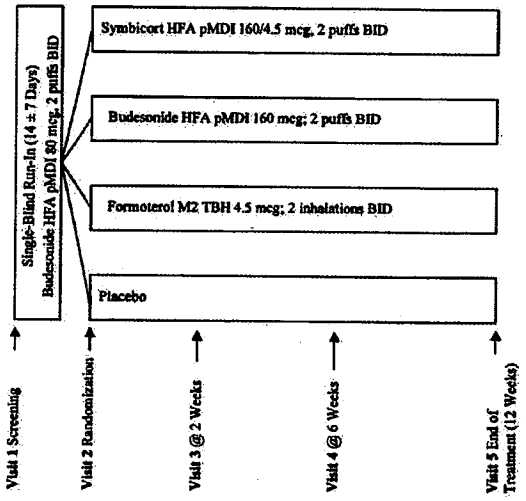
Protocol SD-039-0717: This is a 12-week randomized, double-blind, double-dummy, placebo-controlled trial to compare the safety and efficacy of Symbicort HFA pMDI with its monoproducts, Budesonide HFA pMDI and Formoterol Turbuhaler in adolescents (≥12 years) and adults with moderate to severe asthma. Symbicort, a fixed combination product containing budesonide and formoterol, 160/4.5 µg per puff, respectively, will be administered as two inhalations twice daily. Budesonide (160 µg/puff) or Formoterol (4.5 µg/inhalation) will be administered as two inhalations twice daily. Subjects will undergo a 2-week run-in period during which they will be washed-out of their current asthma therapy and use a single blinded medication (budesonide HFA pMDI, 80 µg/puff). This will be followed by a 12-week randomized double-blind treatment in one of four treatment groups as shown in the diagram below. Each group will consist of approximately 112 patients. Co-primary efficacy variables will be 12-hr serial FEV_1 and withdrawals due to asthma exacerbation.

FIGURE 1
Flow Chart of Study Treatments



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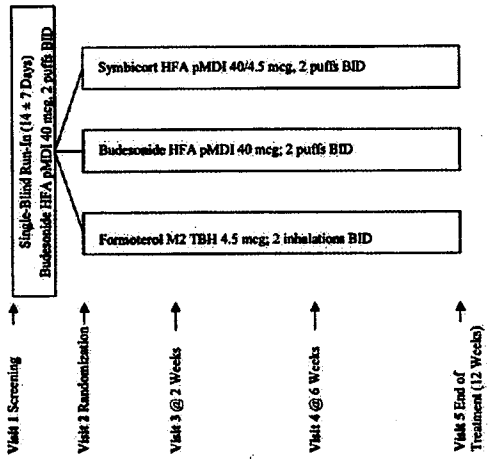
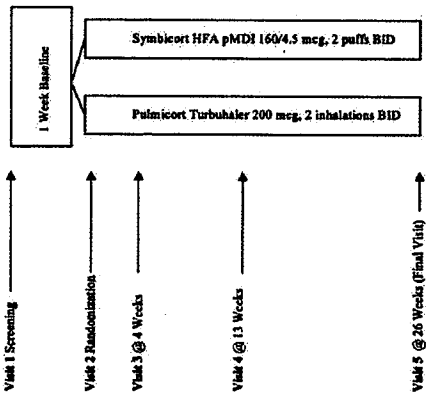
Protocol SD-039-0718: This is a 12-week randomized, double-blind, double-dummy, placebo-controlled trial to compare the safety and efficacy of Symbicort HFA pMDI versus its monoproducts, Budesonide HFA pMDI and Formoterol Turbuhaler, in asthmatic children (6 to 11 years). Symbicort, a fixed combination product, containing budesonide and formoterol, 40/4.5 µg per puff, respectively, will be administered as two inhalations twice daily. Budesonide (40 µg/puff) or formoterol (4.5 µg/inhalation) will be administered as two inhalations twice daily. Subjects will undergo a 2-week run-in period during which they will be instructed to discontinue use of their current asthma therapy and use a single-blinded medication (Budesonide HFA pMDI, 40 µg/puff). This will be followed by a 12-week randomized double-blind treatment in one of four groups as shown in the diagram below. Each group will consist of approximately 135 patients. The primary efficacy variable will be morning peak expiratory flow.

FIGURE 1
Flow Chart of Study Treatments



Protocol SD-039-719: This is a 6-month open-label safety study to evaluate and compare the safety of Symbicort HFA pMDI with Pulmicort Turbuhaler in asthmatic children (6-11 years). Symbicort, a fixed combination product of budesonide and formoterol, 160/4.5 µg, respectively, will be administered as two inhalations twice daily. Pulmicort, 200 µg/inhalation, will be administered as 2 inhalations twice daily. After a 1-week baseline period during which time subjects will continue to use their normally prescribed inhaled corticosteroid, subjects will be randomized to one of two treatment groups as shown in the diagram below. The Symbicort and Pulmicort groups will consist of 100 and 50 patients, respectively. The primary safety variable is reported adverse events.

FIGURE 1
Flow Chart of Study Treatments



Non-U.S. Studies: The sponsor is conducting a 12-month safety and efficacy trial (SD-039-0715) to compare Symbicort HFA pMDI (budesonide/formoterol), 160/4.5 µg, 2 actuations BID with Symbicort Turbuhaler (budesonide/formoterol) 160/4.5 µg, 2 inhalations BID in subjects ≥12 years of age. There will be 300 patients treated with Symbicort HFA pMDI and 150 patients treated with Symbicort Turbuhaler.

II. Additional Study Protocols provided in the EOP2 Package:

Protocol SD-039-0715: the primary objective of this study is to demonstrate that the bronchodilating effect of formoterol administered through the Symbicort pMDI is less than or equal to the bronchodilating effect of formoterol administered through the Oxis turbuhaler, by assessment of 12-hr serial FEV_1. In clinical trials with Symbicort, comparisons of Symbicort to formoterol alone will occur across different drug products. Therefore, it is necessary to demonstrate that differences in pharmaceutical formulations and/or delivery are not the cause of the differences in clinical efficacy that are expected in clinical trials with Symbicort. This is an open-label, active-controlled, single-dose, 4-way cross-over, multicenter, Phase II study to compare the bronchodilating effect of one or four actuations of Symbicort pMDI

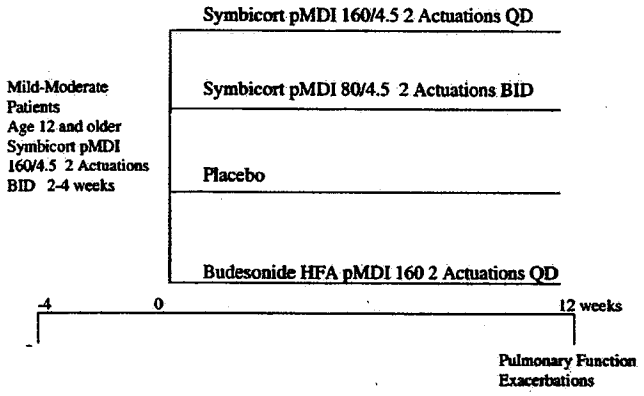
(Budesonide/Formoterol: 40/4.5 µg per actuation) to Oxis M2 turbuhaler (Formoterol: 4.5 µg/actuation) administered once daily in the morning to adults with stable asthma. Male or female subjects, ≥18 years of age, who have a clinical diagnosis of asthma for at least 6 months prior to screening, and are in stable condition, are eligible to enroll in the study. Subjects should have received maintenance asthma treatment with inhaled corticosteroids for at least 3 months prior to screening. During a 7- to 14-day run-in period, subjects will be stabilized on Budesonide pMDI (80 µg/actuation x 2 actuations BID) treatment. Subjects will be randomized and receive each of four, single-dose, crossover treatments, separated by a 2- to 10-day washout period. Subjects will remain on maintenance therapy with Budesonide pMDI throughout the study. Approximately 36 subjects will be enrolled in this clinical trial.

Adjustable Dosing Program (Studies 0725, 0726, 0727, and 0728):

The sponsor proposes to study the efficacy and safety of Symbicort pMDI in two alternative regimens as follows: two actuations once daily (once-daily trials) and four actuations twice a day (higher dosing trials). The once-daily regimen (2 actuations once daily) is intended as a step down therapy in those patients whose asthma is well controlled on Symbicort. The higher dose regimen (four actuations twice a day) is intended for periodic use – not to exceed 4 weeks at a time – as step-up therapy. The step-up therapy could be utilized as initial therapy for patients requiring more aggressive control or for patients previously controlled on Symbicort two actuations BID, who experience a temporary loss of control.

To assess the efficacy of the once daily dosing regimen, the sponsor has proposed to perform two 12-week double blind, double dummy, parallel-group, randomized, placebo and active controlled clinical trials. One trial will be conducted in adults and adolescents (Study 0726) and one trial in children 6-11 years of age (Study 0725). Patients would be stabilized on a dose of Symbicort two actuations BID during the 2 to 4 week run-in period. Qualified patients would then be randomized to one of four treatment groups: (1) the same dose strength of Symbicort, using two actuations QD, (2) the next lower formulation strength of Symbicort at two actuations BID; (3) budesonide pMDI QD or (4) placebo. There will be approximately 115 patients per treatment group.

Figure 12. Study design for Once- daily regimen in adults – Trial 0726

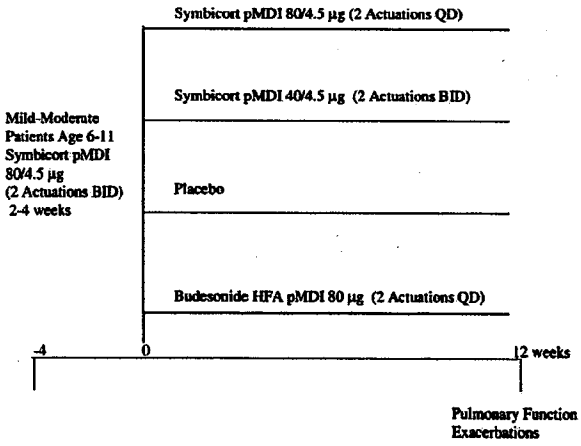


Study 0726

Table 33: Total Delivered Dose and Number of Subjects in each treatment group

Treatment Group	No. of Subjects	Total Daily Delivered Dose (µg Budesonide)	Total Daily Delivered Dose (µg Formoterol)
Symbicort pMDI 160/4.5µg 2 Actuations QD	115	320	9
Symbicort pMDI 80/4.5µg 2 Actuations BID	115	320	18
Budesonide HFA pMDI 160µg – 2 Actuations BID	115	320	0
Placebo	115	0	0

Study 0725



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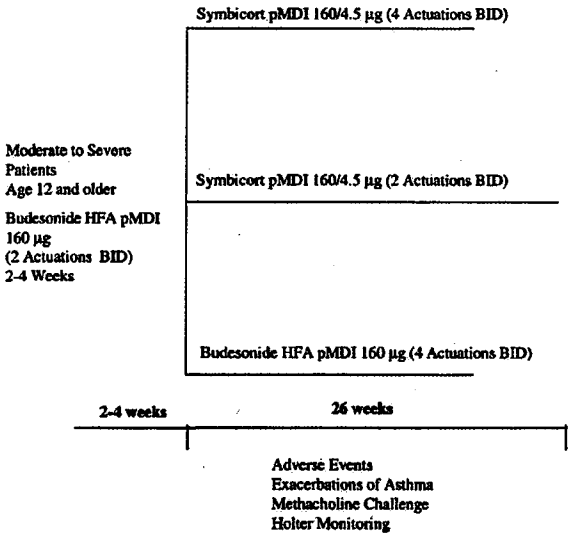
Study 0725

Table 32: Total Delivered Dose and Number of Subjects in each treatment group

Treatment Group	No. of Subjects	Total Daily Delivered Dose (µg Budesonide)	Total Daily Delivered Dose (µg Formoterol)
Symbicort pMDI 80/4.5µg 2 Actuations QD	115	160	9
Symbicort pMDI 40/4.5µg 2 Actuations BID	115	160	18
Budesonide HFA pMDI 80µg – 2 Actuations BID	115	160	0
Placebo	115	0	0

To establish the safety of Symbicort in a higher dose regimen (i.e., 4 actuations BID), the sponsor has proposed to conduct a 6-month safety trial using the highest possible clinical dose. In this double-blind study (Study 0728), asthmatic patients (>12 years of age) will be randomized to one of three treatment groups: (1) Symbicort 160/4.5 µg (4 actuations BID); (2) Symbicort 160/4.5 µg (2 actuations BID); and (3) Budesonide HFA pMDI 160 µg (4 actuations BID). There will be 125 patients in each treatment group. In this trial, all randomized patients at a subset of study centers will participate in serial methacholine challenge testing in order to demonstrate that no increase in bronchial hyperresponsiveness occurs with higher doses of Symbicort. Holter monitoring will also be performed in all patients at a subset of study centers with electronic analysis and reporting of ECG and Holter data.

Figure 13. Study design for Higher Dosing regimen in adults – Study 0728



Study 0728

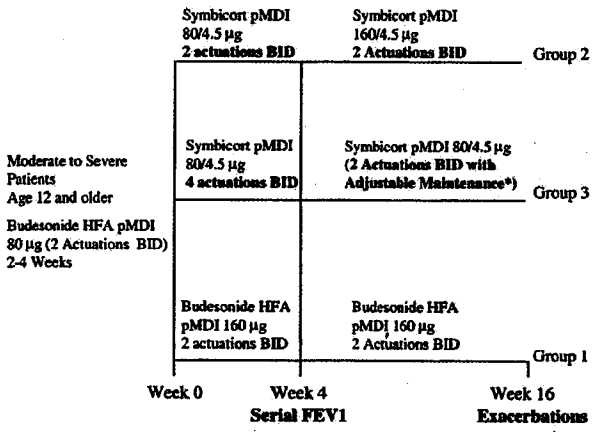
x

Table 34: Total Delivered Dose and Number of Subjects in each treatment group

Treatment Group	No. of Subjects	Total Daily Delivered Dose (µg Budesonide)	Total Daily Delivered Dose (µg Formoterol)
Symbicort pMDI 160/4.5µg 4 Actuations BID	125	1280	36
Symbicort pMDI 160/4.5µg 2 Actuations BID	125	640	18
Budesonide HFA pMDI 160µg – 4 Actuations BID	125	1280	0

Efficacy of Symbicort at higher doses will be assessed in a double-blind, double-dummy, parallel-group, randomized, active controlled 16-week trial (Study 0727). Patients (>12 years of age) previously on a medium to high dose of inhaled corticosteroids will be switched to a medium to low dose of inhaled corticosteroids during a 2 to 4 week run-in period. Those patients symptomatic during the run-in period will be randomized to one of three treatment groups: (1) Budesonide HFA pMDI 160 µg (2 actuations BID), (2) Symbicort 80/4.5 µg (2 actuations BID) or (3) Symbicort 80/4.5 µg (4 actuations BID; adjustable dose group) for 4 weeks. After 4-weeks, the trial would continue for another 12 weeks with the following modifications to the treatment groups: Group 1 would remain on Budesonide HFA pMDI at 160 µg (2 actuations BID); Group 2 receiving Symbicort, would switch from 80/4.5 µg to 160/4.5 (2 actuations BID); and Group 3, the adjustable-dose Symbicort group, would switch from 4 actuations BID to 2 actuations BID. If patients in Group 3 became more symptomatic again after having reduced their Symbicort dose from 4 actuations BID to 2 actuations BID, then the dose could be readjusted back to 4 actuations BID once the patient had remained on 2 actuations BID for at least 2 weeks. There will be 125 patients per treatment group.

Figure 14. Study design for Higher-Dosing Regimen in adults - Study 0727



* After 2 weeks, patients may increase back to 4 puffs BID for 1-4 weeks based upon a pre-set asthma control plan

Trial 0727

Table 35. Total Delivered Dose and Number of Subjects in each treatment group

Treatment Group	No. of Subjects Randomized	Total Daily Delivered Dose (µg)			
		Weeks 1 through 4		Weeks 5 through 16	
		Budesonide	Formoterol	Budesonide	Formoterol
Group 1	125	640	0	640	0
Group 2	125	320	18	640	18
Group 3	125	640	36	320 or 640	18 or 36

Previous clinical experience: Please see review of the Initial Submission.

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

Introduction and drug history:

AstraZeneca LP has proposed to develop Symbicort®, a combination product consisting of a glucocorticoid (budesonide) and a long acting β_2 agonist (formoterol), for treatment of asthma. The proposed product will be available in — dosage levels: — 80 µg budesonide/4.5 µg formoterol per actuation, and 160 µg budesonide/4.5 µg formoterol per actuation. The inhalation device will be a pressurized metered dose inhaler (pMDI). The aerosol formulation contains excipients, povidone K25 (PVP K25) and polyethylene glycol 1000 (PEG 1000), and 1,1,1,2,3,3,3-heptafluoropropane (HFA-227) as the propellant.

The sponsor provided the Initial submission (#000) on October 5, 2001. This submission contained draft clinical protocols for Phase 3 study numbers SD-039-0716, SD-039-0717, SD-039-0718, and SD-039-0719. In November 2001, the Division determined that these clinical trials were reasonably safe to proceed. The sponsor requested an EOP2 meeting in the first quarter of 2002 to discuss their Phase 3 plans. In the EOP2 package, the sponsor provided plans for several additional clinical protocols. For further information, please refer to the review of the initial submission.

Studies reviewed within this submission: Correspondence regarding End of Phase II meeting.

Studies not reviewed within this submission: None.

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

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PHARMACOLOGY/TOXICOLOGY REVIEW

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

Symbicort® is a combination product consisting of a glucocorticoid (budesonide) and a long acting β_2 agonist (formoterol) under development for the treatment of asthma. The proposed product will be available in — dosage levels: — 80 µg budesonide/4.5 µg formoterol per actuation, and 160 µg budesonide/4.5 µg formoterol per actuation. The inhalation device will be a pressurized metered dose inhaler (pMDI). The aerosol formulation contains excipients, povidone K25 (PVP K25) and polyethylene glycol 1000 (PEG 1000), and 1,1,1,2,3,3,3-heptafluoropropane (HFA-227) as the propellant. Clinical trials in children (6-11 years old), adolescents, and adults range from 3- to 6-months. An additional single dose, 4-way crossover trial to address pharmaceutics issues between the Symbicort HFA pMDI and formoterol turbuhaler will be conducted in adults. A budesonide HFA pMDI formulation and a formoterol turbuhaler formulation will be used as comparators to the Symbicort HFA pMDI formulation. In an End of Phase 2 Meeting package submitted in Amendment #017, the sponsor submitted questions with reference to preclinical toxicology. These questions have been listed below with responses to the sponsor. Some questions regarding chronic administration of formoterol have been addressed, but are not intended for communication to the sponsor.

PHARMACOLOGY/TOXICOLOGY

1. Does the Agency concur that the completed/ongoing preclinical pharmacology/toxicology studies with both the monoproducts and the combination support the proposed clinical program?

This section will not be communicated to the sponsor in the EOP2 meeting.

⇒ At the EOP2 meeting for — (formoterol fumarate dihydrate) on August 3, 2000, the sponsor was requested to justify that inhalation toxicity studies with treatment duration of up to 1 month in dogs are adequate to evaluate the toxicity of inhaled formoterol in the non-rodent species. As part of this justification, the sponsor needed to discuss whether the rat or dog was the most appropriate species for evaluating the toxicity of inhaled formoterol. During the discussion, the Division stated that, "chronic inhalation toxicity studies with treatment durations of 6 months in rats and 9-12 months in dogs are generally required for drugs like formoterol. A 12-month oral toxicity study in dogs that AstraZeneca has completed appears to be sufficient to assess the systemic toxicity of formoterol, but it may be inadequate to evaluate its local (respiratory) toxicity in this species." It was agreed that the proposed Phase 3 clinical program with formoterol could start, and the preclinical justification document could be

submitted after the pivotal studies were initiated. To date, the sponsor has never provided this preclinical justification document.

Subchronic and chronic toxicology studies with formoterol have been conducted in both rats and dogs. With the rat, the sponsor has conducted 3-, 6-, and 24-month inhalation studies. With the dog, the sponsor has conducted a 12-month oral study. As noted earlier, the longest inhalation study with formoterol alone in dogs was only 1 month. It should be noted that the sponsor has conducted 3-month inhalation toxicology studies in rats and dogs with both the HFA pMDI and dry powder formulations of budesonide/formoterol combinations.

