

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-912

PHARMACOLOGY REVIEW(S)

INTEROFFICE MEMO

TO: NDA 21912
FROM: C. Joseph Sun, Ph. D., Supervisory Pharmacologist
Division of Pulmonary and Allergy Products
DATE: August 21, 2006

I concur with the pharmacologist's recommendation that pharmacology and toxicology of Arformoterol [(R,R) formoterol] have been adequately studied and the drug product is approvable from a preclinical standpoint.

Pharmacology: Arformoterol is a R,R isomer of racemic formoterol, a selective long-acting beta₂ adrenergic agonist that possesses bronchospamolytic activity. Compared to formoterol, Arformoterol showed greater affinity and selectivity for the beta₂ receptor. Several in vitro and in vivo animal studies using various biologic systems demonstrated that Arformoterol preferentially binds to beta adrenergic receptors and exhibits bronchodilatation effects.

General toxicity: Chronic inhalation toxicity studies up to 6 months in rats and up to 9 months in duration were conducted. In rats, there were no treatment-related target organs of toxicity identified. In dogs, various cardiac findings (sinus tachycardia and ventricular ectopic arrhythmias) were observed. These findings were attributed to the exaggerated pharmacological effects of this beta₂ adrenergic agonist and considered clinically monitorable.

Reproductive toxicity: Arformoterol had no effects on fertility in rats. It was found to be teratogenic in rats and rabbits, typical of beta₂ adrenergic agonists. There was evidence of developmental delays in rats. Therefore a pregnancy category C designation for the compound is appropriate.

Genotoxicity: Arformoterol was not genotoxic in the Ames tests, chromosome aberration assays in mammalian cells and in vivo micronucleus test in mice.

Carcinogenicity: Its carcinogenic potential was evaluated in a 2-year oral study in mice and a 2-year inhalation study in rats. In mice, findings of uterine and cervical endometrial stromal polyps and sarcoma were reported. However, there were no safety concerns for these tumor findings given beta₂ adrenergic agonists, such as formoterol, are known to produce tumors of the female genital tract in rodent. In rats findings of thyroid C-cell adenoma and carcinoma were reported. There was an adequate safety margin based on the drug exposures between the NOAEL in the rat study and the proposed clinical dose. Therefore there were no safety concerns for the tumor findings in both species.

Labeling: Carcinogenesis, mutagenesis and impairment of fertility and pregnancy category C sections have been revised to incorporate the above-mentioned preclinical findings.

There are no outstanding preclinical issues.

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/s/

Joseph Sun
8/24/2006 11:03:41 AM
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-912
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 12/12/05
PRODUCT: Arformoterol Tartrate Inhalation Solution
INTENDED CLINICAL POPULATION: Long term maintenance treatment of bronchoconstriction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema
SPONSOR: Sepracor Inc.
84 Waterford Drive
Marlborough, MA 01752
DOCUMENTS REVIEWED: PharmTox Module (Electronic Submission)
REVIEW DIVISION: Division of Pulmonary and Allergy Products (HFD-570)
PHARM/TOX REVIEWER: Timothy W. Robison, Ph.D.
PHARM/TOX SUPERVISOR: C. Joseph Sun, Ph.D.
DIVISION DIRECTOR: Badrul Chowdhury, M.D., Ph.D.
PROJECT MANAGER: Ladan Jafari

Date of review submission to Division File System (DFS): August 3, 2006

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability
From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval.
- B. Recommendation for nonclinical studies
None
- C. Recommendations on labeling
Recommendations for revisions of the applicant's proposed labeling are attached at the end of the review.

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings
Inhalation toxicology studies with rats up to 6 months in duration provide an adequate safety margin for the clinical dose of 15 µg BID. There were no treatment-related target organs of toxicity.

In inhalation studies with dogs up to 9 months in duration, there were findings of sinus tachycardia at arformoterol doses ≥ 5 µg/kg/day. Various types of ECG abnormalities (i.e., ventricular ectopic arrhythmias) were also observed in dogs treated with arformoterol at doses ≥ 5 µg/kg/day. NOAELs could not be identified for ectopic activity in these studies. Sinus tachycardia and ectopic findings were attributed to the exaggerated pharmacological effects of high inhaled doses of arformoterol. Further, these effects were considered monitorable in a clinical setting.

Arformoterol was not mutagenic or clastogenic in the following tests: mutagenicity tests in bacteria, chromosome aberration analyses in mammalian cells and micronucleus test in mice.

The carcinogenic potential of (R,R)-formoterol was evaluated in a 2-year oral carcinogenicity study with mice and a 2-year inhalation carcinogenicity study with rats. In mice, there were findings of uterine and cervical endometrial stromal polyps and stromal cell sarcoma. There were no safety concerns for these tumor findings in mice given that β_2 -adrenergic agonists, such as (R,R)-formoterol, are known to produce tumors of the female rodent genital tract. In rats, there were findings of thyroid C-cell adenoma and carcinoma. There were no safety concerns for these tumor findings in rats given that there was an adequate safety margin between a dose with no tumor findings in rats and the proposed clinical dose.

Arformoterol had no effects on fertility and reproductive performance in rats. (R,R)-formoterol was found to be teratogenic in rats and rabbits. In addition for rats and rabbits, numbers of viable fetuses and fetal body weights were decreased with high doses of (R,R)-formoterol. There was evidence of developmental delays in rats with high doses of (R,R)-formoterol.

B. Pharmacologic activity

Arformoterol is a selective long-acting beta₂-adrenergic receptor agonist (beta₂-agonist). Compared to racemic formoterol, arformoterol showed greater affinity for both beta adrenergic receptor subtypes and also greater selectivity for the beta₂ receptor. Arformoterol has been extensively characterized in standard *in vivo* and *in vitro* models and has been shown to preferentially bind to beta₂-adrenergic receptors. The pharmacologic effects of beta₂-adrenoceptor agonist drugs, including arformoterol, are at least in part attributable to stimulation of intracellular adenylate cyclase. Increased intracellular cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially mast cells.

C. Nonclinical safety issues relevant to clinical use
None

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-912

Review number: #02

Sequence number/date/type of submission:

#000/December 12, 2005/Initial Submission

March 29, 2006/BP

April 18, 2006/SU

April 27, 2006/BP

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Sepracor, Inc.

84 Waterford Drive

Marlborough, MA 01752

Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison

Division name: Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: August 3, 2006

Drug:

Trade name:

Generic name: Arformoterol Tartrate Inhalation Solution

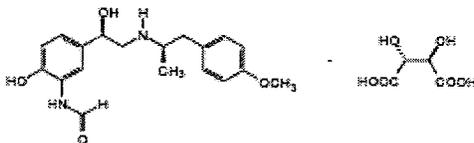
Code name: Arformoterol, (R,R)-Formoterol-L-tartrate

Chemical name: (R,R)-(-)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide-(R,R)-2,3-dihydroxybutanedioate (1:1 salt)

CAS registry number:

Molecular formula/molecular weight: C₂₃H₃₀N₂O₁₀ / MW 494.5

Structure:



Relevant INDs/NDAs/DMFs:

IND 55,302 (Arformoterol, Sepracor, Inc.)

NDA 20-831 (Foradil, Novartis)

Drug class: β_2 -Adrenergic Bronchodilator

Intended clinical population: Arformoterol Tartrate Inhalation Solution is indicated for twice daily (morning and evening) long-term maintenance treatment of bronchoconstriction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema.

Clinical formulation: Arformoterol Tartrate Inhalation Solution is formulated as an isotonic, preservative-free, sterile aqueous solution consisting of arformoterol tartrate in a citrate-buffered saline solution. The formulation contains Citric Acid [] USP and Sodium Citrate [] USP as buffers, and Sodium Chloride USP to [] [] The solution is buffered to a pH of 5.0. Two mL of solution are packaged in low-density polyethylene (LDPE) vials [] [] Each unit-dose vial is individually wrapped with [] [] foil as a sealed pouch. The strength is expressed as the amount of arformoterol free base in the 2 mL vial: 15 µg/2 mL.

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Table 2.3.P.1-1 Arformoterol Tartrate Inhalation Solution 15 µg/ 2 mL, Unit Dose Formula

Component	Quality Standard	Function	Amount/Unit
Arformoterol Tartrate ^a	[] []	Active	[]
Citric Acid, [] []	USP	Buffer Component	[]
Sodium Citrate, [] []	USP	Buffer Component	[]
Sodium Chloride			[]
Total			[]

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^a 0.6967 gm of Arformoterol free base = 1.0 gm Arformoterol Tartrate.

^b The amount in the table is the theoretical amount for the batch. The actual amount charged will be adjusted for purity (total impurities by HPLC, residual solvents, and water, total of which typically ranges [] [] Drug Substance purity is typically [] []

- The recommended dosage of Arformoterol Tartrate Inhalation Solution for COPD patients is 15 mcg administered twice a day (morning and evening) by nebulization. A total daily dose greater than 30 mcg (15 mcg twice daily) is not recommended. Arformoterol Tartrate Inhalation Solution should only be administered by nebulizer by the inhalation route.

Route of administration: Oral Inhalation (Nebulization)

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

Studies reviewed within this submission:

Study Title	Sepracor Document No.
PHARMACOLOGY:	
SAFETY PHARMACOLOGY:	
Cardiovascular Effects:	
In Vitro	
Effects of IN-0304, IN-0475, and IN-0618 on Cloned hERG Channels Expressed in Mammalian Cells.	090-492A1
Effects of IN-0304, IN-0475, and IN-0618 on Action Potentials in Isolated Cardiac Purkinje Fibers.	090-493
Effects of (R,R)-Desformoterol on Cloned hERG Channels Expressed in Mammalian Cells.	090-837
Dogs	
Reanalysis of Corrected QT-Interval (QTc) from Report 090-423: Evaluating the Comparative Effects of (R,R/S,S)-formoterol, (R,R)-Formoterol, and (S,S)-Formoterol on Heart Rate, Blood Pressure, and Lead II Electrocardiogram Following Intravenous Administration to Conscious Dogs.	090-495
PHARMACOKINETICS/TOXICOKINETICS:	
Absorption	
Absorption and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Dogs.	090-527
Distribution	
In Vitro	
Determination of Protein Binding of (R,R)-Formoterol in Human EDTA-Anticoagulated Plasma	090-579
Mice	
[³ H]-(R,R)-Formoterol: Absorption, Distribution, and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Mice.	090-528
Rats	
An Exploratory Distribution Study of [³ H]-(R,R)-Formoterol (without or with an Equal Proportion of Non-radioactive (S,S)-Formoterol) after Single Dose Inhalation Administration to Rats.	090-515 and 090-517
An Exploratory Distribution Study of [³ H]-(S,S)-Formoterol (without or with an Equal Proportion of Non-radioactive (R,R)-Formoterol) after Single Dose Inhalation Administration to Rats.	090-518
Absorption, Distribution, and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses of Male Rats.	090-529
Metabolism	
In Vitro	
An Exploratory In Vitro Metabolism Study of (R,R)-Formoterol and (S,S)-Formoterol in Rat and Human Hepatocyte Suspensions.	090-514
(R,R)-Formoterol and (S,S)-Formoterol: In Vitro Metabolism Study Using Human Cryopreserved Hepatocytes.	090-568A1
Investigation of the Principal Human Cytochromes P450 and UDP-Glucuronosyltransferases Involved in the Microsomal Metabolism of Both (R,R)-Formoterol and (S,S)-Formoterol In Vitro.	090-543a1
UGT Reaction Phenotyping of [³ H]-(R,R)-Formoterol and [³ H]-(S,S)-Formoterol	090-575A1
In Vivo	
Comparative Metabolism of [³ H]-(R,R)-Formoterol in Male Mice, Rats, and Dogs	090-530A1
Excretion	

Mice	
Absorption, Distribution, and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Mice.	090-528
Rats	
Absorption, Distribution, and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Rats.	090-529
Dogs	
Absorption and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Dogs	090-527
TOXICOLOGY:	
Repeat-Dose Toxicity	
Rats	
A 13-Week Inhalation Toxicity Study of a Nebulized Aerosol Formulation of (R,R)-Desformoterol Oxalate in the Albino Rat.	090-840
A 13-Week Inhalation Toxicity Study of a Nebulized Aerosol Formulation of (R,R)-Desformoterol Oxalate in the Albino Male Rat.	090-844
Dogs	
Three-Month Inhalation Toxicity Study of (R,R)-Formoterol and (R,R)-Desformoterol in Dogs (with a One-Month Recovery).	090-836
Qualitative Evaluation of Electrocardiograms Recorded for Three Inhalation Toxicity Studies of (R,R)-Formoterol in Dogs.	090-841
Reproductive and Developmental Toxicology	
Embryofetal Development	
Rabbits	
A Dose Range-Finding Study of the Effects of Racemic Formoterol and (R,R)-Formoterol on Embryo/Fetal Development in Rabbits.	090-835
A Study of the Effects of (R,R/S,S)-Formoterol and (R,R)-Formoterol on Embryo/Fetal Development in Rabbits.	090-834
Prenatal and Postnatal Development	
Study of the Effects of (R,R)-Formoterol on Pre- and Postnatal Development Including Maternal Function in the Rat.	090-832

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Studies submitted to NDA 21-912 (Reviewed under NDA 21-912 and IND 55,302):

NDA 21-912
 Arformoterol Tartrate Inhalation Solution

5 Nonclinical Pharmacology and Toxicology

5 Nonclinical Pharmacology and Toxicology, Table of Contents

Description	Archive copy location folder/file name
1 Integrated Nonclinical Summary	pharmtox\pharmsum.pdf
Pharmacology Study Reports	
1 Pharmacodynamics Reports	
Report 090-432: Study of the Effects of Three Compounds (IN 0228, IN-0229 and IN-0261) in Various In Vitro Assays	pharmtox\pharm\090-432.pdf
Report 090-403: Binding Properties of Eight Compounds at Histaminergic and β -Adrenergic Receptor Subtypes.....	pharmtox\pharm\090-403.pdf
Report 090-409: Binding Properties of Six Compounds in β 1- and β 2-Adrenoceptor Assays.....	pharmtox\pharm\090-409.pdf
Report 090-410: Binding Properties of Nine Compounds in Several Receptor Assays	pharmtox\pharm\090-410.pdf
Report 090-401: Pulmonary Resistance Assay in Guinea Pigs	pharmtox\pharm\090-401.pdf
Report 090-412: Assessing the Comparative Ability of RR,SS-Formoterol, RR-Formoterol and SS- Formoterol to Attenuate the Bronchoconstrictor Response to Histamine or Antigen in Immunized Guinea Pigs	pharmtox\pharm\090-412.pdf
Report 090-434: Effects of (R)-, (S)-, (RS)-Albuterol or (S,S)-Formoterol on the Development of Antigen- Mediated Airway Hyperreactivity in Guinea Pigs. Phase IIA	pharmtox\pharm\090-434.pdf
Report 090-405: Determining the Comparative Effects of Test Compounds on Isolated Guinea Pig Tracheal Strips and Right Atria	pharmtox\pharm\090-405.pdf

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5 Nonclinical Pharmacology and Toxicology

Report 090-408: In Vitro Testing of Formoterol and Its Optically Active Isomers in Guinea Pig Airways ... pharmtox\pharm\090-408.pdf

Report 090-480: Effects of RS-, R- and S-Albuterol and RR,SS-, RR- and SS-Formoterol on Tumor Necrosis Factor-alpha (TNF-alpha) Induced Hyperreactivity in Tracheal Smooth Muscle of the Guinea-Pig In Vitro pharmtox\pharm\090-480.pdf

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Report 090-446: Effects of Enantiomers of B2-Agonists on ACh Release and Smooth Muscle Contraction in the Trachea pharmtox\pharm\090-446.pdf

Report 090-479: Effects of Methacholine, RS-, R- and S-Albuterol, and RR,SS-, RR- and SS-Formoterol on Mucociliary Transport in Calf Trachea pharmtox\pharm\090-479.pdf

Report 090-406: Effect of Formoterol and Its Optically Active Isomers on Histamine Release from Human Leucocytes pharmtox\pharm\090-406.pdf

Report 090-450: Binding Study of (R,S)-Desformoterol and (S,R)-Desformoterol in Human beta-Adrenergic Receptor Binding Assays pharmtox\pharm\090-450.pdf

Report 090-451: Study of Compounds IN-0228 and IN-0290 in Human beta-Adrenergic Receptor Binding Assays pharmtox\pharm\090-451.pdf

Report 090-448: Binding Study of (RR)-Desformoterol Fumarate in beta-Adrenergic Receptor Assays pharmtox\pharm\090-448.pdf

Report 090-439: Evaluation of beta2-Agonists at M1 - M5 Muscarinic Receptor Subtypes pharmtox\pharm\090-439.pdf

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Arformoterol Tartrate Inhalation Solution 5 Nonclinical Pharmacology and Toxicology

Report 090-404: Screening and Evaluation of the Efficacy of Original Compounds on Lipoxygenase and Cyclooxygenase Activity in DMSO-Differentiated Human HL-60.....	pharmtox\pharm\090-404.pdf
Report 090-445: Effects of Four Compounds on Basal IL-5 Secretion by Human PBMC	pharmtox\pharm\090-445.pdf
Report 090-416: Binding Study of Six Original Compounds in Human M1, M2 and M3 Muscarinic Receptor Assays	pharmtox\pharm\090-416.pdf
Report 090-449: Binding Study of Two Compounds in Human β 1- and β 2-Adrenergic Receptor Assays.....	pharmtox\pharm\090-449.pdf
Report 090-415: Characterization of RRSS-, RR-, RS-, SR-, and SS-Formoterol: Pharmacology, and Effects on Signaling and Desensitization of the Human β -Adrenergic Receptors.....	pharmtox\pharm\090-415.pdf
Report 090-481: Study of Four Compounds in Various Cytokine Secretions Using Basal Models	pharmtox\pharm\090-481.pdf
Report 090-484: Study of IN-0712 in the Human β 1- and β 2- Adrenergic Receptor Binding Assays	pharmtox\pharm\090-484.pdf
Report 090-498: Study 890355: In Vitro Pharmacology Human β 1- and β 2- Adrenergic Receptors -Study of IN-0951	pharmtox\pharm\090-498.pdf
Report 090-472: The Effects of Enantiomers of Albuterol and Formoterol on Non-Contractive Function of Human Airway Smooth Muscle Cells	pharmtox\pharm\090-472.pdf
Report 090-486: The Effects of Enantiomers of Albuterol and Formoterol on Indices of Remodeling in Human Lung Fibroblasts	pharmtox\pharm\090-486.pdf
Report 090-490: Comparison of Albuterol, Salmeterol, and Formoterol Activation of Human β 2-Adrenergic Receptors	pharmtox\pharm\090-490.pdf
Report 090-433: Binding Study of Three Compounds (IN-0168, IN-0170 and IN-0247) in Several Receptor Assays	pharmtox\pharm\090-433.pdf

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5 Nonclinical Pharmacology and Toxicology

Arformoterol Tartrate Inhalation Solution

Report 090-478: Study of Four Compounds in Various Inflammation Assays pharmtox\pharm\090-478.pdf

Report 090-482: Study of Compounds IN-0259, IN-0291 and IN-0293 in Various Binding, Enzyme and Ion Transport Assays pharmtox\pharm\090-482.pdf

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Report 090-407: Comparison of R-, S- and RS-Formoterol Binding to Human β_1 and β_2 -Adrenergic Receptors pharmtox\pharm\090-407.pdf

2 Safety Pharmacology Reports

Report 090-423: Evaluating the Comparative Effects of (R;R/S;S)-Efomoterol, (R;R)-Efomoterol and (S;S)-Efomoterol on Heart Rate, Blood Pressure and the Lead II Electrocardiogram Following Intravenous Administration to Conscious Dogs pharmtox\pharm\090-423.pdf

Report 090-495: Reanalysis of the Corrected QT-Interval (QTc) Derived from  Study SEPBR-11105 [Sepracor Document No. 090-423]..... pharmtox\pharm\090-495.pdf

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Report 090-493: Effects of IN-0304, IN-0475 and IN-0618 on Action Potentials in Isolated Cardiac Purkinje Fibers pharmtox\pharm\090-493.pdf

Report 090-492A1: Effects of IN-0304, IN-0475, and IN-0618 on Cloned hERG Channels Expressed in Mammalian Cells pharmtox\pharm\090-492a1.pdf

Report 090-837: Effects of (R,R)-Desformoterol on Cloned hERG Channels Expressed in Mammalian Cells pharmtox\pharm\090-837.pdf

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Arformoterol Tartrate Inhalation Solution

5 Nonclinical Pharmacology and Toxicology

Pharmacokinetics and Drug Metabolism Study Reports

1 Pharmacokinetics/Toxicokinetics and ADME Reports

Report 090-470: Toxicokinetics of Formoterol during a 28-Day Oral Toxicity Study of (R,R)-Formoterol in Mice pharmtox\pk\090-470.pdf

Report 090-460: Toxicokinetics of Formoterol During an Acute Inhalation Tolerance Study of (R,R)-Formoterol in Mice pharmtox\pk\090-460.pdf

Report 090-461: Toxicokinetics of Formoterol during 28-Day Inhalation Toxicity Study of (R,R)-Formoterol in Mice pharmtox\pk\090-461.pdf

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Report 090-463: Toxicokinetics of Formoterol during a 28-Day Inhalation Toxicity Study of (R,R)- and (R,R/S,S)-Formoterol in Rats pharmtox\pk\090-463.pdf

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Report 090-523: Toxicokinetics of (R,R)- and (S,S)-Formoterol after Oral Administration of Racemic Formoterol during an Embryo/Fetal Development Study in Rabbits pharmtox\pk\090-523.pdf

Report 090-464A1: Toxicokinetics of Formoterol and ¹⁴C Ratios during an Acute Inhalation Tolerance Study of (R,R)-, (S,S)- and (R,R/S,S)-Formoterol and (R,R)-Desformoterol in Dogs pharmtox\pk\090-464a1.pdf

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Report 090-465: Toxicokinetics of Formoterol during a 28-Day Inhalation Toxicity Study of (R,R)- and (S,S)-Formoterol in Dogs pharmtox\pk\090-465.pdf

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2 Drug Metabolism Reports

Report 090-452: [³ H]-(R,R)-Formoterol: In Vitro Blood to Plasma Partitioning and Plasma Protein Binding in the Mouse	pharmtox\pk\090-452.pdf
Report 090-528: [³ H]-(R,R)-Formoterol: Absorption, Distribution, and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Mice	pharmtox\pk\090-528.pdf
Report 090-530A1: [³ H]-(R,R)-Formoterol: Comparative Metabolism of [³ H]-(R,R)-Formoterol in Male Mice, Rats and Dogs.....	pharmtox\pk\090-530a1.pdf
Report 090-531: [³ H]-Formoterol: Secretion of Radioactivity in Milk following Administration of [³ H]-(R,R)-Formoterol as Single Oral Doses to Lactating Rats	pharmtox\pk\090-531.pdf
Report 090-526: [³ H]-(R,R)-Formoterol: Placental Transfer Studies of [³ H]-(R,R)-Formoterol during and after Repeated Oral Administration to Pregnant Rats..	pharmtox\pk\090-526.pdf
Report 090-515: [³ H]-(R,R)-Formoterol: An Exploratory Distribution Study of [³ H]-(R,R)-Formoterol after Single Dose Inhalation Administration to Rats.....	pharmtox\pk\090-515.pdf
Report 090-517: [³ H]-(R,R)-Formoterol: An Exploratory Distribution Study of [³ H]-(R,R)-Formoterol after Single Inhalation Doses (Mixed with an Equal Proportion of Non-Radioactive (S,S)-Formoterol) to Rats	pharmtox\pk\090-517.pdf
Report 090-518: [³ H]-(R,R)-Formoterol: An Exploratory Distribution Study of [³ H]-(S,S)-Formoterol after Single Inhalation Doses (Mixed with an Equal Proportion of Non-Radioactive (R,R)-Formoterol) to Rats	pharmtox\pk\090-518.pdf
Report 090-529: [³ H]-(R,R)-Formoterol: Absorption, Distribution, and Excretion Studies of [³ H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Rats	pharmtox\pk\090-529.pdf

