eye	M	0	14/1
	F	0	0
-clear discharge, right eye	M	0	11/1
	F	0	2/1
Oral/Dental			
-gum reddened	M	0	8/2
	F	10/2	1/1

Body weights: There were no treatment-related effects on body weight gain.

Mean body weights for male controls at weeks 0 and 13 were 7.3 and 9.4 kg, respectively, yielding a 28.8% increase of initial body weights. Body weights for male dogs in low, mid, and high dose groups at week 13 were increased by 36.8, 41.6, and 41.3%, respectively, of initial body weights at week 0. Mean body weights for male controls at weeks 14 and 17 (i.e., recovery period) were both 8.8 kg, yielding no change. Body weights for male dogs in the high dose group at week 17 were decreased by 3.9% of body weights at week 14.

Mean body weights for female controls at weeks 0 and 13 were 6.8 and 8.9 kg, respectively, yielding a 30.9% increase of initial body weights. Body weights for female dogs in the low, mid and high dose groups at week 13 were increased by 33.8, 34.3, and 36.5%, respectively, of initial body weights at week 0. Mean body weights for female dogs at weeks 14 and 17 (i.e., recovery period) were 8.1 and 8.3 kg, respectively, yielding a 2.5% increase of initial body weights. Body weights for female dogs in the high dose group at week 17 were decreased by 2.5% of body weights at week 14.

Food consumption: There were no treatment-related effects on food consumption during the exposure (Weeks 0-13) and recovery (Weeks 14-17) periods.

Food consumption for male dogs in mid and high dose groups during week 0-1 was decreased to 86.5 and 70.9% of the control (251 g/animal/day), respectively. Food consumption for male dogs in the high dose group during week 1-2 was decreased to 89.2% of the control (316 g/animal/day). No changes in food consumption were evident for male treatment groups during the remainder of the exposure period.

Ophthalmoscopy: No treatment-related changes were identified in ophthalmic examinations.

Electrocardiography:

Elevated heart rates were evident at all dose levels on the first day of exposure and at weeks 3 and 12, during exposure and at 2- and 4-hr post-exposure (note: the sponsor designated the first week of treatment as week 0). Sinus tachycardia (generally defined as heart rate > 100 bpm) was observed following the first exposure to R,R-formoterol at all dose levels at 2- and 4-hr post-exposure. Electrocardiographic abnormalities consisting of ventricular ectopic patterns were also observed following the first exposure at all dose levels at 4 and 24 hr after dosing. These ectopic patterns (i.e., ventricular escape beat, ventricular escape rhythm, and ventricular premature beat) were clearly treatment-related as they could be attributed to known pharmacological effects of β-adrenergic agonists, although, dose-response relationships were not evident. These ECG abnormalities could be characterized as transient as they were not evident during weeks 3 and 12; however, this is unclear given that in the 9-month study, ectopic changes were observed late in the study. Histopathological examination of the heart revealed no evidence of treatment-related myocardial injury. These ventricular ectopic patterns appeared to be tolerated, although, they were clearly undesirable effects, which could lead to potentially serious adverse events.

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

First Exposure (Week 0):

Sinus tachycardia was evident at 2- and 4-hr post-exposure for male and female dogs at all dose levels. At 4-hr post-exposure, left ventricular premature beats were observed for 1 female (#6862) at the mid dose and 1 male (#6838) at the high dose. At

24-hr post-exposure, a ventricular escape beat was reported for 1 (#6848) of 4 males at the low dose. A ventricular escape rhythm was reported for 1 (#6845) of 4 males at the low dose and 2 (#6842 and #6858) of 4 males at the mid dose. In addition, ventricular premature beats were reported for the 1 male (#6858) at the mid dose observed with a ventricular escape rhythm. These ectopic changes were observed to persist for at least 24 hr after dosing at day 0 monitoring.