In reviewing these studies with rats and dogs, deposited doses achieved in the 3- and 6-month toxicology studies with rats (2-90 µg/kg/day) were significantly higher than those achieved in the 1-month toxicology study with dogs (0.07-2 µg/kg/day). Given the higher deposited doses in rats, studies with these animals are considered more optimal for assessing effects of potential local toxicity. Systemic effects (i.e., increased heart rate and electrocardiographic changes) observed in the 1-month inhalation dog study would most likely limit dosing in dogs. Thus, evaluation of local toxic effects would also be limited. It should be noted that no local toxic effects have been observed in the 3-, 6-, and 24-month inhalation toxicity studies with rats or the 1-month inhalation toxicity study with dogs. In the 6-month inhalation study with rats, the NOAEL of 2.3 µg/kg/day (deposited dose) provides 6.4 and 3.2-fold safety margins for human doses of 18 µg/day (0.36 µg/kg/day) and 36 µg/day (0.72 µg/kg/day), respectively.

The sections below will be communicated to the sponsor in the EOP2 meeting.

Specifically:

Does the Agency concur that toxicology data derived from excipients used in the completed long-term studies with another AstraZeneca compound ~~~~ in rats and dogs support the use of a similar HFA-227 pMDI formulation in the proposed phase 3 clinical program and registration?

⇒ From a preclinical standpoint, data derived for excipients, PVP K-30 and PEG-600, from studies ~~~~~~~~ in rats appear to support the use of a similar formulation with the Symbicort drug product.

Does the Agency concur that toxicology/pharmacokinetic data obtained with budesonide/formoterol pMDI combinations are sufficient to support clinical trials with budesonide HFA-227 pMDI and registration of Symbicort pMDI?

⇒ Given that the Symbicort and budesonide HFA pMDI formulations are essentially identical with the exception of the presence of formoterol in Symbicort, preclinical studies with the Symbicort HFA pMDI formulation are sufficient to support clinical trials with the budesonide HFA pMDI formulation.

CHEMISTRY, MANUFACTURING AND CONTROLS

2. Control of leachables in commercial drug product.

Bullet points will not be shown during the EOP2 meeting; however, responses to the question listed below will be communicated to the sponsor.

- Controlled extraction studies will be performed on the container closure system components that contact the formulation or the patient's mouth (the critical contact parts) to characterize any extractables. Where possible, a qualitative and quantitative assessment of all species detected at levels _____ will be made. The information and methodology derived from the extraction studies will be used to perform a ~~leachables study.~~

- The ~~leachables study will be conducted on the primary stability batches and,~~ where possible, any leachables quantified at a level which would result in a ~~total daily intake (TDI) will be identified. Any leachables quantified at total~~ daily intake will be identified and assessed toxicologically.

- Potential leachables with specific toxicological concerns will be quantified at much lower levels.

- The correlation process will involve demonstrating that leachables can be assigned qualitatively, directly or indirectly (i.e., as addition products of 1 or more reactants), to an extractable. It is proposed that leachables will be controlled in the finished product by routine extractables testing of individual materials or components. The control would be achieved by the inclusion of specifications for extractables limits in the container closure specifications.

- The inclusion of specification limits for particular species in the container closure specifications will be based on either of the following criteria:

  A potential extractable is confirmed as a leachable in the primary stability batches and the species has known toxicological implications.

  A potential extractable is confirmed as a leachable in the primary stability batches and quantified at a level that would result in a total daily intake of ~~—~~

Does the Agency concur with the AstraZeneca strategy where control of leachables in the finished product is achieved by control of extractables from individual materials or components prior to molding or assembly by the component manufacturer?

⇒ Please clarify what batches of the Symbicort HFA pMDI formulation were used in the 90-day preclinical toxicology studies with rats and dogs (i.e., were the cannisters near the expiration date?). This information may assist in determining whether exposure to extractables/leachables in these studies is sufficient for qualification.

⇒ All extractables/leachables should be assessed for mutagenic or carcinogenic potential, including the presence of structural alerts. For extractables/leachables that are carcinogenic or mutagenic, inhalation exposure-based risk assessments should be provided.

General Toxicology Issues:

Subchronic and chronic toxicology studies have been conducted with formoterol in both rats and dogs. Studies with rats include 3-, 6-, and 24-month inhalation toxicology studies. A 1-year oral toxicology study has been conducted with dogs. The longest inhalation toxicology study in dogs was 1 month, but the 6-month inhalation toxicology study in rats is sufficient to bridge the systemic toxicology work-up of formoterol because deposited doses achieved in rats greatly exceed those that could be achieved in dogs, and neither species seemed particularly sensitive to the local effects of formoterol. Studies have adequately evaluated the toxicity of formoterol in terms of local (respiratory) and systemic effects.

Three-month inhalation bridging studies have been conducted with Symbicort (budesonide and formoterol) in both rats and dogs.

Recommendations: None.

Labeling with basis for findings: None.

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

Supervisor signature: Concurrence - _____
Robin Huff, Ph.D.

Non-Concurrence - _____
(see memo attached)

Reviewer: Timothy W. Robison, Ph.D. IND No. 63,394

cc: list:
ZeccolaA
AnthraciteR
HuffR
RobisonT

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

/s/

Timothy Robison
4/4/02 04:34:24 PM
PHARMACOLOGIST

Robin Huff
4/8/02 11:26:49 AM
PHARMACOLOGIST
I concur.

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Appendix 4: Review of IND —— dated March 5, 1997

MAR -5 1997 DF

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 1

IND No.

Serial Nos., Contents and Dates of Submission:

000	Original submission	12/27/93
009	Correspondence	10/14/94
011	6-month rat inhalation toxicity study	10/28/94
012	Rat inhalation carcinogenicity study	01/11/95
016	4 inhalation toxicity studies (3-6 months)	04/18/95
017	Rat teratology study, 5-day dog inhalation toxicity	06/22/95
018	12-month dog toxicity, rabbit reproductive toxicity	11/06/95
020	Mouse oral carcinogenicity study	01/06/96
022	Inhalation reproductive toxicity, 1-month dog toxicity	04/24/96

Information to be conveyed to Sponsor: Yes ( ), No (X).

Reviewer: Luqi Pei, Ph.D. (HFD-570)

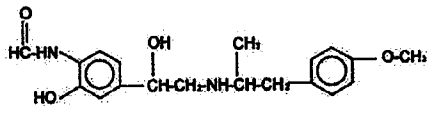
Date of Review Completed: March 5, 1997

Sponsor: Astra Merck Inc.

Drug Name:
Generic Name: Formoterol fumarate dihydrate
Brand Name:
Code Name: D2522

Chemical Names: (R*,R*)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide, (E)-2-butendioate (2:1), dihydrate

Structure:



Formula and Molecular Weight: $(C_{19}H_{26}N_2O_4)_2 \cdot C_4H_4O_4 \cdot H_2O$, MW=840.9

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Formulation:	Use	µg/actuation	% w/w
Lactose	Excipient	600	
Formoterol	bronchodilator	6, 12	

Related INDs and NDAs:

Related DMFs:

Class: β_2-agonist

Indication: Asthma

Route of Administration: Oral inhalation

Proposed Clinical Dose: The initial proposed trial was a multi-center, randomized, double-blind study in 160 adult asthmatic patients at doses of 6, 12, 24 µg, bid for 7 days. This corresponds to 12, 24, and 48 µg/person/day or 0.25, 0.5, and 1.0 µg/kg/day. The current trials (phase 3) use the same doses.

Background:

This IND was originally submitted by the sponsor on December 27, 1993 and was transferred to me on June 19, 1996. A search of the division file did not show any previous pharmacology/toxicology reviews. Dr. Virgil Whitehurst was the original pharmacologist reviewer.

Documents reviewed in the IND.

Pharmacology:

Journal articles:

Adenvier *et al.* Formoterol and salbutamol inhibits bradykinin- and histamine-induced airway microleakage in guinea pigs. *Br J Pharmacol 1992;105:792-798.*

Bibi et al (1990) The effect of inhaled beta-agonist on airway responses to antigen in a canine model of asthma. *J Allergy Clin Immunol*, 85(1):295.

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Decker *et al* (1982). Effects of N-arakyl substitution of beta-agonists in alpha and beta-adrenoceptor subtypes: pharmacological studies and binding assay. *J Pharm Pharmacol, 34:107-*

112.

Erjefalt and Person (1991). Long duration and high potency of antiaxudative effects of formoterol in guinea pig tracheobronchial airways. *Am Rev Respir Dis,* 144:766-791.

Faulds et al (1991). Formoterol: A review of pharmacological properties and therapeutic potential in reversible obstructive airways disease. 42:115-137.

Freyss-Beguin et al. (1983). Comparison of the chronotropic effect and the cyclic AMP accumulation induced by beta 2 agonists in rat heart cell culture. 78:717-723.

Ida H. (1976_a). Comparison of the action of BD40A and some beta-adrenoceptor stimulants on the isolated treachea and atria of the guinea pigs. *Arzveim Forsch Drug Res, 26:839-842.*

Ida H. (1976_b). Cardiorespiratory activities if formoterol and some other beta-adrenoceptor stimulants in conscious guinea pigs. *Arzveim Forsch Drug Res, 26:1337-1340.*

Ida H (1980). General pharmacology of formoterol. *Folia pharmacol japon,* 76:633-654.

Jeppsson *et al (1989).* On the predictive value of experiments in vitro in the evaluation of the effect duration of bronchodilator drugs for local administration. *Pulmonary Pharmacol, 2:81-85.*

Jeppsson et al. (1992). Studies on the interaction between formoterol and salmeterol in guinea pigs trachea in vitro. *Pharmacol Toxicol,* 71:272:277.

Jeppsson et al. (1993). Formoterol and salmeterol and both long-acting compared to terbutaline on the isolated perfused and ventilated guinea pig lung. *Eur J Pharmacol* 257:137-143.

Jeppsson *et al.* (1994). Similar duration of action between formoterol and albuterol in the solated and perfused guinea pig lungs. *Eur J Pharmacol,* 257:134-143

Johansson and Persson (1983). Beta 2-adrenoceptor in guinea pig atria. *J Pharm Pharmacol,* 35:804-807.

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General toxicity of D2522 given by the inhalation route for three months. plasma concentration study. Report No. 843-RD-0295. Submission of 11/27/93, vol 11, p. 191.

General toxicity of D2522 given orally to dogs for one month. plasma concentration study. Report No. 843-RD-0320. Submission of 11/27/93, vol 11, p. 226.

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Toxicology

A. Single doses (Acute) toxicity studies:

1. Acute inhalation toxicity study in rats: Study No. T2514. Submission of 12/27/93. Vol. 1.4, p 141 (Ref. 36).
2. Acute inhalation toxicity study in mice: Study No. T2807. Submission of 1/11/95, vol. 5.1, p 102.
3. Acute oral toxicity study in mice: Study No. T2843. Submission of 1/11/95, vol. 5.1, p 23.
4. Acute oral toxicity study in rats: Study No. T2842 Submission of 1/11/95, vol. 5.1, p 1.
5. Acute oral toxicity in young rats: Study No. T2815. Submission of 1/11/95, vol. 5.1, p 40.

B. Multiple dose Toxicity studies:

1. Inhalation studies:
   a. 5-day dose-range study in rats: Study No. T2460. Resubmission on 4/24/96, vol. 12.1, p 1. 1st submission: vol. 1.5, p 170.
   b. 5-day dose-ranging study in dogs: Study No. T3006, submission of 6/22/95, vol. 8.1, p 96.
   c. 1-month toxicity study in dogs: Study No. T3120. Submission of 4/24/96, vol. 12.5, p 120.
   d. 3-month toxicity study in rats: Study No. T2433. Resubmission on 4/18/95, vol. 7.2, p 1., 1st submission: vol. 1.6, p 1.
   e. 6-month toxicity study in rats: Study No. T2860. Resubmission on 4/18/95, vol. 7.3, p 1., 1st submission: 10/28/94.

2. Oral studies
   a. One-month toxicity study in dogs: Study No. T2579. Resubmission of 4/18/95, vol. 7.5, p 1. 1st submission 12/27/93, vol. 1.7, p 1.
   b. 3-month toxicity study in mice: Study No. T2578. Resubmission on 4/18/95, vol. 7.4, p 1., 1st submission 12/27/93, vol. 1.5, p 1.
   c. 3-month toxicity study in young rats: Study No. T3136, submission of 4/24/96, vol. 12.3, p 1.
   d. 12-month oral toxicity study in dogs: Study No. T3077, submission of 11/6/95, vol. 9.1, p 1.

Reproductive toxicology:

A. Inhalation toxicity studies:
   1. Segment II teratology in rats: T2628. Resubmission on 4/24/96, vol. 12.1, p 88 ; 1st submission: 12/27/93, vol. 1.9, p 26.
   2. 3-month study on the effect of respiratory tract and male reproductive organs in young rats: Study No. T3137, submission of 4/24/96, vol. 12.2, p 1.

B. Oral:
   a. Segment I fertility and reproductive performance study in rats: Study No. T3015, submission of 11/6/95, vol. 9.3, p 112.
   b. Segment II teratology dose-range study in rabbits: Study No. T2710. Resubmission on 4/24/96, vol. 12.5, p 1; 1st submission: 12/27/93, vol. 1.10, p 1. submission of 12/27/93.
   c. Segment II teratology study in rabbits: Study No. T3014, submission of 6/11/95, vol.

9.3, p 1.

d. Segment III (Peri- and postnatal and pregnancy) studies in rats: Study No. T2905, submission of 6/22/95, vol. 8.1 p 1.
e. Testicular effect of 3-month oral formoterol in young rats: Study No. T3160, submission of 4/24/96, vol. 12.4, p 1.

Mutagenicity Studies:

1. Ames Salmonella test: Study Nos. T2388, T2389: submission of 12/27/93, vol. 1.11, p 1 - p 42.
2. Mouse lymphoma cell TK test: Study No. T2397. submission of 12/27/93, vol. 1.11, p 43.
3. Human Lymphocyte chromosomal aberration test: Study No. T2510. Submission of 12/27/93, vol. 1.11, p 70.
4. Rat micromuclei formation test, inhalation: Study No. T2513, vol. 1.10, P 1. Submission of 12/27/93.

Special Toxicity Studies

1. Hemolysis and protein flocculation of human blood in vitro. Study No. T 2296. Vol. 1.8, p45.
2. Vaso-, tissue irritation in dogs, IV, SC, 5 days: T Study No. 2302, submission of 12/27/93, vol. 1.9, p 1 - p 42.

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REVIEW:

I. PHARMACOLOGY

Formoterol is a selective and long-acting beta$_2$ agonist. It has been used as a bronchodilator to treat asthma in several foreign countries. Trough binding to the beta$_2$ adrenoceptors, formoterol exerts pharmacological characteristics such as smooth muscle relaxation, anti-allergy, and inhibiting mediators release. Detailed review of preclinical and clinical pharmacology of the drug are available in the literature. [Tasaka (1986) and Faulds *et al.* (1991)]. The following is a brief summary.

A. Primary Pharmacology

1. Intrinsic activity. By activating β_2-adrenergic receptors, beta-agonists relax contracted-tracheal smooth muscle. Using pre-contracted guinea pig trachea *in vitro*, Ida (1976) studied intrinsic activity of some commonly used beta-agonists. Results from this study are summarized in Table 1. Formoterol was as potent as isoproterenol to relax the pre-contracted tracheal muscles. The pD_2 of these drugs ranged from 6.48 to 8.43.

Table 1. Intrinsic activity of beta-agonists on histamine/methacholine contracted tracheal muscles

	Isoproterenol	Orciprenaline	Tremoterinol	Albuterol	Formoterol
Histamine	1	1	1	1	1
Methacholine	1	0.83	0.89	0.82	1

A major side effect of many beta-agonists in asthma treatment is their cardiac effect - tachycardia. Decker *et al.* (1982) studied receptor selectivity of several commonly used beta-agonists *in vitro*. This was achieved by comparing the concentrations required to induce relaxation of the constricted guinea pig tracheal smooth muscle (β_2) and to induce tachycardia (β_1). Isoproterenol, a non-selective beta-agonist, relaxed tracheal smooth muscle at the same concentrations that induced tachycardia. Formoterol relaxed tracheal muscle at concentrations much lower (204 times lower) than the concentrations that induced tachycardia (Table 2). This indicated that formoterol was highly selective for the beta$_2$-receptor. Källdtröm *et al.* reported that formoterol was 400 times more potent than terbutaline in relaxing human broncheal muscle *in vitro*.

Table 2. Selectivity of beta agonists

	Trachea		Atria		Selectivity
	PD2	Intrinsic activity	PD2 activity	Intrinsic (antilog)	for beta$_2$
Isoproterenol	8.57	1	8.62	1	0.9
ME-454	8.57	0.94	7.86	0.95	8.7
Protokylol	8.20	0.89	8.00	1.15	1.6
Terbutaline	6.43	0.83	5.17	0.89	18
Fenoterol	7.36	0.91	6.16	1.09	16
Salbutemol	7.13	0.91	5.90	0.75	17
Salmefamol	8.23	0.92	6.78	0.82	28
Soterenol	7.59	0.90	6.26	0.56	21
Zinterol	8.53	0.91	6.25	0.70	190
Formoterol	9.29	0.94	6.98	0.94	204

By studying the ability to relax fully or partially contracted guinea pig tracheal smooth muscle (induced by carbachol), Lemoine and Overlack (1992) found that formoterol was a partial agonist of beta$_2$ receptors. EC_{50}, (nM/l) and intrinsic activity are summarized in Table 3. Intrinsic activity of formoterol decreased when the muscle was fully contracted. Intrinsic activity was determined by the ability to activate adenylate cyclase. The partial agonist characteristics of formoterol was later confirmed by Jeppsson *et al.* (1992).

Table 3 Intrinsic activity of beta 2 agonist

Agonist	Isoprenaline	Salbutamol	Fenoterol	Formoterol
Partially contracted (EC50, nM/ml)		20	5.6	0.29
intrinsic activity	1.0	0.67	0.67	0.89
Fully contracted (EC50, nM/ml		120	50	3.6
Intrinsic activity	1.0	0.62	0.62	0.77

2. ***Efficacy.*** Ida (1976) compared efficacy of 4 beta-agonists in preventing chemical- (histamine and methacholine) induced airway constriction in guinea pigs *in vivo*. The Table 4 presents ED_{50} values of some beta-agonists in preventing drug-induced airway constriction. Formoterol was 16 (SC), 40 (PO) and 4 (inhalation) times more potent than albuterol in preventing histamine-induced airway constriction. Formoterol was also more potent than albuterol in preventing methacholine induced- (15 fold) and anaphylactic (13 fold) airway constriction. On the other hand, Tokuyama *et al.* (1991) found that inhaled formoterol was 35 times more potent than salbutamol in inhibiting airway flow obstruction induced by histamine in guinea pigs. Inhalation formoterol was 30 times more potent than oral administration in preventing histamine-induced airway constriction in guinea pigs.

Table 4. ED50s of beta-agonists in preventing drug-induced airway constriction in guinea pigs

	Histamine-induced			Methacholine -induced	Anaphylactic
	SC (µg/kg)	PO (mg/kg)	Inhalation (µg/kg)	SC (µg/kg)	SC (µg/kg)
Isoproterenol	15.7	1.2	4.0	11.2	10.6
Orciprenaline	264	>100	250	457	333
Tremetoquinol	11.3	1.1	3.1	12.4	7.7
Albuterol	33.1	2.4	8.5	27.7	19.0
Formoterol	2.0	0.06	2.6	1.7	1.6

3. ***Potency of isomers.*** Formoterol possesses four isomers: RR, SS, SR, RS. Murase *et al.* (*1978*) studied potency of the four isomers in isolated guinea pig tracheal preparation. Results fromthis study are summarized in Table 5.

Table 5. Relative potency of formoterol enantiomers (isoproterenol is 1.0)

	Alpha	Beta	Potency*
Isoproterenol			1.0
Formoterol			
Racemic 1A			0.1
(-)-1A	R	R	0.08
(+)-1A	S	S	0.31
Racemic 1B			0.91
(-)-1B	S	R	1.1
(+)-1B	R	S	0.2

Data indicated that the SR isomer was the potent enantiomer; however, concentrations of the enantiomers and the inducing agents are unknown. On the other hand, Trofast *et al. (1991)* found that in a isolated carbachol-induced guinea pig trachea model, the RR isomer was the most potent while the SS form was the least potent (Table 6). SR and RS forms were in between. Other studies conducted by the sponsor also showed that the RR isomer is the more potent. Clinical formulations of formoterol are the racemate of RR and SS enatiomers (ratio of 1 to 1).