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5 Nonclinical Pharmacology and Toxicology

Report 090-417: [³H]-(R,R)-Formoterol: In Vitro
 Blood to Plasma Partitioning and Plasma Protein
 Binding in the Rat pharmtox\pk\090-417.pdf

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Report 090-514: An Exploratory In Vitro
 Metabolism Study of (R,R)-Formoterol and (S,S)-
 Formoterol in Rat and Human Hepatocyte
 Suspensions pharmtox\pk\090-514.pdf

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Report 090-525: [³H]-(R,R)-Formoterol: Placental
 Transfer Studies of [³H]-(R,R)-Formoterol during and
 after Repeated Oral Administration to Pregnant
 Rabbits pharmtox\pk\090-525.pdf

Report 090-527: [³H]-(R,R)-Formoterol: Absorption
 and Excretion Studies of [³H]-(R,R)-Formoterol after
 Single Intravenous and Oral Doses to Male Dogs pharmtox\pk\090-527.pdf

Report 090-418: [³H]-(R,R)-Formoterol: In Vitro
 Blood to Plasma Partitioning and Plasma Protein
 Binding in the Dog pharmtox\pk\090-418.pdf

Report 090-411: Metabolism of Three Compounds,
 the Racemate and Two Optically Active Isomers (RR
 and SS) of RR,SS-Formoterol by Bronchial Epithelial
 Cells and Human Liver Cytosol pharmtox\pk\090-411.pdf

Report 090-424: Studies of the Stereoselective
 Metabolism of the β_2 -Agonist Formoterol in Human
 Tissues pharmtox\pk\090-424.pdf

Report 090-419: [³H]-(R,R)-Formoterol: In Vitro
 Blood to Plasma Partitioning and Plasma Protein
 Binding in the Human pharmtox\pk\090-419.pdf

Report 090-425: Metabolic Interactions between
 Albuterol and Two Long-Acting β -Agonists
 (Formoterol, Salmeterol) pharmtox\pk\090-425.pdf

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Report 090-431: Presystemic Intestinal Sulfonation of β_2 -Receptor Agonists in Humans - Phenolsulfotransferase Isoform- and Enantiomer-Specificity	pharmtox\pk\090-431.pdf
Report 090-505: [3 H]-(R)-Albuterol: In Vitro Blood-to-Plasma Partitioning and the Plasma Protein Binding of [3 H]-(R)-Albuterol in Human and the Effect of (S)-Albuterol, (R,R/S,S)-Formoterol and (R,R)-Formoterol on the Protein Binding of (R)-Albuterol	pharmtox\pk\090-505.pdf
Report 090-538: (R,R)- and Racemic (R,R/S,S)-Formoterol: Potential Inhibition of Cytochromes P450 in Human Liver Microsomes	pharmtox\pk\090-538.pdf
Report 090-543A1: (R,R)-Formoterol and (S,S)-Formoterol: Investigation of the Principal Human Cytochromes P450 and UDP-Glucuronosyltransferases Involved in the Microsomal Metabolism of Both (R,R)-Formoterol and (S,S)-Formoterol	pharmtox\pk\090-543a1.pdf
Report 090-568A1: (R,R)-Formoterol and (S,S)-Formoterol: In Vitro Metabolism Study Using Human Cryopreserved Hepatocytes	pharmtox\pk\090-568a1.pdf
┌	
Report 090-579: Determination of Protein Binding of (R,R)-Formoterol in Human EDTA-Anticoagulated Plasma	pharmtox\pk\090-579.pdf
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Report 090-575A1: UGT Reaction Phenotyping of [3 H]-(R,R)-Formoterol and [3 H]-(S,S)-Formoterol	pharmtox\pk\090-575a1.pdf

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Toxicology Study Reports

1 Single-Dose Toxicology Reports

Report 090-818: Acute Inhalation Tolerance Study of (R,R)-Formoterol in Mice.....	pharmtox\tox\090-818.pdf
Report 090-800: Acute Oral Toxicity Study in Rats with RR-, SS-, RR, SS-Formoterol	pharmtox\tox\090-800.pdf
Report 090-801: Acute Intravenous Toxicity Study in Rats with RR- and SS-Formoterol	pharmtox\tox\090-801.pdf
Report 090-815: Comparative Acute Inhalation Tolerance Study of (R,R)-, (S,S)-, (R,R/S,S)-Formoterol and (R,R)-Desformoterol in Rats.....	pharmtox\tox\090-815.pdf
Report 090-808: (R,R)-Formoterol: Single-Dose Intravenous Toxicity Study in Beagle Dogs.....	pharmtox\tox\090-808.pdf
Report 090-809A2: Comparative Acute Inhalation Tolerance Study of (RR)-, (SS)-, and Racemic Formoterol and (RR)-Desformoterol in Dogs.....	pharmtox\tox\090-809a2.pdf

2 Repeat-Dose Toxicology Reports

Report 090-824A1: A 28-Day Oral Toxicity Study of (R,R)-Formoterol in Mice.....	pharmtox\tox\090-824a1.pdf
Report 090-821: A 28-Day Inhalation Toxicity Study of (RR)-Formoterol in Mice.....	pharmtox\tox\090-821.pdf
Report 090-803: 14-Day Oral Toxicity Study in Rats with (R;R)-, (S;S)-, and (R;R/S;S)-Eformoterol.....	pharmtox\tox\090-803.pdf
Report 090-817A1: A 28-Day Inhalation Toxicity Study of (R,R)-Formoterol in Rats.....	pharmtox\tox\090-817a1.pdf
Report 090-840: A 13-Week Inhalation Toxicity Study of a Nebulized Aerosol Formulation of (R,R)-Desformoterol Oxalate in the Albino Rat.....	pharmtox\tox\090-840.pdf
Report 090-827A2: A Six-Month Inhalation Toxicity Study of (R,R)-Formoterol in Rats with a One-Month Recovery Period.....	pharmtox\tox\090-827a2.pdf

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Report 090-802: Oral Dose-Range-Finding Toxicity Study in Dogs with RR-Formoterol	pharmtox\tox\090-802.pdf
Report 090-806: 14-Day Oral Toxicity Study in Dogs with (R,R)-Eformoterol, (S,S)-Eformoterol and (R,R/S,S)-Eformoterol.....	pharmtox\tox\090-806.pdf
Report 090-823: A 14-Day Inhalation Range-Finding Study of (R,R)-Formoterol in Dogs	pharmtox\tox\090-823.pdf
Report 090-816A1: 28-Day Inhalation Toxicity Study of (R,R)-Formoterol in Dogs	pharmtox\tox\090-816a1.pdf
Report 090-830A2: A 13-Week Inhalation Toxicity Study (with Recovery) of (R,R)-Formoterol in Dogs ..	pharmtox\tox\090-830a2.pdf
Report 090-836A1: Three-Month Inhalation Toxicity Study of (R,R)-Formoterol and (R,R)-Desformoterol in Dogs (with a One-Month Recovery)	pharmtox\tox\090-836a1.pdf
Report 090-829A1: A Nine-Month Inhalation Toxicity Study of (R,R)-Formoterol in Dogs	pharmtox\tox\090-829a1.pdf
Report 090-841: Qualitative Evaluation of Electrocardiograms Recorded for Three Inhalation Toxicity Studies of (R,R)-Formoterol in Dogs	pharmtox\tox\090-841.pdf
3 Mutagenicity/Genotoxicity Reports	
Report 090-807: Genotoxicity: Salmonella Typhimurium Reverse Mutation Study	pharmtox\tox\090-807.pdf
Report 090-810: Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells ..	pharmtox\tox\090-810.pdf
Report 090-811A1: Mutagenicity Test on (RR)-Formoterol Tartrate, (SS)-Formoterol Tartrate, (RR,SS)-Formoterol Tartrate, and (RR)-Desformoterol in the In Vivo Mouse Micronucleus Assay	pharmtox\tox\090-811a1.pdf
4 Carcinogenicity Reports	
Report 090-833: A 24-Month Oral (Gavage) Oncogenicity Study of (R,R)-Formoterol in Mice	pharmtox\tox\090-833.pdf

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Report 090-833: A 24-Month Oral (Gavage) Oncogenicity Study of (R,R)-Formoterol in Mice, Data.....	pharmtox\datasets\090-833\define.pdf
Report 090-828A2: A 24-Month Inhalation Oncogenicity Study of (R,R)-Formoterol in Rats	pharmtox\tox\090-828A2.pdf
Report 090-828A2: A 24-Month Inhalation Oncogenicity Study of (R,R)-Formoterol in Rats, Data.....	pharmtox\datasets\090-828\define.pdf
Report 031705a: Tumor Historical Control Data - Summary Incidence Report, CD-1 Mouse.....	pharmtox\tox\wil-031705a.pdf
Report 031705b: Tumor Historical Control Data - Summary Incidence Report, IGS Rat.....	pharmtox\tox\wil-031705b.pdf

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5 Reproductive Toxicology Reports

Report 090-831: A Study of the Effects of (R,R)-Formoterol on Fertility and Early Embryonic Development to Implantation in Rats.....	pharmtox\tox\090-831.pdf
Report 090-813A1: A Dose Range-Finding Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rats	pharmtox\tox\090-813a1.pdf
Report 090-820: A Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rats	pharmtox\tox\090-820.pdf
Report 090-825: A Study of the Effects of (R,R)-Formoterol and Racemic Formoterol on Embryo/Fetal Development in Rats	pharmtox\tox\090-825.pdf
Report 090-812: A Dose Range-Finding Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rabbits	pharmtox\tox\090-812.pdf
Report 090-835: A Dose Range-Finding Study of the Effects of Racemic Formoterol and (R,R)-Formoterol on Embryo/Fetal Development in Rabbits	pharmtox\tox\090-835.pdf
Report 090-819: A Study of the Effects of (R,R)-Formoterol on Embryo/Fetal Development in Rabbits	pharmtox\tox\090-819.pdf

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Report 090-826: A Study of the Effects of (R,R)- Formoterol and Racemic Formoterol on Embryo/Fetal Development in Rabbits	pharmtox\tox\090-826.pdf
Report 090-834: A Study of the Effects of (R,R/S,S)- Formoterol and (R,R)-Formoterol on Embryo/Fetal Development in Rabbits	pharmtox\tox\090-834.pdf
Report 090-832: Study of the Effects of (R,R)- Formoterol on Pre- and Postnatal Development, Including Maternal Function in the Rat.....	pharmtox\tox\090-832.pdf
Report 090-843: Risk Assessment of Reproductive Development of Arformoterol in Rats.....	pharmtox\tox\090-843.pdf

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Formoterol is a potent, selective, and long-acting β_2 -adrenergic agonist that relaxes smooth muscle and exerts bronchodilatory effects in animals and humans. Through G-protein coupling, binding of formoterol to beta receptors activates adenylate cyclase and results in an increase of intracellular cAMP which in turn causes bronchodilation. Formoterol has two chiral centers, which results in four enantiomers (i.e., R,R-, R,S-, S,R-, and S,S-). The active ingredient of Foradil[®] Aerolizer[™] is R,R/S,S-formoterol (equal proportions of the R,R- and S,S-enantiomers). In the present application, Sepracor has proposed to market the (R,R)-enantiomer of formoterol. (R,R)-formoterol has a K_d for the β_2 receptor of 2.9 nM as compared to 3100 nM for (S,S)-formoterol. (R,R)-formoterol has a nearly 40-fold greater selectivity for the human β_2 -receptor as compared to the β_1 -receptor. (R,R)-formoterol was equipotent to isoproterenol in stimulating the generation of intracellular cAMP, whereas (S,S)-formoterol had little activity. In an ovalbumin-sensitized guinea pig model, (R,R)-formoterol produced dose-dependent relaxation of airway resistance induced by histamine or ovalbumin. (R,R)-formoterol significantly inhibited methacholine-induced contraction of guinea pig tracheal smooth muscle (with or without TNF α pretreatment) in a concentration-dependent manner.

Desformoterol, a degradation product of formoterol formed by the loss of the formyl moiety from its formamide group, also binds to beta receptors. The IC_{50} of desformoterol for the β_2 receptor is 35.6 nM as compared to 2.3 nM for (R,R)-formoterol.

In a cardiovascular safety pharmacology study with dogs, (R,R)-formoterol at intravenous doses of 0.03 to 10 μ g/kg increased heart rate and decreased QT interval (uncorrected). QTc interval using Bazett's formula was increased with (R,R)-formoterol at intravenous doses of 0.03 to 10 μ g/kg. However, QTc interval using Van de Water's formula was unchanged with (R,R)-formoterol at intravenous doses of 0.03 to 10 μ g/kg. Bazett's formula is known to over-correct QT interval.

In vitro studies indicated that (R,R)-formoterol, (S,S)-formoterol, and (R,R)-desformoterol at concentrations up to 125 nM had no effects on the hERG channel.

In studies with isolated cardiac Purkinje fibers, (R,R)-formoterol at concentrations up to 12.5 nM had no effects on action potential duration (APD_{50} and APD_{90}), resting membrane potential (RMP), and action potential amplitude (APA). (R,R)-formoterol at concentrations ≥ 1.25 nM with a basic cycle length (BCL) of 2 seconds decreased the maximum rate of rise (dV/dT); however, these effects were not observed with BCL of 0.5 and 1 second. Racemic formoterol at concentrations up to 25 nM had no effects on APD_{50} , APD_{90} , and RMP. Racemic formoterol at a BCL of 2 seconds produced decreases of APA; however, no effects were observed with BCL of 0.5 and 1 sec. Racemic formoterol at concentrations of 2.5, 7.5, and 25 nM decreased dV/dT with BCL of 0.5, 1, and 2 seconds. (S,S)-formoterol at concentrations up to 12.5 nM had no

effects on APD_{50} and APD_{90} . (S,S)-formoterol produced decreases of RMP, APA, and dV/dT . The significance of these changes observed with (R,R)-formoterol, racemic formoterol, and (S,S)-formoterol were unclear.

In general toxicology studies conducted with (R,R)-formoterol in rats and dogs, there was no evidence of treatment-related neurological, pulmonary, renal, or gastrointestinal effects.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

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

Comparison of Albuterol, Salmeterol, and Formoterol Activation of Human β_2 -Adrenergic Receptors (Sepracor Document No. 090-490):

The ability of isoproterenol, racemic mixtures and isolated stereoisomers of albuterol, salmeterol, and formoterol to bind to human β_2 adrenergic receptors was assessed. Confluent and growth arrested human airway smooth muscle cells were used to analyze cAMP production, inositol phosphate production, and calcium flux in the presence of test compounds. The K_d for (R,R)-formoterol was 2.1 nM as compared to values of 4.3 and 4000 nM for (R,R/S,S)-formoterol and (S,S)-formoterol, respectively. None of these compounds promoted a significant increase in inositol phosphate production or calcium flux. The EC_{50} for cAMP production with (R,R)-formoterol was 1.1 nM as compared to values of 5.1 and 3774 nM for (R,R/S,S)-formoterol and (S,S)-formoterol, respectively.

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Drug	K _d (nM) ^a ligand binding	EC ₅₀ (nM) cAMP production	Intrinsic activity
(±) Isoproterenol	123 ± 20	60	1.00
R-Albuterol	357 ± 59	183	0.17
RS-Albuterol	421 ± 20	124	0.16
S-Albuterol	37,000 ± 2000	15,470	0.02
R-Salmeterol	1.03 ± 0.16	3.4	0.13
RS-Salmeterol	0.62 ± 0.04	0.6	0.11
S-Salmeterol	15.5 ± 1.4	ND	0.04
RR-Formoterol	2.1 ± 0.3	1.1	0.53
RRSS-Formoterol	4.3 ± 0.4	5.1	0.65
SS-Formoterol	4,000 ± 447	3774	0.18

Binding Study of (R,R)-Desformoterol Fumarate in β -Adrenergic Receptor Binding Assays (Sepracor Document No. 090-448): IC₅₀ values for (R,R)-desformoterol at the human β_1 and β_2 receptors were 3180×10^{-9} M and 35.6×10^{-9} M, respectively.

The Effects of Enantiomers of Albuterol and Formoterol on Non-Contractive Function of Human Airway Smooth Muscle Cells (Sepracor Document No. 090-486): Granulocyte-macrophage colony stimulation factor (GM-CSF) is a pro-inflammatory cytokine produced by airway smooth muscle (ASM) cells. Effects of enantiomers of albuterol and formoterol on GM-CSF production by ASM cells were examined. R-albuterol, S-albuterol, (R,R)-formoterol, and (S,S)-formoterol were tested at concentrations of 10 nM and 10 μ M. (R,S)-albuterol and R,R/S,S-formoterol were tested at concentrations of 20 nM and 20 μ M. Isoproterenol and propranolol were tested at concentration ranges of 0.5 to 100 μ M. Isoproterenol, R-albuterol and (R,R)-formoterol decreased GM-CSF production. Propranolol, S-albuterol, and (S,S)-formoterol increased GM-CSF production. R,S-Albuterol and R,R/S,S-formoterol had no effects on GM-CSF production. These results suggest beneficial effects of R-albuterol and (R,R)-formoterol and detrimental effects of S-albuterol and (S,S)-formoterol.

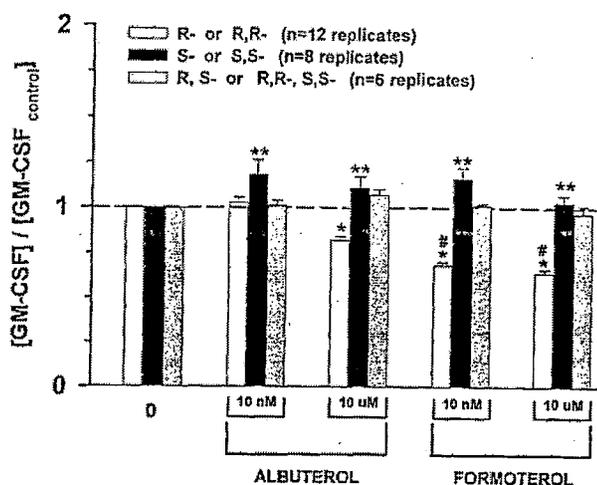


Figure 2. GM-CSF production with either R-enantiomers, S-enantiomers, or racemic (R,S-) versions of beta-agonists. *P<0.05 as compared to control, **P<0.05 S-enantiomer as compared to both control and corresponding R-enantiomer, #P<0.05 R,R- as compared to both R-enantiomers.

The Effects of Enantiomers of Albuterol and Formoterol on Indices of Remodeling in Human Lung Fibroblasts (Sepracor Document No. 090-486): Effects of enantiomers of albuterol and formoterol on collagen expression by cultured human lung fibroblasts were examined. R-albuterol, (R,R)-formoterol, S-albuterol, and (S,S)-formoterol increased expression of Col III α 1 mRNA. These effects were blocked by propranolol indicating the effect is mediated through β adrenoreceptors. Atropine had no effects indicating that muscarinic receptors were not involved in these effects. There were no differences between the R- and S-enantiomers in this assay.

Drug activity related to proposed indication:

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

2.6.2.3 Secondary pharmacodynamics

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

Evaluation of β_2 Agonists at M₁-M₅ Muscarinic Receptor Subtypes (Sepracor Document No. 090-439): Albuterol, fenoterol, formoterol, and salmeterol (racemates and enantiomers) were evaluated for dose-dependent activities as either agonists or antagonists of cloned M₁-M₅ human muscarinic receptor subtypes, expressed in NIH3T3 cells. None of the compounds exhibited activity as agonists or antagonists of M₂ receptors, although a slight trend was observed for (R,R)-formoterol at the highest concentration tested. R, S, and RS-albuterol did not exhibit agonist or antagonist effects against M₁, M₃, M₄, and M₅ receptors. Formoterol (racemate and enantiomers) was apparently not tested at the M₁, M₃, M₄, and M₅ receptors.

2.6.2.4 Safety pharmacology

Neurological effects: Not applicable. Potential neurological effects of (R,R)-formoterol were assessed as a part of general toxicology studies.

Cardiovascular effects: See below and attached reviews of studies submitted under IND 55,302.

In Vitro

Effects of IN-0304, IN-0475, and IN-0618 on Cloned hERG Channels Expressed in Mammalian Cells (Sepracor Document No. 090-492A1).

Methods: In vitro effects of (R,R)-formoterol (IN-0475), (S,S)-formoterol (IN-0618), and racemic formoterol (IN-0304) on hERG channel current (I_{Kr}) were assessed with HEK293 cells using a standard whole cell patch-clamp method. (R,R)-formoterol and (S,S)-formoterol were tested at concentrations of 12.5, 37.5, and 125 nM. Racemic formoterol was tested at concentrations of 25, 75, and 250 nM. Cisapride was used as a positive control.

Results: Neither (R,R)-formoterol nor (S,S)-formoterol at concentrations up to 125 nM had statistically significant effects on hERG channel current. Racemic formoterol at concentrations up to 250 nM had no statistically significant effects on hERG channel current. Racemic formoterol at 250 nM, (R,R)-formoterol at 125 nM, and (S,S)-formoterol at 125 nM decreased hERG currents by 0.8%, 1.7%, and 0.3%, respectively. Cisapride at 90 nM blocked 54.0% of the tail current amplitude.

Effects of IN-0304, IN-0475, and IN-0618 on Action Potentials in Isolated Cardiac Purkinje Fibers (Sepracor Document No. 090-493).

Methods: In vitro effects of (R,R)-formoterol (IN-0475), (S,S)-formoterol (IN-0618), and racemic formoterol (IN-0304) on action potential parameters from Purkinje fibers excised from adult canine ventricles (purpose-bred beagle dogs). (R,R)-formoterol and (S,S)-formoterol were tested at concentrations of 1.25, 3.75, and 12.5 nM. Racemic formoterol was tested at concentrations of 2.5, 7.5, and 25 nM. A positive control, d,l-sotalol was used in these studies. Each test article was evaluated using fibers from each of 4 different animals.

Results: (R,R)-formoterol (IN-0475) at concentrations up to 12.5 nM had no effects on action potential duration (APD₅₀ and APD₉₀), resting membrane potential (RMP), and action potential amplitude (APA). (R,R)-formoterol at concentrations of 1.25, 3.75, and 12.5 nM with a basic cycle length (BCL) of 2 seconds decreased the maximum rate of rise (dV/dT) by 12.2, 11.4, and 11.9%, respectively (see the figure below); however, these effects were not observed with BCL of 0.5 and 1 second. The significance of the change in dV/dT is not clear.