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

Week 0: Time	Diagnosis	Control		Low Dose		Mid Dose		High Dose	
		M	F	M	F	M	F	M	F
2-hr post-exposure	Sinus tachycardia	0/6	0/6	**4/4**	**4/4**	**4/4**	**4/4**	**4/4**	**6/6**
	Not collected	0	0	0	0	0	0	**2**	0
4-hr post-exposure	Sinus tachycardia	-	-	**3/4**	**4/4**	**4/4**	**4/4**	**4/4**	**6/6**
	LV premature beats	-	-	0	0	0	**1/4**	**1/4**	0
	Not Collected	**6**	**6**	0	0	0	0	**2**	0
24-hr post-exposure	Ventricular premature beat	-	-	0	0	**1/4**	0	0	0
	Ventricular escape beat	-	-	**1/4**	0	0	0	0	0
	Ventricular escape rhythm	-	-	**1/4**	0	**2/4**	0	0	0
	Not Collected	**6**	**6**	0	0	0	0	0	0

Heart rate was significantly increased for all male and female treatment groups during exposure and at 2- and 4-hr post-exposure. By 24-hr post-exposure, heart rate had returned to levels observed prior to exposure.

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

Time	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
During saline exposure	98	119	111	146	128	137	161*	139
During test article exposure	122 (124 %)	122 (102%)	**168 (151%)**	**204* (140%)**	**185 (144%)**	**204* (149%)**	**235* (146%)**	**208* (150)**
2 hr post-exposure	103 (105%)	129 (108%)	**210 (189%)**	**210* (144%)**	**201* (157%)**	**202* (147%)**	**216* (134%)**	**203* (146%)**
4 hr post-exposure	-	-	**184 (166%)**	**194 (133%)**	**210 (164%)**	**195 (142%)**	**228 (142%)**	**207 (149%)**
24 hr post-exposure	-	-	120 (108%)	132 (90%)	127 (99.2%)	134 (98%)	147 (91%)	147 (106%)

Ventricular premature beats (VPBs) or complexes (VPCs) are impulses that arise from an ectopic ventricular focus (Miller, M.S. and Tilley, L.P., Electrocardiography, In: Canine and Feline Cardiology, Editor: Fox, P.R., New York, Churchill Livingstone, 1988, pages 43-89). They spread through both ventricles with delay, causing a "bizarre", widened QRS complex. VPBs in dogs comprise the most frequent arrhythmia after sinus tachycardia. Two possible mechanisms for VPBs are reentry and increased automaticity. Occasional VPBs seldom produce clinical signs. A significant reduction in cardiac output is more likely when the animal has pre-existing heart disease. However, in individuals with a significant number of VPBs, there is an increased risk of developing spontaneous lethal ventricular fibrillation (Guyton, A.C. and Hall, J.E., Chapter 13: Cardiac arrhythmias and their electrocardiographic interpretation, In: Medical Physiology 10th Edition, New York, W.B. Saunders Co., 2000, pages 134-142). Spontaneous VPBs were observed in 43 of 3000 (1.4%) dogs examined, although, the type of dog was not specified (Detweiler, D.K. The Dog Electrocardiogram: A Critical Review, In: Comprehensive Electrocardiography Volume 2, Editors: MacFarlane, P.W. and Veitch Lawrie, T.D., New York, Pergamon Press, 1989, pages 1268-1329).

A ventricular escape beat or rhythm may occur under conditions where transmission of cardiac impulses through the A-V junction have been slowed. Purkinje fibers, usually in the ventricular septal portion of the A-V bundle, can develop a rhythm of their own (i.e., ectopic ventricular pacemaker) (Detweiler, D.K. The Dog Electrocardiogram: A Critical Review, In: Comprehensive Electrocardiography Volume 2, Editors: MacFarlane, P.W. and Veitch Lawrie, T.D., New York, Pergamon Press, 1989, pages 1268-1329). When the sinus rate and the discharge rate of the ectopic ventricular pacemaker are similar, the ectopic pacemaker rate may exceed that of the sinoatrial node and "capture" the cardiac rhythm for a series of beats. Such a ventricular escape rhythm will usually be accompanied by bouts of sinus rhythm, single ventricular escape beats and pairs of ventricular escape beats, and fusion beats. Entrance or protection block is not present in ventricular escape rhythms. Conducted sinus impulses thus discharge the ventricular focus. This resets the rhythm of the ventricular ectopic focus, which discharges when the diastolic interval is long enough. Spontaneous incidences of ventricular escape beat or rhythm were not reported.