Table 6. PD_2 values of formoterol isomers in guinea pig tracheal preparations

Batches	RR	RS	SR	SS
1	9.63	8.11	7.30	7.90
2	10.03	8.19	-	-
3	9.89	7.87	7.83	6.96

4. ***Affinity to beta$_2$-receptor.*** Tasaka (1986) reported that the affinity of formoterol to beta$_2$ receptor was 700 times higher that salbutamol in the isolated lung membrane fraction.

*5. **Inhibition of anaphylaxis.*** Formoterol inhibited mouse IgE-mediated passive cutaneous (PCA) and passive peritoneal (PPA) anaphylaxis in rats. Intravenous and oral ED50s in rat PCA were 1.2 and 260 μg/kg respectively. This inhibitory effect was 6.3 and 33 times more potent than salbutamol. The inhibitory effect on anaphylaxis was probably attributed to an inhibition of antigen-induced releases of the mediator such as SRS-A (*Tomioka et al, 1984),* histamine, calcium ionophores, leukeutrien B_4 and interleuken-1β. Compared to albuterol, formoterol is 6 - 200 times more potent, depending on experiment conditions. Adenvier *et al. (1992)* Found that the inhibition of histamine and bradykinin induced vasopermiability by formoterol occurred at higher concentrations than the concentration needed to relax constricted tracheal smooth muscles.

6. Duration of action Nials *et al. (1990)* reported that formoterol was a relatively short-acting agent, being intermediate between salbutamol and salmeterol. Coleman *et al.* (1992) reported that in the electronically stimulated and contracted guinea pig trachea muscle in vitro, 50% recovery of time after drugs were drawn were 3.0, 4.5, 6.7 and 275 minutes for isoproterenol, albuterol, formoterol and salmeterol, respectively. Jeppsson *et al. (1993)* reported that in a isolated perfused and ventilated guinea pig lung model, action duration of aerosol formoterol and salmeterol were also longer than that of terbutaline.

7. Stimulation of mocuciliary activity Lindberg *et al. (1995)* reported that beta-agonists increased ciliary beat frequency *in vitro* in guinea pig trachea preparations and *in vivo* in the rabbit maxillary sinus. Formoterol was 100 times more potent than terbutaline in vitro and 2 fold longer in action duration *in vivo*.

B. Secondary Pharmacology

Tasaka *(1986)* reviewed the safety pharmacology of formoterol. Formoterol may affect [redacted] behavior, hemodynamics, respiratory patterns of muscle contraction, metabolism depending upon the dose of administration in animals. Behaviorally, oral formoterol at doses of ≥ 0.1 mg/kg caused an increases in respiratory rate, an decrease in motor affected responses and miosis, salivation, and ptosis in mice. The same dose levels caused a decrease in appetite and an increase in respiratory rate, exophthalmus, flush of pinna, urination, and defecation were observed in rats. In guinea pigs, the same dose caused hyperirritability, an increase in respiratory rate, lacrimation and flushing. In dogs miosis, salivation, yawning, barking, hypersensitivity and staggering were seen. An increase in respiration rate, ptosis, weak piloerection, and flush of the face were observed in monkeys. In cats at oral doses of ≥ 0.01 mg/kg, formoterol elicited an increase in respiratory rate, yawning, tremor, flushing, nausea, vomiting, lacrimation and ptosis. In dogs at a oral dose of 0.001 mg/kg, formoterol caused tremor, and at 0.003 mg/kg congestion of the eye and lacrimation. Formoterol (1 - 1000 mg/kg, PO) had no effect on convulsion induced by maximal electrochock, muscle relaxant activity and rotarod performance. Formoterol prolonged thiopental sleeping time at oral dose of ≥ 100 mg/kg. Formoterol (1.0 mg/kg, PO) had no effect on electroencephalogram in immobilized cats.

In anesthetized dogs, formoterol lowered diastolic pressure (0.1 μg/kg, ID) and systolic pressure (10 μg/kg, ID). In conscious dogs, formoterol lowered both diastolic and systolic pressures at doses of either 10 μg/kg, PO or 0.1μg/kg, IV. Formoterol induced tachycardia in dogs (1.0 μg/kg, PO), cats (10 μg/kg, PO), guinea pigs (10 μg/kg, PO), and monkeys and rats (30 μg/kg, PO). The effect of formoterol was equal potent to that of isoproterenol. Formoterol (1.0 μg/kg, IV) produced depressed R-wave, ST-depression and decrease of the T-wave amplitude in anesthetized dogs. Formoterol (1.0 μg/kg, IV) also caused a decrease in coronary arteriovenous oxygen difference and an increase in the maximum rate of the rise in left ventricular pressure, myocardial oxygen consumption, cardiac output, and stroke volume.

Formoterol at a dose of 10 μg/kg (ID) caused inhibitions of both the hypertension induced epinephrine (5 μg/kg, IV) and acetylcholine-induced hypotension (0.5 μg/kg, IV) in dogs. Formoterol also inhibit spontaneous motility of the uterus (≥ 10^{-10} g/ml). At doses of ≥ 0.1 μg/kg elicited an increase in pyruvic acid and lactate levels in dogs serum. Blood sugar and free fatty acid levels were also elevated after 10 μg/kg, PO. An isolated guinea pig tracheal strip model showed that SS-formoterol and (S)-terbutaline did not induce airway hyperreactivity (vol. 12.6, p 135 and 160, submitted on 4/26/94).

II. PHARMACOKINETICS

The following summary of pharmacokinetics of formoterol (Table 7) is based on a study by Sasaki *et al.* (*1983*) and reviews of Tasaki (1986) and Faulds (1991).

Table 7. Pharmacokinetics of Formoterol

Parameter	Rat	Dog	Human
Inhal. dose (μg/kg)	90	2.1	0.5
AUC (nmol.h/l)	96.0	21.4	0.31
$t_{½}$ (hour)	1.7	4	1.7 - 2.3
T_{max} (hour)	0.5	1 (oral)	-

In an isolated and perfused guinea pig lung model conducted by the sponsor, steady tissue formoterol concentration was reached one hour after intratracheal and intravenous administration. At the end of the perfusion, 35% and 27% of the perfused dose were recovered for RR and SS, respectively.

Distribution. After oral administration of ^{3}H-formoterol (50 μg/kg), the maximum concentration of radioactivity in tissue was reached at 0.5 hours in rats. The concentration was highest in kidney and liver, followed by plasma, trachea, lung and in the heart in this order. The brain contained the lowest level.

Metabolism Glucoronide conjugation was the major route of formoterol metabolism in rats

and dogs *(Sasaki et al., 1982)* and humans *(Tasake, 1986)*. Major metabolic pathways of formoterol metabolism are presented in Fig. 1. Urine metabolites were identified using thin-layer chromatography, high performance liquid chromatography and mass spectrometry and radiolabeled (^{3}H)- formoterol. In blood both metabolites and the unchanged forms existed. In rats 15 minutes after formoterol administration, the unchanged drug accounted for 64% and 58% of the plasma radioactivity for oral and intravenous route, respectively. Recent study by the sponsor indicated that most mammalians metabolized both the RR and SS enantiomers of formoterol at approximately the same rate (Table 8).

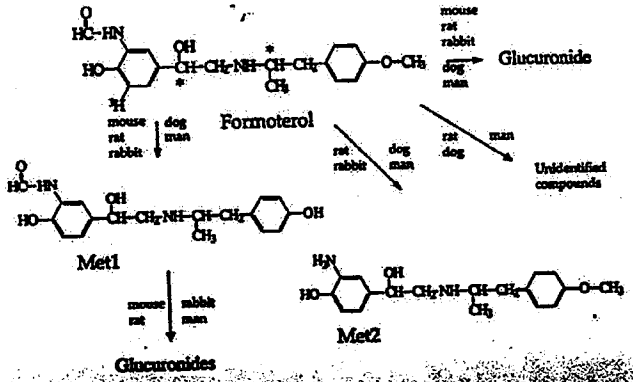

Fig. 1. Proposed metabolic pathways for RR and SS-formoterol.

In addition to glucoronide conjugation, O-demethylation of formoterol was also identified. The metabolic pathways were qualitatively similar across species. The conjugated formoterol metabolites are pharmacologically inactive.

Table 8. The rate of metabolism of RR and SS-formoterol by liver microsomes in vitro (pmol/min/mg protein)*

	Mouse	Rat	Rabbit	Dog	Human
RR	57	213	99	34	5.7
SS	161	206	135	30	7.5

* Substrate concentration = 0.1 μmol/L.

Excretion Formoterol was eliminated through both urine and the bile across species (Table 9). Forty eight hours after oral administration in rats, 48% and 21% of the administered dose were recovered from the bile and urine, respectively. When the bile from of a rat having received formoterol was administered to another rat, 69% of the dose was absorbed by the second rat. This

indicated presence of the enterohepatic circulation. In mice urinary excretion of the unchanged formoterol were 17%, 10% and 3% for IV, IT and PO administration respectively.

Table 9. Urinary and Fecal Excretion of Radioactivity from ^{3}H-Formoterol (0 - 72 hours)

	Mouse			Rat		Dog			Human
Route of adm.	IV	IT	PO	PO	PO	IV	PO	PO	IV	Inhal.
Dose (μg/kg)	100	100	100	50	5000	500	50	100	10	24/subj.
Urine (%)	75±9	82±5	76±7	45±4	24±3	39± 3	36±1	36	42±4	24
Feces (%)	20±8	15±5	2±6	51±7	68±8	56±8	52±1	50	51±3	

Placental crossing: Following oral administration of ^{3}H-formoterol (50 μg/kg) to rat on day 15 of pregnancy, the respective maximal concentration in fetus and placenta were 35% and 38% of the maternal plasma. When formoterol was given to lactating rats, milk and infant tissue contained 39% and 2% of the maternal plasma concentration, respectively.

Bioavailability: Limited data on the bioavailability of formoterol is available. Table 10 presents data from a mouse study and a human study.

Table 10. Bioavailability of Formoterol in Mice and Human

	Mouse	Human
Oral	15.5	9.6
Inhalation		24
Intratracheal	51.8 - 61.3	

After oral administration formoterol was slightly more bioavailable in mice than in human. There was no difference in the bioavailability between the RR and SS enantiomers in mice in either oral or intratracheal administration.

Protein Binding: Protein binding of of formoterol was determined *in vitro* (Table 11)

Table 11. Protein binding (%) of RR and SS-formoterol in vitro

	Rat	Rabbit	Dog	Human
RR	57.7	56.6	54.1	54.1
SS	54.8	45.7	51.2	41.9

* Substrate concentration = 0.5 μmol/L.

Summary: Formoterol is readily absorbed after oral and inhalation administration. Distribution of the drug after inhalation exposure is in the following order: trachea > lung > kidney > liver plasma > heart > brain. Half-lives of the drug after inhalation administration are 2 to 4 hours. Glucoronide conjugation and O-demethylation are the major pathways of formoterol

metabolism. The rate of formoterol metabolism *in vitro is the* following order: rabbits > mouse > dogs > humans based on mg protein. There is no difference in the rate of metabolism between the RR and SS enantiomers. Formoterol was eliminated through both urine and the bile across species. Enterohepatic circulation existed in rats. Bioavailability in mice is slightly higher than that in humans. Protein binding is similar across species. Milk and fetal plasma contains about one-third of the plasma formoterol concentration in lactating and pregnant rats. There are no differences in metabolism, bioavailability and protein binding of the drug between the RR and SS enantiomers.

Toxicokinetics

Toxicokinetics of formoterol was studied in mice, rat and dogs with treatment duration of up to 12 months. The AUC values from a 6-month inhalation study in rats (T2579) is given in Table 12: Data showed that plasma formoterol concentration did not increase over time as the treatment progressed. Other studies gave the same trend. This suggested that formoterol did not accumulate in the body.

Table 12. AUC (nmol.h/l) in a 6-Month Inhalation Study in Rats.

	Males			Females		
Dose (ug/kg/day)	2	15	100	2	15	100
Day 8	-	14.4	60.3	-	14.3	73.6
Day 176	-	19.1	60.2	-	15.4	73.6

Table 13 is a summary of the toxicokinetic data from other toxicity studies. Data indicated that, at the same dose levels, respective plasma formoterol concentrations by inhalation in dogs and rats were approximately 6 and 300 times that by oral exposure. Two factors possibly contributed to the difference: 1) the hepatic metabolism, and 2) oral consumption during inhalation exposure. The relative significance of either of the two factors are unknown.

Table 13. AUC (nmol.h/l) in preclinical toxicological studies.

Route	Species	Duration	Study No.	Dose (ug/kg/day)	Mean AUC (nmol.h/l/ug/kg) By study	by Species	AUC Ratio inhal./oral
Inhalation	Dogs	5 days	T3006	0.07, 0.4, 2.1	3.5		
"	"	1 month	T3120	"	6.5	5	6.1
"	Rats	3 months	T2433	9, 22, 90	2.0		
"	"	"	T3137	2.6, 13, 67	0.82	1.22	297
"	"	6 months	T2860	2.6, 12, 72	1.16		
Oral	Dogs	1 month	T2579	2, 15, 90	0.83	0.82	-
"	"	12months	T3077	0.72, 8.6, 92	0.80		-
"	Rats	3 months	T2578	0.1, 1, 10 mg	0.0041	0.004	-
"	Mice	3 months	T3136	200, 800, 3000	0.01	0.01	-

III. TOXICOLOGY

Toxicology

Single doses (acute toxicity studies):

A. Inhalation studies:

1. Acute inhalation toxicity of formoterol in rats: Study No. T2514.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	91115, Report date -3/26/93
GLP:	Yes
Dose:	3.8 (L), 8.3 (M), 18 (H) mg/kg
Batch No.	100/91

Thirty Wistar rats (5/sex/dose) were exposed to 5.0 - 7.5 mg formoterol/L air in a nose-only exposure chamber for a duration of 13 - 60 minutes. The respective estimated doses were 40, 88 and 199 mg/kg for the inhaled and 3.8, 8.3 and 18 mg/kg for the lung deposition. Surviving animals were sacrificed for macroscopic and microscopic examinations on day 14 post exposure. There was no control group.

Seven animals in the low and high dose group died post exposure. Distribution of the death is shown as the following:

	[illegible]	[illegible]	[illegible]
Male	1/5	0/5	3/5
Female	1/5	0/5	2/5

Formoterol caused a typical response of a beta-agonist: increased heart rate, loss of body weight, decreased motor activity, labored breathing and wheezing, especially in the high dose group. Tachycardia was accompanied by increases in QRS amplitude and epitopic foci. Pathological examinations revealed pulmonary congestion, and cardiac necrosis, fibrosis and cell inflammation.

Conclusion: Inhalation of single dose of formoterol caused a typical beta-agonist response. The lethal dose was as low as 3.8 mg/kg. The target organ of toxicity was the heart.

2. Acute inhalation toxicity of formoterol in mice: Study No. T2807.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	93082
Study dates:	7/29/93 - 8/19/93, Report date - 4/29/94
GLP:	Yes
Dose:	36 (L), 100 (M), 276 (H) mg/kg

Batch No. 105/92

Methods:

Thirty mice (CD-1, 5/sex/dose) were exposed to 0.44 - 3.4 mg formoterol/L air in a nose-only exposure chamber for 60 minutes. The respective estimated doses were 36, 88, 276 mg/kg for the inhaled and 4.2, 7.5, 24 mg/kg for the lung deposition. Surviving animals were sacrificed for macroscopic and microscopic examinations on day 14 post exposure. There was no control group.

Results:

Mortality: None.

Clinical signs (daily): Decreased motor activity was seen 15 - 40 minutes after the exposure in all groups. In addition, rats in the mid and high dose groups showed changes in respiration patterns: decreased frequency and abdominal respiration. Piloerection and partial closed eyes were also seen.

Body weight (day 1, 3, 7, 14): No dose-related effect was seen in mean body weight gain.

Organ weight: Increases in absolute and relative organ weight of the lung and heart were observed in the high dose males.

Pathology: No dose-related effects were seen.

Conclusion: Mice tolerated formoterol well for single inhalation doses up to 24 mg/kg.

B. Non-inhalation exposure studies:

1. Single dose oral toxicity of Formoterol in young rats: Study No. T2815.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	93069
Study dates:	9/30/93 - 11/12/93, Report date - 3/26/92
GLP:	Yes
Dose:	600 (L), 1200 (LM), 2400 (MH), 4800 (H) mg/kg
Batch No.	102/92

Methods:

Young Sprague-Dawley rats (— SPRD, age of 6 - 12 days, 6/sex/dose) were given orally a bolus dose of 600, 1200, 2400, and 4800 mg/kg formoterol, respectively. The vehicle consisted of cellulose (hydroypropylmethyl, 200 g), citric acid monohydrate (30.8 g), sodium phosphate dibasic (90 g), and distilled water (40 kg). Formoterol concentration in the highest dose group was 480 μmol/ml. Surviving animals were terminated on day 14 for gross and microscopic examinations. There was no control group.

Results:

Mortality: Dose-related increase in deaths were observed within 3 days post exposure (Table 14).

Table 14. Mortality incidences of Young Rats after Oral Dose of Formoterol

Dose (mg/kg)	600	1200	2400	4800
Male (death#/total)	0/6	1/6	6/6	6/6
Female (death#/total)	0/6	2/6	6/6	6/6

Animals showed decreased motor activity and changes in respiration pattern and/or frequency, decreased mean body weight, and ectopic foci and ST-T elevation in EKG. The common pathological findings are limited to the heart and lung, especially in the high dose group. The cardiac lesions included necrosis, inflammatory cell reaction, interstitial edema and/or fibrous scarring. Lung lesions were pulmonary inflammation.

Conclusion: Oral formoterol at doses of 600 mg/kg or higher may result in lesions in the lung and heart in rats. The cardiac lesions were typical of β_2-agonists. The LD_{50} of formoterol in rats was between 1200 mg/kg and 2400 mg/kg.

2. Acute oral toxicity in mice: Study No. T2843.

Testing lab: Laboratory of Safety Assessment, AB Astra, Sweden
Study number: 93089
Study dates: 9/30/93 - 10/19/93, Report date - 6/30/94
GLP: Yes
Dose: 2000 mg/kg
Batch No. 105/92, 100/93

NMRI mice (5/sex) were given orally a bolus dose of 2000 mg/kg formoterol and sacrificed on day 14 day for necropsy. Vehicle were the same as in study T2853. No mortalities and gross abnormalities were observed. Abnormal signs included decreased motor activity, salivation, chromodacryorrhea and occasional abdominal respiration.

3. Acute oral toxicity in rats, Study No. T2842.

Testing lab: Laboratory of Safety Assessment, AB Astra, Sweden
Study number: 93069
Study dates: 9/27/93 - 10/15/93, Report date - 6/30/94
GLP: Yes
Dose: 210 (L), 1500 (M), 2000 (H) mg/kg
Batch No. 105/92, 100/93

Sprague-Dawley rats (5/sex/dose) were given orally a bolus dose of 210, 1500, and 2000 mg/kg formoterol respectively. They were killed for necropsies on day 14. Vehicle was the same as the study T2853. The results were similar study T2843, However, decrease in motor activity, salivation, chromodacryorrhea and occasional abdominal respiration were dose-related.

Multiple doses:

A. Inhalation studies:

1. 5-day inhalation dose range toxicity study in rats: T2460.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	90076
Study dates:	5/30/90 - 6/1/90, Report date - 11/25/91, revised on1/26/96
GLP:	Yes
Dose:	0 (C), 0.02 (L), 0.1 (M), 0.5 (H) mg/kg
Batch No.	105/92

Methods:

Young Sprague-Dawley rats —CD, BR; age of age 7 - 8 weeks, 6/sex/dose) were exposed to 3.2 - 99 μg formoterol/L air in a nose-only exposure chamber for 60 minutes. The control group received lactose only (0.2 - 11 mg/L air). The respective estimated doses of formoterol in the treated groups were 0, 0.12, 0.8, 3.7 mg/kg for inhaled and 0, 0.02, 0.1, 0.5 mg/kg for the lung deposition. The estimated mass median aerodynamic diameter (MMAD) was —— Surviving animal were sacrificed at the end of the study for gross and microscopic examinations. Plasma drug concentrations were determined in satellite groups but results were not included in this report.

Results:

Mortality: None.