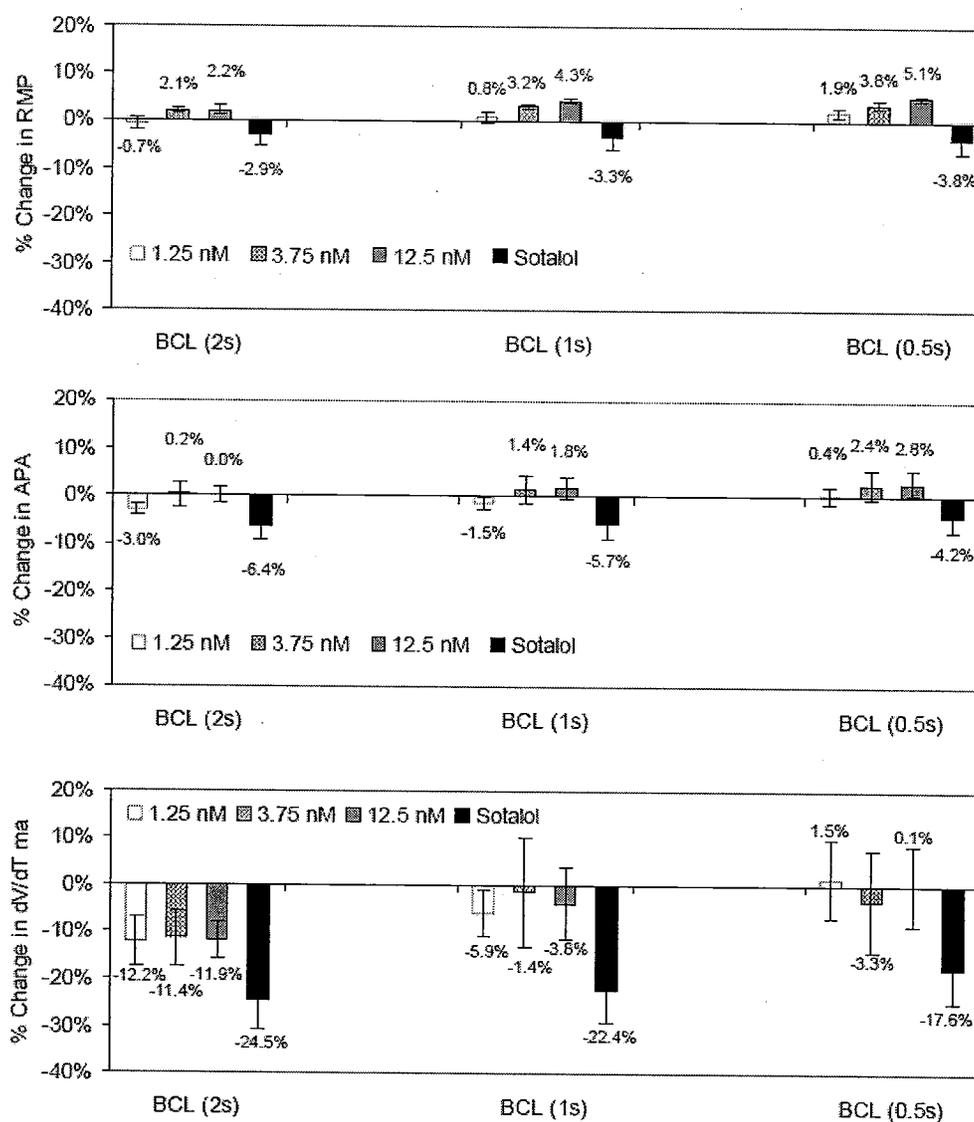


Figure 4. Summary of the average effects of IN-0475 and 100 μ M *dl*-Sotalol on the RMP, APA and dV/dt max at BCLs of 2, 1 and 0.5s .

The % change \pm SEM in the resting Membrane Potential (RMP) (Top Panel), Action Potential Amplitude (APA) (Middle Panel) and dV/dt max (Lower Panel) for each concentration of *dl*-Sotalol (100 μ M) and IN-0475 tested (1.25, 3.75 and 12.5 nM).

Racemic formoterol (IN-0304) at concentrations up to 25 nM had no effects on APD₅₀, APD₉₀, and RMP. Racemic formoterol at a BCL of 2 seconds produced decreases of APA; however, no effects were observed with BCL of 0.5 and 1 sec. Racemic formoterol at concentrations of 2.5, 7.5, and 25 nM decreased dV/dT with BCL of 0.5, 1, and 2 seconds (see figure below).

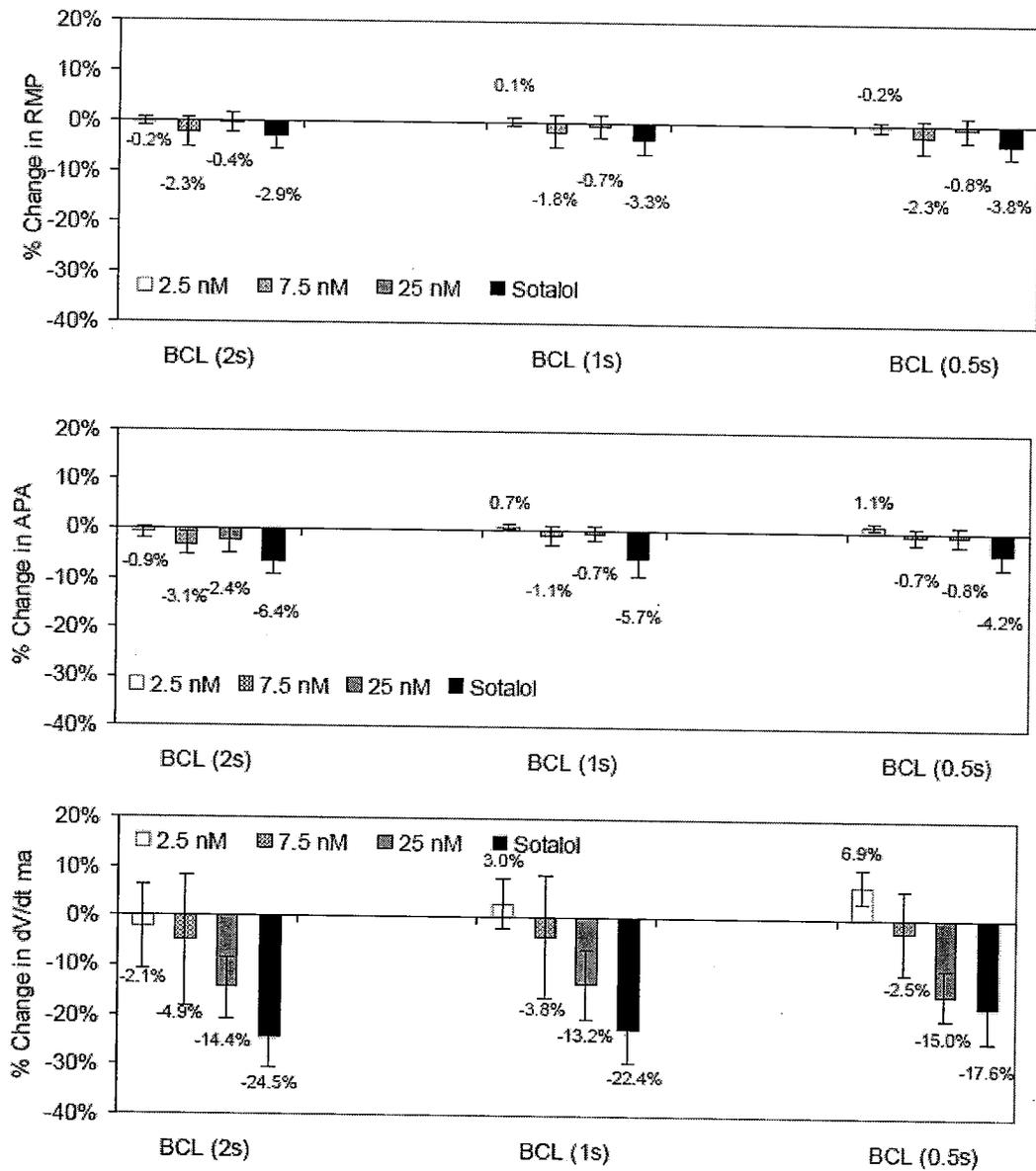


Figure 2. Summary of the average effects of IN-0304 and 100 μ M *d*L-Sotalol on the RMP, APA and dV/dt max at BCLs of 2, 1 and 0.5s .

The % change \pm SEM in the Resting Membrane Potential (RMP) (Top Panel), Action Potential Amplitude (APA) (Middle Panel) and dV/dt max (Lower Panel) for each concentration of *d*L-Sotalol (100 μ M) and IN-0304 tested (2.5, 7.5 and 12.5 nM).

(S,S)-formoterol (IN-0618) at concentrations up to 12.5 nM had no effects on APD₅₀ and APD₉₀. (S,S)-formoterol produced decreases of RMP, APA, and dV/dT (see figures below).

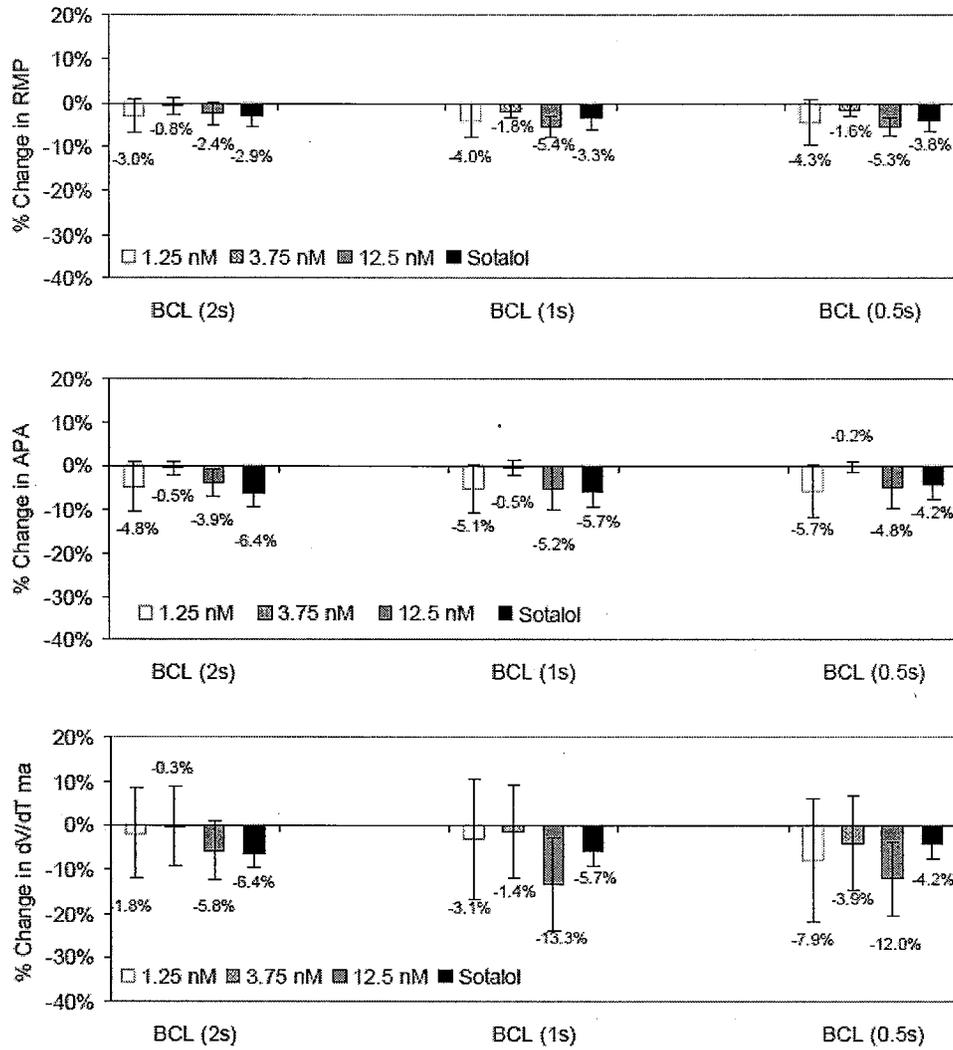


Figure 6. Summary of the average effects of IN-0618 and 100 μM dl-Sotalol on the RMP, APA and dV/dt max at BCLs of 2, 1 and 0.5s .

The % change ± SEM in the resting Membrane Potential (RMP) (Top Panel), Action Potential Amplitude (APA) (Middle Panel) and dV/dt max (Lower Panel) for each concentration of dl-Sotalol (100 μM) and IN-0618 tested (1.25, 3.75 and 12.5 nM).

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Effects of (R,R)-Desformoterol on Cloned hERG Channels Expressed in Mammalian Cells (Sepracor Document 090-837).

Methods: In vitro effects of (R,R)-desformyl formoterol, a degradant of arformoterol, on hERG channel current (I_{Kr}) were assessed with HEK293 cells using a standard whole cell patch-clamp method. (R,R)-desformoterol (Lot numbers MS5249 and MS5262 combined) was tested at concentrations of 12.5, 37.5, and 125 nM. Terfenadine was used as a positive control.

Results: (R,R)-desformoterol at 12.5, 37.5, and 125 nM had no statistically significant effects on hERG channel current, with decreases in hERG channel current of 0.2, 0.3, and 0.5%, respectively, following 10 min exposure (versus 0.9% decrease in vehicle-control treated cells). The positive control, terfenadine (60 nM) blocked 89.3% of tail current amplitude.

Dogs**Reanalysis of Corrected QT-Interval (QTc) from Report 090-423: Evaluating the Comparative Effects of (R,R/S,S)-Formoterol, (R,R)-Formoterol, and (S,S)-Formoterol on Heart Rate, Blood Pressure, and the Lead II Electrocardiogram Following Intravenous Administration to Conscious Dogs (Sepracor Document No. 090-495).**

Methods: The applicant reanalyzed changes of QT interval using Van de Water's correction formula. See the original review of Sepracor Document No. 090-423 for further details of study design.

Results: (R,R)-formoterol at intravenous doses of 0.03 to 10 $\mu\text{g}/\text{kg}$ increased heart rate and decreased QT interval (uncorrected). QTc interval using Bazett's formula was increased with (R,R)-formoterol at intravenous doses of 0.03 to 10 $\mu\text{g}/\text{kg}$. However, QTc interval using Van de Water's formula was unchanged with (R,R)-formoterol at intravenous doses of 0.03 to 10 $\mu\text{g}/\text{kg}$. Bazett's formula is known to over-correct QT interval.

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Figure 3 Effect of Intravenously Administered (R,R)-Formoterol on Heart Rate in Conscious Dogs

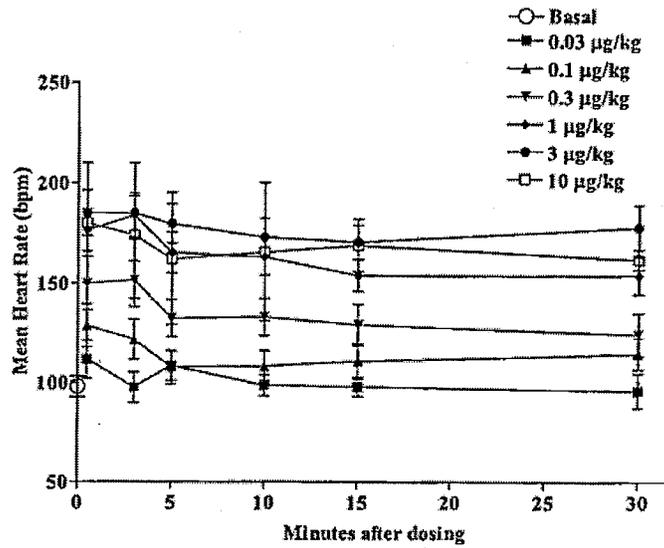


Figure 7 Effect of Intravenously Administered (R,R)-Formoterol on QT-Interval in Conscious Dogs

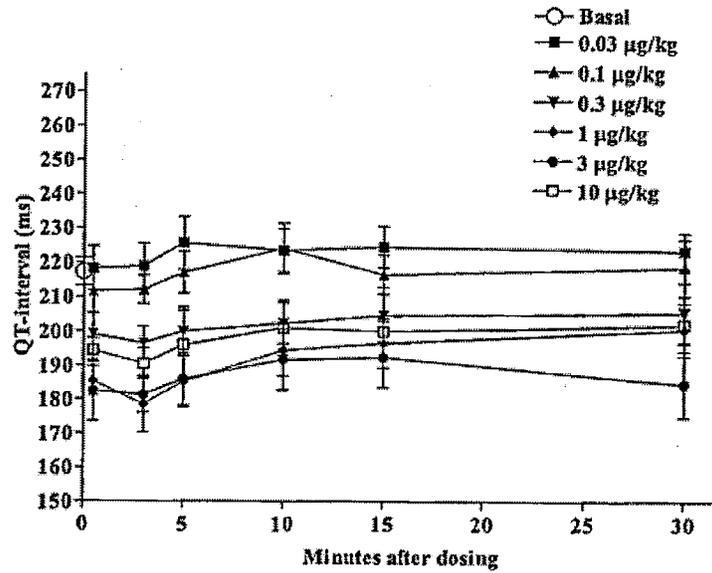
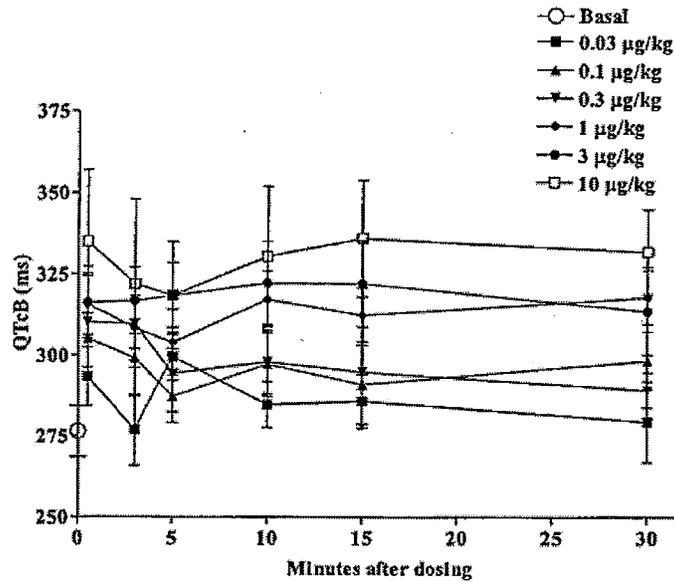


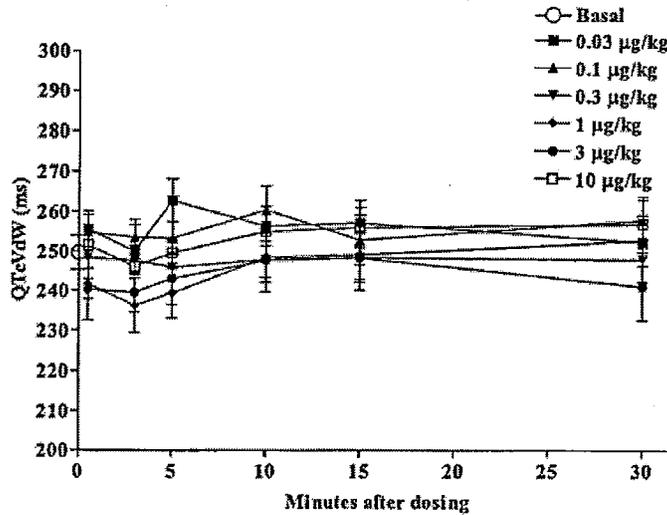
Figure 11 Effect of Intravenously Administered (R,R)-Formoterol on QTc-B in Conscious Dogs



QTc-B = QTc-Bazett = $QT \times \sqrt{HR/60}$. Data were calculated using heart rate and QT-interval data provided in the Final Report for Study SEPBR-11105. All values are presented as Mean \pm S.E.M.

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Figure 15 Effect of Intravenously Administered (R,R)-Formoterol on QTc-VdW in Conscious Dogs



QTcVdW = QTc-Van de Water = $QT - 87(60/HR - 1)$. Data were calculated using heart rate and QT-interval data provided in the Final Report for Study SEPBR-11105. All values are presented as Mean \pm S.E.M.

b(4)

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

Renal effects: Not applicable. Potential renal effects of (R,R)-formoterol were assessed as a part of general toxicology studies.

Gastrointestinal effects: Not applicable. Potential gastrointestinal effects of (R,R)-formoterol were assessed as a part of general toxicology studies.

Abuse liability: No studies of abuse liability were conducted.

Other: No other studies were conducted.

2.6.2.5 Pharmacodynamic drug interactions

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

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not provided.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Studies to assess the pharmacokinetic and toxicokinetic properties of (R,R)-formoterol were conducted in mice, rats, rabbits, and dogs using the intravenous, oral, and inhalation routes. Oral bioavailability of (R,R)-formoterol and its metabolites (i.e., total radioactivity) in mice, rats, and dogs were 49-98%, 28.8-123.3%, and 57-114%, respectively. Following intravenous and oral administration of [³H]-(R,R)-formoterol to mice and rats as well inhalation exposure of rats, radioactivity was rapidly absorbed and widely distributed to tissues. Following inhalation exposure of [³H]-(R,R)-formoterol to rats, radioactivity concentrations were highest in tissues associated with the route of administration (lung tissues and gastrointestinal tract) or routes of elimination (kidney, liver, and gastrointestinal tract). The majority of the (R,R)-formoterol content in EDTA-preserved whole blood from rat, dog, human, and mouse was associated with red blood cells and concentration-independent. The percent of drug bound to rat (37.2-48.9%), dog (35.9-47.8%), human (52.1-64.8%), and mouse (28.2-33.7%) plasma protein was weak and concentration-independent. In studies with pregnant female rats and rabbits that received [³H]-formoterol, radioactivity crossed the placenta and distributed into amniotic fluid and fetal tissues. In studies with lactating female rats that received [³H]-(R,R)-formoterol, distribution of radioactivity into maternal milk was observed. The milk to plasma ratio was 0.713. For mice, rats, and dogs treated with (R,R)-formoterol, analysis of urine by LC/MS/MS found no evidence of chiral inversion. Half-lives of (R,R)-formoterol in mice, rats, and dogs were 2.3-15.9 hr, 2.9-83.3 hr, and 2.9-10.3 hr, respectively. (R,R)-formoterol was extensively metabolized in mice, rats, and dogs. Clearance values for the parent drug, (R,R)-formoterol, in mice, rats, and dogs were high, generally exceeding liver and kidney blood flow, suggestive of a metabolic clearance. Incubation of (R,R)-formoterol with rat or human hepatocytes led to the generation of up to 14 metabolites. Metabolites produced by human hepatocytes

included a glucuronide of desmethylformoterol, a glucuronide of desformoterol, desmethylformoterol, a phenolic glucuronide of formoterol, a hexose conjugate of desformoterol, desformoterol, and a benzylic glucuronide of formoterol. Human hepatic cytochrome P450 isozymes involved in the metabolism of (R,R)-formoterol were determined to be predominantly CYP2D6 and to a lesser extent, CYP2C19. Several UDP-glucuronyl transferase isozymes were found to be involved in the conjugation of (R,R)-formoterol and its metabolites. Studies of the metabolism of [³H]-(R,R)-formoterol in mice, rats, and dogs found 10 potential metabolites. Across species, dose routes/levels, and biological matrices, principal drug-related compounds of interest included the glucuronide of (R,R)-formoterol and unchanged (R,R)-formoterol and to a lesser extent, (R,R)-O-desformyl formoterol and its glucuronide and (R,R)-desformyl formoterol and its glucuronide. For mice and dogs, drug-related radioactivity was primarily excreted in the urine. For rats, drug-related radioactivity was primarily excreted in the feces and biliary excretion was found to play a major role. There was evidence of enterohepatic recirculation in rats. (R,R)-formoterol at 100 nM or (R,R/S,S)-formoterol at 200 nM, with or without pre-incubation, produced no significant inhibition (i.e., ≤7%) of the activities of cytochrome P450 isozymes (i.e., CYP1A2, CYP2A6, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11).

2.6.4.2 Methods of Analysis

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

2.6.4.3 Absorption

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

Absorption and Excretion Studies of [³H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Dogs (Sepracor Document No. 090-527).

Methods: The absorption and excretion of [³H]-formoterol and radiolabeled metabolites were examined in 3 male beagle dogs following an intravenous dose of 3 µg/kg and oral doses of 30 and 300 µg/kg. There was a 7-week washout between treatments. Blood samples, urine, and feces were collected up to 168 hr postdose. Cage washes were collected at 24 hr intervals. Blood, plasma, urine, feces, and cage washes were analyzed for radioactivity concentrations. Blood and plasma levels of (R,R)-formoterol and radioactivity were measured. Urine was examined for evidence of chiral inversion of (R,R)-formoterol.