Week 3: There were no findings in the ECG evaluations at 2, 4, and 24 hr post-exposure. In general, heart rate was significantly elevated for all male and female

treatment groups during exposure and at 2- and 4-hr post-exposure. By 24-hr post-exposure, heart rate had returned to levels observed prior to exposure.

Heart Rate-Week 3 (Values in parentheses are percent of prior to exposure).

Time	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
Prior to exposure	121	129	90	114	125	110	113	110
During saline exposure	113 (93%)	114 (88%)	100 (111%)	120 (105%)	101 (81%)	114 (104%)	97 (86%)	102 (93%)
During test article exposure	123 (102%)	113 (88%)	**138 (153%)**	**166* (146%)**	**161* (129%)**	**164* (149%)**	**146* (129%)**	**155* (141%)**
2 hr post-exposure	125 (103%)	121 (94%)	**195* (217%)**	**197* (173%)**	**159* (127%)**	**155 (141%)**	**163* (144%)**	**156 (142%)**
4 hr post-exposure	-	-	**152 (169%)**	**162 (142%)**	134 (107%)	**142 (129%)**	**149 (132%)**	**139 (126%)**
24 hr post-exposure	-	-	112 (124%)	113 (99%)	104 (83%)	108 (98%)	117 (103%)	111 (101%)

Week 12: There were no findings in the ECG evaluations at 2, 4, and 24 hr post-exposure. Heart rate was significantly elevated for all male and female treatment groups during exposure and at 2- and 4-hr post-exposure. By 24-hr post-exposure, heart rate had returned to levels observed prior to exposure.

Heart Rate-Week 12 (Values in parentheses are percent of prior to exposure).

Time	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
Prior to exposure	123	127	95	109	90	99	91	91
During saline exposure	129 (105%)	112 (88%)	88* (93%)	122 (112%)	87 (97%)	103 (104%)	81* (89%)	92 (101%)
During test article exposure	138 (112%)	120 (94%)	**119 (125%)**	**142 (130%)**	**148 (164%)**	**141 (142%)**	**137 (150%)**	**155 (170%)**
2 hr post-exposure	139 (113%)	113 (89%)	**170 (179%)**	**168* (154%)**	**152 (169%)**	**142 (143%)**	**153 (168%)**	**155 (170%)**
4 hr post-exposure	-	-	**140 (147%)**	**142 (130%)**	**137 (152%)**	**122 (123%)**	**125 (137%)**	**125 (137%)**
24 hr post-exposure	-	-	100 (105%)	112 (103%)	93 (103%)	104 (105%)	83 (91%)	95 (104%)

Week 16 (Recovery): There were no findings in the ECG evaluations. There were no differences in heart rate between the control and high dose groups.

Hematology: A number of small changes in hematology parameters (i.e., red blood cell counts, hemoglobin levels, hematocrit, MCV, MCH, APTT, and white blood cell counts) were observed for male and female treatment groups; however, their toxicological significance appears to be minimal.

Week 1: Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for male dogs in the high dose group were slightly decreased to 97 and 95.2% of control values (65.5 fL and 23.0 pg), respectively. Hemoglobin levels and hematocrit for female dogs in the high dose group were decreased to 85.1 and 85.5% of control values (15.4 g/dL and 44.7%), respectively. Platelet counts for male dogs in the high

dose group were increased to 132.3% of the control (313 x 10^3/µL). White blood cell counts for male dogs in low, mid, and high dose groups were elevated to 129.5-135.2% of the control (8.8 x 10^3/µL). Neutrophil counts for female treatment groups were increased to 146.4-162.5% of the control (5.6 x 10^3/µL). Lymphocyte percentages for male treatment groups were decreased to 62.1-79.3% of the control (29%).