Clinical signs (daily): No treatment-related effects were observed.

Body weight (day -5, 0, 4): A marked increase in body weight gain was observed in both sexes, especially in the females. (♂: 64%-L, 53%-M, 42%-H; ♀: 192%-L, 208%-M and 213%-H).

Food consumption: Normal.

Organ weights (lung and heart only): Increases in absolute (↑10%) and relative (↑12%) lung weights were seen in all treated male groups; however, the increase in relative lung weight was not statistically significant. Increases in absolute (16 - 25%) and relative (11 - 22%) heart weights were seen in the females, with the increase in the mid dose group being most pronounced.

Pathology (heart, nasopharyngeal region, larynx, trachea, carina, lung only): Two males in the high dose group had solitary microfocal leukocyte foci in the heart.

Conclusion: Inhalation of formoterol at doses of 0.5 mg/kg/day for 5 days resulted in cardiac toxicity. This indicated that the heart was the target organ of toxicity.

2. 5-day inhalation dose-ranging study in dogs (T3006).

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	93058
Study dates:	06/07/93 - 06/18/93, Report date - 04/20/95
GLP:	Yes
Dose:	0, 0.07 (L), 0.4 (LM), 2.1 (H) μg/kg/day
Batch No.	100/92

Methods:

Beagle dogs (age of 9 - 12 months, 4/sex/dose) were exposed to 0.44 - 11 μg formoterol/L air in a nose-only exposure chamber for 2.5 - 6 minutes daily for 5 days. The control group received lactose only (0.02 - 0.55 mg/L air). The respective estimated doses of formoterol in the treated groups were 0, 0.5, 2.9 and 15 μg/kg for the inhaled, and 0, 0.07, 0.4, 2.1 μg/kg for the lung deposition. The MMAD was [redacted]. Plasma drug concentration were determined. These animals were sacrificed on day 5 for gross and microscopic examinations.

Results:

Mortality: None.

Clinical signs (daily): Hyperemia of the skin and mucosa as well as increased heart rate were observed (table 15).

Table 15. Percentage of increases in heart rate one hour after formoterol inhalation

Dose (μg/kg/day)	0	0.07	0.4	2.1
Mucosal hyperemia	0	1/8	1/8	2/8
Cutaneous hyperemia	0	0	0	1/8
Heart rate Day 0, first hour	-	↑ 5%	↑ 31%	↑ 79%

No drug-related effects were observed in the following parameters: body weight (daily), food consumption (daily), rectal temperature (days 0, 4), clinical chemistry (Day -2, and 0), necropsy (terminal), and organ weights. The parenthesis indicate times when the observation was made.

Plasma drug concentration (days 0, 3): Plasma $AUC_{0\text{-}4\ h}$ were 0.8 - 1.0 and 6.6 - 9.0 nmol/l.h for the mid and high dose groups, respectively. $AUC_{0\text{-}24\ h}$ (13.1 - 21.4) was available for the high dose group only. No sex difference in plasma drug concentration was observed.

Conclusion: No apparent toxic effect except increased heart rate was observed at doses up to 2.1 μg/kg/day.

3. One-month inhalation toxicity study in dogs: T3120.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	93087
Study dates:	8/27/93 - 09/29/93, Report date - 12/04/95
GLP:	Yes
Dose:	0, 0.07 (L), 0.4 (LM), 2.0 (H) μg/kg/day
Batch No.	100/92

Methods:

Beagle dogs (age of age 7 - 11.5 month, 3/sex/dose) were exposed to 0.02 - 11 μg formoterol/L air in a nose-only exposure chamber for 4.5 - 6 minutes daily for 3 months. The control group received lactose only (0.02 - 0.53 mg/L air). The respective estimated doses of formoterol in the treated groups were 0, 0.5, 2.8 and 15 μg/kg for the inhaled, and 0, 0.07, 0.4, 2.0 μg/kg for the lung deposition. The estimated MMAD was [redacted] f. Plasma drug concentration were determined. Animals were sacrificed for macroscopic and microscopic examinations on day 90.

Results:

Mortality: None.

Clinical signs (daily): Hyperemia of the skin and mucosa were observed. Incidences these observations are shown as following:

Dose (μg/kg/day)	0	0.07	0.4	2.0
Mucosal hyperemia	2	5	24	74
Cutaneous hyperemia	0	0	1	8

Body weight (weekly): Normal.
Food consumption (daily): Normal.
Rectal temperature (weekly): Normal.
EKG (days 0, 7, 21): Dose-dependent tachycardia were seen (Table 16):

Table 16. Percentage of increases in heart rate one hour after formoterol inhalation

Dose (μg/kg/day)	0	0.07	0.4	2.0
Day 0	↑ 7%	↑ 13%	↑ 46%	↑ 76%
Day 21	↑ 2%	↑ 14%	↑ 37%	↑ 77%

Ventricular tachycardia (intermittent ventricular tachycardia) occurred in 3/6 dogs in the high dose group on day 0.

Ophthalmology (weeks -1 & 13): Normal.

Plasma drug concentration (day 0, 6 and 21): Drug concentrations were 0.7 - 1.7 and

13.7 - 23.2 nmol/l.h for the mid and high dose groups, respectively. No sex difference in plasma drug concentration was observed.

Clinical chemistry (weeks -1, 1 and 2):

Hemotology: Dose-related decreases in RBC numbers (↓14% - 18%) and hemoglobin concentrations (↓14% - 20%) were observed in the treated males.

Blood chemistry and urinalysis: Normal.

Clinical pathology (terminal): No treatment-related effects were seen.

Organ weights (lung and heart only): Decreases in relative and absolute spleen weight (↓ 22% - 29%) were observed. Relative heart weight in the high dose groups was decreased by 10% (not statistically significant). There were no changes in the brain and testes weights.

Pathology (heart, nasopharyngeal region, larynx, trachea, carina, lung only): cardiac fibrosis was seen in the mid and high dose groups (Table 17).

Table 17. Incidences of Cardiac Lesions in the One-month Oral Study in Dogs

	Males				Females			
Dose (µg/kg/day)	0	0.07	0.4	2.0	0	0.07	0.4	2.0
Animals examined	3	3	3	3	3	3	3	3
Fibrosis	0	0	1	3	0	1	0	2
Ave. grades	0	0	2	2.3	0	2.0	0	3

Conclusion: The heart is the target organ of toxicity. No NOAEL level was defined by the study. The sponsor claimed that only the cardiac effect (fibrosis) in the high dose is treatment-related. The sponsor claimed that the cardiac changes in the low and mid dose group were not treatment related because it is a frequent observation in the control group. Evaluation of the data, however, showed that none of the 6 control dogs showed any myocardial fibrosis (0/6-C, 1/6-L, 1/6-M and 5/6-H).

4. 3-month inhalation toxicity study in rats: Study No. T2433.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	90106
Study dates:	9/14/90 - 12/18/90, Report date - 9/13/91, revised on 1/23/95
GLP:	Yes
Dose:	0 (C), 9(L), 22 (M), 90 (H) μg/kg
Batch No.	105/92

Methods:

Three groups of Sprague-Dawley rats —CD, BR; age of 10 weeks, 10/sex/dose) were exposed to 2.4 - 25 μg formoterol/L air in a nose-only exposure chamber for 60 minutes daily for 3 months. Another group was given lactose (0.1 - 1.3 mg/L air) to serve as the vehicle control. The respective estimated doses of formoterol in the treated groups were 0, 82, 260 and 870 μg/kg for inhaled and 0, 9, 22 and 90 μg/kg for the lung deposition (from the discussion). The estimated MMAD was [redacted]. These animal were sacrificed on day 91 for necropsy and histological examinations.

Results:

Mortality: One male rat in the high dose group died on day 21. Necropsy revealed cardiac and renal congestion and slight pneumonitis, although no clinical findings were recorded before the sudden death. Contribution of drug treatment could not be excluded due to its cardiac finding. Other 4 (2-L, 1-M, 1-H) died of blood sampling on day 23.

Clinical signs (daily): No treatment-related effects were observed.

Body weight (weekly): Increases in body weight gain were seen in all treated groups (↑10%-L, ↑6%-M, ↑20%-H at week 13).

Food consumption (weekly): Normal.

Heart rate (weeks 0, 1, 4 and 11): Dose-related increases in heart rate were seen: 19%-L, 19%-M and 29%-H).

Clinical chemistry (weeks 3 and 12):

Hemotology: Dose-dependent decreases in platelet numbers (12% - 17%) were seen in both sexes at both monitoring occasions. Increases in hematocrit and reticulocytes were small <10%).

Blood chemistry: Dose-dependent decreases in plasma glucose levels (9% - 32%) were seen in both sexes. The depletion was more pronounced at week 12 than at week 3. Dose-related increase in blood urea concentrations (9% - 25%) was also observed. However, both parameters were within the normal range of the laboratory's references.

Urinalysis: No treatment-related effects were observed.

Organ weights: Increases in absolute (17%) and relative (↑12%) heart weights were seen in treated female groups. The same trend existed in the male although it was less pronounced. Other changes included decreases in brain weight (both sexes) and increases in lung

weight (♀, ↑ 15%). Table 18 lists only changes in absolute organ weights (vs control) because formoterol tends to increase body mass, which results in decreases in relative tissue weights.

Table 18. Changes in Relative Heart and Brain Weight in the 3-month Inhalation Study in Rats.

	Males			Females		
Group	9	22	91	9	22	91
Heart $_{abs.}$ (Δ%)	↑ 13	↑ 10	↑ 6	↑ 17	↑ 11	↑ 17
Brain $_{abs.}$ (Δ%)	↓ 5	↓ 3	↓ 5	↓ 4	↓ 4	↓ 4

Pathology:

Necropsy: No treatment related effects were observed.

Histopathology: Cardiac congestion and granulating myocardial scars were seen in the control and high dose males (1/10-C and 2/10-H). Incidences of pneumonitis were low (1/10♂-H, 2/10♀-L, 1/10♀-M and 1/10♀-H) and lack of dose-response relationship.

Plasma drug levels: See Table 19. (nmol/l.h):

Table 19. AUC (nmol.h/l) in the 3-month Inhalation Study in Rats.

	Males			Females		
Group	L	M	H	L	M	H
Dose (μg/kg/day)	9	22	91	9	22	91
Day 8	28.6	52.3	111.6	22.8	37.6	80.3
Day 84	24.9	43.0	89.4*	26.4	42.3	68.0*

*Exposure concentration of this dose level was only 1/3 of the concentration on day 8.

Conclusion: Granulating myocardial scars were seen in both the control (1/10) and the high dose males (2/10). Low incidences of this observation suggested that myocardial fibrosis (scar) may not be treatment related. But cardiac hypertrophy, along with strong cardiac toxicity of the drug in other studies, suggests that the fibrosis may be a drug-related effect.

5. 6-month inhalation toxicity study in rats: T2860.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	90106
Study dates:	11/25/92 - 6/10/93, Report date - 9/8/94, revised on 1/26/95
GLP:	Yes
Dose:	0 (C), 2.3 (L), 12 (M), 72 (H) μg/kg
Batch No.	100/92

Methods:

Three groups of Sprague-Dawley rats (20/sex/dose) were exposed to 0.71, 3.6 and 24 μg formoterol/L air in a nose-only exposure chamber for 60 minutes/day for 6 months. The control group received lactose (1.4 mg/L air) only. The respective estimated doses of formoterol in the

treated groups were 0, 26, 128 and 852 μg/kg/day for inhaled and 0, 2.3, 12 and 72 μg/kg/day for the lung deposition. The estimated MMAD was . ——— These animal were sacrificed on day 181 for necropsy and histological examinations.

Results:

Mortality: There were no apparent treatment-related mortalities. A total of 7 deaths (6♂ & 1♂) occurred during the study, but many of them (5) died of blood sampling. However, the mortality was slightly higher in the high dose male group (♂: 1-C, 1-L, 1-M & 3-H; 1-H♀) and one of the male rats in this group showed myocardial fibrosis.

Clinical signs (daily): No treatment-related effects were observed.

Body weight (weekly): Dose-dependent increases in body weight gain (week 13) and body weight (week 26) were seen in the females (Table 20). The trend was not as obvious in the males.

Table 20. Body Weight Changes in the 6-month Inhalation Study in Rats

	Male			Female		
Dose (ug/kg/day)	2.3	12	72	2.3	12	72
% Body weight gain (%5) week 13	-	↑ 15	↑ 5	↑ 7	↑ 15	↑ 26
% body weight change week 26	↑ 2	↑ 7	↑ 5	↑ 4	↑ 6	↑ 10

Food consumption (weekly): Dose-related increases were observed in the mid and high dose groups starting from beginning of the experiment. This increase in the low dose group was also frequently statistically significant.

Water consumption (weeks 1, 7, 11 & 25): No treatment-related effects were observed.

Ophthalmology (pre-dosing & month 5): No treatment-related effects were observed.

Heart rate (weeks 1, 6, 12, 15, 18, n=3/sex): Dose-related increases in heart rate were seen: (15%-L, 23%-M and 22%-H).

Clinical chemistry (weeks 5, 13 & 24):

Hemotology: **No apparent treatment-related effects were observed. Occasional statistically changes were seen in several parameters (Hgb, Hct, RBC#, platelet#) were observed. But most changes were within normal reference range of the laboratory.**

Blood chemistry: Decreases in plasma glucose levels (14% - 25%) were seen in both sexes; however, only the females showed a dose-response relationship. Slight increase in blood urea concentration was also observed. All these numbers were within the normal range of the lab's references.

Urinalysis: No treatment-related effects were observed.

Organ weights: Increases in absolute heart weights were seen in both treated males and females (Table 21). Decreases in the weights of the brain, thymus and prostate were seen in males only:

Table 21. Organ Weight Changes in the 6-month Inhalation Study in Rats

	Males			Females		
Group	L	M	H	L	M	H
Dose (ug/kg/day)	2.3	12	72	2.3	12	72
Heart abs. (%)	↑ 12	↑ 13	↑ 11	↑ 8	↑ 19	↑ 15
Brain abs. (%)	↓ 4	↓ 3	↓ 5	↓ 5	-	-
Thymus abs. (%)	↓ 4	↓ 15	↓ 15	-	-	-
Prostate abs. (%)	↓ 8	↓ 7	↓ 10			

Because of the increases in body weight by the drug, decreases in the relative weights of many organs were seen. Emphasis on relative organ weights may be misleading.

Pathology:

Necropsy: No treatment related effects were observed.

Histopathology: Myocardial fibrosis were seen in the mid and high dose groups (2/40-M, 6/40-H). The incidences were much higher in the male than the female (HD: 5♂ vs 1♀). Tubular atrophy of the testes was observed in one mid dose male.

Plasma drug levels: Drug concentration was determined on days 8 and 176 (n=1/sex/group). The limit of quantitation was 0.20 nmol/l when 2.0 ml plasma was used. AUC $_{0\text{-}9\,h}$ values are listed in Table 22 (nmol/l.h):

Table 22. AUC (nmol.h/l) in the 6-month Inhalation Study in Rats

	Males			Females		
Group	L	M	H	L	M	H
Dose (ug/kg)	2.3	12	72	2.3	12	72
Day 8	-	14.4	60.3	-	14.3	73.6
Day 176	-	19.1	60.2	-	15.4	73.6

Conclusion: Cardiac hypertrophy was observed in all the treated groups. Myocardial fibrosis was observed in the middle and high dose groups, especially in the male. The 6-month NOAEL value in rats was 2.3 µg/kg/day.

B. Oral studies

1. One-month oral toxicity study in dogs: T2579.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	92032
Study dates:	5/12/92 to 5/23/93, Report date - 8/31//95
GLP:	Yes
Dose:	0 (C), 2 (L), 15 (M), 100 (H) μg/kg/day
Batch No.	100/91

Methods:

Beagle dogs (3/sex/dose) were given orally formoterol in gelatin capsules daily for one month. The capsule vehicle consisted of 67 - 200 mg lactose and 0.7 - 2.0 mg magnesium stearate and appropriate amount of formoterol. The testing compound was found to be not stable in the vehicle. Formoterol doses were at least 2, 15, and 100 μg/kg/day based on the minimal stability data. Clinical observations and clinical pathology were performed during the study. At the end of the study, animals were sacrificed for pathological examinations.

Results:

Mortality: Not mentioned by the sponsor.

Clinical signs (daily): Hyperemia of the skin and mucosa, periorbital edema, and labored breathing were observed. Incidences these observations are shown in Table 23:

Table 23. Percentage of increases in heart rate one hour after formoterol inhalation

Dose (μg/kg/day)	0	2	15	100
Mucosal/cutaneous hyperemia	2	9	36	95
Periorbital edema	-	-	4	11
Labored breathing	-	1	2	10

Body weight (weekly): A trend of dose-related increase in the body weight was seen.

Food consumption (weekly): Normal.

Rectal temperature (weekly): Normal.

Ophthalmology (weeks -1 & 4): No treatment-related effect was observed.

EKG (days 0 & 21): Sinus tachycardia and ventricular arrhythmia were observed, especially in the high dose groups (Table 24).

Table 24. Percentage of Increases in Heart Rate (vs Control) Two Hours after Oral Formoterol

Dose (μg/kg/day)	0	2	15	100
Heart rate: Day 0	-	↑ 47	↑ 65	↑ 68
Day 21	-	↑ 40	↑ 60	↑ 62
Arrhythmia, ventricular	-	-	1/6	3/6

Clinical pathology: (weeks -1 & 4):

Hemotology: No apparent drug-related effects were observed.

Blood chemistry:

A slight increase in serum urea levels (<20%) was observed in the treated groups. There was no apparent dose-response relationship.

Urinalysis: No treatment-related effects were seen.

Organ weight: Slight decreases in the relative weights of the testes (↓19%), prostate (↓ 50%) and epidydimides (↓19%) were observed in the high dose males.

Pathology:

Gross phathology: Occasional gray-white foci were noted in the heart of the low (1/3) and high dose females (1/3).

Microcopic pathology: Incidences, severity and the affected areas of myocardial fibrosis were dose-related (incidences: 1/6-C, 0/6-L, 2/6-M, 4/6-H).

Toxicokinetics (day 0, 21): Plasma formoterol concentrations are shown Table 25: Formoterol concentrations were slightly higher on day 21 than day 0.

Table 25. Toxicokinetics of Formoterol in One-month Oral Toxicity Study

		Male			Female		
Dose μg/kg/day		2	15	100	2	15	100
C_{max}	Day 0	0.4	2.5	7.8	0.4	2.1	7.8
(nmol/l)	Day 21	0.4	3.0	12.4	0.4	3.4	13.9
AUC 0-24 hr	Day 0	-	7.1 ± 1.5	-	-	10.6 ± 0.2	72 ± 7
(nmol/l).h	Day 21		10.7 ± 4.3	107 ± 6		12.8 ± 4.3	69.2 ± 4.2

[illegible] Data not available

Conclusion: Treatment of dogs with oral formoterol at doses up to 100 μg/kg/day for one month resulted a typical toxicity profile of the β_2-agonists: myocardial fibrosis, tachycardia, peripheral hyperemia, especially in the high dose group. The target organ of toxicity was the heart.

2. 3-month oral toxicity study in mice: T2578.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	91118
Study dates:	9/11/9 - 12/17/91, Report date - 10/8/92, revised 1/26/95
GLP:	Yes
Dose:	0 (C), 0.1 (L), 1.0 (M), 10 (H) mg/kg/day
Batch No.	100/91

Methods:

CD-1 mice (12/sex/dose) were given formoterol by oral gavage for 3 months at the above doses. The vehicle consisted of citric acid monohydrate (30.8 g), sodium phosphate dibasic (67.5 g), sodium chloride (255g) and distilled water (up to 30 kg). Clinical observations and clinical pathology were performed during the study. Animals were sacrificed for pathological examinations at the end of the study. Satellite groups were used to determine plasma drug concentrations.

Results:

Mortality: None.

Clinical signs (daily): Decreased motor activity, increased respiratory frequency and cyanosis were seen in the high dose groups.

Body weight (weekly): A small but statistically significant increase (8%) in mean body weight was observed in the high dose females starting form week 3 and onward.

Food consumption (weekly): Normal or minimal increase was seen in the high dose groups.

Water consumption (weeks 1, 4, & 7 for control and the high dose): No treatment-related effects were seen.

Clinical pathology: (week 12): No treatment-related effects were seen. Two high dose male had a remarkable (17- 20 fold) increases in the serum ASL level, but histological changes were absent.