Results: Following intravenous administration, clearance was 27.7 mL/min/kg, which is comparable to liver and kidney blood flow (30.9 and 21.6 mL/min/kg) and suggests rapid metabolism. Volume of distribution ranged from 5.6 to 6.9 L/kg, which suggests extensive tissue distribution. Half-lives following intravenous and oral dosing ranged 2.9 to 10.3 hr. The applicant considered prolonged half-lives with oral dosing to be artifacts, although it was not determined if enterohepatic recirculation was occurring in dogs.

Oral bioavailability of radioactivity in dogs ranged from 82-85% based upon total radioactivity in plasma and 57-114% based upon freeze-dried radioactivity in plasma.

Systemic bioavailability of (R,R)-formoterol in male dogs following oral administration of [³H]-(R,R)-formoterol ranged from 54 to 60%.

(R,R)-formoterol underwent rapid and extensive metabolism as supported by the high clearance values. Based on mean AUC₁₆₈, (R,R)-formoterol represented less than 1% of the plasma radioactivity, which was also in agreement with the data from both the lower oral dose and the intravenous administration. Again based on mean AUC₁₆₈, (R,R)-formoterol represented approximately 9% of the freeze-dried radioactivity, which was slightly less than both the lower oral dose (13%) and the intravenous administration (15%).

LC/MS/MS analysis of urine samples found no evidence of in vivo chiral inversion of (R,R)-formoterol in dogs. Urine (R,R)-formoterol concentrations were up to 1000-fold higher than peak plasma concentrations.

Pharmacokinetic parameters derived from (R,R)-formoterol concentrations in the plasma of male dogs following intravenous administration of [³H]-(R,R)-formoterol L-tartrate (3 µg/kg)

Animal no.	C ₀	T _{max}	AUC _t	AUC ₁₆₈	AUC	CL	V _z	V _{ss}	λ _z	t _{1/2}
1M	2.38	0	1.1	1.3	1.3 ^a	27.0 ^a	8.6 ^a	6.5 ^a	0.1882 ^a	3.7 ^a
2M	1.12	0	1.1	1.3	1.2	28.6	7.2	5.7	0.2381	2.9
3M	1.60	0	1.2	1.3	1.3	26.7	6.6	5.4	0.2429	2.9
mean	1.70	0	1.1	1.3	1.3	27.7	6.9	5.6	0.2405	2.9 ^b
sd	0.64	0	0.1	0.0	-	-	-	-	-	-

C₀ Calculated concentration at time 0, in ng (R,R)-formoterol/ml

T_{max} Time of maximum concentration, in hours

AUC_t Area under the time-concentration curve up to the last measurable sample point, in ng (R,R)-formoterol·h/ml

AUC₁₆₈ Area under the time-concentration curve up to 168 hours after dose, in ng (R,R)-formoterol·h/ml

AUC Area under the time-concentration curve up to infinity, in ng (R,R)-formoterol·h/ml

CL Plasma clearance, in ml/min/kg

V_z Volume of distribution, in l/kg

V_{ss} Volume of distribution at steady-state, in l/kg

λ_z Terminal rate constant, in hour⁻¹

t_{1/2} Terminal half-life, in hours

sd Standard deviation

^a Parameter could not be calculated in accordance with the acceptance criteria defined in Data Processing, treat with caution and has not been included in any mean calculation

^b Calculated as ln2/mean λ_z

- Not calculated

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Pharmacokinetic parameters derived from (R,R)-formoterol concentrations in the plasma of male dogs following oral administration of [³H]-(R,R)-formoterol L-tartrate(30 and 300 µg/kg)

Dose level	Animal No.	C _{max}	T _{max}	AUC _t	AUC ₁₆₈	AUC	λ _z	t _{1/2}
30 µg/kg	1M ^a	1.93	0.5	5.1	5.1	5.1	0.0943	7.3
	2M	1.29	0.5	6.9	6.9	6.9	0.1118	6.2
	3M	3.43	0.25	8.9	8.9	8.9	0.1095	6.3
	mean	2.36	0.375 ^c	7.9	7.9	7.9	0.1107	6.3 ^d
	sd	-	-	-	-	-	-	-
300 µg/kg	1M ^b	6.93	1.0	33.6	33.6	33.6	0.0562	12.3
	2M	12.4	0.25	79.9	80.0	80.0	0.0910	7.6
	3M	12.0	0.5	69.8	69.8	69.8	0.0439	15.8
	mean	12.2	0.375 ^c	74.9	74.9	74.9	0.0675	10.3 ^d
	sd	-	-	-	-	-	-	-

- C_{max} Maximum concentration, in ng (R,R)-formoterol/ml
- T_{max} Time of maximum concentration, in hours
- AUC_t Area under the time-concentration curve up to the last measurable sample point, in ng (R,R)-formoterol-h/ml
- AUC₁₆₈ Area under the time-concentration curve up to 168 hours after dose, in ng (R,R)-formoterol-h/ml
- AUC Area under the time-concentration curve up to infinity, in ng (R,R)-formoterol-h/ml
- CL Plasma clearance, in ml/min/kg
- λ_z Terminal rate constant, in hour⁻¹
- t_{1/2} Terminal half-life, in hours
- sd Standard deviation
- ^a Dog vomited ca 20% of dose 2h post-dose, excluded from mean calculations
- ^b Dog vomited ca 60% of dose 3h post-dose, excluded from mean calculations
- ^c Value is the mean, due to n = 2
- ^d Calculated as ln2/mean λ_z
- Not calculated

Parameters derived from  data, Tables 10, 15 and Addendum 1

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Systemic availability of radioactivity in male dogs following oral administration of [³H]-(R,R)-formoterol L-tartrate (30 and 300 µg/kg)

Dose level	Analyte	Animal number	F
30 µg/kg	Plasma	1M ¹	0.74
		2M	0.87
		3M	0.85
		Geometric mean	0.82
	Freeze-dried plasma	1M ²	0.42
		2M	0.53
		3M	0.83
		Geometric mean	0.57
300 µg/kg	Plasma	1M ²	0.97
		2M	0.78
		3M	0.81
		Geometric mean	0.85
	Freeze-dried plasma	1M ²	1.48
		2M	1.12
		3M	0.89
		Geometric mean	1.14

- F Systemic availability, where 1 = 100%
- ¹ Dog vomited *ca* 20% of dose 2h post-dose, dose has been adjusted
- ² Dog vomited *ca* 60% of dose 3h post-dose, dose has been adjusted

Systemic bioavailability of (R,R)-formoterol in male dogs following oral administration of [³H]-(R,R)-formoterol L-tartrate (30 and 300 µg/kg)

Dose level	Animal number	F
30 µg/kg	1M ¹	0.47
	2M	0.52
	3M	0.66
	Geometric mean	0.54
300 µg/kg	1M ²	0.62
	2M	0.63
	3M	0.55
	Geometric mean	0.60

- F Systemic bioavailability, where 1 = 100%
- ¹ Dog vomited *ca* 20% of dose 2h post-dose, dose has been adjusted
- ² Dog vomited *ca* 60% of dose 3h post-dose, dose has been adjusted

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2.6.4.4 Distribution

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

Mice

[³H]-(R,R)-Formoterol: Absorption, Distribution, and Excretion Studies of [³H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Mice (Sepracor Document No. 090-528).

Methods: Absorption, distribution, metabolism, and excretion of [³H]-(R,R)-formoterol were examined in male CD-1 mice following an intravenous dose of 1 mg/kg or oral doses of 1 and 10 mg/kg. Blood and plasma (at time points up to 168 hours postdose), selected tissues and organs (at 2, 24 and 48 hours postdose), and urine, feces and respired air (up to 168 hours post-dose) were collected and retained for radioactivity measurement.

Results: Following absorption, radioactivity was rapidly and widely distributed throughout the body tissues. Oral bioavailability of radioactivity ranged from 48 to 66% based upon total radioactivity in plasma and 75 to 98% based upon total radioactivity in freeze-dried plasma. Oral bioavailability of (R,R)-formoterol ranged from 9 to 13% indicating extensive metabolism. Volume of distribution values ranged from 0.751 L/kg with intravenous dosing to 15.492-30.037 L/kg with oral dosing, which were consistent with extensive tissue distribution.

Distribution of radioactivity was relatively similar following intravenous and oral dosing. The highest concentrations of radioactivity in tissues were measured at 2 hr postdose. The kidneys, walls of the small and large intestines, and contents of the small and large intestines contained the largest amounts of radioactivity. Concentrations declined at each of the subsequent sample times (24 and 48 hr postdose) and were mostly still measurable at the last sample time. With the exception of the intestinal walls and contents, the mean tissue:plasma radioactivity concentration ratios were generally 1 or less for all dose groups at 48 hours after the dose.

Clearance of (R,R)-formoterol was rapid with clearance values of 3.697 mL/min/kg with intravenous dosing and 28.372-43.072 mL/min/kg with oral dosing. These values are smaller than liver and kidney blood flow (90 and 65 mL/min/kg, respectively). Comparison of the AUC of formoterol with that of total radioactivity showed that formoterol represented 1.8% of the total plasma radioactivity concentration following intravenous dose administration and less than 1% following oral dose administration suggesting extensive metabolism.

LC/MS/MS analysis of urine samples found no evidence of in vivo chiral inversion of (R,R)-formoterol in mice.

Pharmacokinetic parameters derived from plasma (R,R)-formoterol concentrations in male mice following the administration of single doses of [³H]-(R,R)-formoterol L-tartrate

Dose route	Dose level (mg/kg)	C _{max}	T _{max}	AUC ₁₆₈	AUC	λ _z	t _{1/2}	CL	V _z	V _{ss}	F
iv	1	95.7 ^a	0	100.5	100.5	0.2955	2.3	3.697	0.751	0.367	1
oral	1	3.2	0.25	8.4	8.4	0.1668	4.2	43.072	15.492	-	0.09
oral	10	56.9	0.5	126.6	126.4	0.0436	15.9	28.372 ^b	39.037 ^a	-	0.13

a Predicted value at time zero (C₀)

b Parameter is CL/F

c Parameter is V_z/F

Units:

Dose mg formoterol tartrate/kg

C_{max} ng/mL

T_{max} hours

AUC₁₆₈, AUC ng·h/mL

λ_z hours⁻¹

t_{1/2} hours

CL mL/min/kg

V_z, V_{ss} L/kg

F Fraction of the dose systemically available

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Pharmacokinetic parameters derived from whole-blood and plasma radioactivity concentrations in male mice following the administration of single oral doses of [³H]-(R,R)-formoterol L-tartrate

Dose level (mg/kg)	Analyte	C _{max}	T _{max}	AUC	AUC ₁₆₈	AUC	λ _z	t _{1/2} ^b	Systemic bioavailability (F)
1	Plasma (total radioactivity)	487.9	0.25	2572	2572	2684	0.0155	44.7	0.48
	Plasma (after freeze-drying)	476.1	0.25	1372	1394	1395 ^a	0.0311 ^a	22.3 ^a	0.75
	Plasma (volatile radioactivity)	15.9	6	1178	1178	1313	0.0135	51.2	-
	Whole-blood (total radioactivity)	287.2	0.25	1923	1923	2096	0.0122	56.9	-
10	Plasma (total radioactivity)	5444	0.5	34727	34727	36782	0.0139	49.8	0.66
	Plasma (after freeze-drying)	5349	0.5	17592	18134	18131	0.0799	8.7	0.98
	Plasma (volatile radioactivity)	190	6	16593	16593	19229	0.0119	58.5	-
	Whole-blood (total radioactivity)	3172	0.5	23580	23580	25305	0.0134	51.6	-

a The parameter could not be calculated in accordance with the pre-defined acceptance criteria (see DATA ACQUISITION AND PROCESSING) and must be treated as an approximation

b Calculated as ln(2)/λ_z

Units:

C_{max} ng equivalents free-base/g

T_{max} hours

AUC, AUC₁₆₈, AUC ng equivalents free-base·h/g

λ_z hours⁻¹

t_{1/2} hours

F fraction (no units)

Rats

An Exploratory Distribution Study of [³H]-(R,R)-Formoterol (without or with an Equal Proportion of Non-Radioactive (S,S)-Formoterol) after Single Dose Inhalation Administration to Rats (Sepracor Document No. 090-515 and 090-517).

Methods: [³H]-(R,R)-formoterol (without or with an equal proportion of non-radioactive (S,S)-formoterol) was administered as a liquid aerosol to male Sprague-Dawley rats for 30 min and achieved an average inhalation dose of 150 or 120 µg/kg, respectively. After exposure, 3 rats were housed in metabolism cages for collection of urine at 0-2, 2-6, and 6-24 hr postexposure. Remaining rats, used for assessments of pharmacokinetics and tissue distribution, were sacrificed (3/time point) at 0, 0.5, 1, 2, 4, 6, and 24 hr postexposure. The following organs were collected for assessment of tissue distribution of radioactivity: brain, bronchi, carina, gastrointestinal tract, kidneys, larynx, liver, lungs, pharynx, and trachea. Radioactivity in blood, plasma, urine, and tissues was measured. Metabolic profiling of selected urine and plasma samples was conducted by HPLC.

Results: Tissue concentrations of radioactivity were highest immediately after completion of exposure and generally declined thereafter. Radioactivity concentrations were highest in tissues associated with the route of administration (lung tissues and gastrointestinal tract) or routes of elimination (kidney, liver, and gastrointestinal tract).

Metabolites profiling of selected urine and plasma samples resolved 4 radioactive peaks (F1, F2, F3, and F4). F1, F2, F3, and F4 were identified as ³H-water, the O-demethylation product of (R,R)-formoterol, a glucuronide of (R,R)-formoterol, and unchanged (R,R)-formoterol, respectively.

The presence of unlabeled (S,S)-formoterol appeared to have little effect on results.

An Exploratory Distribution Study of [³H]-(S,S)-Formoterol After Single Inhalation Doses (Mixed with an Equal Proportion of Non-Radioactive (R,R)-Formoterol) to Rats (Sepracor Document No. 090-518).

Methods: [³H]-(S,S)-formoterol (mixed with an equal proportion of non-radioactive (R,R)-formoterol) was administered as a liquid aerosol to male Sprague-Dawley rats for 30 min and achieved an average inhalation dose of 147 µg/kg. After exposure, 3 rats were housed in metabolism cages for collection of urine at 0-2, 2-6, and 6-24 hr postexposure. Remaining rats, used for assessments of pharmacokinetics and tissue distribution, were sacrificed (3/time point) at 0, 0.5, 1, 2, 4, 6, and 24 hr postexposure. The following organs were collected for assessment of tissue distribution of radioactivity: brain, bronchi, carina, gastrointestinal tract, kidneys, larynx, liver, lungs, pharynx, and trachea. Radioactivity in blood, plasma, urine, and tissues was measured. Metabolic profiling of selected urine and plasma samples was conducted by HPLC.

Results: Tissue concentrations of radioactivity were highest immediately after completion of exposure and generally declined thereafter. Radioactivity concentrations

were highest in tissues associated with the route of administration (lung tissues and gastrointestinal tract) or routes of elimination (kidney, liver, and gastrointestinal tract).

Metabolites profiling of selected urine and plasma samples resolved 4 radioactive peaks (F1, F2, F3, and F4). F1, F2, F3, and F4 were identified as ^3H -water, the O-demethylation product of (S,S)-formoterol, a glucuronide of (S,S)-formoterol, and unchanged (S,S)-formoterol, respectively.

The presence of unlabeled (R,R)-formoterol appeared to have little effect on results.

Absorption, Distribution, and Excretion Studies of [^3H]- (R,R)-Formoterol after Single Intravenous and Oral Doses to Male Rats (Sepracor Document number 090-529).

Methods: The absorption and distribution of [^3H]- (R,R)-formoterol and radiolabeled metabolites were examined in male Sprague-Dawley rats. [^3H]- (R,R)-formoterol was administered at an intravenous dose of 10 mg/kg or oral doses of 1, 10, and 100 mg/kg. Whole-blood and plasma (at time points up to 168 hr postdose), selected tissues and organs (at 2, 24, and 48 hr postdose), and urine, feces, cage wash, and respired air (up to 168 hr postdose) were collected for radioactivity measurement. An additional 48-hr excretion balance study was conducted with bile duct-cannulated rats that received [^3H]- (R,R)-formoterol at an intragastric dose of 1 mg/kg. Urine was examined for evidence of chiral inversion of (R,R)-formoterol.

Results: Following an intravenous dose of 10 mg/kg or oral dose of 100 mg/kg, animals appeared lethargic and exhibited signs of irregular respiration.

[^3H]- (R,R)-Formoterol was rapidly and extensively absorbed following oral administration. Oral bioavailability of total radioactivity (i.e., R,R-formoterol + metabolites) ranged from 69 to 104.9% for freeze-dried radioactivity and 28.8 to 123.3% for total plasma radioactivity. Oral bioavailability of (R,R)-formoterol with doses up to 100 mg/kg was low (i.e., <9.1%) due to extensive metabolism. Terminal half-lives were prolonged with oral doses of (R,R)-formoterol at 10 and 100 mg/kg, possibly due to enterohepatic recirculation. Clearance of radioactivity following an intravenous dose of 10 mg/kg was 84.4 mL/min/kg, which exceeds liver and kidney blood flow (55.2 and 36.8 mL/min/kg) and is suggestive of metabolic clearance. Estimates of volume of distribution ranged from V_{ss} at 11.5 L/kg to V_z at 217 L/kg indicating extensive tissue distribution of (R,R)-formoterol and its metabolites.

LC/MS/MS analysis of urine samples found no evidence of in vivo chiral inversion of (R,R)-formoterol in rats.

Comparison of the AUC of (R,R)-formoterol with that of total radioactivity indicated that (R,R)-formoterol represented 2.7% of the total plasma radioactivity exposure following intravenous bolus dose administration and 0.2-0.5% following oral dose administration.

Furthermore, unchanged (R,R)-formoterol represented a lower percentage of total plasma radioactivity AUC with increasing oral dose.

Following intravenous or oral administration, radioactivity was rapidly and widely distributed throughout the body tissues. However, following each dose level and route of administration, tissue radioactivity concentration declined rapidly between 2 and 48 hr postdose. By 48 hr postdose, tissue radioactivity concentrations corresponded very closely to the corresponding plasma concentration. Tissue distributions of radioactivity were relatively similar following intravenous and oral administration. At 2 hr postdose, the highest concentrations of radioactivity were observed in the kidney, urinary bladder, stomach wall, small intestine wall, and large intestine wall. With oral dosing, high concentrations were also observed in the liver. Urinary and biliary excretion were the major routes of elimination, which explain the high levels of radioactivity in these tissues.

Pharmacokinetic parameters derived from plasma radioactivity concentrations in male rats following the administration of single intravenous doses of [³H]-(R,R)-formoterol L-tartrate (10 mg/kg)

Analyte	C ₀	T _{max}	AUC _t	AUC ₁₆₈	AUC	λ _z	t _{1/2}
Plasma freeze-dried radioactivity	2944	-	8536	8567	8725 ^a	0.0174 ^a	39.9 ^a
Plasma total radioactivity	3140	-	50750	50750	56710	0.0130	53.4
Plasma volatile radioactivity	555.4 ^b	6	42170	42170	48700	0.0120	57.8

a The parameter could not be calculated in accordance with the defined acceptance criteria (see DATA ACQUISITION AND PROCESSING) and must be treated as an approximation

b Value is C_{max} and not C₀

Units:

C₀ ng equivalents free-base/g
 T_{max} hours
 AUC_t, AUC₁₆₈, AUC ng equivalents free-base-h/g
 λ_z hours⁻¹
 t_{1/2} hours

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Pharmacokinetic parameters derived from plasma radioactivity concentrations in male rats following the administration of single oral doses of [³H]-(R,R)-formoterol L-tartrate

Dose (mg/kg)	Radioactivity	C _{max}	T _{max}	AUC _t	AUC ₁₆₈	AUC	λ _z	t _{1/2}	F
1	Freeze-dried	290.3	0.25	576.8	579.7	599.3 ^a	0.0112 ^a	61.7 ^a	0.690
	Total	294.5	0.25	1432	1432	1601	0.0111	62.6	0.288
	Volatile	12.21	2	852.3	852.3	1019	0.0109	63.7	
10	Freeze-dried	1108	1	6853	6895	7233 ^a	0.0108 ^a	64.1 ^a	0.813
	Total	1282	1	37970	37970	43320	0.0119	58.3	0.771
	Volatile	410.4	6	31080	31080	36760	0.0113	61.5	
100	Freeze-dried	9568	1	88680	88680	94110 ^a	0.0080 ^a	86.3 ^a	1.049
	Total	12560	2	589000	589000	690300	0.0113	61.2	1.233
	Volatile	5895	12	500300	500300	617700	0.0100	69.5	

a The parameter could not be calculated in accordance with the defined acceptance criteria (see DATA ACQUISITION AND PROCESSING) and must be treated as an approximation

Units:
 C_{max} ng equivalents free-base/g
 T_{max} hours
 AUC_t, AUC₁₆₈, AUC ng equivalents free-base-h/g
 λ_z hours⁻¹
 t_{1/2} hours

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Pharmacokinetic parameters derived from plasma (R,R)-formoterol concentrations in male rats following the administration of single doses of [³H]-(R,R)-formoterol L-tartrate

Dose route	Dose level (mg/kg)	C _{max}	T _{max}	AUC ₁₆₈	AUC	λ _z	t _{1/2}	CL	V _z	V _{ss}	F
iv	10	1469 ^a	0	1389	1389	0.0233	29.7	84.4	217	11.5	1
oral	1	1.98	0.5	6.642	6.621	0.2359	2.9				0.049
oral	10	15.3	2	107.3	107.6	0.0083	83.3				0.078
oral	100	369	1	1253	1254	0.0188	36.8				0.091

a Predicted value at time zero (C₀)

Units:
 Dose mg formoterol tartrate/kg
 C_{max} ng/mL
 T_{max} hours
 AUC₁₆₈, AUC ng-h/mL
 λ_z hours⁻¹
 t_{1/2} hours
 CL mL/min/kg
 V_z, V_{ss} L/kg

Tissue radioactivity concentrations in male rats following the administration of single intravenous doses of [³H]-(R,R)-formoterol L-tartrate (10 mg/kg)

Results are expressed as ng equivalents free-base/g

Sample	2 h		24 h		48 h	
	Mean	sd	Mean	sd	Mean	sd
Plasma	1527	273	318.3	134.4	330.5	38.6
Whole blood	1103	234	452.3	109.6	500.7	35.3
Blood cells	1109	207	443.0	82.6	559.2	26.6
Brain	304.9	80.2	407.9	100.6	283.9	24.6
Eye	896.0	189.7	411.7	119.8	301.0	30.0
Heart	2146	483	452.8	110.5	314.4	33.0
Kidney	2457.6	1738.6	1374	319	1033	42.7
Urinary bladder	1233.0	1405.9	411.6	193.4	208.0	22.5
Liver	2638	341	958.0	283.3	379.2	64.6
Lung	5326	1733	625.7	128.2	331.0	33.6
Pancreas	6308	1502	535.5	120.2	337.4	36.9
Spleen	3051	790	544.6	89.3	374.6	5.6
Adrenal glands	3428	1108	1374	163	678.7	52.8
Lactimal glands	7648	994	523.7	50.1	345.1	10.5
Lymph nodes	2547	293	417.3	154.7	288.0	37.6
Placental gland	8736	1303	1249	790	338.3	100.2
Salivary gland	2705	1674	744.0	44.8	289.4	33.0
Thyroid	2548	372	443.5	87.5	311.3	55.0
Thyroid	4747	2721	6122	3835	289.9	49.2
Epithelium	2131	466	485.1	74.6	300.3	34.1
Pituitary	2397	727	425.2	48.4	295.0	38.8
Testis	978.9	36.7	784.0	63.2	619.5	39.6
Uterus mesenteric	7847	8239	331.6	307.6	320.3	39.8
Fat (perirenal)	723.3	186.4	379.5	70.0	300.5	31.5
Muscle (diaphragm)	2341	373	425.5	87.8	388.4	29.9
Skin	2130	261	467.2	365	383.9	52.7
Stomach wall	3669.0	2043.0	1050	365	297.7	151.3
Small intestine wall	7560.0	1383.0	3263	1729	423.2	173.1
Large intestine wall	3984.0	5706	2821	951	418.6	288.6

sd Standard deviation

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2.6.4.5 Metabolism

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

In Vitro

An Exploratory In Vitro Metabolism Study of (R,R)-Formoterol and (S,S)-Formoterol in Rat and Human Hepatocyte Suspensions (Sepracor Document No. 090-514).