Week 4: MCV and MCH for male dogs in the high dose group were decreased to 95.7 and 95.75% of control values (65.9 fL and 22.5 pg), respectively. Hemoglobin levels and hematocrit for female dogs in the high dose group were decreased to 89.6 and 88.8% of control values (16.3 g/dL and 48.0%), respectively. Activated partial thromboplastin times (APTT) for female treatment groups were increased to 110.9-115.2% of the control (9.2 sec). Lymphocyte percentages and counts for male treatment groups were decreased to 66.7-87.9% and 63.6-87.9% of control values (33% and 3.3 x 10^3 µL).

Week 12: Red blood cell counts for male dogs in mid and high dose groups were decreased to 88.9 and 93% of the control (7.28 x 10^6/µL), respectively. Hemoglobin levels for male dogs in mid and high dose groups were decreased to 85 and 88% of the control (16.7 g/dL), respectively. Hematocrits for male dogs in mid and high dose groups were decreased to 85 and 88.1% of the control (48.8%), respectively. MCV for male dogs in mid and high dose groups were decreased to 96 and 94.9% of the control (67.0 fL), respectively). MCH for male dogs in mid and high dose groups were decreased to 95.6 and 94.8% of the control (22.9 pg). Hemoglobin and hematocrit values for female dogs in the high dose group were decreased to 85.7 and 86.6% of control values (16.8 g/dL and 48.7%), respectively. APTTs for female treatment groups were increased to 109.7-115% of the control (9.3 sec).

Week 17 (Recovery): MCV for male dogs in the high dose group was decreased to 95% of the control (67.3 fL). APTT for female dogs from the high dose group was increased to 110% of the control (8.9 sec).

Clinical chemistry: Increased potassium levels were evident for male treatment groups throughout the exposure period. Increased potassium were evident for female dogs in the high dose group during weeks 1 and 4 and all female treatment groups during week 12. Alanine aminotransferase activity was increased for male dogs in the high dose group during week 1. Triglyceride levels were decreased for male dogs in mid and high dose groups during weeks 4 and 12. Triglyceride levels were decreased for female dogs in mid and high dose groups during week 4 and the high dose group during week 12. Creatinine levels were elevated for male treatment groups during weeks 4 and 12. Given the lack of histopathological findings, these serum chemistry changes would appear to have little or no toxicological significance.

Week 1: Potassium levels for male dogs in low, mid, and high dose groups were increased to 111.3, 116.8, and 122.4% of the control (4.06 mEq/L), respectively. Potassium levels for female dogs in the high dose group were increased to 107.8% of the control (4.72 mEq/L). Phosphate levels for male dogs in low, mid, and high dose groups were increased to 109, 107.5, and 111.9% of the control (6.7 mg/dL), respectively. The albumin/globulin (A/G) ratio for male dogs in mid and high dose

groups was decreased to 84.7 and 85.3% of the control (1.70), respectively. Alanine aminotransferase activity for the male dogs in the high dose group was increased to 177.4% of the control (31 U/L). Triglyceride levels for female dogs in mid and high dose groups were decreased to 80.6 and 71% of the control (31 mg/dL)

Week 4: Potassium levels for male dogs in low, mid, and high dose groups were increased to 116.4, 116.4, and 110.2% of the control (4.51 mEq/L), respectively. Potassium levels for female dogs in the high dose group were increased to 110% of the control (4.82 mEq/L). Creatinine levels for male treatment groups were increased to 125-150% of the control (0.4 mg/dL). Triglyceride levels for male dogs in mid and high dose groups were decreased to 84.4 and 68.8% of the control (32 mg/dL), respectively. Triglyceride levels for female dogs in mid and high dose groups were decreased to 84.8 and 78.8% of the control (33 mg/dL), respectively.