Hemotology: No treatment-related effects were seen.

Organ weight: Both absolute and relative weights of the spleen (28%) and liver (10%) was statistically significantly increased in the high dose females. The absolute and relative weight of adrenal glands was also decreased (26%) in the mid and high dose females. Brain weight was not determined.

Pathology:

Gross phathology: No treatment-related effects were seen.

Microcopic pathology: No apparent treatment-related effects were seen.

Toxicokinetics (days 14, 90): Plasma formoterol concentration was determined by HPLC with electrochemical detection. The lower limit of quantitation was 0.15 nmol/L. The results (Table 25.) showed that AUCs between day 14 and 90 similar.

Table 26. $AUC_{0-24\,h}$ (nmol.h/l) of Formoterol in 3-month Oral Toxicity Study in Mice

	Male			Female		
Dose (mg/kg/day)	0.1	1.0	10	0.1	1.0	10
Day 14	-	11.6	104.8	-	8.8	80.8
Day 90	-	10.7	> 72.6	-	9.1	89.5

- Data not available.

4. 3-month oral toxicity study in young rats: T3136.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	94031
Study dates:	5/18/94 to 8/31/94, Report date - 1/12/96
GLP:	Yes
Dose:	0.2 (L), 0.8 (M), 3 (H) mg/kg
Batch No.	100/93

Methods:

Young Sprague-Dawley rats (aged 16 days, 10/sex/dose) were given formoterol by oral gavage at the above doses for 12 months. The vehicle consisted of cellulose (hydroypropylmethyl, 200 g), citric acid monohydrate (30.8 g), sodium phosphate dibasic (90 g), and distilled water (40 kg). Formoterol stability data were acceptable. Clinical observations and clinical pathology were performed during the study. Animals were sacrificed for pathological examinations at the end of the study. Satellite groups were used to determine plasma drug concentrations.

Results:

Mortality: No treatment-related death was seen. One female rat in the high dose groups died of dosing technique on day 2.

Clinical signs (daily): No treatment-related effects were seen.

Body weight (every 3 days for first 3 weeks, then weekly): No treatment-related effects were seen. Poor randomization resulted in lower body weight in group 2 throughout this study.

Food consumption (weekly): Normal or minimal increase was seen in the high dose groups.

Water consumption (every 4 weeks): No treatment-related effects were seen.

Rectal temperature: Normal.

Ophthalmology (month 3): No treatment-related effect was observed.

Clinical pathology: (month1 and 3):

Hemotology: Slight dose-dependent increases in leukocytes (↑10%-L, ↑18%-M, ↑27%-H) were seen at week 12. Other observations were either sporadic or lacking of dose-response relationship. They were considered of little toxicological significance.

Blood chemistry. **Slight** Increases in the serum levels of glucose, urea and phosphate levels were observed (Table 27):

Table 27. Blood Chemistry Changes in the 1-month Oral Study in Dogs

		Male			Female		
Dose (mg/kg/day)		0.2	0.8	3.0	0.2	0.8	3.0
Glucose (Δ %):	Week 3	↓ 10	↓ 16	↓ 17	↓ 17	↓ 21	↓ 23
	Week 12	**↓ 24***	↓ 34	↓ 31	↓ 15	↓ 19	↓ 23
Urea (Δ %):	Week 3	↑ 14	↑ 14	↑ 20	↑ 23	↑ 30	↑ 25
	Week 12	↑ 11	↑ 19	↑ 25	↑ 15	↑ 26	↑ 31
P (Δ %), Week 12		↑ 4	↑ 4	↑ 10	0	↑ 5	↑ 10

* Bold indicates statistically different from the control.

Urinalysis: No treatment-related effects were seen.

Organ weight: Absolute heart weight was increased in the mid and high dose groups while both absolute and relative organ weights of the testis and brain were decreased (Table 28). Statistical differences were apparent in the high dose groups.

Table 28. Organ weight Changes in the 1-month Oral Study in Dogs

		Male			Female		
Dose (mg/kg/day)		0.2	0.8	3.0	0.2	0.8	3.0
Heart (Δ %):	Absolute	↑ 2	↑ 8	**↑ 14***	↓ 2	↑ 9	↑ 7
	Relative	0	↑ 5	↑ 9	↓ 4	0	↑ 2
Testis (Δ %):	Absolute	↓ 4	↓ 7	**↓ 21**			
	Relative	↓ 5	↓ 11	**↓ 25**			
Brain (Δ %):	Absolute	↓ 5	↓ 3	↓ 4	↓ 5	↓ 3	↓ 5
	Relative	↓ 3	↓ 5	↓ 8	↓ 6	↓ 12	↓ 11

* Bold indicates statistically significantly different from the control.

Pathology:

Gross phathology:

Testis: Dose-dependent increases in the incidence of small testes in the male. (0/10-C, 0/10-L, 1/10-M, 3/10-H.)

Microcopic pathology:

Heart: Myocardial granular scars were noted in the mid and high dose groups. (Incidences: 0/20-C, 0/20-L, 1/20-M, 3/20-H.)

Testes: The males showed testicular tubular atrophy (incidences: 1/10-C, 4/10-L, 1/10-M, 7/10-H), and spermatic debris (incidences: 0/10-C, 2/10-L, 1/10-M, 4/10-H) oligospermia in the epididymides (incidences: 0/10-C, 2/10-L, 1/10-M, 3/10-H)

Toxicokinetics (days 7, 91): Blood samples were collected before dosing and at 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing. Plasma formoterol concentration was determined by HPLC with electrochemical detection. The lower limit of quantitation was 0.15 nmol/L. The results (Table 29.) showed that half-life and C_{max} of the drug could be increased after repeated administration. This suggested that accumulation occurred after the repeated administration.

Table 29. Toxicokinetics of Formoterol in 3-month Oral Toxicity Study

		Male			Female		
Dose (ug/kg/day)		0.2	0.8	3.0	0.2	0.8	3.0
C_{max}	Day 7	0.3	0.6 ± 0.5	2.4 ± 0.2	-	0.7 ± 0.1	2.6 ± 0.4
(nmol/l)	Day 91	-	1.5 ± 0.5	4.0 ± 0.7	-	0.7 ± 0.2	3.1 ±0.6
$AUC_{0-8\,hr}$	Day 7	-	2.6	25.5	-	3.0	26.0
(nmol/l).h	Day 91	-	5.2	47.5	-	3.7	37.1

- Data not available.

Conclusion: Oral exposure of formoterol in young rats may induce myocardiac lesion (myocardial granular scar) at doses of 0.8 mg/kg/day or higher and testicular tubular atrophy at 3 mg/kg/day.

7. 12-month oral toxicity study in dogs: Study No. T3077.

Testing lab: Laboratory of Safety Assessment, AB Astra, Sweden
Study number: 92032
Study dates: 5/12/92 to 5/23/93; Report date - 8/31/95
GLP: Yes
Dose: 0.72 (L), 8.6 (M), 92 (H) µg/kg
Batch No. 100/91, 100/92, LOT#:1536:4-7DSD

Methods:

Forty beagle dogs were given oral formoterol in gelatin capsules daily for 12 months. The capsule vehicle consisted 67 - 200 mg lactose and 0.7 - 2.0 mg magnesium stearate and appropriate amount of formoterol. The testing compound was found to be not stable in the vehicle. Administered doses were the calculated minimal formoterol doses. Clinical observations and clinical pathology were performed during the study. At the end of 12 months, animals were sacrificed for pathological examinations.

Results:

Mortality: Not mentioned by the sponsor.

Clinical signs (daily): Formoterol caused a dose-related mucosal and cutaneous hyperemia. The incidences of mucosal hyperemia were 0%-C, 1%-L, 7%-M, 39%-H,

respectively. This lasted for up to 8 hours post dosing. Formoterol also causes dose-related white transversal lines in the claw keratin (0%-C, 6%-L, 47%-M, 60%-H) starting from 3 months of the exposure. However, the incidence declined over time and were seen in the high dose group only from 7 months onward (4 - 8%).

Body weight (weekly): Normal.

Food consumption (weekly): Normal.

Rectal temperature: Normal.

Ophthalmology (predosing, months 6, 12): No treatment-related effect was observed.

EKG (day 0 and months 3, 6, 9, 12): Sinus tachycardia was observed in all treated groups during the entire study period. Ventricular ectopic extrasystoles were seen in mid (1 dog) and high dose (7 dogs) groups on day 0, but was absent during the rest monitoring.

Clinical pathology: (day 0 and months 3, 6, 9, 12):

Hemotology: Occasional slight but statistically significant changes were seen in leukocyte numbers (↓ 6 - 18%), hemoglobin concentrations, hematocrit, erythrocytes and so on. The differences were small and sporadic. They were of little toxicological significance.

Blood chemistry:

A slight increase in serum glucose levels (25%) was observed in the high dose males at week 49. Increases in serum creatinine levels (22 - 53%) were noted in all doses in males and the mid and high doses in the female starting from week 10, but only the increases in the females were dose-dependent. Increases in serum urea (30 - 50%) were seen in the mid and high doses in males starting from week 10 and in the females staring from week 22. Creatinine and urea levels increased above the control in a dose-related manner (0/10-C, 1/10-L, 4/10-M, 5/10-H). An examination of family history showed that most dogs with high creatinine and urea levels were within the same family tree. Also, the magnitude of the increase was very small. They were considered of little toxicological significance. Other observations were either sporadic or lack of dose-response relationship and were of little toxicological significance.

Urinalysis: No treatment-related effects were seen.

Organ weight: No treatment-related effects were seen.

Pathology:

Gross phathology: Occasional gray-white foci were noted in the dorsal papillary muscle of a high dose female.

Microcopic pathology: Incidences and severity of myocardial fibrosis were dose-related (incidences: 0/10-C, 2/10-L, 3/10-M, 4/10-H; severity: 0-C, 1-L, 1.3-M, 2.3-H, with 3 being the most severe). Acinar atrophy of pancreas was seen in the high dose group only (3/10-H).

Toxicokinetics (day 0, months 1, 6, 12): Blood samples were collected before dosing and at 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing. Plasma formoterol concentration was determined by HPLC with electrochemical detection. The lower limit of quantitation was 0.15 nmol/L. The results (Table 30) showed that half-life and C_{max} of the drug could be increased after repeated administration; however, AUC was not remarkably different. The consistence of AUC value suggested that little accumulation occur after the repeated administration.

Table 30. Toxicokinetics of Formoterol in 12-month Oral Toxicity Study

		Male			Female		
Dose (µg/kg/day)		0.72	8.6	92	0.72	8.6	92
C_{max}	Day 0	0.15-0.23	0.63-3.1	3.5 - 15.2	0.15-0.34	0.6 - 4.8	4.8 - 16.3
(nmol/l)	Month 12	-	-	8.7 - 21.2	-	-	6.8 - 34.4
$AUC_{0\text{-}8\ hr}$	Day 0	-	5.3 - 6.0	57.6 -61.6	-	7.3 - 8.1	$67.3_{(1\ month)}$
(nmol/l).h	Month 12	-	-	-	-	-	78.4
$t_{½}$ (hr)	Day 0	-	3.2	6.2	-	1.7	6.5
	Month 12	-	3.8	8.3	-	8.0	5.3

- Data not available.

Conclusion: Treatment of dogs with formoterol at doses up to 12 months resulted a typical toxicity profile of the β_2-agonists: myocardial fibrosis, ventricular cardiac ectopics, tachycardia, peripheral hyperemia, and discoloration of the claw keratin and slight decreases in hematocrit and hemoglobin concentration, especially in the high dose group.

Reproductive toxicology:

A. Inhalation:

1. Segment II teratology study in rats: Study No. T2628.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	92064
Study dates:	8/25/92 - 10/02/92, Report date - 3/17/93, revised on 2/1/96
GLP:	Yes
Dose:	0 (C), 0.37 (L), 7.7 (M), 91 (H) µg/kg
Batch No.	100/92

Methods:

Three groups of Sprague-Dawley rats (— CD, BR; age of 11 - 12 weeks, 23/dose) were exposed to 0.21 - 64 µg formoterol/L air for a duration of 30 minutes in a nose-only exposure chamber on days 6 - 15 of the pregnancy. The vehicle control group received lactose only (3.2 mg/L air). The respective estimated doses of formoterol in the treated groups were 0, 1.6, 38, 690 µg/kg for inhaled and 0, 0.37, 7.7, 91 µg/kg for the lung deposition. The estimated aerodynamic mass median diameter (MMAD) was ——— . Clinical observations and clinical pathology laboratory analysis were made during the study. Dams were sacrificed for necropsy on day 21 of pregnancy. The following parameters were examined: #corpora leutea, placental weights,

#implantation sites, sex and #viable fetus, #dead fetus, resorption sites, fetal weights, litter weights and external abnormalities.

Results:

Maternal examinations:

Mortality (twice daily): None.
Clinical signs (daily): No treatment-related effects were observed.
Body weight (weekly): Dose-dependent increases in body weight gain (5%-L, 14%-M, 18%-H) starting from the beginning of the exposure.
Food consumption (every 3 days): A slight increase (7%) in food consumption was seen in the high dose group on days 9 - 15.
Heart rate (days 6 and 14): Dose-dependent tachycardia was seen on both occasions (6%-L, 19%-M, 15%-H).
Pathology: One high dose dam had a large spleen and markedly large left kidney. This may be incidental.
Placental weight: No treatment-related effect was observed. Statistical increases in mean placental weights were seen in all treated animals (7%-L, 5%-M, and 9%-H). However, the absolute placental weight (0.48 g) in the high groups was still within the historic control of the lab. In addition, there was no clear dose-response relationship.

Fetal examinations:
Litter response: No treatment-related effect was seen.
External examinations: No treatment-related effect was seen.
Visceral examinations: No treatment-related effect was seen.
Skeletal examinations: Dose-related increases in partially ossified or not ossified odontoid were noted (49-C, 45-L, 57-M, and 94-H). However, ossification is a very variable parameter.

2. 3-month inhalation study on respiratory tract and male reproductive system: Study No. T3137.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	92064
Study dates:	1/27/95 - 5/3/95, Report date - 1/11/96
GLP:	Yes
Dose:	0 (C), 2.6 (L), 13 (M), 67 (H) μg/kg
Batch No.	311/92

Methods:

Three groups of Wistar rats (—— , SPF; age of 16 days, 6/sex/dose) were exposed to 1.3, 7.4, 36 μg formoterol/L air for a duration of 30 minutes once daily in a nose-only exposure chamber for 3 months. Another group of animals received lactose only (3.2 mg/L air) and served

as the vehicle control group. The respective estimated doses of formoterol in the treated groups were 0, 12, 75, 350 μg/kg for the inhaled and 0, 2.6, 13, 67 μg/kg for the lung deposition. The estimated aerodynamic mass median diameter (MMAD) was [redacted]. Clinical observations and clinical pathology laboratory analysis were made during the study. At the end of the treatment, animals were sacrificed for gross and microscopic examinations. Another 4 groups of animals (n = 6/sex/dose) were sacrificed on week 3 for gross and microscopic examinations. Additional groups (13 satellite groups) were used for interim analysis of blood drug concentration and microscopic examinations of male reproductive organs (week 1 only). Most parameters listed in the results section were derived from animals in the groups 1 - 4 only.

Results:

Mortality (daily): None.

Clinical signs (daily): Alopecia was seen in the high dose females. (0/12-C, 0/12-L, 1/12-M, 6/12-H).

Body weight (1-2/week): Dose-dependent increases in body weight gain (8%-L, 17%-M, 20%-H at week 12) were seen in the males, only the increase in the mid and high dose groups were statistically significant. The same trend exsited in the females (15%-L, 11%-M, 10%-H), but none were statistically significant.

Food consumption (1 - 2 weeks): Increases in food consumption (8%-L, 12%-M, 15%-H at week 12) were seen in the males. Slight increases in food consumption was seen in the females (13-5%).

Water consumption (daily on weeks 2, 6, 9): normal.

Heart rate (n = 3, weeks 6, 11): Dose-dependent tachycardia was seen on both occasions (-5%-C, 9%-L, 9%-M, 31%-H).

Rectal temperature (weekly from week 6): normal.

Clinical pathology (weeks 4 and 12):

Hematology: Slight but dose-dependent increases in RBC numbers (3 - 7%) and hemoglobin (3 - 7%) were observed in the females at week 12.Remarkable increases in white blood cell numbers (0 - 80%) were seen both sexes at week 12.

Clinic chemistry: No treatment related effect was observed.

Pathology (terminal):

Organ weights: Increased heart weight, decreases testis and brain weight were apparent in all treated groups (Table 30).

Table 30. Organ Weight Changes in the 3-month Oral Study in Young Rats

	Male			Female		
Dose (mg/kg/day)	2.6	13	67	2.6	13	67
Heart (Δ %): Week 3, Absolute	↑ 17	↑ 17	↑ 24	↑ 9	↑ **29***	↑ **31**
Relative	↑ 15	↑ 13	↑ 21	↑ 7	↑ **31**	↑ **30**
Week 12, Absolute	↑ 16	↑ **37**	↑ **28**	↑ 13	↑ 11	↑ **34**
Relative	↑ 10	↑ **20**	↑ 10	↑ 1	↑ 4	↑ **26**
Testis /ovary (Δ %)	testis	testis	testis	ovary#	ovary	ovary
Week 1, Absolute	↓ 13	↓ **16**	↓ **18**			
Relative	↓ **12**	↓ 9	↓ 4			
Week 3, Absolute	↓ 4	↑ 6	↑ 6			
Relative	↓ 5	↑ 3	↑ 3			
Week 12, Absolute	0	↑ 1	↓ 3	↑ 13	↑ **24**	↑ **36**
Relative	↓ 5	↓ **12**	↓ **16**	↑ 11	↑ **16**	↑ **27**
Brain (Δ %): Week 3, Absolute	↓ 2	↓ 4	↓ 4	↓ 2	↓ 6	↓ 5
Relative	↓ 2	↓ 6	↓ 5	↓ 4	↓ 5	↓ 6
Week 12, Absolute	↓ 2	↑ 1	0	↓ 5	↓ 2	↓ 3
Relative	↓ 7	↓ **12**	↓ **14**	↓ 4	↓ 8	↓ 9

* bold indicates the value is statistically significantly different from the control.
\# with an ovary cyst.

Microscopic examinations (control and the high dose only; the testis and epidydimides were examined in all doses): No treatment-related effect were seen. Minimal testicular atrophy (1♂-M) was considered as incidental.

Plasma drug concentrations. The respective plasma concentrations of formoterol for low, mid and high doses were 2.2, 10.1 and 47.5 nmol/l in males and 1.6, 10.2 and 32.3 nmol/l in females. The AUCs were 2.3 - 3.4, 7.5 -25.6 and 45 - 86 nmol/l.h, respectively.

Comment: Decreases in brain weight may be something of concern in the future.

B. Oral:

1. Oral segment I fertility and reproductive performance study in rats: T3015.

Testing lab:
Study number: ARA 192/942424
Study dates: 8/25/1993 - 11/10/1993. Report date - 5/4/95
GLP: Yes
Dose: 0 (C), 0.2 (L), 3.0 (M), 15 (H) mg/kg/day
Batch No. 1533-1, 100/93

Methods:

Four groups of SPF Sprague-Dawley rats — :CE — BR VAF/Plus strain, 32♀ and 16♂/dose) were given by oral gavage formoterol once a day to study the drug's effect on fertility and reproductive performance. Formoterol dose levels were 0, 0.2, 3 and 15 mg/kg/day, respectively. The vehicle (cellulose) was the same as previously described (T2815). The females were treated from 2 weeks before mating to day 19 of the pregnancy. One half of the females were sacrificed on day 20. Fetus were delivered by C-section for fetal examinations. The other half of the dams were allowed to give births and sacrificed on day 21 post partum. The males were treated from 9 weeks prior to mating till the time of the sacrifice (total 25 weeks treatment). Due to a slightly lower pregnancy rate in Group 4 animals, additional 64 untreated females were mated with the control and high dose males for comparison. These females were killed on day 15 of pregnancy for litter response examinations. Clinical observations and clinical pathology were performed during the study. Satellite groups were used to determine drug plasma levels.

Results:

Maternal examinations:

Mortality (daily): Four deaths (all in the treated groups) occurred during the study (Table 31).