Methods: Metabolism of radiolabeled and non-radiolabeled (R,R)-formoterol and (S,S)-formoterol by male Sprague-Dawley rat and human hepatocytes were assessed. Incubation periods were 0, 1, 4, and 16 hr. Metabolites were detected and identified with HPLC with radioactivity detection and LS/MS/MS.

Results: Up to 14 metabolites were formed by human and rat hepatocytes incubated with (R,R)-formoterol and (S,S)-formoterol. Three metabolites, peaks 4 (desmethyl formoterol), 9 (desformyl formoterol), and 11 (dehydroxylated formoterol), were prevalent in incubations with human hepatocytes. Peaks 2 (glucuronide) and 10 (glucuronide) were prevalent in incubations with rat hepatocytes. Peaks 3 (glucuronide), 4, 9, and 12 (unknown) were also formed in significant levels in rat hepatocyte incubations. Peak 1 (³H-H₂O) was observed in all incubations.

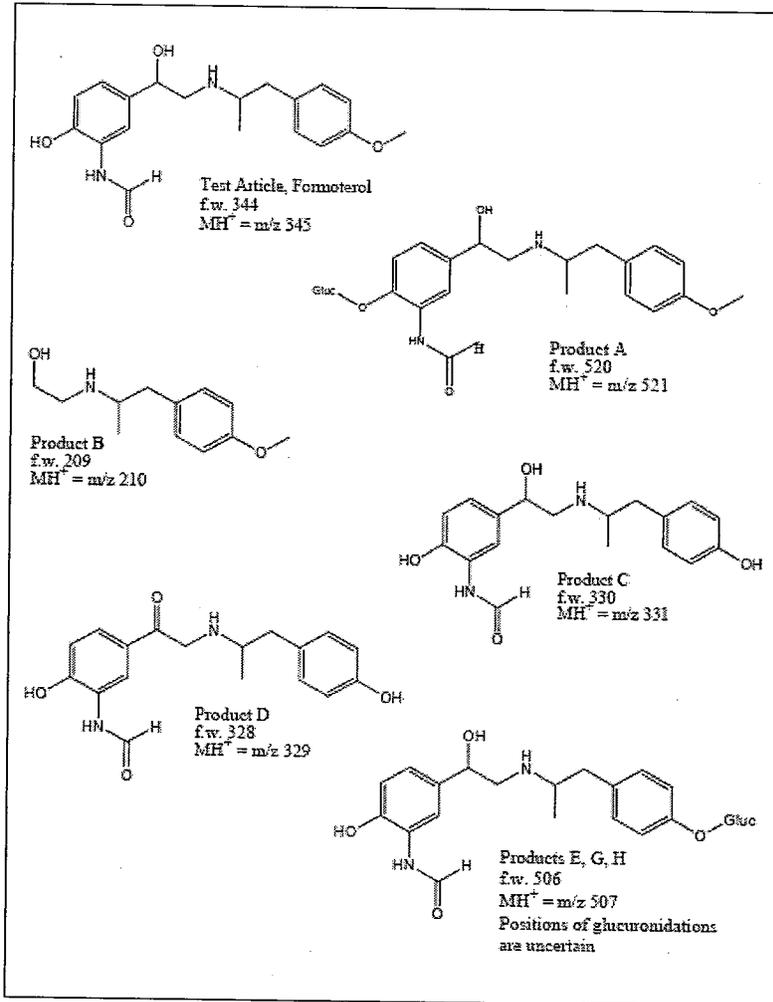
Table 1: Putative Identity of HPLC/RAM and LC/MS/MS metabolite Peaks

LC/RAM Peak	LC/RAM Relative Retention Time	Assigned Structure	LC/MS Relative Retention Time
1	0.143	³ H-H ₂ O	
14	0.224	?	
15	0.267	?	
2	0.349	E	0.288
3	0.408	G	0.381
4	0.503	C	0.501
5	0.599	?	
6	0.612	?	
7	0.721	H	0.720
9	0.869	F	0.807
?		O	0.858
?		L	0.891
10	0.912	A	0.922
11	1.073	D	1.061
12	1.180	?	
?		P	1.293
13	1.580	?	

HPLC/RAM peaks and LC/MS/MS peaks were separated using Gradient Condition 2. The relative retention time of the test article is 1.0 by definition.

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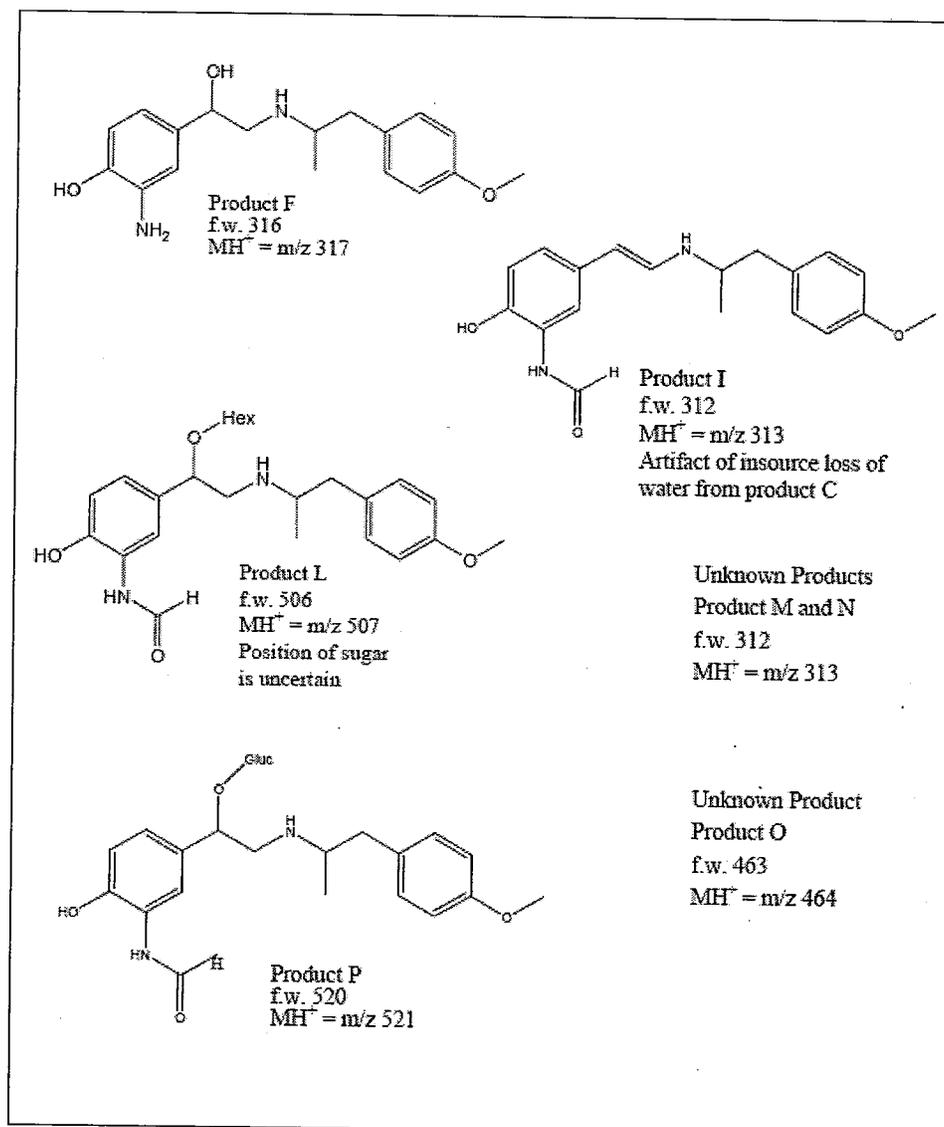
Figure 1: Proposed Structures for Possible Metabolic Products



Proposed metabolite structures from LC/MS/MS analysis of nonradiolabeled (R,R)- and (S,S)-fomoterol bulk incubations with human and rat cryopreserved hepatocytes

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Figure 1 (cont'd)



(R,R)-Formoterol and (S,S)-Formoterol: In Vitro Metabolism Study Using Human Cryopreserved Hepatocytes (Sepracor Document No. 090-568A1).

Methods: The *in vitro* metabolism of [³H]-(R,R)-formoterol and [³H]-(S,S)-formoterol was assessed using human cryopreserved hepatocytes pooled from 4 individual donors. Metabolites were separated and identified using a combination of HPLC with radioactivity detection and LC/MS/MS.

Results: Incubation of [³H]-(R,R)-formoterol and [³H]-(S,S)-formoterol with human hepatocytes led to the formation of up to 7 and 9 metabolites, respectively, as shown in the tables below.

Metabolism of [³H]-(R,R)-formoterol (100 µM) by human cryopreserved hepatocytes after 18 hours incubation 2: identification of metabolites by LC-MS/MS

Metabolite fraction	RAM R _t (min)	% ROI		MH ⁻	MS/MS R _t (min)	Proposed identity
		U	D			
MF-1	10.1	0.6	0.5	507	9.65	Glucuronide of desmethylformoterol
MF-2	21.5	0.1	0.1	493	21.61	Glucuronide of desformoterol
MF-3	22.9	1.6	1.9	331	22.32	Desmethylformoterol
MF-4	27.3	2.8	2.4	521	26.91	Phenolic glucuronide of formoterol
MF-5	30.5	0.9	1.0	479	29.95	Hexose conjugate of desformoterol
MF-6	37.7	0.7	0.7	317	37.20	Desformoterol
MF-7	40.2	1.4	1.4	521	40.11	Benzylic glucuronide of formoterol
Parent	41.1	16.2	13.4	345	40.70	Formoterol

% ROI % of total injected radioactivity associated with this region of the radiochromatogram from which the control value has been subtracted

U Untreated sample

D Sample following incubation with β-glucuronidase/sulphatase enzyme

MH⁻ m/z of the molecular ion of the metabolite

Metabolism of [³H]-(S,S)-Formoterol (100 µM) by human cryopreserved hepatocytes after 18 hours incubation 2: identification of metabolites by LC-MS/MS

Metabolite fraction	RAM R _t (min)	% ROI		MH ⁻	MS/MS R _t (min)	Proposed identity
		U	D			
MF-1	10.2	4.0	3.3	507	9.78	Glucuronide of desmethylformoterol
MF-2	15.2	0.3	0.2	491	15.05	Glucuronide of dehydroxylated desmethylformoterol
MF-3	21.8	0.2	0.2	-	-	-
MF-4	22.8	3.2	3.1	331	22.47	Desmethylformoterol
*	*	*	*	521	**	Phenolic glucuronide of formoterol
MF-5	27.5	3.6	3.5	315	27.47	Dehydroxylated desmethylformoterol
MF-7	30.6	1.1	1.0	479	30.26	Hexose conjugate of desformoterol
MF-8	34.4	0.1	0.1	505	33.26	Glucuronide of dehydroxylated formoterol
MF-9	37.7	2.2	0.5	317	37.25	Desformoterol
*	*	*	*	521	**	Benzylic glucuronide of formoterol
Parent	41.1	23.2	21.7	345	40.81	Formoterol
*	*	*	*	329	42.49	Dehydroxylated formoterol

% ROI % of total injected radioactivity associated with this region of the radiochromatogram from which the control value has been subtracted

U Untreated sample

D Sample following incubation with β-glucuronidase/sulphatase enzyme

MH⁻ m/z of the molecular ion of the metabolite

- Not detected by Mass Spectrometer

* Not detected by β-RAM

** Only Q1 data available

Investigation of the Principal Human Cytochromes P450 and UDP-Glucuronosyltransferases Involved in the Microsomal Metabolism of Both (R,R)-Formoterol and (S,S)-Formoterol In Vitro (Sepracor Document No. 090-543a1).

Methods: Human hepatic cytochrome(s) P450 and UDP-glucuronosyltransferase(s) (UGT) involved in the metabolism of (R,R)-formoterol and (S,S)-formoterol *in vitro* were characterized. [³H]-(R,R)- and [³H]-(S,S)-formoterol were first incubated with human liver microsomes pooled from six individual donors in the presence of NADPH. Incubations were also conducted in the presence of chemical inhibitors of CYP2A6 (8-methoxypsoralen), CYP2B6 (thio-TEPA), CYP2C8 (trimethoprim), CYP2C9 (sulphaphenazole), CYP2C19 (tranylcypromine), and CYP2D6 (quinidine). Studies were also conducted with liver microsomes from 16 individual donors. Microsomes containing expressed human P450 reductase, cytochrome b5 and either CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6, using baculovirus as a vector were obtained to identify which specific cytochrome 450 isozyme(s) were involved in the metabolism of (R,R)- and (S,S)-formoterol. Microsomes containing expressed human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 or UGT2B15, using baculovirus as a vector were obtained to determine which UDP-glucuronoyl transferase(s) were involved in the conjugation of (R,R)- or (S,S)-formoterol and their metabolites. Metabolites were separated and identified using a combination of HPLC with radioactivity detection, LC/MS, NMR, and LC/MS/MS.

Results:

Pooled human liver microsomes:

Incubation of [³H]-(R,R)- or [³H]-(S,S)-formoterol with human liver microsomes pooled from 6 donors yielded one major metabolite, designated MF-1 (R,R- or S,S-O-desmethyl formoterol). Kinetic analysis using a single enzyme model of the Michaelis-Menten equation calculated a V_{max} of 25.48 pmoles/min/mg protein and a K_m of 7.79 μ M for MF-1 formation from (R,R)-formoterol. A V_{max} of 76.91 pmoles/min/mg protein and a K_m of 41.44 μ M for MF-1 formation from (S,S)-formoterol were determined.

Liver microsomes from 16 individual donors:

Incubation of [³H]-(R,R)- or [³H]-(S,S)-formoterol with liver microsomes from 16 individual donors found statistically significant correlations for MF-1 formation from [³H]-(R,R)-formoterol and CYP2D6 ($r = 0.962$, $\rho = <0.0001$) or [³H]-(S,S)-formoterol and CYP2D6 ($r = 0.780$, $\rho = 0.0004$).

Chemical inhibitors:

The formation of MF-1 from [³H]-(R,R)-formoterol by pooled human liver microsomes was inhibited to the greatest extent by quinidine, tranylcypromine and 8-methoxypsoralen. These results implicated CYP2D6 and CYP2C19.

The formation of MF-1 from [³H]-(S,S)-formoterol by pooled human liver microsomes was inhibited to the greatest extent by tranylcypromine and 8-methoxypsoralen, with

quinidine inhibiting MF-1 formation to a lesser extent. These results implicated CYP2D6 and CYP2C19.

Human CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, AND CYP2D6

After a 30-min incubation with [³H]-(R,R)- or [³H]-(S,S)-formoterol, only CYP2C19 and CYP2D6 had formed significant quantities of MF-1.

b(4)

Incubation with Microsomes and UDP-Glucuronyl Transferase:

Incubation of pooled human liver microsomes with UDPGA and [³H]-(R,R)- or [³H]-(S,S)-formoterol produced a metabolite, MF-3 (phenolic glucuronide), that was susceptible to a mixed β -glucuronidase/ aryl sulphatase preparation. MF-3 was determined to be the glucuronide of O-desmethyl formoterol. However, when [³H]-(R,R)- or [³H]-(S,S)-formoterol were incubated with UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15 and UDPGA, there was no evidence for the metabolism of either compound.

b(4)

UGT Reaction Phenotyping of [³H]-(R,R)-Formoterol and [³H]-(S,S)-Formoterol (Sepracor Document No. 090-575A1).

Methods: The in vitro metabolism of [³H]-(R,R)-formoterol and [³H]-(S,S)-formoterol was assessed to determine the principle uridine 5'-phosphoglucuronosyltransferase (UGT) enzyme(s) responsible for the formation of glucuronide conjugates. Techniques to identify the major UGTs included incubations with cDNA-expressed human UGTs, UGT-specific chemical inhibitors, and pooled human liver microsomes.

Results:

Incubation of [³H]-(R,R)-formoterol or [³H]-(S,S)-formoterol with human liver microsomes led to the formation of two glucuronide metabolites, designated as M1 (phenolic glucuronide) and M2 (benzylic glucuronide). Desformyl formoterol, a degradant of formoterol, was also identified in incubations. For (R,R)-formoterol, the kinetics of M1 formation demonstrated K_m and V_{max} values of 1410 μ M and 788 pmol/min/mg protein, respectively. For (S,S)-formoterol, the kinetics of M1 formation demonstrated K_m and V_{max} values of 864 μ M and 2216 pmol/min/mg protein, respectively.

The major metabolite (M1) of [³H]-(R,R)-formoterol was predominantly produced by UGT2B17, followed by 1A9, 1A7, 1A1 and 2B7. UGT1A9 and 2B7 were the major contributors of M2 formation from (R,R)-formoterol. Metabolite M1 from [³H]-(S,S)-formoterol was formed mainly by UGT1A1, 1A3, 1A7, 1A9, 2B7 and 2B17. Four UGTs catalyzed the formation of M2 from (S,S)-formoterol. The amount of M1 formed from [³H]-(S,S)-formoterol was highest with UGT1A1 and 2B17 in addition to variable amounts from six other UGTs.

To further characterize the contribution of UGT1A1 involvement in the metabolism of [³H]-(R,R)-formoterol and [³H]-(S,S)-formoterol, the specific chemical inhibitor bilirubin, was used. The results demonstrate that bilirubin was able to inhibit the formation of M1

and M2 from [^3H]-(*R,R*)-formoterol and [^3H]-(*S,S*)-formoterol by approximately 30% at a substrate concentration.

For the three UGTs evaluated kinetically, UGT2B17 was found to have the highest intrinsic clearance with respect to M1, the primary glucuronide metabolite for both (*R,R*)- and (*S,S*)-formoterol.

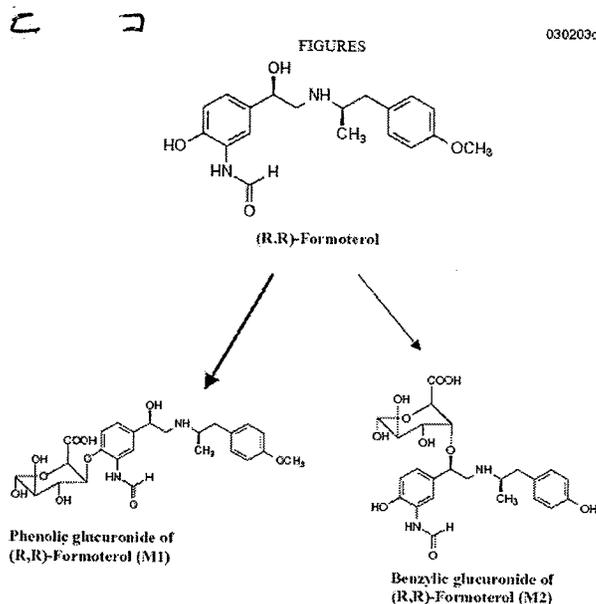


Fig. 1. Glucuronide Metabolites of Formoterol

(Only the (*R,R*)-formoterol enantiomer is shown)

In Vivo

Comparative Metabolism of [^3H]-(*R,R*)-Formoterol in Male Mice, Rats, and Dogs (Sepracor Document No. 090-530A1).

Methods: Metabolism of [^3H]-(*R,R*)-formoterol was examined in male mice, rats, and dogs. Mice received an intravenous dose of 1 mg/kg or oral doses of 1 and 10 mg/kg. Rats received an intravenous dose of 10 mg/kg or oral doses of 1, 10, and 100 mg/kg. Dogs received an intravenous dose of 3 $\mu\text{g}/\text{kg}$ and oral doses of 30 and 300 $\mu\text{g}/\text{kg}$. Blood samples, urine, and feces were collected up to 168 hr postdose. Bile samples were collected from rats up to 48 hr postdose. Plasma, urine, and bile samples were treated with β -glucuronidase/sulphatase. Metabolites in plasma, urine, bile, and feces were separated and identified through a combination of HPLC with a radioactivity detector, LC/MS, and LC/MS/MS.

Results: After examination of radiochromatograms, 10 regions of interest (designated M1- M10) were located. Regions M4, M8 and M10 had retention times corresponding to

(R,R)-O-desmethyl formoterol, (R,R)-desformyl formoterol, and (R,R)-formoterol, respectively. Across species, dose routes/levels, and biological matrices, the main regions of interest containing radioactivity were M6 (glucuronide of (R,R)-formoterol) and M10 ((R,R)-formoterol), and to a lesser extent, M2, M4 and M8. Generally after treatment with mixed β -glucuronidase/sulphatase, the radioactivity in region M6 was reduced and to lesser extents, M1, M2 and M3, while radioactivity was increased in region M10 and to a lesser extent M8. The table below contains two designations for M4 given that these two compounds could not be separated.

Identification of the major HPLC metabolite profiling regions of interest

Analyte	LC-MS retention time (minutes)	Corresponding HPLC region of interest
(R,R)-Formoterol (1)	35 – 39	M10
(R,R)-Desformylformoterol (2)	30 – 33	M8
(R,R)-O-Desmethylformoterol (3)	17 – 18	M4
Glucuronide of (1)	21 – 22	M6
Glucuronide of (2)	17 – 18	M4
Glucuronide of (3)	4 – 8	M2/M3

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Dog Urine: After intravenous dosing, radioactivity in regions M6 (glucuronide of (R,R)-formoterol) and M10 ((R,R)-formoterol) dominated profiles, but radioactivity was also present in regions M2, M5, M8 ((R,R)-desformyl formoterol) and M9 in smaller quantities. After enzyme treatment, almost all radioactivity was associated with M10 ((R,R)-formoterol). Similar results were obtained with oral dosing. Unlike the urine of mice or rats, dog urine contained little evidence of (R,R)-O-desmethyl formoterol or its glucuronide conjugate.

Dog Plasma: After intravenous dosing, most radioactivity was initially in region M10 ((R,R)-formoterol), with a small amount of radioactivity in region M6 (glucuronide of (R,R)-formoterol) and smaller amounts in regions M4 and M8 ((R,R)-desformoterol). By 15 to 30 min after dosing, radioactivity in regions M6 (glucuronide of (R,R)-formoterol) and M10 was approximately equal. By 1-2 hours, most radioactivity was in region M6 with lower amounts in regions M4, M8, and M10. By 8-12 hr, radioactivity in regions M6 and M10 were approximately equal. After treatment with β -glucuronidase/suphatase, most radioactivity was associated with region M10, with smaller amounts in regions M4, M6 and M8. Similar results were obtained after oral dosing.

Dog Feces: All feces extract profiles were similar with radioactivity in region M10 ((R,R)-formoterol) predominating, with relatively small amounts of radioactivity in regions M4 ((R,R)-O-desmethyl formoterol) and M8 ((R,R)-desformoterol).