Week 12: Potassium levels for male dogs in low, mid, and high dose groups were increased to 104.1, 102.5, and 107.9% of the control (4.84 mEq/L), respectively. Potassium levels for female dogs in low, mid, and high dose groups were increased to 107.7, 104.2, and 124.2% of the control (4.56 mEq/L), respectively. Creatinine levels for male dogs in low, mid, and high dose groups were increased to 140, 160, and 160% of the control (0.5 mg/dL). Urea nitrogen levels for male dogs in mid and high dose groups were increased to 127.6 and 117.1% of the control (15.2 mg/dL), respectively. Urea nitrogen levels for female treatment groups were increased to 112.5-118.4% of the control (15.2 mg/dL). Triglyceride levels for male dogs in mid and high dose groups were decreased to 88.6 and 68.6% of the control (35 mg/dL), respectively. Triglyceride levels for female dogs in the high dose group were decreased to 75% of the control (32 mg/dL). Aspartate aminotransferase activities for female treatment groups were elevated to 117.2-124% of the control (29 U/L).

Week 17 (Recovery): Urea nitrogen levels for female dogs in the high dose group were increased to 134.7% of the control (15.0 mg/dL). Triglyceride levels for female dogs in the high dose group were increased to 129.2% of the control (24 mg/dL).

Urinalysis: Differences in urinary specific gravity and total volume were evident between control and treatment groups, for both male and female dogs, prior to the start of treatment, which persisted through the treatment and recovery periods. These differences were not attributable to drug treatment

Organ weights: Organ weight changes were observed for the lungs, spleen, and adrenal glands, although, there were no correlations to histopathological findings.

Lungs: Absolute lung weights for female treatment groups at primary necropsy were reduced to 82.1-90.3% of the control (93.00 g). Relative lung weights for female dogs at low, mid, and high dose groups were reduced to 90.25, 82.1, and 83.7% of the control (1.036%), respectively. Absolute lung weights for female dogs in the high dose group at the recovery necropsy were reduced to 88.6% of the control (76.27 g).

Spleen:

Absolute and relative spleen weights for male treatment groups at the primary necropsy were decreased to 84.2-89.7% and 75.4-82.3% of control values (65.63 g and 0688%), respectively. Absolute spleen weights for female dogs in mid and high dose groups at the primary necropsy were 88.7 and 80% of the control (53.05 g), respectively. Relative spleen weights for female dogs in mid and high dose groups at the primary necropsy were decreased to 89.3 and 77.8% of the control (0.590%), respectively.

Absolute spleen weights for male and female dogs in the high dose group at the recovery necropsy were reduced to 83.9 and 67.1% of controls (54.62 and 58.22 g), respectively. Relative spleen weights for male and female dogs in the high dose group at the recovery necropsy were reduced to 76.4 and 73.2% of controls (0.615 and 0.714 g), respectively.

Adrenal glands: Relative adrenal gland weights for male dogs in the low, mid, and high dose groups at the primary necropsy were decreased to 75, 83.3, and 66.7% of the control (0.012%), respectively.

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

Histopathology: There were no target organs of toxicity. There were low incidences of findings for the mesenteric lymph nodes, lungs, nasal cavity (levels 1 and 5), parathyroid gland, pancreas, larynx, and eyes, primarily confined to the high dose group, although, any causal relationships to treatment were not clear. The sponsor reported that microscopic examination of tissues was not performed for tissues collected at the recovery necropsy since no test article-related microscopic changes were observed at the primary necropsy.

Histopathological findings following 92 days of consecutive treatment in dogs that received (R,R)-formoterol at inhaled doses of 5, 40, and 70/100 µg/kg/day (deposited doses of 0, 1, 8, and 14 µg/kg/day). This table includes only animals sacrificed at scheduled termination.

Tissue/Organ	Sex	Control	Low Dose	Mid Dose	High Dose
Mesenteric lymph node					
-hemorrhage, minimal	M	0/4	0/4	0/4	2/3
	F	0/4	1/4	2/4	0/3
-sinus histiocytosis, minimal	M	0/4	0/4	0/4	1/3
	F	0/4	0/4	0/4	0/3
Lungs					
-alveolar macrophages, minimal	M	0/4	0/4	1/4	0/4
	F	0/4	0/4	1/4	1/4
-peribronchial lymphoid hyperplasia, minimal	M	0/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	1/4
Nasal level 1					
-edema	M	0/4	0/4	0/4	1/3
	F	0/4	0/4	0/4	0/3
Nasal level 5					
-corpora amylacea, minimal	M	0/4	0/4	0/4	1/3
	F	0/4	0/4	0/4	1/3
Parathyroid					
-cyst, minimal	M	0/4	1/4	1/3	1/2
	F				
Pancreas					
-atrophy, islet cells	M	0/4	0/4	0/4	1/3
	F				
Larynx					
-B papilloma, squamous cell present	M	0/4	0/4	0/4	0/3
	F	0/4	1/4	0/4	0/3
Eyes/Optic Nerve					
-retinal dysplasia, minimal;	M	0/4	0/4	0/4	0/3
	F	0/4	0/4	0/4	1/3