Table 31. Mortality in the Oral Segment I Fertility Study in Rats

Dose (mg/kg/day)	0	0.2	3.0	15	15 (satellite)
Death (incidences)	-	1/16♂	1/16♂	-	2/40♀
Time (week)	-	9	18	-	day 13, week 3

Clinical signs (daily): Treatment related signs in both sexes included salivation and splayed posture. Others were observed in the males only: swollen face/bulging eyes in all doses, dose dependent pink scrotal sac, brown stained muzzle and red ear/feet. (Table 32)

Table 32. Clinical Signs in the Oral Segment I Fertility Study in Rats

	Males				Females			
Dose (mg/kg/day)	0	0.2	3.0	15	0	0.2	3.0	15
n/group	16	16	16	16	32	32	32	32
Salivation			3	16				9
Splayed posture		2	16	16			6	30
Swollen face/bulging eyes		15	16	16				
Dark pink scrotal sac		4	16	16				
Red ear/feet		1	16	16				
Ungroomed/ brown stained coat				11				

Body weight (every other day): Body weight gains increased dose-dependently during early part of the treatment in the males and lactation period in the females. Absolute body weights decreased in the late part of the treatment in the males. During pregnancy, female body weight remain essentially the same among all groups. These changes are expressed as percentage of changes in Table 33:

Table 33. Body Weight Changes in the Oral Segment I Fertility Study in Rats

	Males			Females		
Dose (mg/kg/day)	0.2	3.0	15	0.2	3.0	15
Week 1	↑ 27	↑ 55	↑ 55	↑ 31	↑ 200#	↑ 246
Week 7	↑ 11	↑ 13	↑ 18	NC	NC	NC*
Week 9	↑ 9	↑ 7	↑ 1	↑ 14$	↑ 50	↑ 90
Week 25	↑ 17	↑ 8	↑ 9			

* = NC apparent changes. # = statistically different from the controls
$ = end of lactation period.

Food consumption (every other day): dose-related increases in food consumption in both sexes (↑ 2 - 10%)

Clinical chemistry (day 15): No treatment-related effects were observed.

Plasma drug levels: Results are summarized in Table 34.

Table 34. Formoterol AUC_{0-t} (nmol/l.h), n = 2

	Females			Males		
Dose (mg/kg/day)	0.2	3.0	15	0.2	3	15
Day 2 (pregnancy)	-	28.4	358	-	-	-
Day 15 (pregnancy)	-	24.1	435	-	-	-
Day 15 (post partum)	-	8.9	145	-	-	-
Month 6	-	-	-	-	38.0	682

- = Not determined or below the limit of quantitation

Mating and Performance: Formoterol induced dose-dependent decreases in pregnancy rate of the female; however, the drug may act through its impairment to male fertility as indicated in Table 35:

Table 35. Mating & Performance in the Oral Segment I Fertility Study in Rats

		both ♂ & ♀ treated				♂ treated only	
	Dose (mg/kg/day)	0	0.2	3.0	15	0.2	3.0
Males:	N/group	16	16	16	16	16	16
	Fail to induce pregn.		1	1			
	induced pregn.only in 1 /2♀			5		1	6
Females:							
	N/group	32	32	32	32		
	Sperm at mating	31	31	25	23		
	Non-pregnant			2	7		
	Sperm in smear			1	2		
	No evidence of mating			1	5		
	Total litter loss			1	4		
	Reared young to day 21	16	16	14	9		
	Pregnancy rate (%)	100	100	94	78		
	Duration of pregn. 21-22 d	15	11	14	6		
	22-23 d	4	-	7			

Pathology (♂ - week 25): Atrophy of seminiferous tubes was seen in the low (2/16) and high (1/16) males.

Organ weight (testes and epididymides only, ♂- week 25): Slight decrease (5%) in the weight of these organs were noted in the high dose male group.

Litter examinations: The following parameters were examined: #corpora leutea, placental weights, #implantation sites, sex and #viable fetus, #dead fetus, resorption sites, fetal weights, litter weights and external abnormalities.

Litter response: See Table 36:

Table 36. Litter Responses in the Oral Segment I Fertility Study in Rats

Interim sacrifice (day 20)

	both ♂ & ♀ treated				♂ treated only	
Dose (mg/kg/day)	0	0.2	3.0	15	0	15
Dam w/ live young	16	16	15	12	31	26
Implantation's/dam	16.1	15.9	16.3	16.3	17.4	14.5*
Preimplantation loss (%)	9.0	10.2	11.4	11.3		
Postimplantation loss (%)	4.7	6.5	8.4	8.3		
Dam w/ embryonic deaths 0	1	3	3			
Litter weight (g)	55.2	56.3	56.6	50.1		
Placental weight (g)	7.7	7.4	8.1	9.3		
Litters with malformations1	2	1	5			

Table 36. Litter Responses in the Oral Segment I Fertility Study in Rats (continued)

At weaning

	both ♂ & ♀ treated			
Dose (mg/kg/day)	0	0.2	3.0	15
Number of litters born	16	16	15	13
Number of litters weaned	16	16	14	9
Implantation sites/dam	17.3	15.3	15.1	13.8
Total young born/dam	16.2	14.6	13.8	**11.4***
Live young/dam (at birth)	16.0	14.3	13.4	**10.0**
(Day 21) 15.8	13.7	12.2	6.1	
Litter weight (g) (at birth)	99.9	91.5	**83.0**	**58.0**
pup weight (g) (at birth)	6.3	6.4	6.2	5.9
(at day 4)	10.7	11.0	10.4	**8.1**
Implantation loss (litters)	2	2	4	6
Pup loss at day 21 (litters)	0	0	5	**10**
Dam w/ embryonic deaths	0	1	3	3
Fetal examinations:				
Total malformations (litters)	0	2	2	5
External examinations:				
Craniorachischisis	0	1	0	0
Visceral examinations:				
Ubilical hernia (litters)	0	0	2	2
Skeletal examinations:				
brachygnathia (litters)	0	0	0	2
Others	0	2	0	1

* Bold indicates statistically different from the control.

4. Segment II teratology dose-range study in rabbits: Study No. T2710.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	93025
Study dates:	3/30/93 - 5/14/93, Report date - 12/24/93, revised on 2/1/96
GLP:	Yes
Dose:	0 (C), 0.2 (L), 60 (ML), 125 (MH), 300 (H) mg/kg/day
Batch No.	1533-1, 100/92

Methods:

Four groups of pregnant rabbits (New Zealand White, 6/dose) were given by oral gavage formoterol once a day during 6 - 18 of the pregnancy to determine doses for the definitive teratology study. Formoterol dose levels were 0.2, 60, 125 and 300 mg/kg/day, respectively. The vehicle (cellulose) was the same as previously described (T2815). Clinical observations and clinical pathology were performed during the study.

Results:

Maternal examinations:

Mortality (daily): No treatment-related deaths were observed. Two preterminal sacrifices (L and H groups each) were due to technical errors.

Clinical signs (daily): Vaginal bleeding, decreases in water intake and feces were seen in the high dose group (Table 37). The vaginal bleeding could be the result of abortion.

Table 36. Clinical observations in the Oral Segment II Dose-range Study in Rats

Dose (mg/kg/day)	0	0.2	60	125	300
↓ feces (doe#)	3	1	2	3	6
↓ water intake	3	1	2	1	6
Vaginal bleeding (incidence)	2	0	2	2	12
BW gain (%)	-	↑ 75	↓ 12	↓ 29	↓ 181*

* statistically different from the control

Body weight (days 1, 6, 10, 14, 19 and 24): Dose-dependent decreases in body weight gains were seen on days 6 - 10. (See above table)

Food consumption (days 6, 14 19, 24 and 28): Decreased food consumption on days 6 - 14 in the high dose group. (See above table).

Water intake (daily by visual inspection): decreases water intake was observed in the high dose group (1/6-C vs 5/6-H).

Clinical chemistry (day 15): No treatment-related effects were observed.

Pathology (day 29): No treatment-related effects were observed.

Fetal examinations: The following parameters were examined: #corpora lutea, placental weights, #implantation sites, sex and #viable fetus, #dead fetus, resorption sites, fetal weights, litter weights and external abnormalities.

Litter response (Table 38):

Dose (mg/kg/day)	0	0.2	60	125	300
Litter weight (g)	346	395	340	243	199*
Fetal weight (g)	40.2	40.5	37.0	36.4	36.9
Fetus #	8.6	9.8	9.2	6.7	5.4
Placental weight (g)	5.5	6.0	5.6	6.2	7.2
Intra-uterine deaths (mean)	1.4	0.3	2.8	3.3	5.4
Abnormalities					
Fetus w/minor defect (%)#	2.3	0	3.6	23	30
Fetus w/major defects (%)	0	0	2	10	22

Table 38. Litter Response in the Segment II Dose-range Study in Rats *(continued)*

Dose (mg/kg/day)	0	0.2	60	125	300
External examinations:					
Edemas body	0	0	0	0	3
Visceral examinations:					
Enlarged heart	0	0	0	1	3
Enlarged aortic arch	0	0	0	6	9
Enlarged pulmonary artery	0	0	1	8	13
Skeletal examinations:					
Brachydactyly	0	0	0	7	3
Flexed forepaw	1	0	1	0	3

* Bold indicates statistically different from the control. # Minor defects were primarily edema and cutaneous hemorrhage, while the major defects were enlarged heart/aortic arch, and brachydactyly

Conclusion: Significant maternal toxicity and fetal effect were observed in the 125 and 300 mg/kg/day group. Signs of toxicity included decreased body weight, and water and food intake, and abortion. The dose of 60 mg/kg/day was tolerated well by the dams and can be used as the top dose for the definitive studies.

5.a. Segment II teratology study in rabbits: T3014.

Testing lab: [redacted]
Study number: T93025
Study dates: 9/6/93 - 2/15/94; Report date - 5/4/95
GLP: Yes
Dose: 0 (C), 0.2 (L), 3.5 (M), 60 (H) mg/kg/day
Batch No. 1533-1, 100/92

Methods:

Pregnant rabbits (New Zealand White, n = 16/dose) were given by oral gavage formoterol once a day during days 6 - 18 of the pregnancy to study teratogenicity of the drug. Formoterol dose levels were 0, 0.2, 3.5 and 60 mg/kg/day, respectively. The vehicle (cellulose) was the same as previously described (T2815). Clinical observations and clinical pathology were performed during the study. Does were sacrificed on day 29 for maternal and fetal examinations. Satellite groups were used to determine drug plasma levels.

Results:

Maternal examinations:

Mortality (daily): No treatment-related deaths were observed.

[redacted]

Clinical signs (daily): No treatment-related effects were observed.

Body weight (every other day): Body weight gains increased dose-dependently during the treatment and persisted till time of sacrifice:

Dose (mg/kg/day)	0	0.2	3.5	60
BW gain $_{day\ 19}$ (%)	-	+44	+57	+73
BW gain $_{day\ 29}$ (%)	-	+29	+21	+41

Food consumption (every other day): No apparent effect were seen.

Clinical chemistry (day 15): No treatment-related effects were observed.

Plasma drug levels (days 12 and 18): Results are summarized in the following table:

	Formoterol AUC (nmol/l.h)		
Dose (mg/kg/day)	0.2	3.5	60
Day 12	4.4 ± 0.7	84.4 ±33.4	2130 ± 348
Day 18	11.2 ± 2.2	125.6 ± 60	3576 ± 379

Pathology (day 29): No treatment-related effects were observed.

Fetal examinations: The following parameters were examined: #corpora leutea, placental weights, #implantation sites, sex and #viable fetus, #dead fetus, resorption sites, fetal weights, litter weights and external abnormalities. Results are summarized in Table 39.

Table 39: Litter Response in the Segment II Study in Rats

Litter response				
Groups	0	0.2	3.5	60
Litters	11	15	15	15
Post implantation loss (%)	6.1	6.5	7.8	10.5
Placental weight (g)	42.7	49.9	43.1	44.8
Mean placental weight (g) 5.5	5.3	5.0	5.9	
Late embryonic deaths				
Litter involved	2	3	6	7
Incidences	3	5	8	15
(%)	0.3	0.3	0.5	1.0
External examinations: Not mentioned by the sponsor.				
Visceral examinations:				
mean % fetus/litter w/ abn.	1.9	8.2	14.0	29.8
Anomalous cervicothoracic Artery (litters)	0	5	5	6
Atelectic lungs (litters)	0	0	1	1
Liver				
Abnormal lobes	0	0	1	2
Subcapsular cysts (litters)	1	1	0	7
(Fetuses)	1	1	0	23
Skeletal examinations:				
Satural cranial bones (litters)	1	1	3	4
Asym. bipartite sternbrae (%)	1.1	4.1	4.2	9.4

Comment: An inaccuracy was found in the incidences of embryonic deaths of summary. Specifically, the total incidences of embryonic deaths in the control group did not add up. This was illustrated in Table 40. However, the error is trivial and does not appear to compromise the entire study.

Table 40. Embryonic Deaths in the Oral Segment II teratology Study in Rats

Number of embryonic death /litter	0	1	2	3	≥4	total (litters)
Early	8	2	1	0	0	11
Late	9	1	1	0	0	11
Total		3* (1)	2	0(1)	0	

* Bold indicates correct summary.

7. Segment III reproductive toxicity study in rats: Study No. T2905.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	93081
Study dates:	8/16/93 - 3/14/93, Report date - 4/27/95
GLP:	Yes
Dose:	0 (C), 0.2 (L), 0.8 (M), 3.4 (H) mg/kg/day
Batch No.	105/92

Methods:

Four groups of female Sprague-Dawley rats (F0 generation, age of 11 - 12 weeks, 30/dose) were given by oral gavage formoterol during day 6 of the pregnancy onward and the lactation period (days 6 - 43 post coitus) to study its effect on delivery and post natal development. Formoterol dose levels were 0, 0.2, 0.8, 3.4 mg/kg, respectively. The vehicle compositions were previously described (2815). Clinical observations were made during the pregnancy and lactation periods. Pregnant dams were allowed to give birth naturally. Litter parameters (i.g. litter size, litter weight, general development) were recorded. Twenty litters of the F1 generation (4 pups/sex/litter) were then subjected to before and after weaning examinations. The pre-weaning examination parameters included pinna unfolding, tooth eruption, eye opening, surface righting, auditory startle, puitary reflex. Pups (F1 generation) were weaned on day 47 post coitus. Some of them (1 litter/sex/dose) were further subjected to post weaning examinations: Rota-rod performance (age 25 days), water-maze test (age of 30 days), motor activity (age of 2 months) and mating performance (age of 3 months). After mating, pregnant F1 females were allowed to give birth naturally and their litter weight were recorded.

Results:

a. Maternal Responses:

(1). F0 generation:

Mortality (daily): None.

Clinical signs (daily): No treatment-related effects were observed.

Body weight (once/3 days during pregnancy and weekly post partum): Dose-dependent increases in body weight gains starting from the day 9 onward:

Table 41. Body Weight Changes in the Oral Segment II Study in Rabbits

Dose (ug/kg/day):	0.2	0.8	3.4
Day 15	↑ 6%	↑ 9%	↑ 10%
Day 43	↑ 8%	↑ 11%	↑ 12%

Note: the low dose group had significant higher body weight than the control at day 0 of the pregnancy (5%).

Food consumption (every 6 days during pregnancy and weekly post partum): Dose-related increases (5% - 10%) in food consumption were seen in the treated group on days 6 - 21.

Pregnancy rate: Slight lower pregnancy rates (6%) were seen in all treated groups, but there was no dose response relationship.

Gestation period, parturition, nursing and lactation: No treatment-related effects were observed.

Necropsy: No treatment-related effects were observed.

(2). F1 Generation:

Mortality (daily): No treatment-related effects were seen. Four rats of one litter (3423/93) were either died or sacrificed because of moribund conditions. Autopsies of two of them revealed bilaterally severely enlarged, pale cystic kidneys. Microscopic examinations revealed multiple prominent cystic dilations of the cortical and medullar collecting tubes. These results (especially from one litter) suggest that they may not be treatment-related.

Clinical signs (daily): No treatment-related effects were observed.

Body weight (weekly): No treatment-related effects were observed from 3 weeks post partum onward.

Food consumption: No treatment-related effects were observed.

Mating performance: Decreased mating performance in the low and mid dose groups were seen. This was indicated by incidences of negative vaginal smear of paired females on day 11 of the mating period (1/20-C, 8/19-L, 8/19-M, 1/17-H). These non-pregnant females become pregnant when they were paired with other males (mating period of day 12 - 17).

Pregnancy rates: Slight lower pregnancy rates (6%) were seen in all treated groups, but there was no dose response relationship.

Gestation, parturition, nursing and lactation periods: No treatment-related effects were observed.

Necropsy: No treatment-related effects were observed.

(3) **F2 Generation:**
No treatment-related effects were seen.

b. **Litter Responses:**

F1: Slight increase in total litter loss in the mid and high dose groups were seen (Table 42). Slight increases in pup loss were seen in all treated groups, but none were statistically significant:

Table 42. Litter Response (F1) in the Oral Segment II Study in Rabbits.

Dose (µg/kg/day):	0	0.2	0.8	3.4
Total litter loss	1/30	1/30	3/30	3/30
Pup loss				
At birth (%)*	2.5	5.6	8.4	6.4
Post partum (%)	9.3	15.9	22.0	19.9
Day 26 post coitus	11.0	21.6	28.8	27.8
Day 26-43 post coitus (%)	0	0	0.6	1.3

* Cumulative losses.

Pup weight: Slight decreases (6%) in pup weight at day 43 post coitus were seen, but a dose-response relationship was not apparent.

Functional/behavioral tests: No treatment-related effects were observed.

F2 Generation: No treatment-related effects were observed in these parameters: implantation loss, litter size, pup loss, pup weight gain.

9. Testicular effect of 3-month oral formoterol in young rats: Study No. 13160.

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	94146
Study dates:	3/7/95 to 6/8/95, Report date - 1/26/96
GLP:	Yes
Dose:	0, 0.03 (L), 0.2 (ML), 0.8 (MH), 3 (H) mg/kg/day
Batch No.	311/92

Method

Three groups of young male Sprague-Dawley rats (aged 16 days, 12/dose) were given by oral gavage formoterol once a day for 3 months to examine testicular findings in the previous study (T3136). Formoterol dose levels were 0.03, 0.2, 0.8, and 3.0 mg/kg respectively. The vehicle consisted of cellulose (hydroypropylmethyl, 5 mg), citric acid monohydrate (0.77 mg), sodium phosphate dibasic (2.25 mg), and distilled water (ad 1 ml). Clinical observations and clinical pathology were performed during the study. At the end of 3 months, animals were

sacrificed for pathological examinations. Saltutamol (batch no. 72274010; given twice daily) was used as a reference. Daily salbutamol doses were with 20 and 50 mg/kg, respectively. Satellite groups were used to determine plasma drug concentrations.

Results:

Mortality: No treatment-related mortalities were observed. Two pre-terminal deaths (1-MH and 1-H on day 50 and 34, respectively) occurred. The high dose animal had plauritis and pneumonitis and the mid dose animal had stomach hemorrhage (4 mm in diameter). These deaths were not considered as treatment-related by pathologist.

Clinical signs (daily): No treatment-related effects were seen.

Body weight (every 3 days for first 3 weeks, then weekly): Transient increases in body weight were seen in the treated groups in the first two weeks (Table 43). The satellite animal also showed the same effect except that the dose-response relationship was more obvious; increases in percentage were: 7%-L, 13%-ML, 16%-MH, 14%-H.

Table 43. Percent of body weight changes in the 3-month testes study of formoterol#

Dose	Formoterol				Salbutamol	
(mg/kg/day)	0.03	0.2	0.8	3	20	50
Week 1	0	↑ 6%*	↑ 7%	↑ 4%	↑ 2%	0
Week 2	0	↑ 4%	↑ 5%	↑ 2%	↑ 7%	↑ 5%

From the main study

* Bold indicates this value is statistically significantly different from the control.

Food consumption (weekly): Minimal increases in food consumption were seen in the high dose groups by the end of the study.

Water consumption (weeks 2, 6, 10.): Decreases in water consumption (2 - 4%) were seen in all treated groups, including the albuterol treated groups. However, only half of the observations were statistically decreased, mostly in the low and mid low groups.

Rectal temperature (1-2/week): Transient and dose-related increases in body temperature were seen in the beginning of the study in most treated groups (Table 44). This effect was absent from day 7 onward.