Rat Urine: Initially after intravenous dosing, most radioactivity was associated with region M10 ((R,R)-formoterol). There was some radioactivity in regions M4 and M6 (glucuronide of (R,R)-formoterol) and relatively small amounts in regions M2 (glucuronide of (R,R)-O-desmethyl formoterol), M3 and M8 ((R,R)-desformoterol). During 6-24 hours and continuing to 48 hr, region M6 contained most radioactivity, with lower levels in regions M2, M4, M10, M3, and M8. After treatment with β -

glucuronidase/suphatase, most radioactivity was in region M10 ((R,R)-formoterol), with some in regions M4 and M8. Similar results were obtained with oral administration.

Rat Plasma: Up to 2 hr after intravenous administration, most radioactivity was observed in regions M6 (glucuronide of (R,R)-formoterol) and M10 ((R,R)-formoterol), with smaller levels in regions M2 (glucuronide of (R,R)-O-desmethyl formoterol), M4, and M8 ((R,R)-desformoterol). From 4 to 12 hr after dosing, most radioactivity was observed in region M6, with smaller amounts of radioactivity in regions M2, M4, M8 and M10. After treatment with β -glucuronidase/sulphatase, levels of radioactivity present in region M10 increased. The levels of radioactivity in regions M4 and M8 were also increased, while levels in regions M2 and M6 were reduced. Similar results were obtained with oral dosing.

Rat Feces: All feces extract profiles were similar with radioactivity in region M10 ((R,R)-formoterol) predominating, with a relatively small amount of radioactivity in region M4 ((R,R)-O-desmethyl formoterol). Glucuronides were not present and presumably deconjugated in the intestines by endogenous bacteria.

Rat Bile: After oral administration, most radioactivity was in region M6 (glucuronide of (R,R)-formoterol) with lower levels in regions M1, M4, and M10 ((R,R)-formoterol).

Mouse Urine: After intravenous administration, most radioactivity was associated with regions M6 (glucuronide of (R,R)-formoterol) and M10 ((R,R)-formoterol), and lower levels in regions M2 (glucuronide of (R,R)-O-desmethyl formoterol), M4, M3, and M8 ((R,R)-desformoterol). After treatment with β -glucuronidase/sulphatase, region M10 contained most radioactivity, although the level of radioactivity in region M4 increased. Similar results were obtained with oral dosing.

Mouse Plasma: After intravenous administration, most radioactivity was in region M6 (glucuronide of (R,R)-formoterol), with smaller amounts in regions M2 (glucuronide of (R,R)-O-desmethyl formoterol), M4 and M10 ((R,R)-formoterol). After treatment with β -glucuronidase/suphatase, the level of radioactivity present in region M10 increased. The level of radioactivity in region M4 also increased. Similar profiles were obtained with oral dosing.

Mouse Feces: All mouse fecal extract profiles were similar with most radioactivity in region M10 ((R,R)-formoterol) and a smaller level of radioactivity in region M4 (R,R)-O-desmethyl formoterol). Glucuronides were not present and presumably deconjugated in the intestines by endogenous bacteria.

Mouse Bile: After intravenous or oral dosing, most radioactivity was in region M6 (glucuronide of (R,R)-formoterol) with lower levels in regions M2 (glucuronide of (R,R)-O-desmethyl formoterol) and M10 ((R,R)-formoterol). After treatment with β -glucuronidase/suphatase, the levels of radioactivity in region M10 ((R,R)-formoterol) and M4 increased.

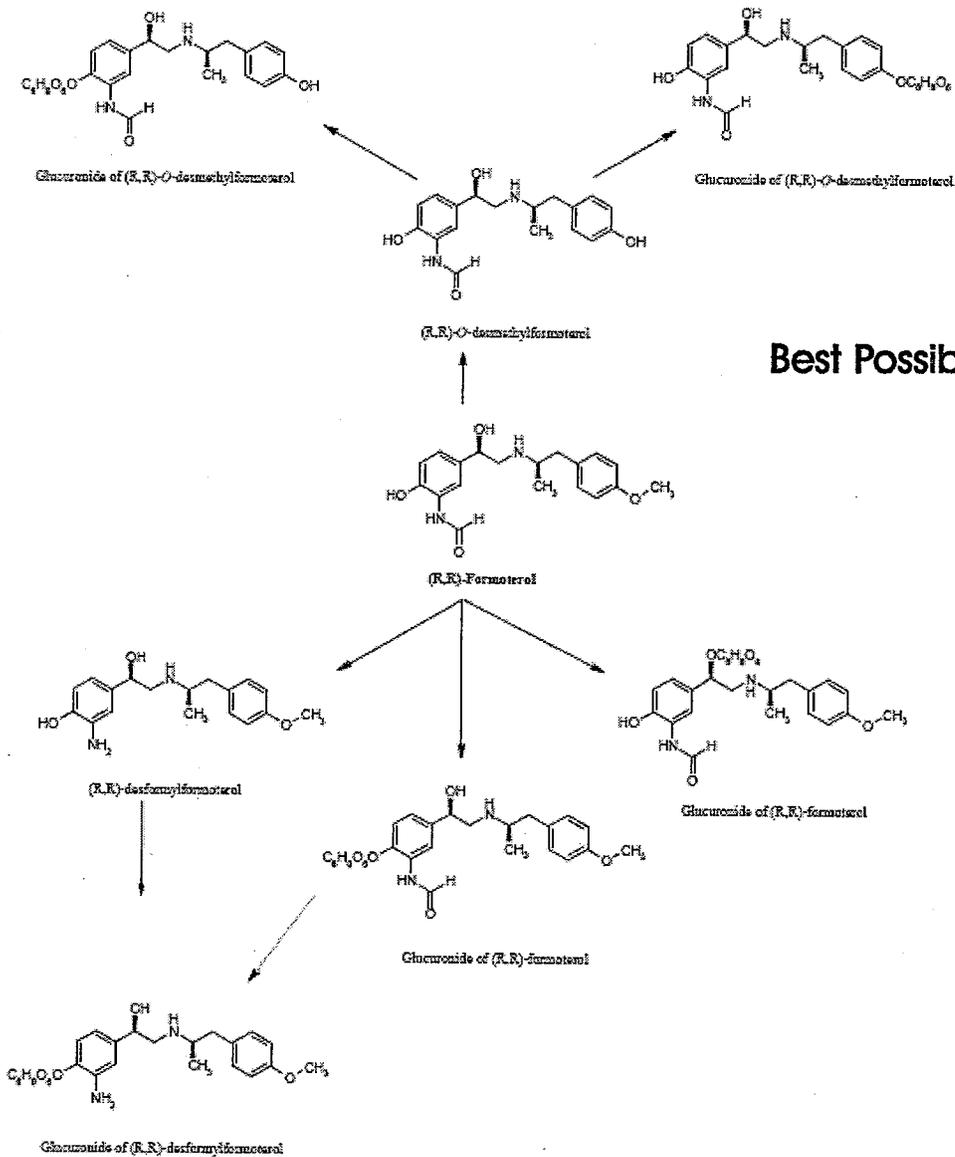
Analytes present in mass spectrometry analysis of selected urine and bile samples from dog, rat and mouse

Analytes	Retention time (minutes)	Dog		Rat		Mouse	
		Urine	Urine	Urine	Bile	Urine	Urine
		Oral	Oral	Oral	Oral	Intravenous	Oral
		30 µg/kg 0 - 6 hours	300 µg/kg 0 - 6 hours	100 mg/kg 0 - 6 hours	10 mg/kg 0 - 3 hours	1 mg/kg 6 - 24 hours	10 mg/kg 0 - 6 hours
(R,R)-Formoterol (1)	35 - 39	Y	Y	Y	Y	Y	Y
(R,R)-Desformylformoterol (2)	30 - 33		Y	Y			
(R,R)-O-Desmethylformoterol (3)	17 - 18			Y			Y
Glucuronide of (1)	21 - 22	Y		Y	Y	Y	Y
Glucuronide of (2)	17 - 18			Y		Y	Y
Glucuronides* of (3)	4 - 8			Y			Y

Y Denotes presence of the compound in the mass spectrum
 * This region includes two phenolic glucuronides of (R,R)-O-desmethylformoterol

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Proposed biotransformation pathways of [³H]-(R,R)-formoterol



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2.6.4.6 Excretion

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

Mice

[³H]-(R,R)-Formoterol: Absorption, Distribution, and Excretion Studies of [³H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Mice (Sepracor Document No. 090-528).

Methods: Absorption, distribution, metabolism, and excretion of [³H]-(R,R)-formoterol were examined in male CD-1 mice following an intravenous dose of 1 mg/kg or oral doses of 1 and 10 mg/kg. Blood and plasma (at time points up to 168 hours postdose), selected tissues and organs (at 2, 24 and 48 hours postdose), and urine, feces and respired air (up to 168 hours post-dose) were collected and retained for radioactivity measurement.

Results: Radioactivity was primarily excreted in urine (45-50% dose) with lesser amounts in the feces (32 -43% dose).

Mean recovery of radioactivity from male mice up to 168 hr following intravenous or oral administration of [³H]-(R,R)-formoterol

Treatment group	Urine	Feces	Cage wash	Expired air	Carcass	Total
1 mg/kg Intravenous	45.41	32.00	9.05	2.47	0.67	89.60
1 mg/kg Oral	47.39	38.19	5.09	0.92	0.59	92.18
10 mg/kg Oral	49.47	42.90	4.29	0.87	0.50	98.03

Rats

Absorption, Distribution, and Excretion Studies of [³H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Rats (Sepracor Document number 090-529).

Methods: The absorption, distribution, and excretion of [³H]-(R,R)-formoterol and radiolabeled metabolites were examined in male Sprague-Dawley rats. [³H]-(R,R)-formoterol was administered at an intravenous dose of 10 mg/kg or oral doses of 1, 10, and 100 mg/kg. Whole-blood and plasma (at time points up to 168 hr postdose), selected tissues and organs (at 2, 24, and 48 hr postdose), and urine, feces, cage wash, and respired air (up to 168 hr postdose) were collected for radioactivity measurement. An additional 48-hr excretion balance study was conducted with bile duct-cannulated rats that received [³H]-(R,R)-formoterol at an intragastric dose of 1 mg/kg.

Results: Following intravenous or oral administration of [³H]-(R,R)-formoterol, radioactivity was primarily excreted in the feces. Biliary excretion was determined to be a major route of excretion.

Mean recovery of radioactivity from male rats up to 168 hr following intravenous or oral administration of [³H]-(R,R)-formoterol

Treatment group	Urine	Feces	Cage wash	Expired air	Carcass	Total
10 mg/kg Intravenous	33.10	51.16	0.94	1.16	1.59	87.95
1 mg/kg Oral	24.62	67.89	0.48	0.24	0.37	93.60
10 mg/kg Oral	47.99	59.88	1.23	0.90	1.60	111.59
100 mg/kg Oral	25.51	66.31	0.88	1.82	2.45	96.95

Mean recovery of radioactivity from bile duct-cannulated male rats up to 48 hr following intragastric administration of [³H]-(R,R)-formoterol

Treatment group	Urine	Feces	Cage wash	Bile	Carcass	Total
10 mg/kg Intravenous	24.89	12.63	0.58	53.3	3.00	94.40

Dogs

Absorption and Excretion Studies of [³H]-(R,R)-Formoterol after Single Intravenous and Oral Doses to Male Dogs (Sepracor Document No. 090-527).

Methods: The absorption and excretion of [³H]-formoterol and radiolabeled metabolites were examined in 3 male beagle dogs following an intravenous dose of 3 µg/kg and oral doses of 30 and 300 µg/kg. There was a 7-week washout between treatments. Blood samples, urine, and feces were collected up to 168 hr postdose. Cage washes were collected at 24 hr intervals. Blood, plasma, urine, feces, and cage washed were analyzed for radioactivity concentrations.

Results: Radioactivity was primarily eliminated in the urine

Mean recovery of radioactivity from male dogs following intravenous and oral administration of [³H]-(R,R)-Formoterol.

Treatment Group	Urine	Feces	Cage wash	Total
3 µg/kg IV	39.16	24.89	1.53	65.58
30 µg/kg Oral	37.39	29.76	1.43	70.28
300 µg/kg Oral	46.13	22.51	1.50	74.68

2.6.4.7 Pharmacokinetic drug interactions

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

2.6.4.8 Other Pharmacokinetic Studies

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

2.6.4.9 Discussion and Conclusions

The following information regarding the pharmacokinetic and toxicokinetic properties of (R,R)-formoterol should be conveyed in the product labeling.

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2.6.4.10 Tables and figures to include comparative TK summary

Species comparison of pharmacokinetic/toxicokinetic parameters of (R,R)-formoterol

Parameter	Mouse	Rat	Dog	Human
t _{1/2} for (R,R)-formoterol, hr	2.3 (IV) 4.2-15.9 (Oral)	29.7 (IV) 2.9-83.3 (Oral)	2.9 (IV) 6.3-10.3 (Oral)	25.6 (Inhalation)
V _d , L/kg	0.751 (IV) 15-39 (Oral)	11.5-217	5.6-6.9	
CL, mL/min/kg	3.697 (IV) 28-43 (Oral)	84.4	27.7	
Oral bioavailability of (R,R)-formoterol, %	9-13%	4.9-9.1	54-60	
Oral bioavailability of (R,R)-formoterol-related radioactivity, %	48-98	28.8-123.3	57-114	

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not provided by the applicant.

2.6.6 TOXICOLOGY**2.6.6.1 Overall toxicology summary****General toxicology:****Acute toxicity studies with (R,R)-formoterol in mice, rats, and dogs:**

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 high dose group and unexposed control groups that were sacrificed two days after exposure. Cardiomyopathy, characterized by small aggregates of mononuclear inflammatory cells in foci of myofiber degeneration (vacuolated and/or hyalinized sarcoplasm) in the inner third of the left ventricle was observed for mice treated with (R,R)-formoterol at the high dose.

In an acute toxicity study, rats received an oral dose of either (R,R)-, (S,S)-, or R,R/S,S-formoterol at 1000 mg/kg. The minimum lethal oral dose of formoterol was >1000 mg/kg in rats.

In an acute toxicity study, rats received an intravenous dose of (R,R)-formoterol at 10, 50, 75, or 100 mg/kg or (S,S)-formoterol at 10, 50, or 75 mg/kg. Incidences of death were 6 of 6 for 100 mg/kg (R,R)-formoterol, 4 of 6 for 50 mg/kg (S,S)-formoterol, and 5 of 6 for 75 mg/kg (S,S)-formoterol. (S,S)-formoterol appeared to be more toxic than (R,R)-formoterol.

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

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

In an acute intravenous toxicity study, beagle dogs received either (R,R)-formoterol at 3 µg/kg or R,R/S,S-formoterol at 6 µg/kg. There were no deaths or clinical signs of toxicity.

Repeat dose toxicity studies with (R,R)-Formoterol:

Mice

In a 28-day nose-only inhalation toxicity study with (R,R)-formoterol in mice, total doses were 0, 100, 400, and 800 µg/kg/day. Deposited doses were estimated to be 0, 10, 40, and 80 µg/kg/day, respectively. Body weight gains were increased in all male and female treatment groups. Target organs of toxicity were the spleen, kidneys, and skin. Incidences of extramedullary hematopoiesis were increased in all male and female treatment groups. Incidences of basophilic tubules in the kidneys were increased in all male treatment groups. Incidences of lymphoid interstitial infiltrate in the kidneys were increased in all male treatment groups. The incidence of chronic active inflammation of the skin was increased for all male treatment groups; however, high incidences of this finding were observed in female control and treatment groups. A NOAEL was not identified in this study based upon histopathological findings in the spleen, kidneys, and skin.

In a 28-day oral toxicology study with mice, (R,R)-formoterol was administered at doses of 5, 15, 50, and 150 mg/kg/day. Target organs of toxicity were the heart, salivary gland, thymus, liver, and kidneys. Minimal to mild cardiomyopathy consisting of focal or multifocal lesions sequestered around the papillary muscles in the left ventricle was observed in males at 150 mg/kg/day and females at 50 and 150 mg/kg/day. Minimal to severe generalized hypertrophy of serous acinar cells in the mandibular and parotid salivary glands was observed at doses of 50 and 150 mg/kg/day. Thymic atrophy (i.e., decreased thickness of thymic cortex) was observed for males at 15, 50, and 150 mg/kg/day. Minimal to moderate glycogen deposition in the liver was observed at higher

incidences in treatment groups. This appeared to correlate with increased liver weights for females. A higher incidence of minimal to mild basophilic cortical tubules was in the kidneys was observed in all male treatment groups.

Rats

In a 28-day inhalation toxicity study with (R,R)-formoterol in rats, total doses were 104, 424, and 784 µg/kg/day. Using a deposition factor of 0.09, deposited doses were calculated to be 9.4, 38.2, and 70.6 µg/kg/day, respectively. The NOAEL was identified as 38.2 µg/kg/day. Increases in heart rate were evident for all treatment groups during the daily exposure period; however, there was no evidence of a dose-response relationship. Target organs of toxicity were heart, kidneys, lungs, nasal cavity, testes, and epididymides. The incidence of cardiomyopathy, characterized by multifocal myofiber degeneration and infiltration of mononuclear cells, was increased for male rats at in the high dose group. The incidence of lymphoid infiltration in the kidneys was increased for females in the high dose group. The incidence of vascular mineralization in the lung was increased for males and females combined in the high dose group. Incidences of non-suppurative inflammation in the nasal cavity level 2 were increased for all treatment groups, although this finding might be considered monitorable. Incidence of degeneration in the testes and intratubular cell debris in the epididymides were increased for males in the high dose group. These findings in the heart, kidneys, lungs, nasal cavity, testes, and epididymides were not confirmed in the 6-month inhalation toxicology study with rats that received (R,R)-formoterol at deposited doses up to 77 µg/kg/day.

In a 6-month inhalation toxicology study, 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 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. Thymic hemorrhage was a sporadic finding related to the euthanasia or tissue collection processes and not a direct test article-related effect.

In a 13-week nose-only inhalation toxicology study, rats were exposed to (R,R)-desformoterol, a degradant of (R,R)-formoterol, at target inhaled doses of 3.1, 8.6, and 26.9 µg/kg/day. Using a deposition factor of 0.1, pulmonary deposited doses were calculated to be 0.3, 0.86, and 2.7 µg/kg/day, respectively. Histopathological findings were observed in the cecum, heart, adrenal gland, and prostate. Increased incidences of minimal to slight inflammation with resulting secondary and reactive hyperplasia were observed in the cecal mucosa for all male and female treatment groups. The finding was described as follows: lamina propria was expanded by increased numbers of inflammatory cells, primarily mononuclear, with intestinal crypts exhibiting greater basophilia and cellularity and an increased mitotic activity. The incidence and severity of this finding displayed no dose-response relationship. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal

limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696). The incidence of mononuclear infiltration in the heart was increased for male and female rats in the high dose group; however, lower dose groups were not examined. Incidence of cortical vacuolation in the adrenal glands and inflammation in the prostate were increased for males in the high dose group as compared to the concurrent control. It is noted that these findings were not test article-specific and incidences were relatively high in the concurrent control group. There was no evidence of local toxicity in the respiratory tract tissues. A NOAEL was not established in the present study based upon an increased incidence of mononuclear cell infiltration in the heart for male and female rats in the high dose group. There was no histopathological examination of lower dose groups. There were no such findings in the heart from a second 13-week inhalation toxicology study with rats (Study 090-844) that received (R,R)-desformoterol at total inhaled doses of 1.02 and 3.65 µg/kg/day.

In a 13-week inhalation toxicology study with rats, (R,R)-desformoterol, a degradant of arformoterol, was administered at total inhaled doses of 1.02 and 3.65 µg/kg/day. Deposited doses were estimated to be 0.1 and 0.37 µg/kg/day, respectively. Histopathological findings of potential significance were observed in the cecum, colon, jejunum, and lung. In the cecum, incidences of minimal hemorrhage were increased for both treatment groups. The relationship of this finding to treatment was unclear given that there were no similar observations in study 090-840. Inflammation with mucosal hyperplasia was observed in all groups with the highest incidence observed in the control group. Hemorrhage was observed in the colon, jejunum, and lung. In the lung and colon, incidences of hemorrhage were increased in the high dose group. In the jejunum, incidences of hemorrhage were increased in both the low and high dose groups; however, a dose-response relationship was not observed. The relationships of these findings to treatment were unclear given that there were no similar observations from study 090-840. Consideration of the present study along with results of study 090-840 where (R,R)-desformoterol was administered at total inhaled doses up to 30 µg/kg/day suggests that findings in the cecum, colon, jejunum, and lung were unrelated to treatment and the NOAEL could be identified as 0.37 µg/kg/day. The deposited dose of 0.37 µg/kg/day for the high dose group provides a \square (R,R)-desformoterol \square found in the clinical dose of arformoterol. b(4)

Dogs

In a 14-day inhalation range-finding study, dogs (2/sex/group) received (R,R)-formoterol at doses of 0, 70, 100, and 200 µg/kg/day using a mask-type nose-only apparatus. Using a deposition factor of 0.20, deposited doses were estimated to be 0, 14, 20, and 40 µg/kg/day, respectively. During the 14-day exposure period, the exercise program was suspended due to time constraints resulting from the exposure regimen. In addition, formoterol, a β_2 -adrenergic agonist, is known to produce large increases of heart rate as well as arrhythmias. Continued exercise periods might have compromised the health of study animals. Clinical signs included body flushed and reddened ears for all male and female treatment groups. Tremors were observed for male and female dogs in the 200 µg/kg/day group. Slight losses of body weight were observed for male dogs in the 100 and 200 µg/kg/day groups. Food consumption was reduced for male

treatment groups. White blood cell counts were decreased for all male and female treatment groups. Phosphorus levels were decreased for females in the 100 and 200 $\mu\text{g}/\text{kg}/\text{day}$ groups. Triglyceride levels were decreased for males in the 100 $\mu\text{g}/\text{kg}/\text{day}$ group and males and females in the 200 $\mu\text{g}/\text{kg}/\text{day}$ group. Increased heart rate was observed during and following exposure in all male and female treatment groups. Sinus tachycardia was observed at 2- or 4-hr postdose. Ectopic ventricular activity was generally observed at 24-hr postdose. Paroxysmal ventricular escape rhythms with or without paroxysmal ventricular tachycardia was observed for one male in the 70 $\mu\text{g}/\text{kg}/\text{day}$ group and one male and one female each in the 100 and 200 $\mu\text{g}/\text{kg}/\text{day}$ groups. In addition, one male in the 100 $\mu\text{g}/\text{kg}/\text{day}$ group has ventricular escape beats. Relative heart weights were increased for male treatment groups. The target of toxicity was the heart. Degeneration of the myocardium was observed in all treatment groups. Incidences of mineralization of the myocardium were increased for males in the 70 and 200 $\mu\text{g}/\text{kg}/\text{day}$. A NOAEL was not identified based upon histopathological findings and ectopic activity in the heart at all doses.

In a 28-day inhalation toxicity study with (R,R)-formoterol in dogs, total doses were 5.8, 21.8, and 41.4 $\mu\text{g}/\text{kg}/\text{day}$. Using a deposition factor of 0.17, deposited doses were calculated to be 1, 3.7, and 7 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The NOAEL was identified as 3.7 $\mu\text{g}/\text{kg}/\text{day}$. Heart rate was increased in male and female treatment groups during the exposure period and at 2- and 4-hr post exposure, although, there was no evidence of dose-response relationships. Histopathological changes observed for male dogs at the high dose included dilatation of the medullary renal tubules in the kidneys, cytoplasmic vacuolation of the periportal region in the liver, non-suppurative inflammation in the nasal cavity (Level 2), and vacuolation of the germinal epithelium in the testes. There were no significant histopathological findings in female treatment groups. These histopathological findings were not confirmed in the 13-week or 9-month inhalation toxicology studies with dogs that received (R,R)-formoterol at comparable and higher doses.

In 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 $\mu\text{g}/\text{kg}/\text{day}$. Using a deposition factor of 0.20, deposited doses were calculated as 0, 1, 8, and 14/20 $\mu\text{g}/\text{kg}/\text{day}$, 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 $\mu\text{g}/\text{kg}/\text{day}$ 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 (i.e., flushing of the body surface and facial area and reddening of the ears and gums). 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. Sinus tachycardia was observed following the first exposure to (R,R)-formoterol at all dose levels at 2- and 4-hr post-exposure. Electrocardiographic abnormalities consisting of ventricular ectopic patterns were also observed following the first exposure at all dose levels at 4 and 24 hr after dosing. These ectopic patterns (i.e., ventricular escape beat, ventricular escape rhythm, and ventricular premature beat) were clearly treatment-related as they could be attributed to known pharmacological effects of β_2 -adrenergic agonists, although, dose response relationships were not evident. These ECG abnormalities could be characterized as transient as they were not evident during weeks 3 and 12; however, this is unclear given that in the 9-month study, ectopic changes were observed late in the study. Histopathological examination of the heart revealed no evidence of treatment-related myocardial injury. These ventricular ectopic patterns appeared to be tolerated, although, they were clearly undesirable effects, which could lead to potentially serious adverse events. Animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm.

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

Pretest and post-exposure electrocardiograms recorded for dogs in the 14-day, 13-week, and 9-month inhalation toxicology studies were re-evaluated for the presence of cardiac rhythm changes by two veterinary cardiologists. Sinus tachycardia was a common findings in dogs treated with (R,R)-formoterol at doses ≥ 5 $\mu\text{g}/\text{kg}/\text{day}$. Various types of ECG abnormalities (i.e., ectopic findings) were also observed in dogs treated with (R,R)-formoterol at doses ≥ 5 $\mu\text{g}/\text{kg}/\text{day}$. The applicant noted that NOAEL could not be identified for ectopic activity with particular regard to the 13-week and 9-month inhalation toxicology studies; however, they have asserted that an exposure threshold for ectopic findings can be identified within the low dose groups that received 5 $\mu\text{g}/\text{kg}/\text{day}$. Using C_{max} or AUC data from the study week 0 toxicokinetic evaluations from the 13-week and 9-month studies, the five 5 $\mu\text{g}/\text{kg}/\text{day}$ group animals with ectopic findings (i.e., Male #6845, Male #6848, and Female #6864 in the 13-week study and Female #6915 and Female #6924 in the 9-month study) had C_{max} values >250 pg/mL and AUC values >2000 $\text{pg}\cdot\text{hr}/\text{mL}$. The applicant's designation of a threshold for ectopic activity within the 5 $\mu\text{g}/\text{kg}/\text{day}$ groups of the 13-week and 9-month inhalation toxicology studies appears potentially arbitrary and inappropriate. All animals were considered at risk for ectopic activity. As noted in original study reviews, ECG monitoring periods were short and could have missed ectopic activity. Further, animal exercise periods were discontinued throughout the treatment period due to concerns regarding drug-induced changes of heart rate and rhythm. AUC and C_{max} data is known to vary from day to day (or measurement to measurement), which could significantly shift the designated threshold. Exposure margins between animals with and without ectopic activity were small. All dogs were young with no pre-existing cardiac problems, which minimized potential adverse effects of ectopic activity. The designation of threshold may have little value for the COPD patient population, which generally have pre-existing cardiac problems.

In a 13-week inhalation toxicology study, dogs were exposed to 40 $\mu\text{g}/\text{kg}/\text{day}$ (R,R)-formoterol in the presence or absence of (R,R)-desformoterol. The primary objective of this study was to qualify the degradant, (R,R)-desformoterol at a level in the present study. Pulmonary deposited doses for (R,R)-formoterol and (R,R)-desformoterol were 8.50-9.50 $\mu\text{g}/\text{kg}/\text{day}$ and 0.52-0.60 $\mu\text{g}/\text{kg}/\text{day}$, respectively. A concurrent negative control group received citrate-buffered isotonic saline on a comparable regimen. Additional dogs were included in treatment groups for a 4-week recovery period, although no concurrent control dogs were included. The secondary objective of this study was to assess the toxicity of potential leachables from (R,R)-formoterol unit dose vials (UDV), although specifications for leachables were not provided. A solution of (R,R)-formoterol from UDVs that had been stored for an extended time was used to prepare the test article formulation with appropriate concentrations of bulk (R,R)-formoterol and (R,R)-desformoterol. No specification of leachables was provided. Increased heart rates were observed in both the formoterol only and formoterol + desformoterol groups, although there was no evidence of ectopia as observed in the 13-week and 9-month inhalation toxicology studies with dogs. It should be noted that ECG measurements were not conducted on the first day of exposure as done in the 13-week and 9-month inhalation toxicology studies with dogs. Histopathological findings were observed in the heart, epididymides, testes, lung, and

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mesenteric lymph node, although these findings were not observed in the 13-week and 9-month inhalation toxicology studies with dogs that received (R,R)-formoterol alone at comparable and higher doses. Findings were generally comparable between the formoterol only and formoterol + desformoterol groups. Administration of desformoterol in the presence of formoterol produced no additional toxic effects. There were concerns about the design of this study given that only one dose of (R,R)-desformoterol was tested and the cardiac toxicity of (R,R)-formoterol in dogs might confound interpretation of the study. There were no apparent differences in the toxic profiles between the (R,R)-formoterol alone and (R,R)-formoterol + — (R,R)-desformoterol groups.

Genetic toxicology:

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

Carcinogenicity:

Mice received (R,R)-formoterol at oral doses of 0, 1, 5, and 25 mg/kg/day for periods up to 104 weeks. The applicant did not have ECAC concurrence for dose selection. The applicant did not contact the Division prior to early termination of groups. Survival was significantly decreased for males and females in the 25 mg/kg/day group. Thus, the maximum tolerated dose (MTD) was exceeded at 25 mg/kg/day. Due to decreased survival for males in the 25 mg/kg/day group, the applicant elected to sacrifice this group at week 77 when survival was 34%. The applicant elected to sacrifice females in the 25 mg/kg/day group during week 92 when survival was 33%. The applicant elected to sacrifice males in the 5 mg/kg/day group during week 95 when survival was 35%. Surviving females in control groups 1 and 2 and the 1 and 5 mg/kg/day groups were sacrificed during week 102 when survival was approximately 36, 39, 43, and 38%, respectively. Surviving males in control groups 1 and 2 and the 1 mg/kg/day group were sacrificed during week 104 when survival was approximately 42, 36, and 39%, respectively. A maximum tolerated dose was achieved based upon decreased survival for males and females at 25 mg/kg/day; however, surviving males in the 25 mg/kg/day group were inappropriately sacrificed up to 6 months early and the duration of treatment appeared to be insufficient. The treatment period appeared to be sufficient for females at 25 mg/kg/day (sacrificed at week 92) and males at 5 mg/kg/day (sacrificed at week 95). Histopathological examination of tissues was complete for males at 5 and 25 mg/kg/day and females at 25 mg/kg/day. The incidences of uterine endometrial stromal polyps, combined incidences of uterine endometrial stromal polyps and stromal cell sarcoma, and combined incidences of uterine and cervical endometrial stromal polyps and stromal cell sarcoma were significantly increased for female treatment groups. It is noted that tumor incidences for the high dose group were lower than the low and mid dose groups due to decreased survival and early termination of surviving animals at the high dose. Based upon tumor findings in the uterus and cervix combined, (R,R)-formoterol is tumorigenic in female mice at oral doses ≥ 1 mg/kg. Systemic exposure to (R,R)-formoterol in females at 1 mg/kg/day was 70-fold of exposure at the clinical dose of 15 μ g BID. Given that (R,R)-formoterol was not genotoxic, observed tumors most likely developed through non-genotoxic mechanisms. Treatment with β_2 -adrenergic

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agonists is known to produce tumors in the female rodent genital tract. In studies with albuterol, salmeterol, and racemic formoterol (R,R- and S,S-enantiomers), there were findings of uterine leiomyomas and/or leiomyosarcomas. (R,R)-formoterol produced tumors in the uterus and cervix as observed with other β_2 -adrenergic agonists, although the tumor type differed. Leiomyomas and/or leiomyosarcomas are tumors derived from uterine smooth muscle (myometrial muscle), whereas endometrial stromal polyps and stromal cell sarcoma are derived from the endometrial stroma. There are no safety concerns for tumor findings in mice treated with (R,R)-formoterol given that β_2 -adrenergic agonists are known to produce tumors in the female rodent genital tract.

Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 $\mu\text{g}/\text{kg}/\text{day}$ for periods up to 104 weeks. The applicant did not have ECAC concurrence for dose selection. The applicant did not contact the Division prior to early termination of groups. There was a statistically significant decrease in the survival rate for male rats in the 400 $\mu\text{g}/\text{kg}/\text{day}$ group. Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups. The applicant sacrificed all surviving males in control group 1 and the 400 $\mu\text{g}/\text{kg}/\text{day}$ during weeks 91 and 92. All surviving females in control group 1 and the 400 $\mu\text{g}/\text{kg}/\text{day}$ group were sacrificed during weeks 90 and 91, and all remaining females in the 100 $\mu\text{g}/\text{kg}/\text{day}$ group were sacrificed during week 92. The remaining females in control group 2 and the 40 and 200 $\mu\text{g}/\text{kg}/\text{day}$ groups were sacrificed during weeks 100 and 101. The males in control group 2 and the 40, 100, and 200 $\mu\text{g}/\text{kg}/\text{day}$ groups were exposed for 104 weeks. Absolute body weight was decreased for male rats in the 400 $\mu\text{g}/\text{kg}/\text{day}$. Absolute body weight for female rats in the 400 $\mu\text{g}/\text{kg}/\text{day}$ was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 $\mu\text{g}/\text{kg}/\text{day}$ group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 $\mu\text{g}/\text{kg}/\text{day}$ group suggests a maximum tolerated dose was also achieved at this dose. For the ovaries and oviducts, incidences of cyst(s) were significantly increased for female treatment groups at doses ≥ 40 $\mu\text{g}/\text{kg}/\text{day}$. For the thyroid gland, combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 $\mu\text{g}/\text{kg}/\text{day}$ groups as compared to controls. The combined incidence of c-cell adenoma and carcinoma for females at 100 and 200 $\mu\text{g}/\text{kg}/\text{day}$ exceeded mean incidences from the historical control background data of the testing laboratory. Increases at 100 and 200 $\mu\text{g}/\text{kg}/\text{day}$ were significant using trend analysis. However, only the increase at 200 $\mu\text{g}/\text{kg}/\text{day}$ was statistically significant by pairwise comparison. Based upon increased incidences of thyroid C-cell adenoma and carcinoma in female treatment groups, (R,R)-formoterol is tumorigenic in rats. Given that (R,R)-formoterol was not genotoxic, tumors developed through non-genotoxic mechanisms. Systemic exposure in rats that received 40 $\mu\text{g}/\text{kg}/\text{day}$ (R,R)-formoterol, where there were no treatment-related tumor findings, was approximately 35.9 to 55.5 times systemic exposure with a clinical dose of 15 μg BID. There are no safety concerns for tumors findings in rats treated with (R,R)-formoterol given that there is a sufficient safety margin.

Reproductive toxicology:

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 F₀ 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.

In a dose range finding 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.

In a 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 resorptions, 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.

In a 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. 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 447.8 and 368.1 times the AUC observed with the clinical dose of 15 μ g BID, respectively; however, it should be noted that 1 mg/kg/day is not a NOAEL.

In a dose range finding 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 groups 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, adactyly, was observed at 80 mg/kg/day. Adactyly was observed at an incidence that exceeded concurrent and historical controls. Post-implantation loss was increased at 80 mg/kg/day.

In a 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. Three female rabbits at 80 mg/kg/day and one female at 40 mg/kg/day aborted between days 23 and 29 of gestation. It is noted that abortion is a fairly common observation for pregnant female rabbits (range of 0 to 33.3%). Decreased defecation was observed in a dose-related manner for all treatment groups. Post-implantation loss at 80 mg/kg/day was increased when compared to the

control. This increase was attributed to higher early and late resorptions. There was a corresponding decrease in the number of viable fetuses per dam at 80 mg/kg/day. Fetal body weight at 40 and 80 mg/kg/day was decreased as compared to the control. Total malformations (i.e., external, visceral, and skeletal) at 20, 40, and 80 mg/kg/day were increased in a dose-related manner to 10(5), 11(9), and 26(12), respectively, as compared to 3 fetuses (2 litters) for the control. Treatment-related external malformations were observed with 40 and 80 mg/kg/day (R,R)-formoterol. Adactyly, syndactyly, and umbilical herniation of the intestine were observed at 80 mg/kg/day. Findings of syndactyly and umbilical herniation were limited to limited to single fetuses. Brachydactyly and short tails were observed at 40 and 80 mg/kg/day. Findings of short tail were limited to single fetuses at 40 and 80 mg/kg/day. Adactyly was observed at a dose of 80 mg/kg/day in the dose range finding study. Treatment-related visceral malformations were observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. A malpositioned right kidney was observed with 20, 40, and 80 mg/kg/day (R,R)-formoterol. Bulbous aorta was observed at 40 and 80 mg/kg/day. Lobular dysgenesis of the lungs, interventricular septal defect, cardiomegaly, and common truncus were observed at 80 mg/kg/day. Findings of cardiomegaly and common truncus were limited to single fetuses. Skeletal malformations consisted of single findings of costal cartilage anomaly, metatarsals fused, and skull bones fused. 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 skeletal variations, sternbrae 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, although oviduct cysts and dark red areas on the liver or mesenteric cyst(s) were not observed in a dose-related manner. Cysts in the liver were observed in fetuses at 40 and 80 mg/kg/day.

In a 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 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.

In a dose range finding embryofetal development study, 6 artificially-inseminated rabbits/group received either racemic formoterol at oral doses of 5, 10, 20, 40, 80, and 160 mg/kg/day or (R,R)-formoterol at oral doses of 10, 20, or 40 mg/kg/day from gestation days 7 to 20. A vehicle-control group received 0.5% carboxymethylcellulose. One female in the 160 mg/kg/day racemic formoterol group aborted on gestation day 24. Incidences of decreased defecation were increased for females receiving racemic formoterol or (R,R)-formoterol at doses ≥ 10 mg/kg/day. Decreased defecation for the 80

and 160 mg/kg/day racemic formoterol groups was attributed to decreases of food consumption. Gravid uterus weights were decreased for dams in the 160 mg/kg/day racemic formoterol group. Numbers of viable pups/dam were decreased for dams in the 160 mg/kg/day racemic formoterol group. Resorptions (early, late, and total) and post-implantation losses were increased for dams in the 160 mg/kg/day racemic formoterol group. Combined fetal weights were decreased for the 80 and 160 mg/kg/day racemic formoterol groups and the 40 mg/kg/day (R,R)-formoterol group. External examination of pups found 1 pup (1/26 = 3.8%) in the 40 mg/kg/day (R,R)-formoterol group with a cleft palate.

In an embryofetal development study, artificially-inseminated female rabbits received either 160 mg/kg/day R,R/S,S-formoterol, 80 mg/kg/day (R,R)-formoterol, or 0.5% carboxymethylcellulose from gestation days 7 to 22. The incidence of decreased defecation was increased in the 160 mg/kg/day R,R/S,S-formoterol group. Post-implantation losses were significantly increased in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. This resulted in significant decreases in numbers of viable pups in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Gravid uterus weights and fetal weights were also decreased in the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Incidences of visceral malformations, malpositioned kidney, bulbous aorta, and interventricular septal defect, were increased in both the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Incidences of external malformations, ectrodactyly and brachydactyly, were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Single incidences of spina bifida were observed in both treatment groups. Incidences of skeletal malformations, sternebrae fused, vertebral anomaly with or without associated rib anomaly, and sternebra(e) malaligned, were increased in the 160 mg/kg/day R,R/S,S-formoterol group. Sternebra(e) malaligned was observed in both treatment groups. Increased incidences of skeletal variations, sternebra(e) malaligned (slight or moderate), 13th full rib, and sternebra(e) with thread-like attachment, were increased in both the 160 mg/kg/day R,R/S,S-formoterol and 80 mg/kg/day (R,R)-formoterol groups. Skeletal variations, 27 presacral vertebrae and hyoid body and/or arch(es) unossified, were observed in the 80 mg/kg/day (R,R)-formoterol group. R,R/S,S-formoterol at 160 mg/kg/day and (R,R)-formoterol at 80 mg/kg/day were teratogenic in rabbits.

In a pre- and post-natal development study, time-mated F₀ female rats received (R,R)-formoterol at oral doses of 0, 1, 5, and 10 mg/kg/day from gestation day 6 to postnatal day 20. One female in the 10 mg/kg/day group was euthanized on gestation day 21 due to complications during parturition. This female had total litter loss. Lengths of gestation for F₀ female rats in the 1, 5, and 10 mg/kg/day groups were slightly prolonged. Survival was decreased for F₁ pups in the 5 and 10 mg/kg/day groups on postnatal days 0 and 1 and birth to postnatal day 4. Decreased fetal survival was primarily attributed to 2 females in the 5 mg/kg/day group and 1 female in the 10 mg/kg/day group with total litter loss from birth to postnatal day 4. No significant effects on fetal survival were observed at later time points. Umbilical hernia, a malformation observed in teratology studies with (R,R)-formoterol, was observed for 1 pup in the 10 mg/kg/day group.

Incidences of missing (presumed cannibalized), subcutaneous hemorrhage, pale in color, and cyanotic were increased for pups in all (R,R)-formoterol treatment groups. Absolute body weights for male and female pups in the and mid high dose groups were decreased on postnatal days 1, 14, 17, and 21 as compared to control values; however, body weight gains for F₁ male and female pups from postnatal days 0 to 21 (i.e., lactation or weaning period) were unaffected. Clinical observations of F₁ males and females during the post-weaning period found increased incidences of prominent annular rings over the entire length of the tail for the 5 and 10 mg/kg/day groups on postnatal days 35, 42, 49, and 56; however, this finding did not persist. During the post-weaning period, absolute body weights for F₁ male and female pups in the mid and high doses group were generally lower than corresponding control values; however, body weight gains for F₁ male rats from postnatal day 21 to week 22 and F₁ female rats from postnatal day 21 to week 17 were unaffected by treatment. Acquisition of balanopreputial separation appeared to be delayed for male pups receiving the high dose of 10 mg/kg/day, although all male pups from treatment groups acquired separation. Acquisition of vaginal patency appeared to be delayed for female pups receiving the high dose of 10 mg/kg/day, although all female pups from treatment groups acquire vaginal patency. Measurements of acoustic startle responses found that mean V_{max} values were increased for F₁ male pups in the 5 and 10 mg/kg/day groups on postnatal day 20 and F₁ males in the 1, 5, and 10 mg/kg/day groups on postnatal day 60. V_{max} values were increased for F₁ females in all treatment groups on postnatal days 20 and 60; however, lack of dose-response relationships, particularly on postnatal day 60, suggests that there was no treatment-related effect. Differences of swimming ability, mean times to escape from the Biel maze and mean numbers of errors committed during trials 1-10 (days 2-6 of the Biel maze procedure), and/or when probed for memory, mean time to escape and mean number of errors (day 7 of the Biel maze procedure) were observed between control and treatment groups; however, toxicological significance was unclear. Male and female F₁ rat mating and fertility indexes were unaffected by treatment. No treatment-related effects were observed for the F₂ generation fetuses. Delays of prenatal and postnatal development were potentially present for (R,R)-formoterol high dose group that received 10 mg/kg/day.

2.6.6.2 Single-dose toxicity

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

2.6.6.3 Repeat-dose toxicity

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

Rats

Study title: A 13-Week Inhalation Toxicity Study of Nebulized Aerosol Formulation of (R,R)-Desformoterol Oxalate in the Albino Rat.

Key study findings:

- In a 13-week nose-only inhalation toxicology study, rats were exposed to (R,R)-desformoterol at target inhaled doses of 3.1, 8.6, and 26.9 µg/kg/day. Using a deposition factor of 0.1, pulmonary deposited doses were calculated to be 0.3, 0.86, and 2.7 µg/kg/day, respectively.
- Histopathological findings were observed in the cecum, heart, adrenal gland, and prostate.
- Increased incidences of minimal to slight inflammation with resulting secondary and reactive hyperplasia were observed in the cecal mucosa for all male and female treatment groups. The finding was described as follows: lamina propria was expanded by increased numbers of inflammatory cells, primarily mononuclear, with intestinal crypts exhibiting greater basophilia and cellularity and an increased mitotic activity. The incidence and severity of this finding displayed no dose-response relationship. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696).
- The incidence of mononuclear infiltration in the heart was increased for male and female rats in the high dose group. Lower dose groups were not examined to establish a NOAEL for this finding. There were no such findings in a second 13-week inhalation toxicology study with rats (Study 090-844) that received (R,R)-desformoterol at total inhaled doses of 1.02 and 3.65 µg/kg/day (deposited doses of 0.1 and 0.37 µg/kg/day, respectively). The NOAEL will be established in Study 090-844.
- Incidence of cortical vacuolation in the adrenal glands and inflammation in the prostate were increased for males in the high dose group as compared to the concurrent control. It is noted that these findings were not test article-specific and incidences were relatively high in the concurrent control group. There were no such findings for the adrenal gland in a second 13-week inhalation toxicology study with rats (Study 090-844). A NOAEL for prostate inflammation was established in Study 090-844.
- There was no evidence of local toxicity in the respiratory tract tissues.

Study no.: Sepracor Document number 090-840

Conducting laboratory and location:

Date of study initiation: May 31, 2004 (Dosing started)

GLP compliance: Yes.

QA report: yes (X) no ()

Drug, lot #, and % purity: (R,R)-Desformoterol oxalate, batch/lot number MM7448 (Purity, 97.26%)

Methods

Doses: Total inhaled doses were 3.1, 8.6, and 26.9 µg/kg/day. Using a deposition factor of 0.1, pulmonary deposited doses were calculated to be 0.3, 0.86, and 2.7 µg/kg/day, respectively. Rats were exposed by nose-only inhalation 20 min per day for 91 days.

The overall theoretical achieved doses of (R,R)-desformoterol were recalculated based on an active moiety of 64.63%. The recalculated values were as follows:

Group	Sex	RMV (L/min)	Active Conc'n (µg/L)	Exposure Duration (min)	Deposition Fraction ^a	Mean Body Weight (kg)	Calculated Achieved Dose (µg/kg/day)
2	Male	0.252	0.250	20	1	0.430	2.9
	Female	0.165				0.254	3.2
							3.1 ^b
3	Male	0.258	0.694	20	1	0.442	8.1
	Female	0.160				0.245	9.1
							8.6 ^b
4	Male	0.264	2.192	20	1	0.455	25.4
	Female	0.166				0.256	28.4
							26.9 ^b

^a 100% deposition is assumed.

^b Mean of Males and Females.

Standard aluminum cylindrical flow-through nose-only inhalation chambers were utilized in this study. Each chamber was of modular design with 20 separate ports in each row into which the conical front section of a polycarbonate restraint tube was inserted. The top section of the inhalation chamber has an opening for inlet air into which the test or control articles were introduced. The bottom section of the chamber had a corresponding air extraction port.

Actual chamber concentrations of aerosol were measured at least once for all groups each day from a sampling port within the animal breathing zone using a gravimetric method. Chamber concentrations of active ingredient (of all filters collected during treatment) were determined at least twice weekly by chemical analysis of the deposit collected on the gravimetric filters.

Particle size distribution analysis was performed for each test and control article chamber at least once weekly using a cascade impactor. Filters were analyzed with a gravimetric method for Group 1. Filters were analyzed with a chemical method using a HPLC for treated Groups 2, 3, and 4 for active ingredient. Mass median aerodynamic diameters and geometric standard deviations were determined from filters collected on the cascade impactor.

Group	Target dose, µg/kg/day	MMAD (µm) ± GSD determined analytically
2	3.1	1.6 ± 2.0
3	8.6	1.8 ± 2.1

4	26.9	1.7 ± 2.1
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Nominal chamber concentrations were calculated from the airflow through the chamber and the quantities of test or control article used.

Achieved doses ($\mu\text{g}/\text{kg}/\text{day}$) were calculated with the following formula:
 (Respiratory minute volume (L/min) x Active concentration ($\mu\text{g}/\text{L}$) x Treatment time (min))/BW (kg)

Species/strain. Male and female Sprague-Dawley CD-1 (CD[®][SD] IGS BR) rats were obtained from [redacted] [redacted]

Number/sex/group or time point (main study). 15 rats/sex/group

Route, formulation, volume, and infusion rate. Vehicle and drug solutions were administered by nose-only inhalation. Control animals were exposed to an aerosol of the vehicle-control, citrate buffered saline, pH 4.5. The dose formulation was initially prepared approximately daily, and after establishing the stability (at study week 1), was formulated weekly. The control and test atmospheres were generated into the chamber air inlet using Pari LC Plus nebulizers supplied with predried compressed air. The test article was dissolved in the vehicle (target pH 4.5) and the target formulation concentrations were 0, 50, 120, and 300 $\mu\text{g}/\text{mL}$ for Groups 1, 2, 3, and 4, respectively.

Satellite groups used for toxicokinetics or recovery. None

Age. At the start of treatment, animals were 7 weeks old.

Weight. At the start of treatment, body weight ranges were 219 to 282 g for males and 148 to 199 g for females.

Unique study design or methodology (if any). In a 13-week nose-only inhalation toxicology study, rats were exposed to (R,R)-desformoterol at target inhaled doses of 0, 3.1, 8.6, and 26.9 mg/kg/day.

Observations and times:

Mortality. Animals were observed twice daily for mortality.

Clinical signs. Animals were observed twice daily for clinical signs. A detailed examination was conducted once per week.

Body weights. Body weights were measured once per week during the treatment period.

Food consumption. Food consumption was measured once per week during the treatment period.

Ophthalmoscopy. Not performed.

EKG. Not performed.

Hematology. Blood samples for measurement of hematology parameters were collected at study termination.

Clinical chemistry. Blood samples for measurement of clinical biochemistry parameters were collected at study termination.

Urinalysis. During week 13, urine samples were collected from individual animals placed in metabolism cages overnight.

Gross pathology. All animals were euthanized on the same day after 91 days of treatment. In order to minimize autolytic change, a complete gross pathology examination of the carcass was conducted immediately on all animals which were

b(4)

euthanized. The order of necropsy was as follows: control (Group 1), high dose (Group 4), mid dose (Group 3), and low dose (Group 2). Tissues and organs were collected and placed in neutral buffered 10% formalin with the exception of the epididymides, eyes, optic nerve, and testes, which were fixed in Zenker's fluid.

Organ weights: Absolute and relative weights were determined for the adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, spleen, testes, thymus, thyroid lobes and parathyroid glands.

Histopathology: Tissues were prepared for histopathological examination by embedding in paraffin wax, sectioning, and slide staining with hematoxylin and eosin. For the control and high dose groups, all tissues were examined. For the low and mid dose groups, respiratory tract tissues (lungs, larynx, pharynx, trachea, tracheobronchial lymph node, tracheal bifurcation (with mainstem bronchi) and nasal cavities and sinuses) and all gross lesions were examined. Based upon findings in the ceca from the high dose group, the ceca from low and mid dose groups were examined. A peer review pathologist examined the slides of the cecum.

Toxicokinetics: Not performed.

Results

Mortality: None.

Clinical signs: Incidences of scab on the interscapular skin were increased for male and female treatment groups. Incidences of hypersensitivity (hyperactivity) were increased for males in the mid and high dose groups. There were recurring clinical observations of muzzle staining red observed in all groups including the control, although incidences may have been slightly higher for males in the mid and high dose groups.

Clinical signs (15 rats/sex/group)

Clinical signs	Males				Females			
	0	3.1	8.6	26.9	0	3.1	8.6	26.9
Skin scab/interscapular	2	4	10	4	1	4	6	5
Hypersensitivity (hyperactivity)	1	0	2	4	0	0	2	0

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

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

Hematology: Observed differences in neutrophil, monocyte, and large unstained cell counts between control and treatment groups appeared to have little or no toxicological significance. Neutrophils were increased for male treatment groups. Monocyte counts were decreased for female treatment groups, although a dose-response relationship was not observed. Monocyte counts were slightly increased for males in the high dose group. Large unstained cell counts were increased for female treatment groups. However, large unstained cell counts were decreased for male treatment groups.

Hematology parameters

Hematology parameters	Males				Females			
	0	3.1	8.6	26.9	0	3.1	8.6	26.9
Neutrophils, %	10.05	13.87 (138%)	13.45 (134%)	13.18 (131%)				
Neutrophils, 10 ³ /μL	0.635	0.733 (115%)	0.789 (124%)	0.930 (147%)				
Monocytes %					1.64	1.35 (82%)	1.39 (85%)	1.12 (68%)
Monocytes 10 ³ /μL	0.095	0.093	0.089	0.113 (119%)	0.063	0.045 (71%)	0.055 (87%)	0.45 (71%)
Large unstained cells %					1.02	1.17 (115%)	1.16 (114%)	1.30 (128%)
Large unstained cells 10 ³ /μL	0.095	0.071 (75%)	0.082 (86%)	0.077 (81%)	0.036	0.041 (114%)	0.046 (128%)	0.051 (142%)

Clinical chemistry: Decreased triglyceride levels for male treatment groups and increased potassium levels for females in the high dose group appeared to have little or no toxicological significance. Triglyceride levels for male treatment groups were decreased to 70.7-78.5% of the control (61.5 mg/dL). Potassium levels were increased for females in the high dose group to 112.7% of the control (4.412 mmol/L).

Urinalysis: There were no treatment-related effects on urinalysis parameters.

Gross pathology: Potential treatment-related gross pathological findings were observed in the kidney, lung, mandibular lymph node, prostate, spleen, thymus, and pituitary; however, there were no correlations to histopathological findings.

Necropsy findings (15 rats/sex/group)

Organs/Tissues	Males				Females			
	0	3.1	8.6	26.9	0	3.1	8.6	26.9
Kidney -area dark	0	0	0	1	0	0	0	1
Lung -foci dark	0	0	1	1	0	0	0	0
Mandibular LN -foci dark	0	0	0	1	0	0	1	0
Prostate -area pale -mass	0 0	0 0	0 0	0 0				
Spleen -cyst	0	0	0	1	0	0	0	0
Thymus -foci dark	1	0	1	2	0	1	0	0
Pituitary -enlargement	0	0	0	0	0	0	0	1

Organ weights: There were no treatment-related changes of organ weights.

Histopathology: Histopathological findings were observed in the cecum, heart, adrenal gland, and prostate.

Increased incidences of minimal to slight inflammation with resulting secondary and reactive hyperplasia were observed in the cecal mucosa for all male and female treatment groups. The lamina propria was expanded by increased numbers of inflammatory cells, primarily mononuclear, with intestinal crypts exhibiting greater basophilia and cellularity and an increased mitotic activity. The incidence and severity of this finding displayed no dose-response relationship. The historical control incidence of this finding in male and female rats from the testing laboratory was 1.53% (range of 0 to 20% for males and 0 to 10% for females). The incidence for the con-current control was significantly higher than the historical control. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696).¹

The incidence of parasites in the colon was increased for the high dose group. Histopathological examination determined that cross sections of parasites found in the colon were morphologically compatible with pinworm nematodes. These nematodes are seen in the intestinal lumen with no specific inflammatory response. No immunosuppressive effects of formoterol or desformoterol have been observed. These findings were possibly a colony-specific finding and not attributed to treatment with desformoterol.

The incidence of mononuclear infiltration in the heart was increased for male and female rats in the high dose group. Lower dose groups were not examined to establish a NOAEL for this finding.

Incidence of cortical vacuolation in the adrenal glands and inflammation in the prostate were increased for males in the high dose group as compared to the concurrent control. It is noted that these findings were not test article-specific and incidences were relatively high in the concurrent control group.

Incidences of tubular basophilia, pyelitis/pyelonephritis, and inflammation in the kidneys were increased for rats in the high dose groups. It is noted that these are low incidence findings and appear to be consistent with age-related changes in the kidneys for Sprague-Dawley rats.

There were additional findings in several other organs with unclear relationship to treatment.

Histopathology findings in rats that received (R,R)-desformoterol at inhaled doses of 0, 3, 10, and 30 µg/kg/day

Organs/Tissues	Sex	0	3.1	8.6	26.9
Cecum -inflammation	M	1/15	10/15	8/15	10/15
	F	5/15	8/15	10/15	11/15
Colon -parasite	M	0/15	-	-	1/15
	F	0/15	-	-	5/15
Heart					

-infiltration, mononuclear cell	M	1/15	-	-	4/15
	F	0/15	-	-	2/15
Adrenal					
-vacuolation, cortical	M	4/15	-	0/1	7/15
	F	0/15	-	-	0/15
Prostate					
-inflammation	M	8/15	-	-	11/15
Kidney					
-basophilia, tubular	M	0/15	0/1	0/2	2/15
	F	0/15	-	-	2/15
-pyelitis/pyelonephritis	M	0/15	0/1	0/2	1/15
	F	1/15	-	-	2/15
-inflammation	M	0/15	0/1	0/2	1/15
	F	0/15	-	-	2/15
Lung					
-infiltration, mixed cells	M	0/15	0/15	0/15	1/15
	F	0/15	0/15	0/15	1/15
Eye					
-atrophy, retina	M	0/15	-	-	1/15
	F	0/15	-	-	1/15
Liver					
-hyperplasia, bile duct	M	0/15	-	0/2	1/15
	F	0/15	0/2	0/2	0/15
-infiltration, mononuclear cell	M	0/15	-	0/2	1/15
	F	0/15	0/2	0/2	0/15
Lacrimal gland					
-infiltration, mononuclear cell	M	0/15	-	-	1/15
	F	0/15	0/1	-	0/15
Mandibular LN					
-granuloma	M	0/15	-	-	1/15
	F	0/15	-	-	0/15
Spleen					
-cyst, capsular	M	0/15	0/1	-	1/15
	F	0/15	-	-	0/15
Trachea					
-hemorrhage	M	0/15	-	-	1/15
	F	0/15	-	-	0/15
Thyroid					
-cyst: ultimobranchial/	M	0/15	-	-	0/15
thyroglossal	F	0/15	-	-	1/15

Study title: A 13-Week Inhalation Toxicity Study of a Nebulized Aerosol Formulation of (R,R)-Desformoterol Oxalate in Albino Male Rats.

Key study findings:

- In a 13-week inhalation toxicology study with rats, (R,R)-desformoterol, a degradant of arformoterol, was administered at total inhaled doses of 1.02 and 3.65 µg/kg/day. Deposited doses were estimated to be 0.1 and 0.37 µg/kg/day, respectively.

- Histopathological findings of potential significance were observed in the cecum, colon, jejunum, and lung; however, their relationships to treatment were unclear given that there were no similar observations in study 090-840 where (R,R)-desformoterol was administered at total inhaled doses up to 30 µg/kg/day.

- In the cecum, incidences of minimal hemorrhage were increased for both treatment groups. The relationship of this finding to treatment was unclear given that there were no similar observations in study 090-840. Inflammation with mucosal hyperplasia was observed in all groups with the highest incidence observed in the control group. In study 090-840, increased incidences of minimal to slight inflammation with resulting secondary and reactive hyperplasia were observed in the cecal mucosa for all male and female treatment groups. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696).

- Hemorrhage was observed in the colon, jejunum, and lung. In the lung and colon, incidences of hemorrhage were increased in the high dose group. In the jejunum, incidences of hemorrhage were increased in both the low and high dose groups; however, a dose-response relationship was not observed. The relationships of these findings to treatment were unclear given that there were no similar observations from study 090-840.

- An examination of this study in isolation indicates that a NOAEL was not identified based upon histopathological findings in the cecum and jejunum for both treatment groups and the lung for the high dose group. Consideration of the present study along with results of study 090-840 where (R,R)-desformoterol was administered at total inhaled doses up to 30 µg/kg/day suggests that findings in the cecum, colon, jejunum, and lung were unrelated to treatment and the NOAEL could be identified as 0.37 µg/kg/day. The deposited dose of 0.37 µg/kg/day for the high dose group provides a safety margin for level of (R,R)-desformoterol found in the clinical dose of arformoterol.

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Study no.: Sepracor Document number 090-844

Conducting laboratory and location:

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Date of study initiation: September 22, 2005 (Start of dosing)

GLP compliance: Yes.

QA report: yes (X) no ()

Drug, lot #, and % purity: (R,R)-Desformoterol Oxalate, batch/lot number MM7488 (Purity, 94.2%)

Methods

Doses: Doses of (R,R)-desformoterol are shown in the table below. (R,R)-desformoterol doses were based on the free base form of drug (i.e., adjusting for the salt content, chiral form, and impurity). Control rats were exposed to sodium citrate buffered oxalate solution (pH 4.5).

Group	RMV L/min	Active Conc µg/L	Exposure Duration min	Mean BW Kg	Target · Inhaled Dose µg/kg/day	Calculated Achieved Dose µg/kg/day	Pulmonary Deposited Dose* µg/kg/day
2	0.248	0.174	10	0.4215	1	1.02	0.10
3	0.248	0.413	15	0.4205	3	3.65	0.37

* A deposition factor of 10% was used to calculate pulmonary deposited doses.

Standard aluminum cylindrical flow-through nose-only inhalation chambers were utilized in this study. Each chamber was of modular design with 20 separate ports in each row into which the conical front section of a polycarbonate restraint tube was inserted. The top section of the inhalation chamber has an opening for inlet air into which the test or control articles were introduced. The bottom section of the chamber had a corresponding air extraction port.

Actual chamber concentrations of aerosol were measured at least once for all groups each day from a sampling port within the animal breathing zone using a gravimetric method. Chamber concentrations of active ingredient (of all gravimetric filters collected on each day) during treatment were determined at least twice weekly by chemical (HPLC) analysis of the deposit collected on the gravimetric filters.

Particle size distribution analysis was performed for each test and control article chamber at least once weekly using a cascade impactor. Filters were analyzed with a gravimetric method for Group 1. Filters were analyzed with a chemical method using a HPLC for treated Groups 2 and 3 for active ingredient. Mass median aerodynamic diameters and geometric standard deviations were determined from filters collected on the cascade impactor.

Group	Target dose, µg/kg/day	MMAD (µm) ± GSD determined analytically
1	0	1.5 ± 1.9
2	1	1.8 ± 1.9
3	3	1.6 ± 1.9

Nominal chamber concentrations were calculated from the airflow through the chamber and the quantities of test or control article used.

Achieved doses (µg/kg/day) were calculated with the following formula:
 (Respiratory minute volume (L/min) x Active concentration (µg/L) x Treatment time (min))/BW (kg)

Species/strain: Male Sprague-Dawley CD \surd CD[®][SD]IGS BR] rats were obtained from \surd

Number/sex/group or time point (main study): 15 male rats/group

Route, formulation, volume, and infusion rate: \surd was administered by nose-only inhalation using a nebulized aerosol formulation. The control and test atmospheres were generated into the chamber air inlet using Pari LC Plus nebulizers supplied with predried compressed air. The concentration of the test article in the atmosphere was controlled by varying the solution concentration (target concentrations of 25 and 50 $\mu\text{g}/\text{mL}$ for Groups 2 and 3, respectively) and maintaining a constant dosing duration of 10 and 15 min, respectively.

Satellite groups used for toxicokinetics or recovery: None.

Age: At the start of treatment, rats were approximately 6 weeks old.

Weight: At the start of treatment, the body weight range was 185 to 225 g.

Unique study design or methodology (if any): In a 13-week inhalation toxicology study with rats, (R,R)-desformoterol, a degradant of arformoterol, was administered at total inhaled doses of 1.02 and 3.65 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses were estimated to be 0.1 and 0.37 $\mu\text{g}/\text{kg}/\text{day}$, respectively. This study was conducted as a follow-up to an earlier 13-week inhalation toxicology study with rats that received (R,R)-desformoterol at total inhaled doses of 0, 3, 10, and 30 $\mu\text{g}/\text{kg}/\text{day}$ (Sepracor Document number 090-844).

Observations and times:

Mortality: Animals were observed for moribundity/mortality twice per day.

Clinical signs: Animals were observed prior to exposure and approximately 1 hr following each exposure during the treatment period.

Body weights: Body weights were measured weekly during the treatment period.

Food consumption: Food consumption was measured weekly during the treatment period.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: Blood samples for determination of hematology parameters were collected at study termination.

Clinical chemistry: Blood samples for determination of clinical biochemistry parameters were collected at study termination.

Urinalysis: Urinalysis was performed during week 13. Urine samples were collected from individual animals placed in metabolism cages overnight.

Gross pathology: All animals were euthanized on the same day. In order to minimize autolytic change, a complete gross pathology examination of the carcass was conducted immediately for each animal euthanized. The order for necropsy was as follows: control (Group 1), high dose (Group 3), and low dose (Group 2). For all animals, 3 femoral bone marrow smears were prepared; however, smears were retained but not evaluated given that there were no test article-related effects in the hematology and histopathologic assessments.

Organ weights: Absolute and relative organ weights were determined for the adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, pituitary gland, prostate, spleen, testes, and thymus.

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Histopathology: All tissues for all animals were prepared for histopathological examination by embedding in paraffin wax, sectioning and slide staining with hematoxylin and eosin and examined.

Toxicokinetics: Not performed.

Results

Mortality: None.

Clinical signs: There were no treatment-related clinical signs. There were slight increased incidences of a few nonspecific clinical signs, although relationships to treatment were questionable in most cases due to lack of a dose-response.

Clinical signs, n = 15 males/group

Clinical sign	Control	1 µg/kg/day	3 µg/kg/day
Hypersensitive	2	2	5
Fur thin cover/Fore paw	5	6	9
Skin dry/Tail	7	6	10
Skin scab/Tail	5	9	9

Body weights: There were no treatment-related differences of body weight gain.

Food consumption: There were no treatment-related differences of food consumption.

Hematology: Monocyte counts for the low and high dose groups were increased to 127.5 and 117.6% of the control ($0.091 \times 10^3/\mu\text{L}$), respectively. Basophil counts for the low and high dose groups were decreased to 79.2 and 81.1% of the control ($0.037 \times 10^3/\mu\text{L}$), respectively. Differences were small and dose-response relationships were not present; thus, it is unclear if these were treatment-related effects.

Clinical chemistry: Indirect bilirubin levels for the high dose group were increased to 134.2% of the control (0.051 mg/dL). Triglyceride levels for the low and high dose groups were decreased to 79.2 and 81.1% of the control (57.2 mg/dL), respectively.

Urinalysis: There were no treatment-related changes of urinalysis parameters.

Gross pathology: Gross pathological findings were observed in the cecum, colon, epididymis, jejunum, liver, prostate, stomach, testis, and adrenal. Findings in the cecum appear to correlate with histopathological findings of hemorrhage and inflammation. Findings in the colon and jejunum appear to correlate with histopathological findings of hemorrhage. Findings in the liver appear to correlate with histopathological findings of tension lipidosis and/or inflammation. The finding in the prostate appears to correlate with histopathological findings of inflammation. The finding in the testis appears to correlate with the histopathological finding of atrophy of the seminiferous epithelium. The finding in the epididymis appears to correlate with the histopathological finding of oligo/aspermia. There did not appear to be any corresponding histopathological findings for gross pathological findings in the stomach and adrenal.

Gross pathological findings, n = 15 males/group

Organ/Tissue	Control	Low dose	High dose
Cecum			
-area dark	0	0	3
-foci dark	0	1	1
Colon			
-area dark	0	1	2
-foci dark	0	1	2
Jejunum			
-area dark	3	9	8
Liver			
-area pale	1	2	3
Prostate			
-area pale	0	0	1
Testis			
-small	0	0	1
Epididymis			
-small	0	0	1
Stomach			
-area pale	0	0	1
Adrenal			
-foci pale	0	0	1

Organ weights: There were no treatment-related changes of absolute or relative organ weights.

Histopathology: Histopathological findings of potential significance were observed in the cecum, colon, jejunum, and lung; however, there relationships to treatment were unclear given that there were no similar observations in study 090-840 where (R,R)-desformoterol was administered at total inhaled doses up to 30 µg/kg/day.

In the cecum, incidences of minimal hemorrhage were increased for both treatment groups. The relationship of this finding to treatment was unclear given that there were no similar observations in study 090-840. Inflammation with mucosal hyperplasia (minimal to slight in severity) was observed in all groups with the highest incidence observed in the control group. This change was characterized by increased numbers of inflammatory cells, primarily mononuclear in the lamina propria, with intestinal crypts exhibiting greater basophilia and cellularity and an increased mitotic activity (mucosal hyperplasia). In study 090-840, increased incidences of minimal to slight inflammation with resulting secondary and reactive hyperplasia were observed in the cecal mucosa for all male and female treatment groups. From literature references, a slight increase in inflammatory cells in the lamina propria would be considered within normal limits (Handbook of Toxicology 2nd Edition, CRC Press, Page 696).

Hemorrhage was observed in the colon, jejunum, and lung. In the lung and colon, incidences of hemorrhage were increased in the high dose group. In the jejunum, incidences of hemorrhage were increased in both treatment groups; however, a dose-

response relationship was not observed. The relationship of these findings to treatment was unclear given that there were no similar observations from study 090-840.

For the prostate, the incidence of inflammation was slightly increased in the high dose group. However, for study 090-840, the incidence of prostate inflammation in the control group was 8 of 15 (53.3%). The incidence in the present study appears to be within the spontaneous background range.

Additional histopathological findings were observed in the duodenum, ileum, aorta, kidney, larynx, liver, pancreas, testis, epididymis, and urinary bladder; however, incidences were low and/or dose-response relationships were not present. Hemorrhage was observed in the duodenum and ileum, each with an incidence of 1 of 15.

Histopathological findings

Organ/Tissue	Control	Low dose	High dose
Cecum			
-inflammation with mucosal hyperplasia	6/15	2/15	2/15
-hemorrhage, multifocal	0/15	3/15	4/15
Colon			
-hemorrhage, multifocal	0/15	0/15	2/15
Jejunum			
-hemorrhage, focal-multifocal	4/15	7/15	5/15
Lung			
-hemorrhage, focal-multifocal	2/15	0/15	4/15
Prostate			
-inflammation, focal-multifocal	3/15	3/15	5/15
Duodenum			
-hemorrhage, multifocal	0/15	1/15	0/15
Ileum			
-hemorrhage, multifocal	0/15	1/15	0/15
Aorta			
-inflammation	0/15	0/15	1/15
Kidney			
-basophilia, tubular	0/15	0/15	2/15
-cyst	0/15	0/15	1/15
-pyelitis/pyelonephritis	0/15	0/15	1/15
Larynx			
-granuloma	0/15	0/15	1/15
Liver			
-tension, lipidosis	1/15	0/15	2/15
-inflammation	0/15	0/15	1/15
Pancreas			
-epithelial hyperplasia	0/15	0/15	1/15
Testis			
-atrophy, seminiferous, epithelium, diffuse, unilateral, Grade 4	0/15	0/15	1/15
Epididymis			
-oligo/aspermia, unilateral, Grade 4	0/15	0/15	1/15
Urinary bladder			
-hyperplasia, transitional cell	0/15	0/15	1/15