Toxicokinetics:

For the high dose group, the dosage level was decreased from 100 to 70 µg/kg/day on day 3 for males and day 22 for females. For male treatment groups, AUC and C_{max} values for formoterol at weeks 0, 4, and 12 increased in an approximate proportional manner with dose. For female treatment groups, AUC and C_{max} values for formoterol on day 0 increased in an approximate proportional manner with dose. However, mean AUC values for formoterol in female dogs from mid and high dose groups during weeks 4 and 12 were approximately equal. Further, the mean C_{max} value for formoterol in female dogs from the mid dose group during week 12 was greater than that observed for female dogs in the high dose group.

Plasma desformoterol/formoterol ratios in male and female dogs from low, mid, and high dose groups at day 0 and weeks 4 and 12 were ≤0.06 and 0.03, respectively.

Table 5. Mean Formoterol Pharmacokinetic Parameters Following Inhalation of 5, 40, and 100/70 µg/kg/day (R,R)-Formoterol for 13 Weeks in Male and Female Dogs

Formoterol Inhalation (µg/kg/day)/ Time Point	Male Dogs						Female Dogs					
	$AUC_{(0.5\text{-}24h)}$ (ng·h/mL)		C_{max} (pg/mL)		T_{max} (h)		$AUC_{(0.5\text{-}24h)}$ (ng·h/mL)		C_{max} (pg/mL)		T_{max} (h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
(R,R)-Formoterol												
5												
Day 0	1930	1210	254	117	1.0	0.7	1590	478	195	59.7	0.9	0.3
Week 4	2160	1240	285	181	2.1	2.6	2470	1400	249	121	0.8	0.3
Week 12	1120	369	115	74.4	2.0	2.7	1490	646	23.1	93.0	0.6	0.3
40												
Day 0	5800	1970	984	371	0.5	0.0	7270	2100	1280	349	0.5	0.0
Week 4	10100	1810	1510	282	1.1	0.6	27900	10200	2330	906	2.6	2.4
Week 12	12300	3030	1390	209	1.1	0.6	7640	1420	1840	278	1.6	0.8
100/70[a]												
Day 0	27500[b]	13000	4620[b]	1920	0.7[b]	0.3	24600[b]	6990	3840[b]	988	0.7[b]	0.3
Week 4	19100[b]	6670	2990[b]	1310	0.6[b]	0.2	28100[c]	12700	3400[c]	1590	0.9[c]	0.7
Week 12	19800	3110	1920	332	0.8	0.3	9490[c]	3160	1190[c]	230	1.2[c]	0.8

Note: N = 4 unless otherwise stated.

[a] The dose for male and female animals in the 100 µg/kg/day group was lowered to 70 µg/kg/day on study Day 3 and Day 22, respectively.

[b] N = 6.

[c] N = 5.

Summary of individual study findings:

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

Treatment-related deaths occurred at the high dose for one male and one female, which were both euthanized in extremis on days 87 and 21, respectively. Treatment-related clinical signs were evident for dogs treated with (R,R)-formoterol at all dose levels. Incidences of flushing of the body surface and facial area (i.e., vasodilation) and reddening of the ears and gums were observed to increase in a dose-related manner at 1 hr after treatment with (R,R)-formoterol. Female dogs at 70/100 µg/kg/day had an increased occurrence of clear discharge from the eyes at 1 hr after exposure. The incidences of injected sclera in the right and left eyes was increased in male and female treatment groups at 1 hr after dosing. Male dogs at 70/100 µg/kg/day had an increased occurrence of wet clear material around the mouth at 1 hr after exposure. During the recovery period, low incidences of flushed facial area, injected sclera in the eyes, clear discharge in the eyes, and reddened gum persisted for dogs from the high dose group, although, it was unclear if these observations had any relationship to earlier drug treatment.