Table 44. Increases in body temperature (1 hour post dosing) in the 3-month testes study of formoterol (in cellos degrees)

Dose	Formoterol				Salbutamol	
(mg/kg/day)	0.03	0.2	0.8	3	20	50
Day 0	0	↑ 0.2*	↑ 0.5	↑ 1.0	↑ 0.3	↑ 0.7
Day 2	0.1	↑ 0.4	↑ 0.9	↑ 1.1	↑ 1.0	↑ 1.0

* Bold indicates that value is statistically significantly different from the control.

Testes location (2-3 times/week in the first 5 weeks, weekly thereafter): Normal. However, testes in the treated groups were distinctly visible and the scrotum were hyperemic (relative frequencies: 8%-C, 16%-L, 36%-ML, 24%-MH, 42%-H). These were also seen with the albuterol treated animals (49% and 40% for the low and high dose respectively).

Pathology:

Necropsy: No treatment-related effects were observed.
Organ weights: See Table 45. There was no apparent changes in testis weight on day 8.

Table 45. Percent of organ weight changes in the 3-month testes study of formoterol[#]

Dose	Formoterol				Salbutamol	
(mg/kg/day)	0.03	0.2	0.8	3	20	50
Testes (absolute)	0	↓ 1%	↑ 1%	↓ 7%*	↑ 5%	↓ 2%
Brain (absolute)[@]	0	↓ 2%	↓ 3%	↓ 5%	↓ 4%	↓ 4%
Heart	↓ 5%	↑ 3%	↑ 13%	↑ 11%	↑ 15%	↑ 19%
Lung	0	↑4%	↑ 1%	↑ 11%	↑ 17%	↑ 24%

From the main study
* Statistically significantly different from the control.
@ There was also a trend of dose -related decrease in relative brain weight, but none were statistically significant.

Histopathology (testis, epididymides and organs with relevant gross changes): No treatment-related effects were found. Testes atrophy were sporadically seen and lack of dose-response relationship: 1/12-C, 1/12-L, 5/12-ML, 0/12-MH and 1/12-H). Brain was not examined.

Plasma drug concentrations (day 7 and 91): The $AUC_{0-6\,h}$ was available for the high dose groups only (Table 46).

Table 46. Plasma drug levels ($AUC_{0-6\,h}$ nmol/Lh)

Dose	Formoterol			
(mg/kg/day)	0.03	0.2	0.8	3
Day 7	-	-	2.4	12.7
Day 91	-	-	3.9	11.8

- below detection limit.

Comment: Similar to the previous study (T3137), dose-related decreases in absolute brain weight were seen. Significance of this observation is unknown at present.

Mutagenicity Studies:

1. **Ames Salmonella/Mammalian microsome reverse tests: Study Nos. T2388, T2389.**

Testing Lab:	Astra Safety Assessment, Södertäjie, Sweden
Study No.:	T2388, T2389
Study Dates:	Feb. 18, 1991 to ?
GLP:	Yes
Testing material:	Formoterol, batch 131/90, 100/91

Mutagenicity of D7193 was tested in *Salmonella typhirium* in the presence and absence of an externally supplied metabolic activation system (S-9). The bacterial strains were *S. typhirium* TA 1535, TA1537, TA1538, TA98, TA100. The liver S9 fraction was from Aroclor 1254 induced rat. Four studies were conducted. The respective concentrations of formoterol for the two studies were 0, 124 - 129, 414 - 430, 1240 - 1290, 4140 - 4300 and 12400 - 12900 µg/plate. Dose selections were acceptable under the OECD guidelines (*OECD Guidelines for the Testing of Chemicals. Revised draft, September 1995*). The positive control are aminoanthracene (5.0 µg/plate), sodium azide (0.5 µg/plate), 2-nitrofluorene (0.5 µg/plate) and 2-aminoacridine (75.0 µg/plate). Solvent controls were water, dimethylsulfoxide, or ethanol. One plate per test system was used as a sterility control. Criteria for the positive results was statistically significant increase in revertant colonies by Dunnett's test for multiple comparison with one tail-distribution at $p \leq 0.01$ level. Positive findings are listed in Table 47.

Table 47. Qualitative Findings in the Bacterial Mutagenicity Assays

Salmon. strain	S9 Fraction	Experiment No.	Estimated formoterol (µg/plate) 130	430	1300	4300	13000
T1535	-	1	-	-	-	*	**
	-	2	-	-	-	*	**
	-	3	-	-	-	*	-
	-	4	-	-	-	-	-
T1538	+	1	-	-	-	-	*
	+	2	-	-	-	**	**
	+	3	-	-	-	-	*
	+	4	-	-	-	**	**
T1537	+	4	-	-	-	-	**

* significant at $P < 0.05$, ** significant at $P < 0.01$.

Formoterol induced increases in the number of revertant colonies occasionally in Salmonella strain T1535, T1537 and T1538 at very high concentrations (13 mg/plate). The concentration was twice the currently accepted levels (5 mg/plate). At this high concentration, physiological factors other than mutagenic effect of the testing compound may give rise to a positive result. At lower concentration that slightly lower than the currently accepted level (4.3

mg/plate), only the strain T1538 in the presence of the activating system showed positive results half of the time. All other test strains showed no evidence of mutagenicity in the presence or absence of the external metabolic activation system. The positive controls in each study yielded typical results. Hence, formoterol may be weakly mutagenic in T1538 Salmonella strain under the experimental conditions.

2. L5178Y Mouse Lymphoma cell thymidine kinase locus mutagenicity test: Study No. T2397.

Testing Lab:	Astra Safety Assessment, Södertälje, Sweden
Study Dates:	January 28, 1991 to unspecified.
GLP:	Yes
Testing material:	Formoterol, batch 131/90, 100/91

Genotoxic potential of formoterol was evaluated in the *in vitro* L5178Y mouse Lymphoma cell thymidine kinase forward mutation assay using Fisher's $F_{10}P$ medium. Concentrations of D7193 ranged from 12, 10.6, 9.3, 8.0, to 6.8 mg/ml in the absence and presence of an externally supplied metabolic activation (S-9) system. Dose selections were acceptable under the OECD guidelines (*OECD Guidelines for the Testing of Chemicals. Revised draft, September, 1995*). Positive controls were 4-nitroquinoline-N-oxide (0.25 µg/ml) and 9, 10-dimethyl-1,2-benzantracene (2.5 µg/ml) without S-9 and 7, 12-dimethylbenz (-) anthracene with S-9 from Aroclor 1254 induced rat. The negative control was dimethyl sulfoxide (vehicle). Criteria for a positive response included: 1) two fold increase in mutation frequency that is statistically significant at toxicity levels done to 10% to growth. 2) spontaneous mutation frequency in the negative control remains the historical range. 3) significant dose-response relationship. Results showed that formoterol did not induce an increase in the number of revertant colonies in the presence, nor in the absence of the external metabolic activation system. The positive controls yielded typical results. Hence, formoterol was classified as negative in this L5178 Y mouse lymphoma cell thymidine kinase locus mutagenicity assay under the experimental conditions.

3. Human Lymphocyte chromosomal aberration test: Study No. T2510.

Testing Lab:	Astra Safety Assessment, Södertälje, Sweden
Study Dates:	Oct. 10, 1991 to Feb., 6, 1992
GLP:	Yes
Testing material:	Formoterol, Batch No. 110/91

Formoterol was evaluated in an *in vitro* chromosomal aberration assay utilizing lymphocytes from a healthy human volunteer. The test material was assayed at respective concentrations ranging from 0.1, 0.3, 0.6 and 0.9 mmol/l in the absence of an externally supplied metabolic activation (S-9) system and 0.9, 2, 3, 4 and 5 mmol/l in the presence of S9-system. The analyzable doses were the three low dose levels in both experiments. These selections were acceptable; however, only the mid dose level were analyzed in the second experiment of the non-S9-system. The positive control was methyl methanesulfonate (0.22 mmol/ml) and cyclophosphamide. The negative control was the culture medium and dimethyl sulfoxide. Criteria for a positive response included: 1) a significant, dose-dependent increases in the

frequency of cells with aberration. 2) aberration frequency in the positive control is significantly higher than the background frequency. No significant increase in cells with aberrations was observed at any of the tested concentrations. The positive control chemical induced significantly higher incidences of abnormal cell. Hence, formoterol was considered to be non-mutagenic in the *in vitro* chromosomal aberration assay utilizing human lymphocytes.

4. Rat micromuclei formation test of formoterol after inhalation exposure: Study No. T2513.

Testing Lab:	Astra Safety Assessment, Södertäjie, Sweden
Study Dates:	Nov. 18, 1991 to ?
GLP:	Yes
Testing material:	Formoterol, Batch No. 100/91
Animals:	Wistar Crl:(Wi) BR, inhalation, 5/sex/treatment/dose/time

Mutagenicity of formoterol was evaluated in an *in vivo* rat micronucleus assay. Wistar rats were given by nose-only inhalation formoterol at dose levels of 8.2 and 15.8 mg/kg in lactose. The negative control group received lactose only while the positive group received methyl methanesulfate (24 hour only). Animals (n = 3 - 5/time point) were sacrificed respectively at 24, 48 and 72 hours after dosing. Bone marrow smears were prepared and examined for the presence of micronucleated polychromatic erythrocytes (PCE). The criteria for a positive response was a statistically significant increase in PCE number by Kruskal-Wallis' mean rank test ($p \leq 0.05$). Results indicated formoterol did not cause any statistically significant increase in the incidences of the PCE at the doses and time intervals. Treatment of the positive control did significantly increase the incidences of PCE ($p \leq 0.001$). Hence, formoterol was classified as non-clastogenic in rat micronuclei formation assay under the experimental conditions.

Summary of mutagenicity studies: Genotoxicity of formoterol was tested in *Salmonella typhirium* TA1535, TA1537, TA1538, TA98, TA100; in L5178Y mouse lymphoma cell thymidine kinase forward mutation assay; in the *in vivo* rat micronucleus assay and the *in vitro* human lymphocytes chromosome aberration assay. Formoterol at high concentrations (4300 mg/plate) was weakly mutagenic in Salmonella strain T1538 in the presence of S9 liver fraction under the experimental conditions. The drug was not mutagenic in mutagenic or clastogenic in any of the other assays.

Special Toxicity Studies:

1. Hemolysis and protein flocculation of human blood in vitro: Study no. T2296.

Testing Lab:	Astra Safety Assessment, Södertäjie, Sweden
Study Dates:	Sept. 30, 1991 to Dec. 17, 1991
GLP:	Yes
Testing material:	formoterol, Batch No. 138/91

Hemolytic potential of formoterol to human blood and its compatibility with human blood

were evaluated *in vitro*. Clinically intended formulations of formoterol (0, 33 and 98 mg/ml) were added *in vitro* in proportion to human blood or plasma from a 47 year old female volunteer. The proportions of formoterol solution to blood and plasma were 1:100, 3:100 and 10:100, respectively. After incubating at 37°C for 10 - 15 minutes, absorbency (for hemolysis) and turbidity (for protein flocculation) of the assay solution were measured by a spectrophotometer. For hemolysis potential assay, red blood cells were removed by centrifugation prior to the absorbency determination. No increase in turbidity, nor in absorbency was found at any of the tested concentrations. Hence, formoterol was considered to be compatible with, and not hemolytic to human blood.

2. Vaso-, tissue irritation in dogs, IV, SC, 5 days: Study No. T2302.

Study Dates:	Mar. 29, 1990 to Apr. 9, 1990
Testing Lab:	Astra Safety Assessment, Södertälje, Sweden
GLP:	Yes
Testing material:	Formoterol, Batch No. D90-0334/OW 900315

Tissue irritation and vasoconstriction effects of formoterol were studied by subcutaneous and intravenous administration in beagle dogs (1/sex/dose) for 5 days. The respective daily doses of formoterol were 50 and 200 µg/kg for IV and 5 and 20 µg/kg for SC. The vehicle control consisted of citric acid (0.07%), disodium hydrogen phosphate (0.9%), sodium chloride (0.9%) and water. All intravenous dose was given as 5 minute infusion. Immediately after the IV infusion, the same subject was given its SC. The animals were sacrificed 3 days after the last dose. Microscopic examinations were performed in the following tissues: intravenous deposition site, subcutaneous injection site, axially lymph nodes and lungs. Clinical signs, food and water consumption, body temperature, organ weights and gross pathology were recorded. Injection sites, lung and axially lymph node were examined microscopically.

Slight reactive changes were seen at the subcutaneous injection site in all dogs treated with formoterol. The severity of the change was dose-dependent. Changes (1/2-C, 2/2-L, and 2/2-H) included hemorrhage, perivascular infiltration of leukocytes, granulation and microfocal necrosis. There was no apparent changes in the regional lymph nodes. Nor were there changes at the intravenous injection sites. In conclusion, formoterol was slightly irritant given subcutaneously.

OVERALL SUMMARY AND EVALUATION

A. SUMMARY

Pharmacology

Formoterol is a selective long-acting beta$_2$-agonist that possesses potent bronchospasmolytic activity. Formoterol is 20 - 70 times more potent than albuterol in relaxing guinea pig tracheal strip preparations. It is 70 - 400 times more potent than albuterol in relaxing isolated human bronchus *in vitro*. Formoterol overcomes receptor occupation by maximal concentrations of albuterol and causes additional relaxation of strongly contracted strips of tracheal or bronchial smooth muscles. Aerosol formoterol (DE50 = 2.6 µg/kg) is more potent than albuterol (4 fold) and salbutamol (35 fold) in preventing histamine-induced airway constriction in guinea pigs.

Studies in guinea pigs, both *in vivo* and *in vitro*, show that duration of bronchospasmolytic effect of formoterol is intermediate between albuterol and salmeterol. An *in vitro* study shows that 50% of recovery time after removal of the beta agonists are 3, 5, 7 and 275 minutes for isoproterenol, albuterol, formoterol and salmeterol, respectively. Action duration formoterol in increasing ciliary beat frequency *in vivo* is 2 times of terbutaline.

Formoterol is highly selective to β_2-adrenoceptor. Formoterol is over 200 times more selective for the β_2 receptor than isoproterenol, a non-selective beta-adrenoceptor agonist. Formoterol's affinity to beta$_2$ receptor is 700 times higher than salbutamol. However, formoterol (like salbutamol) is a partial agonist for the beta$_2$ receptor. The intrinsic activity of formoterol in activating adenylate cyclase is slightly lower that of isoproterenol. Formoterol is a racemate of the RR and SS enantiomers with a ratio of one to one. The RR isomer is 1000 times more potent than the SS in relaxing guinea pig trachea in vitro.

In addition to its bronchodilatory activity, formoterol inhibits mediator release, decreases vasopermiability and increases ciliary beat frequency in guinea pig trachea. It inhibits mouse IgE-mediated passive cutaneous anaphylaxis and passive peritoneal anaphylaxis in rats, with ED50s of 1.2 and 260 µg/kg for IV and PO respectively. This inhibitory effect is 6 to 33 times more potent than salbutamol. Formoterol is 100 times more potent than terbutaline in increasing ciliary beat frequency *in vivo*. However, the non-bronchodilatory activity of beta agonists provided little clinical relevance in the treatment of asthma as the patients develop tolerance rapid (M. A. Giembycz, *TIPS, 1996;17:331-336*).

Safety Pharmacology

Safety pharmacology of oral or IV formoterol was evaluated in mice, rats, guinea pigs, cats, dogs and monkeys. Formoterol exhibited characteristics of beta-agonists similar to hexoprenaline. Formoterol may affect behavior, hemodynamics, respiratory patterns muscle contraction, and metabolism depending upon the dose of administration in animals (Table 49). Cardiovascular effects are the most predominant in all species studied.

Table 48. Secondary Pharmacological Effects of Formoterol in Laboratory Animals

	Dose (µg/kg and route of administration)*					
	Mouse	Rat	Guinea pig	Cat	Dog	Monkey
Tachycardia		30	10	10	1.0	30
↑ respiration rate	100	100	100	10		100
↓ motor activity	100					
Hyperreactivity/irritability			100			
Salivation	100			10		100
Tremor					1.0	
Lacrimation& eye congestion				3.0	3.0	
Exophthalmus		100				
Urination & dedecation		100				
Face flushing			100	10		100
Ptosis	100			10		100
Vomiting				10		
↓R, ST, in ECG					1.0 IV	
Diastolic pressure					0.1 ID	
Systolic pressure					10 ID	

* Oral doses unless specified.

Pharmacokinetics

Formoterol is readily absorbed after oral and inhalation administration. The peak concentrations are reached 0.5 - 1 hour after oral and intratracheal administration. There is no apparent tissue accumulation of the drug after prolonged treated although Tasaki (1986) reported a slow rise in tissue concentrations in first 14 - 21 days. Distribution of the drug after inhalation exposure is in the following order: trachea > lung > kidney > liver plasma > heart > brain. Half-lives of the drug after inhalation administration are 2 (humans) to 4 hours (dogs). Glucoronide conjugation and O-demethylation are the major pathways of formoterol metabolism in animals and humans. The rats have the highest the rate of formoterol metabolism *in vitro*, followed by rabbits, mouse and dogs while the humans have the lowest rate based on mg protein. Formoterol was eliminated through both urine and the bile across species. Most urinary formoterol is in the metabolized form, but a small portion exists as the unchanged form in mice. A portion (69%) of the biliary excreted glucoronide conjugates is reabsorbed through enterohepatic circulation in rats. Bioavailability in mice is slightly higher than that in humans. Protein binding (RR: 54 % - 58%) is similar in rat, rabbits, dogs and human. Milk and fetal plasma contains about one-third of

the plasma formoterol concentration in lactating and pregnant rats. There is no differences in bioavailability, protein binding and the rate of metabolism between the RR and SS enantiomers.

Toxicology

General and special toxicity of formoterol has been evaluated *in vivo* by oral and inhalation routes of administration and *in vitro* testing. The general toxicity studies ranged from single dose in mice and rats, to one year repeated dosing in dogs and to 2-year carcinogenicity studies in rodents. Reproductive toxicity was tested in rats and rabbits.

1. General toxicity:

This summary of the general toxicity studies is divided into acute and multiple dose toxicity studies. Each section is further grouped according to the route of administration: inhalation and non-inhalation studies.

a. single dose (acute) toxicity:

Inhalation toxicity studies:

In an acute inhalation toxicity study, Wistar rats were given nose-only formoterol at dose of 3.8, 8.3 and 18 mg/kg (lung deposition) in within 60 minutes. Mortality occurred at the low and high dose groups. The LD50 was estimated to be between 3.8 and 18 mg/kg. Animals showed increased heart rate, decreased motor activity and labored breathing. Tachycardia was accompanied by epitopic foci and increases in QRS amplitude in EKG. Necropsy at 2 weeks after the exposure revealed cardiac necrosis and fibrosis. The target organ of toxicity was the heart.

In another acute inhalation toxicity study, CD-1 mice were given nose-only a single formoterol dose of 4.2, 7.5 and 24 mg/kg in within 60 minutes. No mortality occurred. Similar to the rats, mice also showed decrease motor activity and labored breathing. Necropsy at 2 weeks after the exposure revealed increased organ weights of the heart and lungs.

Non-inhalation inhalation studies:

In an acute oral toxicity study, young Sprague-Dawley rats were given by gavage a bolus dose of formoterol (600, 1200, 2400 and 4800 mg/kg). Mortality was observed at 1200 mg/kg and higher. The LD50 was between 1200 - 2400 mg/kg. Similar to the inhalation studies, rats showed increased heart rate, decrease motor activity and labored breathing. Tachycardia not observed due to the very high baseline possibly resulted from contamination. EKG showed epitopic foci and SR-T elevation. Necropsy at 2 weeks after the exposure revealed cardiac necrosis, fibrosis and cell inflammation.

In another acute oral toxicity study, adult Sprague-Dawley rats were given 210, 1500 and

2000 mg/kg formoterol. Animals showed also decreased motor activity and labored breathing. However, no mortality, and gross and microscopical changes were observed.

In another acute oral toxicity study NMRI mice receiving 2000 mg/kg formoterol showed again decreased motor activity, labored breathing and salivation. No mortality, gross and microscopical changes were observed.