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

Study title: A Nine-Month Inhalation Toxicity Study of (R,R)-Formoterol in Dogs.

Key study findings:

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

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

♦ A NOAEL was not established in this study. Atrial (supraventricular) premature depolarization was observed at all doses during week 38.

♦ Heart rate and electrocardiogram were evaluated on day 0 and during weeks 26 and 38. On day 0 at 2- and 4-hr post-exposure, elevated heart rates (and sinus tachycardia) were evident for male and female dogs at all dose levels. At 4-hr post-exposure, ventricular tachycardia was evident for 1 female dog in the high dose group. At 24-hr post-exposure, ventricular tachycardia and R on T depolarizations were evident for this dog. Further at 24-hr post-exposure for 1 male dog in the high dose group, ventricular premature beat, slow couplets, rapid premature tachycardia, and bigeminy were observed. During week 38, atrial (supraventricular) premature depolarization was observed for 1 female dog in the mid dose group prior to dosing, 1 female dog in the

low dose group at 2-hr post-exposure, and for 1 female dog in the high dose group at 24-hr post-exposure. Exercise periods were discontinued at the beginning of the study due to concerns regarding drug-induced changes of heart rate and rhythm.

♦ There were no target organs of toxicity.

Study no: Sepracor Document number 090-829, 2001
Volume #, and page #: Volumes 1-8, Pages 1-2611
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: October 20, 1999 (First exposures for male and female dogs were December 16 and 17, 1999, respectively). Please note that this study was initiated approximately 10 days after the start of the 13-week dog study.
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: (R,R)-Formoterol-L-tartrate, Lot number 113098A (Purity, 99.03%). Based on formula weight calculations, (R,R)-formoterol free base constituted 69.6% of the mass of the (R,R)-formoterol tartrate test article.
Formulation/vehicle: The vehicle used during acclimation and saline aerosol exposures and in the formulation of the test article solutions was 0.9% Sodium Chloride for Injection USP.

Methods (unique aspects)**:** Male and female beagle dogs (4 dogs/sex/group) received (R,R)-formoterol by nose-only aerosol inhalation at target inhaled doses of 0 (0.9% saline), 5, 40, or 70 µg/kg/day (deposited doses of 0, 1, 8, and 16 µg/kg/day, respectively) for 15 min/day for 273 consecutive days. The sponsor designated the first week of treatment as week 0. Exercise periods were discontinued at the start of the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm.
Dosing: b(4)
Species/strain: Beagle dogs were obtained from [redacted] Animals were received on November 18 and 22, 1999.
#/sex/group or time point (main study): 4 dogs/sex/group
Satellite groups used for toxicokinetics or recovery: None.
Age: Animals were approximately 6.5 months old at the start of treatment.
Weight: Body weight ranges were 6.1 to 8.4 kg for male dogs and 5.1-6.9 kg for female dogs.

Doses in administered units: See table below. All doses were expressed as free base equivalents.

Group	Test Article	Daily Exposure Duration (min)	Target Inhaled Dose µg/kg/day	Number of animals	
				Males	Females
1	Saline	15	0	4	4
2	(R,R)-Formoterol	15	5	4	4
3	(R,R)-Formoterol	15	40	4	4
4	(R,R)-Formoterol	15	70	4	4

The estimated dosage (as inhaled dose in µg/kg) for each male and female group of dogs was calculated from the exposure using the following equation:

Inhaled Dose (µg/kg) = Exposure concentration (µg/L) x mean minute volume to mean body weight ratio (L/min/kg) x Exposure duration (min)

Exposure concentrations were determined by chemical analysis of test atmosphere samples that were collected on filters during the 15-min animal exposure periods. The actual exposure concentration for each group (by sex) was used with the exposure duration of 15 min and the group mean minute volume/body weight ratio to calculate an inhaled dose.