Summary of acute toxicity: Single oral dose of 2000 mg/kg formoterol is not lethal in adult mice and rats. Inhalation exposure of formoterol is more toxic. The LD50 values of inhalation formoterol are 3.8 - 18 gm/kg and > 24 mg/kg for rats and mice, respectively. The young animals seem to be more susceptible. The oral LD50 value in young rats is 1200 - 2400 mg/kg. The target tissue of toxicity is the heart. Signs of intoxication include tachycardia, labored breathing and decreased motor activity.

b. Multiple dose toxicity:

Inhalation studies:

There were a total of five inhalation toxicity studies of formoterol in rats (3) and in dogs (2). Study duration ranged from 5 days to one month in dogs and to 6 months in rats, respectively. Because of the uniqueness of the pulmonary anatomy and physiology, each study has three dose levels (i.e., inhaled dose, total body burden and lung burden). Only the lung burden (deposition) doses are used as the index of actual exposure, based on the marked difference in LD_{50} values of formoterol between the oral, intravenous and inhalation administration (IV-72 mg/kg vs PO-6700 mg/kg in rats).

In a 5-day dose-range study (2460), Sprague-Dawley rats (n= 6/sex/dose) received 0.02, 0.1 and 0.5 mg/kg/day formoterol in lactose for five days and were sacrificed at the end of exposure for gross and pathological examinations. A marked increase in body weight gain was seen in all treated groups. Increased organ weight of the lung and the heart were observed in females. Two male rats in the high dose group had solitary microfocal leukocyte foci in the heart.

In another 5-day dose-range study (T3006), beagle dogs (n= 4/sex/dose) received formoterol in lactose nose-only for five days. The respective doses were 0.5, 2.9, and 15 µg/kg/day for the inhaled and 0.07, 0.4 and 2.1 ug/kg for the lung deposition. Animals were sacrificed at the end of study for gross and pathological examinations. Dose dependent increase in heart rate and hyperemia of the skin and mucosa were observed. No drug-related effects were observed in the body weight, food consumption, clinical pathology, necropsy, organ weights microscopic examinations. The 5-day NOAEL was 2.1 µg/kg.

In a one-month inhalation toxicity study (T3120), beagle dogs (n= 3/sex/dose) received nose-only formoterol in lactose for 5 minutes daily for 30 days. The respective doses were 0.5, 2.9, and 15 µg/kg/day for the inhaled and 0.07, 0.4 and 2.1 ug/kg for the lung deposition. Dose-dependent increase in heart rate occurred in both sexes. Slight decreases in RBC numbers and

hemoglobin concentration were observed in males only. Gross and microscopic examinations at the end of exposure revealed increased incidences of cardiac fibrosis, especially in the high dose group and decreased absolute spleen weight in the females. However, the decreases in the relative spleen weight was not statistically significant. The NOAEL levels was 0.4 µg/kg/day.

In a 3-month inhalation toxicity study (T2433), Sprague-Dawley rats (n= 10/sex/dose) received nose-only formoterol in lactose for 60 minutes daily for 90 days. The respective doses were 0, 82, 260 and 870 µg/kg/day for the inhaled and 0, 9, 22 and 90 ug/kg for the lung deposition. Dose-dependent increases in the heart rate and body weight, and decreases in plasma glucose levels were observed. Slight increases in hematocrit, reticulocyte numbers and plasma urea level were also seen. Organ weight analysis indicated increased absolute heart weight and slight increase in brain weight in all the treated animals. Histological examinations revealed a marginal increase in the incidence of myocardial scars in the high dose males. NOAEL was 22 µg/kg/day for the lung deposition.

In a 6-month inhalation toxicity study (T2860), Sprague-Dawley rats (n= 20/sex/dose) received nose-only formoterol in lactose for 60 minutes for 180 days. The respective doses of 0, 26, 128 and 852 µg/kg/day for the inhaled and 0, 2.3, 12 and 72 ug/kg for the lung deposition. Changes observed were similar to the 3-month study: dose dependent increase in heart rate, body weight, plasma urea levels, heart weight and slight decreases in plasma glucose levels brain weight (♂ only). Increase in the incidence of myocardial fibrosis were observed in the mid and high dose males. The NOAEL was 2.3 µg/kg/day.

Oral:

In a one-month oral study (T2579), beagle dogs (n= 3/sex/dose) were given by gavage formoterol at doses of 0, 2.15, 15 and 100 µg/kg/day in gelatin capsules for 30 days. The vehicle consisted of lactose and magnesium stearate. Animals were sacrificed at the end of study for gross and pathological examinations. In addition to the mucosal/cutaneous hyperemia and tachycardia previously seen in the inhalation studies, labored breathing and increases in body weight gain were observed. There were no apparent changes in heart weight, but necropsy revealed occasional gray-white foci in the heart in the low and high dose females. Microscopic examinations revealed dose-related increases in the incidences of cardiac fibrosis in the mid and high dose groups. In addition, decreased organ weight of the male reproductive system (testes, prostate and epididymides) were observed in the high dose group. The NOAEL was 15 µg/kg/day.

In a 3-month oral study (T2578), CD-1 mice (n= 12/sex/dose) were given by gavage formoterol at doses of 0, 0.24, 2.4 and 24 mg/kg/day for 90 days. Significant increase in mean body weight was seen in the high dose group. Increased liver and spleen weights and decrease in adrenal weights were observed in the high dose females. No apparent histological changes were observed. The NOAEL was 24 mg/kg/day.

In another 3-month oral study (T3136), young Sprague-Dawley rats (n= 10/sex/dose)

were given by gavage formoterol at doses of 0, 0.2, 0.8 and 3.0 mg/kg/day for 90 days. significant decreases in blood glucose and slight increase in serum urea were observed as early as week 3 and the severity of the change was dose-dependent. The high dose group also showed a slight increase in blood phosphorus, increases in the heart weight and the incidences of myocardial fibrosis. Dose-related decreases in the testes were observed in the mid and high dose males but testes tubular atrophy were observed in the low and high dose males. Brain weights were decreased in all groups but a dose-response relationship was lacking. The NOAEL was 0.8 mg/kg/day.

In a 12-month oral study (T3077), beagle dogs (n= 5/sex/dose) were given formoterol in gelatin capsule at doses of 0, 0.72, 8.6, 92 µg/kg/day for 1 year. In addition to hyperemia and tachycardia previously seen in all treated groups, ventricular ectopic extrasystoles were seen in mid and high dose groups at the beginning of the study. Occasionally slight but statistically significant changes were seen in leukocyte numbers, hemoglobin concentrations, hematocrit, erythrocytes. A slight decrease in serum glucose levels was observed in the high dose males only at week 49. One high dose female at necropsy showed occasional gray-white foci in the dorsal papillary muscle. The incidences and severity of myocardial fibrosis were dose-related. Acinar atrophy of pancreas was seen in the high dose group only. No clear NOAEL value was established.

2. Reproductive toxicities:

Inhalation Toxicity Studies:

In a segment II teratology study (T2628), pregnant Sprague-Dawley rats (23 /dose) were given nose-only (30 minutes) formoterol in lactose at doses of 0, 0.4, 7.7 and 91 µg/kg/day during days 6 - 15 of the pregnancy. Dose-dependent increases in body weight gain and food consumption were seen at the beginning of the treatment. Minimal tachycardia was seen in mid and high dose group. No apparent abnormalities were observed in the responses of the litters, external, visceral and skeletal examinations.

A 3-month inhalation toxicity study (T3137) was conducted in young male rats to address the concerns on the lung and male reproductive system. This study was prompted by the occasional abnormalities observed in the male reproductive system and/or performance in other toxicity study (T2579, T3136, T3015). Young Wistar rats (n= 6/sex/dose) were given formoterol in lactose nose-only (30 min/day) for 90 days. The respective doses were 0, 12, 75 and 350 ug/kg/day for the inhaled and 0, 2.6, 13 and 67 µg/kg for the lung deposition. Interim analysis were conducted at weeks 1 and 3. Statistically significant increases in body weight were seen in the mid and high dose males. A trend of body weight increase was apparent in the females. Tachycardia was seen in both sexes. Organ weight analysis indicated increased absolute heart weight, slight decrease in brain weight in the mid and high dose males and decreases in testis weight in all treated males at the beginning of the treatment. Histological examinations of the male reproductive organs of the control and the high dose groups revealed no apparent abnormalities.

Oral Toxicity Studies:

In an oral segment I fertility and male reproductive performance study (3015), Sprague-Dawley rats (32♀ + 16 ♂/dose) were given by gavage formoterol at doses of 0, 0.2, 3 and 15 mg/kg/day. The respective treatment duration were two weeks before mating, through mating and to day 19 of the pregnancy in females; and 9 weeks prior to mating to the time of the sacrifice in the males (total treatment duration of 25 weeks). One half of the dams were sacrificed on day 20 and their fetus were delivered by C-section for fetal examinations. The other half of the dams were allowed to give birth and sacrificed on day 21 post partum. Because of a lower pregnancy rate in high dose group females, additional untreated females (32/group) were mated with the control and high dose males, respectively, for comparison. These females were killed on day 15 of pregnancy for litter response examinations. The results showed that the mid and high dose groups had increased mean body weight, salivation, splayed posture, swollen face and dark pink scrotal (males). Impairment of male fertility and/or reproductive performance was indicated by their failing to induce pregnancy in paired female partners in both treated and untreated groups. The mid dose group showed decreased litter and pup loss. In addition to the litter and pup loss, the high dose group also showed slight increases in post implantation loss (and/or embryonic deaths) and placental weight. The fetus of high dose group showed low incidences of ubilical hernia and brackygnathia.

In a segment II teratology dose-range study (T2710), New Zealand white rabbits (6/dose) were given formoterol (0.2, 60, 125, 300 mg/kg/day) during days 6 - 18 of the pregnancy. The pregnant dams showed significant decreases in body weight gain during the treatment. The highest dose females actually suffered a weight loss. Fetal examinations on day 29 revealed the following responses in the two high dose groups: decreased litter weight, decreased fetal weight, decreased number of live fetus, increased intrauterine deaths, the enlarged heart, aortic arch and pulmonary arteries, and brachydactylies. These two high doses were considered the maternal toxic doses.

In a segment II teratology study (3014), New Zealand white rabbits (16/dose) were given formoterol (0.2, 3.5 and 60 mg/kg/day) during days 6 - 18 of the pregnancy. The pregnant dams were sacrificed on day 29 of the pregnancy. Dose related-increased mean body weight was seen in all treated groups. Fetal examination revealed abnormal cervicothoracic arteries, subcapsular cysts of the liver and assymetric bipartite sternbrae in the high dose group.

In a segment III reproductive and developmental toxicity study (T2095), female Sprague-Dawley rats (F0 generation, 30/dose) were given by oral gavage formoterol during day 6 of the pregnancy onward and though the lactation period. Pregnant dams were allowed to give birth naturally. Formoterol dose levels were 0, 0.2, 0.8, 3.4 mg/kg for the low, mid and high dose groups, respectively. Physical maturation of the F1 generation (pre-weaning, 4 pups/sex/litter) was assessed by pinna unfolding, tooth eruption, eye opening, surface righting, auditory startle, pupil reflex. Function and behavior of the F1 generation (post weaning, 1 litter/sex/dose) was assessed by Rota-rod performance, water-maze test, motor activity and mating performance.

Reproductive performance of the F1 generation was assessed by allowing them to mate and to litter. No clinical signs were observed during the treatment. Increased mean body weight was seen in all treated groups starting from treatment day 3. Increased food consumption was seen in the latter part of the treatment. A slight increase in pup loss was seen in the mid and high dose groups of the F1 generation. No abnormalities were observed in the other parameters.

A 3-month oral toxicity study (3160) was conducted in rats to further study (replicate) testicular effects of the drug in male reproductive system. This study was prompted by the observation of the decreased testis size in the 3-month inhalation toxicity study in the young male rats (3137). Young male Sprague-Dawley rats (age 16 days at the start of the treatment) were given orally formoterol 0, 0.03, 0.2, 0.8 and 3 mg/kg/day for 90 days. The vehicle consisted of hydroypropylmethyl cellulose, citric acid monohydrate, sodium phosphate dibasic. Animals were sacrificed for pathological examinations at the end of the treatment. Albuterol (20 and 50 mg/kg/day) was used as a reference. No clinical signs of toxicity were observed. Transient increases in the mean body weight occurred in the beginning of the treatment. The following were seen in both high dose formoterol and all albuterol groups: scrotal hyperemia, increased organ weights of the heart and lung, decreased absolute brain and slight increases in the body temperature. Significant decrease in testis weight was observed in the high dose formoterol group only. Scrotal hyperemia was also seen in the 0.8 mg/kg/day group. Histologic examinations showed occasional occurrence of testis tubular atrophy, but a dose-response relationship was lacking.

3. Mutagenicity:

Genotoxicity of formoterol was tested in *Salmonella typhirium* TA 1535, TA1537, TA1538, TA98, TA100, in L5178Y mouse lymphoma cell thymidine kinase forward mutation assay, in the *in vivo* rat micronucleus assay and the *in vitro* human lymphocytes chromosome aberration assay. Formoterol at high concentrations (4300 mg/plate) was weakly mutagenic in Salmonella strain T1538 in the presence of S9 liver fraction. The drug was not clastogenic in those assays.

4. Special Toxicity:

Formoterol clinical formulations were not hemolytic to human blood in vitro, nor it did induce protein flocculation when mixed with human serum. The drug was slightly irritating when given subcutaneously.

5. Carcinogenicity:

Carcinogenic potential of the formoterol was evaluated in mice (oral) and rats (inhalation). The respective dose levels were 0.1, 0.5 and 2.5 mg/kg/day in mice and 0, 4.7, 22 and 130 µg/kg/day in rats. Hard copy results of these studies have been submitted to the Agency; however, electronic format of the data is unavailable. The statistical reviewer is awaiting for his evaluation. Nonetheless, a preliminary review of the carcinogenicity data indicated that dose-

related incidences of leiomyoma was associated with the formoterol treatment in both rats and mice. This is not surprising because leiomyoma has been recognized as class effect for beta agonists and is considered irrelevant to the human population (Kelly et al., *J Amer Col Toxicol, 1993;12:13-21*). There were no other apparent treatment-related tumor findings. Final review and evaluation of the formoterol carcinogenicity will be carried out in consultation with the statistician reviewers.

6. Literature Review:

Yoshida *et. Al.* [*1983*] reported the following findings in the acute, subacute and chronic toxicity of formoterol in mice, rats and dogs: The LD50s of formoterol in mice and rats are summarized in Table 50. Pulmonary edema and myocardial necrosis were observed in animals that died pre-terminally.

Table 50. LD_{50} of Formoterol (mg/kg)

Route	IV	IP	SC	PO
Mice	100	190	1050	3130
Rats	72	225	655	6700

Multiple dose exposure toxicity of formoterol was evaluated for the duration of up to 6 months. A five-week oral study showed that formoterol treatment in rats resulted in myocardial fibrosis, increases in heart weights, and slight/reversible increases in serum AST, ALT and urea and decreases in blood glucose levels. Formoterol dosage was 3, 12 and 60 mg/kg/day. The cardiac lesions were seen in all dose groups and a dose-response relationship was apparent. These cardiac lesions was not readily reversible. After 10 weeks of recovery severe lesions were still apparent: (2/6-L, 6/6-M, 6/6-H).

A 6-month oral study in rats showed similar changes in the heart (≥ 0.3 mg/kg/day): myocardial fibrosis, increases in heart weights, slight and reversible increases in serum AST, ALT and urea and decreases in blood glucose levels. Formoterol dosage was 0.003, 0.03, 0.3, 3 and12 mg/kg/day, respectively. The NOEAL was 0.03 mg/kg/day.

A 6-month oral study in dogs again showed formoterol induced myocardial fibrosis at doses of 0.1 mg/kg/day or higher. Formoterol dosage was 0.001, 0.01, 0.1 and 10 mg/kg/day, respectively. Animals of pre-terminal deaths showed myocardial necrosis. The NOEAL was 0.01 mg/kg/day.

The effect of formoterol on fertility and reproductive performance was also investigated by the Japanese company previously. Sato and Kaneko (*folia pharmacol japon, 1984;27:257-65*) reported that Formoterol did not affect fertility and reproductive performance in rats. Sprague-Dawley rats (20/sex/dose) were dosed by oral gavage formoterol at doses of 0, 0.2, 6.0,

30 and 60 mg/kg/day. The dose schedules were 7 weeks before mating and through mating (two weeks) in the males; and the mating period plus the first week of pregnancy in the females. The vehicle was 0.5% methycellulose. The dams were sacrificed on day 20 for fetal examinations. Deaths occurred at the 2 highest dose levels. Increases in the organ weights of the heart, parotid and submaxillary glands were observed in the males. There were no apparent effect on the fertility parameters and litter responses.

Sato (*falia pharmacol japon, 198427:239-249*) studied teratogenic effects of oral formoterol in rats. Pregnant Sprague-Dawley rats (30/dose) treated with formoterol (0, 0.2, 6 and 60 mg/kg/day) during days 7-17 of pregnancy gave an increase in the incidences of undulate ribs in the high dose group. There were no apparent external and visceral abnormalities observed. Increased body weight and heart weight were also recorded. There were also slight increases in the organ weights of the spleen, liver, kidney and ovary. Hexoprenaline (6 and 60 mg/kg/day) as a control caused similar responses.

Sato (*falia pharmacol japon, 1984;27:251-256*) also studied teratogenic effect of oral formoterol in rabbits. Pregnant Japanese white rabbits (10/dose) were given formoterol (0, 0.2, 60 and 500 mg/kg/day) during gestation days 6 - 18. Dams were sacrificed on day 29 for fetal examinations. The control group received 0.5% methylcellulose. Hexopremaline (60 and 500 mg/kg/day) was used as a positive control. Decreases in mean plantation and pup viability were seen in the high dose group; however, no significant changes were observed in external, visceral and skeletal examinations.

Sato and Saegusa (*falia pharmacol japon, 1984;27:375-385*) studied the effect of oral formoterol on delivery and post natal development in rats. Four groups of female Sprague-Dawley rats were given by oral gavage formoterol during days 17 - 43 post coitus. Formoterol dose levels were 0, 0.2, 6.0 and 30 mg/kg respectively. The mid and high dose groups showed increases in the incidences of stillbirth, pup loss, and increases in pup body weight. Food and water intake was also decreased during the lactation period. No functional and developmental changes were observed in the F1 generation. No teratogenic effect was observed in both F1 and F2 generations.

B. EVALUATION

Pharmacology: Formoterol is a highly selective long-acting β_2-agonist that possesses potent bronchospasmolytic activity. As a bronchodilator, formoterol has been approved for clinical use in several countries. Japan first approved its oral administration in 1986. Subsequently, its metered dose inhalers were approved in Switzerland (1990), Netherlands (1992) and Denmark (1993), South Africa, (1994), Spain (1994) and the United Kingdom (1996). Formoterol is over 200 times more selective to beta$_2$ receptor than isoproterenol, a non-selective beta-adrenoceptor agonist. The high selectivity of formoterol presumably minimizes its cardiac effect compared to other β_2-adrenoceptor agonists.

In addition to its bronchodilatory action, formoterol may also exhibit secondary pharmacological effect in animals regardless of the route of administration and species. Formoterol exhibited characteristics of beta-agonists (i.e. hexoprenaline) that may affect behavior, hemodynamics, respiratory patterns, muscle contraction, and metabolism depending upon the dose of administration in animals. Among them, cardiovascular and hemodynamic effects are the most predominant and occur at relatively low doses. These effects include tachycardia, decreased blood pressure and changes in EKG. Other effects occurring at higher doses include decreases in blood glucose levels, increased serum pyruvic acid and lactate levels, decreased intestinal propulsion, depresses subtetanic contractions of uterus, and inhibition of gastric acid secretion induced by tetragastrin.

Formoterol is readily absorbed after oral and inhalation administration. The peak concentrations are reached 0.5 - 1 hour after oral and intratracheal administration. Distribution of the drug after inhalation exposure is in the following order: trachea > lung > kidney > liver > plasma > heart > brain. Half-lives of the drug after inhalation range 2 to 4 hours. Glucoronide conjugation and O-demethylation are the major pathways of metabolism. Formoterol is eliminated through both urine and the bile and an enterohepatic circulation may exists. Bioavailability after inhalation in animals is unknown. Approximately a half of the drug in the plasma is protein-bond. Formoterol readily crosses placenta to reach the fetus and is excreted into the milk. Pharmacokinetic profile of formoterol is similar between rats, dogs and humans.

Toxicology: General and special toxicities of formoterol has been evaluated *in vivo* and *in vitro* investigating its acute and chronic toxicity, genotoxicity, reproductive toxicity, tissue irritation and rodent carcinogenicity.

Acute toxicity: Signs of acute formoterol toxicity include tachycardia, decreased motor activity, labored breathing and salivation. The LD50 values in mice and rats after various routes of administration are presented in Table 51: