

pathological examination identified a dose-dependent increase in the incidence of small testes (0/10-C, 0/10-L, 1/10-M, and 3/10-H). Target organs of toxicity were the heart and testes. In the heart, there were findings of myocardial granular scars in the mid and high dose groups (incidences: 0/10-C, 0/10-L, 1/10-M, and 3/10-H). In the testes, there were findings in all dose groups that consisted of 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) and oligospermia in the epididymides (incidences: 0/10-C, 2/10-L, 1/10-M, 3/10-H). Formoterol-induced testicular toxicity was observed in the present study for all dose groups and was observed in other studies conducted by the present sponsor as well as by other sponsors (NDA 20-831, 6/12-month inhalation toxicology study with rats).

In a previous 3-month oral toxicology study with young rats that received formoterol at doses of 0.2, 0.8, and 3.0 mg/kg/day, there were treatment-related findings of testicular tubular atrophy, and spermatic debris, and oligospermia in the epididymides. In the present study, the sponsor used male rats of the same age that received oral doses of formoterol at 0.03, 0.2, 0.8, and 3.0 mg/kg/day to determine if previous findings could be replicated. Potential treatment-related deaths may have occurred with formoterol at doses of 0.8 and 3 mg/kg/day. In the formoterol and salbutamol treatment groups, there was a statistically significant increase in the frequency of observations of testes that were distinctly visible with a hyperemic scrotum. A corresponding decrease in the frequency of observations of testes not visible was also observed in all treatment groups with the exception of the 0.03 mg/kg/day formoterol group. There was no delay in the descent of the testes in treatment groups. The observation of testes that were distinctly visible with a hyperemic scrotum was attributed to a possible β -adrenergic effect (dilatation of systemic veins). Histopathological examination of tissues was limited to the testes, epididymides, and gross lesions. Incidences of tubular atrophy in the testes and spermatic debris in the epididymides for formoterol groups displayed no relationship to treatment. The appearance of atrophy was generally unilateral and subcapsular in distinction to the severe bilateral lesions with germ cell depletion (Sertoli cell-only pattern) observed in a previous study with young rats. This repeat study failed to replicate the drug-induced lesions in the testes and epididymides of an earlier study; however, the testicular toxicity of formoterol in rats has been replicated in several studies by the present sponsor as well as by other sponsors (NDA 20-831, 6/12-month inhalation toxicology study with rats).

In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group. Target doses of formoterol fumarate dihydrate for the low, mid, and high dose groups were 0.090, 0.280, and 0.890 mg/kg/day, respectively. Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control). Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively. These doses of excipients were significantly higher than those used in 3-month inhalation bridging

toxicology studies with Symbicort HFA pMDI in rats and dogs. The target organ of toxicity for formoterol was the heart. In the heart, the incidence and severity of myocyte degeneration were increased for male and female rats in the high dose group. The Grade 2 myocyte degeneration in the region of the papillary muscle for one female rat was characteristic of cardiac lesions produced by β -adrenergic agonists. Changes were evident in the lungs for vehicle-control group as compared to the air-control group. The incidence and severity of alveolar histiocytosis, pneumonitis, and congestion in the lungs were increased for the vehicle-control group as compared to the air-control group. During final review of this study under the NDA, there was concern that these findings could potentially be indicative of local toxicity induced by the vehicle (PVP K-25 and PEG-1000). However, the sponsor and independent pathologist re-examined these lung tissue slides and results indicated no evidence of local toxicity. See addendum to review under General Toxicology Studies conducted with Formoterol.

In a 6-month inhalation toxicology study with rats, formoterol was administered at doses of 0, 26, 128, and 852 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses were 0, 2.3, 12, and 72 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Decreased plasma glucose levels were observed in both sexes; however, only females showed a dose-response relationship. Increased absolute heart weights were observed in both male and female treatment groups, which is suggestive of cardiac hypertrophy. Myocardial fibrosis was observed for males and females in the mid (2/40) and high (6/40; 5 males and 1 female) dose groups. Tubular atrophy of the testes was observed in one mid dose male. The NOAEL was identified as the low dose based upon findings of myocardial fibrosis in the mid and high dose groups.

In a 1-month inhalation toxicology study with dogs, formoterol was administered at doses of 0, 0.5, 2.8, and 15 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses were 0, 0.07, 0.4, and 2.0 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Cardiac fibrosis was observed in males at the mid and high doses and in females at the low and high doses. Based upon findings of cardiac fibrosis, a NOAEL was not identified.

In a 1-month oral toxicology study with dogs, formoterol was administered at doses of 0, 2, 15, and 100 $\mu\text{g}/\text{kg}/\text{day}$. Increased heart rate was observed in all treatment groups on days 0 and 21. Ventricular arrhythmias were observed in the mid (1/6) and high (3/6) dose groups. Myocardial fibrosis was observed with dose-related incidences and severity (incidences: 1/6-Control, 0/6-Low, 2/6-Mid, and 4/6-High). The NOAEL was identified as the low dose based upon histopathological findings of myocardial fibrosis and occurrences of ventricular arrhythmias in the mid and high dose groups.

In a 12-month oral toxicology study with dogs, formoterol was administered at doses of 0, 0.72, 8.6, and 92 $\mu\text{g}/\text{kg}/\text{day}$. Sinus tachycardia was observed in all treatment 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 not observed at later ECG monitoring during the study period. Myocardial fibrosis was observed with dose-related incidences and severities (incidence: 0/10-Control, 2/10-Low, 3/10-Mid, and 4/10-High; severity: 0-Control, 1-Low, 1.3-Mid, and 2.3-High with a grade of 3 as the most severe).

Acinar atrophy of the pancreas was observed in the high dose group (3/10). A NOAEL was not identified based upon findings of myocardial fibrosis at all doses.

In a 1-month toxicology study, dogs received a formulation of formoterol HFA pMDI containing excipients, povidone K-25 (PVP K-25) and polyethylene glycol 1000 (PEG-1000), and propellants, HFA-227 and HFA-134a. Air-control and vehicle-control groups were included in the study. The vehicle-control group received PVP K-25, PEG-1000, HFA-227, and HFA-134a. Given that a close-fitting face mask and aerosol delivery tube system was used to deliver the aerosol directly into the buccal cavity of the dog, the deposition factor was assumed to be 100%. Doses of formoterol were 0.508, 3.01, and 15.3 µg/kg/day. Doses of PVP K-25, PEG-1000, HFA-227, and HFA-134a in the vehicle-control and high dose formoterol groups were 1.02, 102, 25500, and 76500 µg/kg/day, respectively. The target organ of toxicity for formoterol was the heart. Histopathological findings in the heart were confined to the high dose formoterol group and consisted of focal left ventricular myocytolysis and focal left ventricular mononuclear cell infiltration in 1 male (#27), multifocal myocardial left ventricular fibrosis, multifocal mononuclear cell infiltration, and chronic extramural coronary arteritis in 1 male (#26), and chronic extramural coronary arteritis in 1 female (#28). There was no evidence of local toxicity in the lung produced by the vehicle. The NOAEL was the mid dose of 3.01 µg/kg/day.

The sponsor has conducted subchronic and chronic toxicology studies with formoterol in both rats and dogs under IND — 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 studies 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.

General Toxicology Studies conducted with Budesonide:

Single Dose:

Acute Toxicity: In mice the minimal lethal inhalation dose was 100 mg/kg. In rats there were no deaths at an inhalation dose of 68 mg/kg. In mice the minimal lethal oral dose was 200 mg/kg. In rats, the minimal oral lethal dose was less than 100 mg/kg.

Repeat Dose:

See NDA 20-441 for single and repeat dose general toxicology studies conducted with budesonide.

Genetic toxicology:**Formoterol:**

The genotoxic potential of formoterol was assessed in the in vitro bacterial reverse mutation assay with *Salmonella typhimurium* tester strains, TA1535, TA1537, TA1538, TA98, and TA100; in the L5178Y mouse lymphoma cell thymidine kinase forward mutation assay; in the in vivo rat micronucleus assay; and the in vitro human lymphocytes chromosomal aberration assay. Formoterol was negative in the in vitro bacterial reverse mutation assay. Increased revertant colony counts were observed for TA1538 in the presence of S9 liver fraction; however, a 3-fold elevation over the vehicle-control was not achieved. Statistical analysis of the in vitro bacterial reverse mutation assay is generally not considered appropriate. Further, the significance of increased revertant colony counts for TA1538 was questionable given that TA98 was negative. The only difference between strains TA1538 and TA98 is that TA98 possesses an error-prone repair system (i.e., TA98 should be more sensitive). Strain selection for the bacterial reverse mutation assay was inadequate. The standard set of strains used in bacterial mutation assays should include strains that will detect point mutations at A-T sites, such as TA102 or *Escherichia coli*/WP2 uvrA. Neither strain was included in the present study. The drug was not clastogenic in the in vitro mouse lymphoma assay and in vivo rat micronucleus assay. It is noted that racemic formoterol was negative in in vitro bacterial reverse mutation assays reported in NDA 20-831 (Novartis, Foradil®).

The mutagenic potential of _____ a degradant of formoterol, was assessed in the in vitro bacterial reverse mutation assay. The study was inadequate with regard to selection of tester strains as noted for formoterol above. Further, the number of plates per dose \pm S9 was inadequate (i.e., only 1 plate per dose \pm S9). _____ at high doses up 12300 or 11600 μ g/plate \pm S9 produced no increases of revertant colony counts for tester strains TA1535, TA100, TA1538, TA98, or TA1537. _____ contains a structural alert (i.e., aromatic amine) that is similar to the structural alert in the parent compound, formoterol (i.e., aromatic amine with a formyl group). Genetic toxicity testing with the parent compound, formoterol, is considered sufficient for _____. In 2-year carcinogenicity studies with formoterol, there were findings of ovary and/or uterine leiomyomas as observed with other beta-agonist drugs.

Budesonide:

Budesonide was not mutagenic or clastogenic in six different test systems: Ames Salmonella /microsome plate test, mouse micronucleus test, mouse lymphoma test, chromosome aberration test in human lymphocytes, sex-linked recessive lethal test in *Drosophila melanogaster*, and DNA repair analysis in rat hepatocyte culture. See NDA 20-441 for further details of studies conducted with budesonide.

Carcinogenicity:

Formoterol (See attached reviews under IND _____):

To evaluate the carcinogenic potential of formoterol fumarate, the sponsor conducted a 2-year oral carcinogenicity study with Swiss mice and a 2-year inhalation carcinogenicity study with Sprague-Dawley rats.

Swiss mice [_____ 60/sex/dose] received formoterol by oral gavage at doses of 0.1, 0.5, and 2.5 mg/kg/day for 2 years. Two additional groups received the vehicle only and served as controls. Plasma formoterol AUC in the high dose group was 65 times the human AUC. A dose-related and statistically significant increase ($P < 0.0001$) in the incidences of leiomyomas in the uterus were found in all female treatment groups (Incidences: 4/120-C, 7/60-LD, 11/60-MD, and 13/60-HD). The same trend was also apparent in the preterminally sacrificed animals (Incidences: 3/73-C, 2/33-LD, 6/41-MD, and 7/38-HD). Leiomyomas are known to be associated with β_2 -agonist treatment of female mice.

In the mouse carcinogenicity study, the sponsor reported that AUC values in the high dose group (2.5 mg/kg/day) ranged from 5.1 to 24 nmol·hr/mL; however, no reliable AUC data was obtained from the low dose (0.1 mg/kg/day) and mid dose (0.5 mg/kg/day) groups. The sponsor reported the human AUC obtained with the proposed therapeutic dose of formoterol in clinical trials was measured to be 0.31 nmol·hr/mL (these were clinical trials with formoterol only). Examination of the data indicated that mice in the mid dose group had plasma formoterol concentrations at higher levels based on single time point estimates than humans. Thus, it would be expected that there would be little difficulty in detecting the drug in plasma of mice from the low and mid dose groups, provided that assays for both mice and human samples were similar. The Executive CAC (April 14, 1998) recommended further clarification of the toxicokinetic data from the sponsor. The sponsor provided a response on April 29, 1998. The sponsor stated that the inability to calculate AUC data for mice was due to the use of different analytical methods for rodents and humans. The assays for mouse plasma samples, conducted in 1992, had a lower sensitivity and selectivity than the improved assay for human samples (1995), which had a 30-fold lower detection limit.

Wistar rats (50/sex/dose, the review mistakenly states Sprague-Dawley rats) received formoterol by nose-only inhalation at doses of 0, 4.7, 22, and 130 $\mu\text{g}/\text{kg}/\text{day}$. Two additional groups received lactose only to serve as controls. Considering the particle size distribution and collection efficiency of the respiratory tract, the corresponding mean body burdens of formoterol fumarate were 0, 1.9, 9.0, and 58 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The deposition in the tracheobronchial and pulmonary regions was estimated at approximately 9% of the inhaled dose. Plasma formoterol AUC in the high dose group was 50 times the human AUC. The only detected neoplastic finding was a non-statistically significant increase in the incidence of ovary and uterine leiomyomas (one each). No such tumor was found in the control, low, and mid dose groups. The (combined) incidence of leiomyomas (4%) in the high dose group was much higher than

the historic background incidence from the testing laboratory of 0.052% (4/7748). Leiomyomas in the female reproductive organs in rats are a typical β_2 -agonist effect and considered a drug-related effect.

Budesonide:

See NDA 20-441 for further details of carcinogenicity studies conducted with budesonide:

In a two-year study in Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of gliomas in male rats at an oral dose of 50 $\mu\text{g}/\text{kg}$. No tumorigenicity was seen in male and female rats at respective oral doses up to 25 and 50 $\mu\text{g}/\text{kg}$. In two additional two-year studies in male Fischer and Sprague-Dawley rats, budesonide caused no gliomas at an oral dose of 50 $\mu\text{g}/\text{kg}$. However, in the male Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of hepatocellular tumors at an oral dose of 50 $\mu\text{g}/\text{kg}$. The concurrent reference corticosteroids (prednisolone and triamcinolone acetonide) in these two studies showed similar findings.

In a 91-week study in mice, budesonide caused no treatment-related carcinogenicity at oral doses up to 200 $\mu\text{g}/\text{kg}$.

Reproductive toxicology:

Reproductive toxicology studies conducted with Symbicort (budesonide/formoterol):

Teratology study with Symbicort: In embryofetal development study, three groups of 24 mated female rats were exposed to a combination of budesonide and formoterol in a Symbicort HFA pMDI formulation by nose-only inhalation at actual doses of 2.5 + 0.14, 12 + 0.66, and 80.0 + 4.4 $\mu\text{g}/\text{kg}/\text{day}$ from days 6 to 16 of gestation. Deposited doses of budesonide and formoterol for low, mid, and high dose groups were 0.24 + 0.014, 1.01 + 0.057, and 6.8 + 0.39 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Target doses selected were identical to those used in the 3-month inhalation toxicology study with the Symbicort HFA pMDI formulation in rats. The pMDI formulation contained excipients, PVP K-25 and PEG 1000, and the propellant, HFA-227. The pMDIs used in this study were approximately 4 months beyond their documented shelf life. A control group was exposed to air only.

Body weight gains for female rats in the low, mid, and high dose groups from days 6 to 16 of gestation were decreased to 87.6, 85.5, and 33% of the control, respectively. Maternal toxicity was evident for the high dose group.

Post-implantation losses were slightly increased for dams in mid and high dose groups, although there were no treatment-related effects on numbers of live fetuses per dams.

The Symbicort HFA pMDI formulation was found to be teratogenic for mid and high dose groups. A major external malformation, umbilical hernia, was observed for 1 fetus (0.4%) at the mid dose and 2 fetuses at the high dose (0.9%). These incidences exceeded the mean historical control incidence of 0.01%. A major visceral malformation, aortic arch: right sided, was observed for 1 fetus in the high dose group. For sternebra, a major malformation, one or more fused, was observed for 1 fetus in the high dose group. These findings were each confined to one fetus and their relationship to treatment was unclear given that maternal toxicity was evident for the high dose group. Incidences of incomplete ossification and not ossified were increased for the high dose group as well as possibly lower dose groups. The incidences of a rudimentary 14th right rib were increased for treatment groups.

Reproductive toxicology studies conducted with Formoterol:

Combined Fertility, Reproductive Performance, and Embryofetal Development Study in Rats: There were treatment-related clinical signs suggestive of toxicity for males at oral doses of 0.2, 3, and 15 mg/kg/day and females at 3 and 15 mg/kg/day. Decreased fertility and/or reproductive performance were observed for males in the 15 mg/kg/day group. Formoterol produced teratogenic effects with findings of umbilical hernia in the 3 and 15 mg/kg/day groups and brachygnathia and malrotated right hindlimb in the 15 mg/kg/day group. Embryoletality was observed at doses of 3 and 15 mg/kg/day. Placental weights were increased in the 15 mg/kg/day group. Litter and pup loss was observed at 3 and 15 mg/kg/day. Pup body weights were decreased at 15 mg/kg/day.

Inhalation teratology study in rats: In an inhalation teratology study, formoterol was not teratogenic at inhaled deposited doses up to 91 µg/kg/day.

Oral teratology study in rabbits: In an oral teratology study, incidence of anomalous cervicothoracic arteries were increased for all treatment groups, although a dose-response relationship was not present. The incidence of subcapsular cysts on the liver was increased at 60 mg/kg/day (23/115 = 20%). Incidences of satural cranial bones were increased at 3.5 and 60 mg/kg/day. Incidences of asymmetric bipartite sternebrae for the low, mid, and high dose groups were increased to 4.1, 4.2, and 9.4%, respectively, as compared to 1.1% for the control. Formoterol was teratogenic at 60 mg/kg/day in rabbits based upon findings of subcapsular cysts on the liver. There were no teratogenic findings in rabbits at doses of 0.2 and 3.5 mg/kg/day.

Pre- and postnatal development study in rats: In a pre- and postnatal development study, incidences of total litter loss for mid and high dose groups were both 3 of 30 as compared to 1 of 30 for the control group. Cumulative litter loss from birth to postpartum day 26 for the low, mid, and high dose groups was increased to 21.6, 28.8, and 27.8%, respectively, as compared to 11.0% for the control group; however, there was no evidence of a dose-response relationship. Litter loss after postpartum day 26 was minimal. Pup body weights were slightly lower for treatment groups as compared to the control group. There were no treatment-related effects on the physical, functional, and

behavioral development of the F₁ generation. There were no treatment-related effects on the reproductive capacity of the F₁ generation.

See attached reviews for further details of reproductive toxicology studies conducted with formoterol under IND _____.

Reproductive toxicology studies conducted with Budesonide:

Fertility and Reproductive Performance: In rats, budesonide had no effect on fertility at subcutaneous doses up to 80 µg/kg. However, it caused a decrease in prenatal viability and viability in the pups at birth and during lactation, along with a decrease in maternal body-weight gain, at subcutaneous doses of 20 µg/kg and above. No such effects were noted at 5 µg/kg

Teratology Studies: As with other corticosteroids, budesonide was teratogenic and embryocidal in rabbits and rats. Budesonide produced fetal loss, decreased pup weights, and skeletal abnormalities at subcutaneous doses of 25 µg/kg in rabbits and 500 µg/kg in rats. In another study in rats, no teratogenic or embryocidal effects were seen at inhalation doses up to 250 µg/kg.

See NDA 20-441 for further details of reproductive toxicology studies conducted with budesonide.

Special toxicology:

Formoterol in vitro did not produce hemolysis or protein flocculation. Potential tissue irritation and vasoconstriction properties of formoterol were assessed in beagle dogs following subcutaneous and intravenous administration. Formoterol produced dose-dependent slight reactive changes (e.g., hemorrhage, perivascular infiltration of leukocytes, granulation, and microfocal necrosis) at subcutaneous injections; however, no effects were observed at intravenous injection sites.

See attached reviews for further details of studies conducted with formoterol under IND _____ . See NDA 20-441 for studies conducted with budesonide.

2.6.6.2 Single-dose toxicity

See attached reviews of single dose toxicology studies conducted with formoterol under IND _____. See NDA 20-441 for studies conducted with budesonide.

Rats

Study title: Symbicort (Budesonide + Formoterol): Single Dose Inhalation (Powder) Toxicity Study in the Rat.

Key study findings:

▶ In a single dose 1- hr inhalation toxicology study, rats were exposed to air or Symbicort (97 mg/kg budesonide+3 mg/kg formoterol) and observed for 14 days after exposure. Deposited doses of budesonide and formoterol were 7.9 and 0.24 mg/kg, respectively.

▶ There were no deaths. Body weight gains for male rats treated with Symbicort were decreased to 40% of the air-control. Female rats treated with Symbicort lost 8.3% of initial body weight gain. These effects on body weight were attributed to the action of budesonide.

▶ Decreased absolute and relative weights of the spleen, thymus, and adrenal glands were observed for male and female rats treated with Symbicort. These changes are attributed to the pharmacological action of budesonide.

Study no.: 02218

Conducting laboratory and location: AstraZeneca R&D Sodertalje
SE-151 85 Sodertalje
Sweden

Date of study initiation: November 5, 2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Symbicort (Budesonide + Formoterol fumarate dihydrate), batch 1916/02. This formulation was a micronized, mixed dry powder containing budesonide and formoterol

Methods

Doses: See table below

Actual dose of Symbicort (mg/kg)

Agent	Aerosol concentration µg/L	Duration of exposure (min)	Inhaled dose mg/kg	Deposited dose ^a mg/kg
Budesonide	-	68	97	7.9
Formoterol	-	68	3	0.24
Symbicort ^b	2023.206	68	100	8.1

a. The pulmonary deposition factor calculated by the sponsor was estimated to be 8%. MMAD values for budesonide and formoterol were _____ respectively.

b. Approximately 2 mg of aerosol mass per liter of air was the highest technical and practical target concentration that could be achieved with the inhalation system and with the aerosol generator operating at its maximum performance. The maximum dose of Symbicort that could be administered within 1 hr was 100 mg/kg Symbicort.

Species/strain. Male and female Wistar _____, rats

Number/sex/group or time point (main study): 5 rats/sex/group

Route, formulation, volume, and infusion rate. Symbicort or air was administered by nose-only inhalation. The exposure by the inhalation route was performed using nose-only flow-past exposure chambers. The test compound was compressed to a powder cake before use and a dry particle aerosol was produced by the use of a modified dust feeder mechanism. A chamber with 40 animal ports was used for dosing. During exposure the rats were restrained in polycarbonate tubes with their snouts protruding into the chamber. The actual aerosol concentration was measured on-line during each exposure by the use of a light-scatter monitor. Durations of exposure for air- and Symbicort-treated rats were 60 and 68 min, respectively. Aerosol concentrations of budesonide and formoterol were measured with gravimetric and chemical methods.

The particle size distribution of the aerosol was determined once prior to the start and on the day of dosing. Samples were taken using a cascade impactor. Samples were collected on non-coated stainless steel substrates. The amount of Symbicort on each stage of the impactor was determined with a gravimetric method. Actual amounts of budesonide and formoterol were determined with a HPLC method. Mass median aerodynamic diameter and geometric standard deviation values for budesonide and formoterol were determined by calculation.

Satellite groups used for toxicokinetics or recovery. None

Age. On the day of treatment, animals were approximately 8 weeks old.

Weight. On the day of treatment, body weight ranges were 190-240 g for males and 180-210 g for females.

Unique study design or methodology. Symbicort was administered to rats by a single dose nose-only inhalation.

Observations and times:

Mortality. Animals were observed for mortality/moribundity once or twice daily.

Clinical signs. Animals were observed for clinical signs before dosing, at least once during the intervals of 0-1, 1-3, 3-6, and 6-24 hr, and then daily up to 14 days after dosing.

Body weights. All animals were weighed immediately before dosing and on days 2, 3, 4, 7, 10, and 15.

Food consumption. Not performed

Ophthalmoscopy. Not performed

EKG. Not performed

Hematology. Not performed

Clinical chemistry. Not performed

Urinalysis. Not performed

Gross pathology. All animals were necropsied on day 15. The lung, spleen, kidneys, liver, thymus, heart, and adrenals were collected for possible microscopic examination.

Organ weights. Absolute and relative organ weights were determined for the brain, heart, adrenals, lungs, thymus, kidneys, liver, and spleen.

Histopathology. Not performed.

Toxicokinetics. Not performed.

Results

Mortality: None.

Clinical signs: After exposure to Symbicort, there 24 observations of thin for 4 male rats from days 3 to 9 and 38 observations of thin for 5 female rats from days 3 to 11. There were 3 observations of trembling during handling for 2 male rats from days 7 to 13 and 4 observations for 3 female rats from days 1 to 15. Increased respiratory rate was observed for 2 male rats at 1 hr after dosing with Symbicort. A slight decrease of motor activity was observed for 1 male rat at 1 hr after dosing with Symbicort. Staining of the nostrils was observed for 1 female rat at 1 hr after dosing with Symbicort.

Body weights: Body weight gain for the male rats over the 14-day observation period after a single exposure to Symbicort was decreased to 40% of the air-control. Over the 14-day observation period after a single exposure to Symbicort, female rats lost 8.3% of the initial body weight. Body weight effects were attributed to budesonide.

Gross pathology: Not reported.

Organ weights: Decreased absolute and relative weights of the spleen, thymus, and adrenal glands were observed for male and female rats treated with Symbicort. These changes are attributed to the pharmacological action of budesonide.

Absolute and relative organ weights

Organ	Male rats		Female rats	
	Air	Symbicort	Air	Symbicort
Spleen g	0.60480	0.47680* (79%)	0.53680	0.35560* (66%)
Spleen %BrW	33.14	26.48* (80%)	29.51	20.15* (68%)
Thymus %	0.53120	0.22560* (43%)	0.48400	0.08020* (17%)
Thymus %BrW	29.13	12.61* (43%)	26.59	4.58* (17%)
Adrenal glands g	0.05440	0.04240* (78%)	0.07400	0.05100* (69%)
Adrenal glands %BrW	2.98	2.36* (79%)	4.06	2.89* (71%)

Dogs

Study title: Symbicort (Budesonide + Formoterol): Single Dose Inhalation (Powder) Toxicity Study in the Dog.

Key study findings:

► In a single dose nose-inhalation toxicology study, one group of 2 male and 2 female beagle dogs were exposed to Symbicort (737 µg/kg budesonide + 22 µg/kg) for 6.8 min. Deposited doses of budesonide and formoterol were 117 and 3.3 µg/kg, respectively.

► There were no deaths. Clinical signs after dosing included mucosal redness, body tremor, vomiting, loose stools, increased salivation, nasal catarrh, abdominal respiration, and redness of intact skin.

► Sinus tachycardia was observed for all dogs from immediately after dosing to 4-hr postdose. Ventricular tachycardia was observed for male #2 at 24- and 48-hr postdose and female #4 at 24-hr postdose. Male #1 was observed with 3 monofocala VES at 24-hr postdose.

Study no.: 02219

Conducting laboratory and location: AstraZeneca R&D Sodertalje
SE-151 85
Sodertalje, Sweden

Date of study initiation: November 19, 2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Symbicort (Budesonide + Formoterol fumarate), batch number 1916/02. Symbicort was provided as a mixed dry powder containing particles of budesonide and formoterol

Methods

Doses: The dogs were exposed by nose-only inhalation, at the reported maximum technically achievable concentration corresponding to _____ particulate aerosol mass per liter of air. The test compound was compressed to a powder cake, which was administered as a dry particle aerosol using a _____ dust feeder. Test material was scraped from a cake through rotation against a scraper blade and was dispersed in clean compressed air at a constant flow of 10 L/min. The concentration of the aerosol was adjusted, when appropriate, by changing the speed of rotation. Tubes carried the aerosol to a nose-only arrangement in which the nose of the dog was fitted into a molded mask with a nostril cut-out. The actual aerosol concentration of Symbicort was measured on-line during each exposure by use of a light-scatter monitor, which had been calibrated to the quantities of budesonide and formoterol. The duration of exposure time was 6.8 min.

Compound	Concentration, µg/L		Dose, µg/kg		Deposited dose ^a , µg/kg
	Target	Actual	Target	Actual	
Budesonide	485	396	730	737	117
Formoterol	15	12	20	22	3.3
Symbicort	500	408	750	759	120

a. The sponsor used a deposition factor of 0.15.

The particle size distribution of the aerosol was determined once prior to the start and on the day of dosing. Samples were taken using a _____ cascade impactor. Samples were collected on non-coated stainless steel substrates. The amount of Symbicort on each stage of the impactor was determined with a gravimetric method. Actual amounts of budesonide and formoterol were determined with a HPLC method. Mass median aerodynamic diameter and geometric standard deviation values for budesonide and formoterol were determined by calculation.

Compound	MMAD, μm	GSD
Budesonide		2.29
Formoterol		1.94

Species/strain. Male and female beagle dogs were obtained from _____

Number/sex/group or time point (main study). The study consisted of one group of 2 male and 2 female dogs. A concurrent control group was not included.

Route, formulation, volume, and infusion rate. Symbicort was administered as a micronized dry powder aerosol by nose-only inhalation.

Satellite groups used for toxicokinetics or recovery. None.

Age. On the day of dosing, males were 8.5 to 9 months old and females were 8 to 8.5 months old.

Weight. On the day of dosing, body weight ranges were 9.7-9.9 kg for males and 7.9-8.2 kg for females.

Unique study design or methodology. Symbicort was administered to dogs by a single dose nose-only inhalation.

Observations and times:

Mortality. Animals were observed daily.

Clinical signs. Clinical signs were observed daily. Particular attention was directed to animals during dosing and the first hr postdose. Complete clinical examinations were performed on all animals before dosing started, on day 8, and on the day of necropsy. Rectal temperature was measured before dosing started, at 1, 4, and 48 hr postdose, and on days 4 and 8.

Body weights. Body weights were measured before dosing started, on day 8, and on the day of necropsy. There was no concurrent control group for comparison.

Food consumption. Food consumption was measured daily. There was no concurrent control group for comparison.

Ophthalmoscopy. Not performed.

EKG. Electrocardiographic recordings were recorded on 3 occasions before the start of dosing, on day 1 before dosing, immediately after dosing, 0.5, 1, 4, 24, and 48 hr postdose, and on days 4, 7, and 14. The dogs were held in right lateral recumbence. Leads I, II, III, aVR, aVL, aVF, V1, V2, V3, and V6 were simultaneously recorded. Heart rate, P wave amplitude, and RR, PR, QRS, and QT intervals were determined. QTc intervals were calculated using van der Water's formula. Visual examinations of all ECG recordings were conducted to discern any pathological disturbances in the conducting mechanism.

Hematology: Blood samples for measurement of hematology parameters were collected twice before dosing started and on days 2 and 15 postdose.

Clinical chemistry: Blood samples for measurement of clinical chemistry parameters were collected twice before dosing started and on days 2 and 15 postdose.

Urinalysis: Not performed.

Gross pathology: Dogs were sacrificed on day 15 and submitted to necropsy examination. Tissue samples were collected for microscopic examination from the trachea, heart, spleen, kidneys, lungs, bronchial lymph node, thymus, larynx, liver, axillary lymph node, adrenals, and nose; however, no tissues were processed to slides in this study.

Organ weights: Absolute and relative organ weights were measured for the brain, heart, adrenals, lungs, thymus, kidneys, liver, and spleen. There was no concurrent control group for comparison.

Histopathology: Not performed.

Toxicokinetics: Blood samples for determinations of plasma budesonide and formoterol concentrations were collected before dosing and at 0.083, 0.5, 2, 4, 6, and 24 hr postdose; toxicokinetic analysis was not performed.

Other: Tidal volumes and respiratory frequencies were recorded during each daily inhalation exposure.

Results

Mortality: None.

Clinical signs: Clinical signs consisted of mucosal redness (females #3 and #4 on day 1), body tremor (male #1 on day 1), vomiting (female #3 on day 1), loose stools (female #3 on days 2 and 11 and female #4 on day 4), increased salivation (males #1 and #2 on day 1), nasal catarrh (male #1 on day 1), abdominal respiration (male #1 on day 1), and redness of intact skin (males #1 and #4 on day 1). There were no effects on rectal temperature.

Body weights: All animals gained weight from days -6 to 15; however, there was no concurrent control group for comparison.

Food consumption: Food consumption from days -16 to 14 ranged from 88 to 257 g/day for male #1, 139 to 259 g/day for male #2, 195 to 208 g/day for female #3, and 98 to 207 g/day for female #4.

EKG: Heart rate from immediately after dosing to 4-hr postdose was increased to 184.5-207.4% of the baseline (104.5 bpm). The RR interval from immediately after dosing to 4-hr postdose was decreased to 46-52.6% of the baseline (0.195 sec). Observed increase of heart rate or decrease of RR interval was classified as sinus tachycardia. Ventricular tachycardia was observed for male #2 at 24- and 48-hr postdose and female #4 at 24-hr postdose. Male #1 was observed with 3 monofocala VES at 24-hr postdose. There were no effects on P wave amplitude or the PR, QRS, QT, and QTc intervals as compared to baseline values.

Hematology: White blood cell counts on day 2 were increased to 143% of baseline ($9.7 \times 10^9/L$) at day -9. Neutrophil counts on day 2 were increased to 190.5% of baseline ($12 \times 10^9/L$) at day -9. Lymphocyte counts on day 2 were decreased to 44.6% of baseline ($1.24 \times 10^9/L$) at day -9. No eosinophils were evident on day 2 as compared to baseline ($0.175 \times 10^9/L$) at day -9. These effects were reversible by day 15.

Clinical chemistry: Alkaline phosphatase (ALP) activity on day 2 was increased to 356% of the baseline (129.8 U/L) at day -9. Cholesterol levels on days 2 and 15 were increased to 165.6 and 162.3 of baseline (3.02 mmol/L), respectively. Potassium levels on day 2 were increased to 109% of baseline (4.83 mmol/L). Triglyceride levels on day 2 were decreased to 83.4% of baseline (0.603 mmol/L). Calcium levels on day 2 were slightly decreased to 94.1% of baseline (2.83 mmol/L) at day -9. Changes of ALP activity, potassium, triglycerides, and calcium were reversible by day 15, however, increased cholesterol levels remained elevated at day 15.

Gross pathology: There were no reported gross pathological findings. No concurrent control group was available for comparison.

Other: There were no effects on tidal volume or breathing frequency.

2.6.6.3 Repeat-dose toxicity

See attached reviews of repeat dose toxicology studies conducted with formoterol under IND — See NDA 20-441 for studies conducted with budesonide.

Rats

Study title: Testicular Effects of Formoterol Fumarate (D2522) Given Orally to Young Rats for 3 Months, Using Salbutamol Sulphate as a Reference Compound.

Key study findings:

- ▶ In a previous 3-month oral toxicology study with young rats that received formoterol at doses of 0.2, 0.8, and 3.0 mg/kg/day, there were findings of testicular tubular atrophy (incidences: 1/10-C, 4/10-L, 1/10-M, 7/10-H), and spermatid debris (incidences: 0/10-C, 2/10-L, 1/10-M, 4/10-H) and oligospermia in the epididymides (incidences: 0/10-C, 2/10-L, 1/10-M, 3/10-H). In the present study, the sponsor used male rats of the same age that received oral doses of formoterol at 0.03, 0.2, 0.8, and 3.0 mg/kg/day to determine if previous findings could be replicated. Salbutamol was studied at doses of 10 and 25 mg/kg BID.
- ▶ Potential treatment-related deaths may have occurred with formoterol at doses of 0.8 and 3 mg/kg/day.

► In the formoterol and salbutamol treatment groups, there was a statistically significant increase in the frequency of observations of testes that were distinctly visible with a hyperemic scrotum. A corresponding decrease in the frequency of observations of testes not visible was also observed in all treatment groups with the exception of the 0.03 mg/kg/day formoterol group. There was no delay in the descent of the testes in treatment groups. The observation of testes that were distinctly visible with a hyperemic scrotum was attributed to a possible β -adrenergic effect (dilatation of systemic veins).

► Histopathological examination of tissues was limited to the testes, epididymides, and gross lesions. Incidences of tubular atrophy in the testes and spermatic debris in the epididymides for formoterol groups displayed no relationship to treatment. The appearance of atrophy was generally unilateral and subcapsular in distinction to the severe bilateral lesions with germ cell depletion (Sertoli cell-only pattern) observed in a previous study with young rats.

► This repeat study failed to replicate the drug-induced lesions in the testes and epididymides of an earlier study; however, the testicular toxicity of formoterol in rats has been replicated in several studies by the present sponsor as well as other sponsors (NDA 20-831, 6/12-month inhalation toxicology study with rats).

Study no.: T3160

Volume #, and page #: Module 4, Pages 1 to 378

Conducting laboratory and location: Astra
Safety Assessment
S-151 85 Sodertalje, Sweden

Date of study initiation: February 22, 1995

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Formoterol fumarate dehydrate \longrightarrow , batch 73274010
(Purity, \longrightarrow)

Methods

Doses: Formoterol was administered at oral doses of 0, 0.03, 0.2, 0.8, and 3 mg/kg/day. The reference, salbutamol was administered at oral doses of 10 and 25 mg/kg BID with 6 hr between the two daily doses.

Dose, mg/kg/day	Toxicology (3-month treatment) Group #	Toxicokinetics	
		3-month treatment Group #	7-day treatment Group #
0 (Vehicle)	1	8	15
0.03 Formoterol	2	9	16
0.2 Formoterol	3	10	17
0.8 Formoterol	4	11	18
3.0 Formoterol	5	12	19
2 x 10 Salbutamol	6	13	20
2 x 25 Salbutamol	7	14	21

Species/strain: Pregnant female Sprague-Dawley rats were obtained from _____ approximately 9 to 13 days before delivery. The pups were kept with their dams until they were approximately 25 days old. Male rats were then separated from their dams and placed two per cage.

Number/sex/group or time point (main study): 12 males/group

Route, formulation, volume, and infusion rate: Vehicle and drug solutions were administered by oral gavage. The vehicle for formoterol dose groups was 5 mg/mL hydroxypropyl methylcellulose, 0.77 mg/mL citric acid monohydrate, 2.25 mg/mL disodium hydrogen phosphate, dihydrate in distilled water. The vehicle for salbutamol sulphate dose groups was physiological saline.

Satellite groups used for toxicokinetics or recovery: 12 males/group (separate groups of animals were used for toxicokinetic measurements on day 7 and after 3 months of treatment)

Age: At the start of treatment, male rats in main study and satellite groups were 16-17 and 15-17 days old, respectively.

Weight: At the start of treatment, body weight ranges for male rats in main study and satellite groups were 38-49 g and 36-49 g, respectively.

Unique study design or methodology: This study was intended to examine the effects of formoterol fumarate on the testes of young rats (16-17 days old at the start of the study). The objective of the study was to determine if the findings of testicular atrophy observed in a previous 3-month study in rats could be replicated. Salbutamol sulphate was included in the study as a reference compound.

Observations and times:

Mortality: Animals were observed twice daily for moribundity/mortality.

Clinical signs: Animals were observed daily for clinical signs in connection with dosing. A detailed clinical examination of each animal before dosing was performed once per week during the first month and thereafter every second week.

Observations of the location of the testes were conducted in order to monitor descent of the testes to the scrotum. Observations were conducted at 5 hr after dosing. From week 2 (after pups were separated from the dam) and up to week 5, the testes locations were recorded 2 or 3 times per week. From week 5 and until termination, the testes locations were recorded every other week. The locations of the testes were recorded as testes not visible, testes visible, and testes distinctly visible and scrotum hyperemic. The last alternative indicates that the testes are in the scrotum, clearly visible and protruding, with a hyperemic scrotum.

Rectal temperatures were measured for the control and all treatment groups on days 0, 2, 9, and 14 and for the control, high dose formoterol, and high dose salbutamol groups on days 0, 2, 7, 14, 24, 30, 35, 44, 58, 72, and 86. On day 0, rectal temperatures were recorded before dosing and 1 hr after dosing. On days 2, 7, and 9, rectal temperatures were recorded before dosing and at 2, 3, 4, and 5 hr after dosing. On days 14, 24, 30, 35, 44, 58, 72, and 86, rectal temperatures were recorded before dosing and at 3 hr after dosing (after the first 2 weeks, the post-dose recording was conducted at 3 hr after

dosing to avoid a temperature elevation caused by stress in connection with the dosing procedure).

Body weights. Body weights were measured on days 0, 3, 7, 10, 14, and 17 and thereafter once per week.

Food consumption. Food and water consumption were not recorded until after weaning. From week 2, food consumption was measured weekly for animals in each cage. From week 2, water consumption was measured every fourth week for animals in each cage.

Ophthalmoscopy. Not performed.

EKG. Not performed.

Hematology. Not performed.

Clinical chemistry. Not performed.

Urinalysis. Not performed.

Gross pathology. Rats in main study groups were sacrificed after 3 months of treatment and submitted to necropsy examination. Tissue samples for microscopic examination were taken from all rats and preserved in a buffered, neutral 3.7% formaldehyde solution with the following exceptions: eyes were preserved in methanol formaldehyde acetic acid fixative and testes and **epididymides in Bouin's fixative.**

Organ weights. Relative and absolute organ weights were measured for the brain, lungs, liver, heart, thymus, spleen, adrenals, kidneys, testes, and prostate.

Histopathology. Histopathological examination of tissues was limited to the testes, epididymides, and gross lesions, which were embedded in Histowax, sectioned at 4-6 μm , stained with hematoxylin and eosin and the PAS stain, and examined by light microscopy. In addition, specimens from all testes were embedded in glycol-methacrylate, sectioned at 2 μm , and stained with hematoxylin and phloxine and toluidine blue; however, these specimens were not examined.

Toxicokinetics. Blood samples for measurement of plasma drug concentrations were collected from satellite groups on day 7 and after 3 months of treatment at 0.5, 1, 3, 6, and 12 hr after dosing for formoterol groups and at 0.5, 1, 3, 6, 7, 9, and 24 hr after dosing for salbutamol groups. Formoterol and salbutamol were measured by liquid chromatography methods with electrochemical detection. The limits of quantification for formoterol and salbutamol were 0.25 and 1.0 nmol/L when 1.0 mL plasma was used. Plasma drug concentrations for the 0.03 mg/kg/day formoterol group were not measured due to lack of toxicity. It should be noted that plasma drug concentrations were undetectable for the 0.2 mg/kg/day group. For animals sampled on day 7 (Groups 15-21), a necropsy examination restricted to the testes and epididymides was conducted. Animals sampled after 3 months of treatment (Groups 8-14) were discarded without examination.

Results

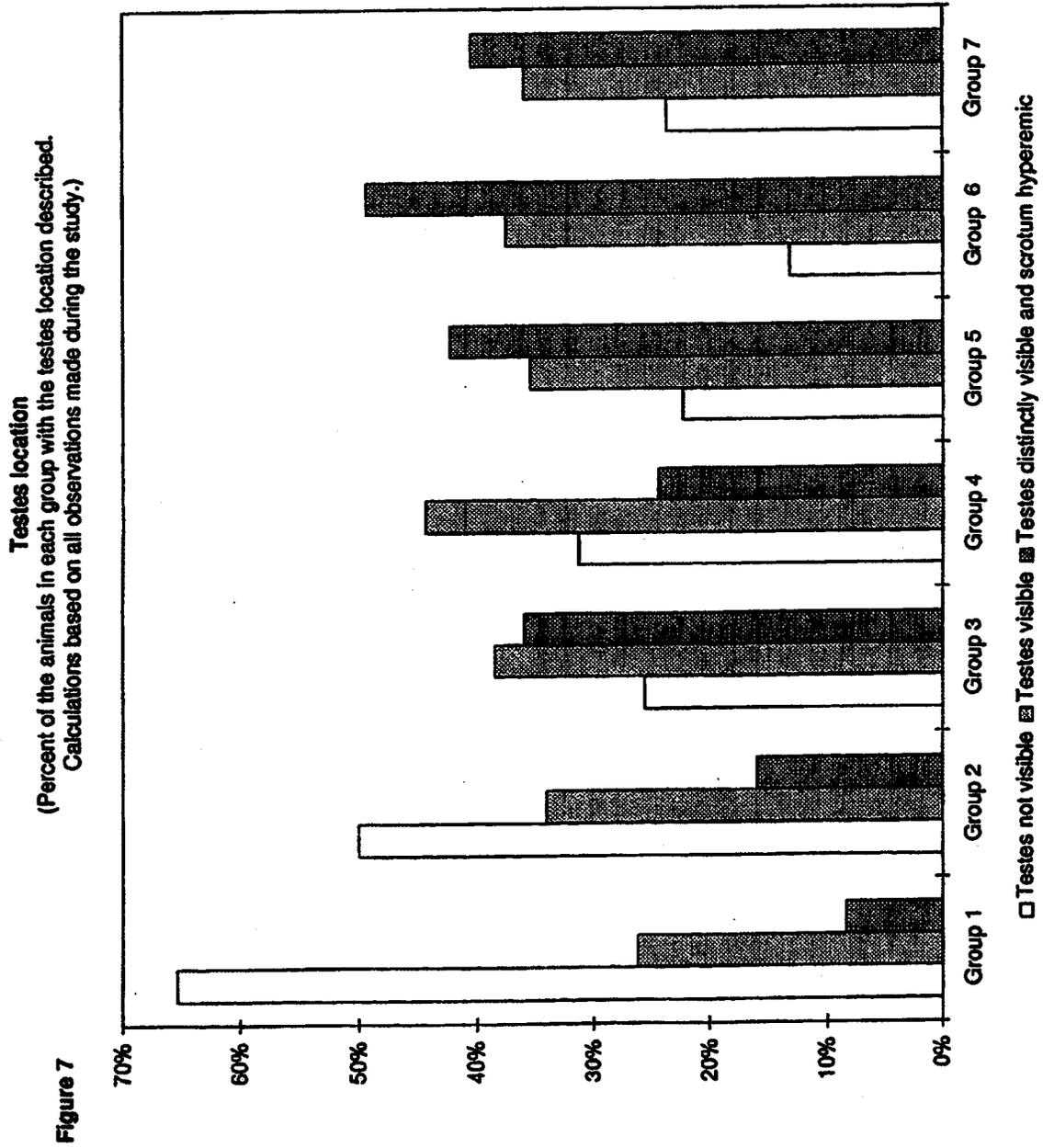
Mortality: Potential treatment-related deaths may have occurred with formoterol at doses of 0.8 and 3 mg/kg/day. One male (#7049/94) in the 0.8 mg/kg/day group was found dead on day 50 of the study. There were no histopathological findings to suggest a cause of death. One male (#7056/94) in the 3 mg/kg/day group was found dead on day 34 of the study. Clinical signs for this animal prior to death included cyanosis, dyspnea, and a body temperature of 30.9°C. Histopathological findings were observed

in the lungs and consisted of partially purulent pleuritis, Grade 4 and multifocal interstitial pneumonia with blood congestion.

Two deaths occurred in toxicokinetic groups. One male (#7129/94) that received formoterol at 3 mg/kg/day (Group 12) was found dead on day 13. One male (#7142/94) that received salbutamol at 25 mg/kg BID was sacrificed in a moribund condition on day 2. The sponsor attributed both deaths to gavage errors.

Clinical signs: In the formoterol and salbutamol treatment groups, there was a statistically significant increase in the frequency of observations of testes that were distinctly visible with a hyperemic scrotum (see figure and table below). A corresponding decrease in the frequency of observations of testes not visible was also observed in all treatment groups with the exception of the 0.03 mg/kg/day formoterol group. There was no delay in the descent of the testes in treatment groups. The observation of testes that were distinctly visible with a hyperemic scrotum was attributed to a possible β -adrenergic effect (dilatation of systemic veins).

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Testes location (relative frequency of animals in each group with respective testes location) on days 17, 20, 22, 24, 27, 29, 31, 34, 36, 37, 38, 64, and 78

Location of testes	Vehicle control	Formoterol, mg/kg/day				Salbutamol, mg/kg BID	
		0.03	0.2	0.8	3	10	25
Testes not visible	0.65	0.50	0.26	0.31	0.22	0.13	0.24
Testes visible	0.26	0.34	0.38	0.44	0.35	0.38	0.36
Testes distinctly visible and scrotum hyperemic	0.08	0.16	0.36	0.24	0.42	0.49	0.40

Slight decreases of water consumption were observed for treatment groups during the dosing period; however, the toxicological significance of these differences was unclear. Water consumption for males in the 0.8 and 3 mg/kg/day formoterol groups during week 2 were decreased to 87.3 and 86.1% of the control (33.8 mL/animal/day), respectively. Water consumption for formoterol treatment groups during week 6 was decreased to 84.6-90.7% of the control (48.6 mL/animal/day). No dose-related effects on water consumption were evident during week 10.

The sponsor contended that formoterol-induced increases of body temperature may have been involved in testicular atrophy observed in previous studies. In the present study, slight elevations of rectal body temperature ($\leq 2.8\%$) were observed for formoterol treatment groups during the first week of the present study; however, for the remainder of the dosing period, there were only sporadic deviations of rectal body temperature with no apparent relationship to treatment. Thus, a continuous or prolonged formoterol-induced alteration of body temperature could not be demonstrated.

Body weights: There were no treatment-related effects on body weight gain from weeks 0 to 13 of the dosing period.

Food consumption: There were no treatment-related effects on food consumption from weeks 2 to 12 of dosing period.

Gross pathology: The relationship of gross pathological findings to treatment was unclear. Reddish discoloration of the lungs was observed for 1 of 12 males in the formoterol high dose group. Discoloration of the liver was observed for 4 of 12 males in the salbutamol high dose group as compared to 2 of 12 control males.

Organ weights: Absolute and relative heart weights were increased for formoterol groups at doses ≥ 0.8 mg/kg/day and salbutamol group. Absolute and relative lung weights were increased for formoterol and salbutamol groups, although differences in formoterol groups were minimal ($<10\%$). Absolute and relative spleen weights were increased for formoterol and salbutamol groups.

Absolute and relative organ weights

Organ	Vehicle Control	Formoterol mg/kg/day				Salbutamol, mg/kg BID	
		0.03	0.2	0.8	3	10	25
Heart, g	1.87	1.76	1.93	2.12 (113%)	2.08 (111%)	2.17* (116%)	2.24* (120%)
Heart, %BW	0.377	0.368	0.398	0.440 (117%)	0.419 (111%)	0.445* (118%)	0.445* (118%)
Lungs, g	1.88	1.93	1.95	1.90	2.01 (107%)	2.21* (118%)	2.34* (125%)
Lungs, %BW	0.377	0.404 (107%)	0.403 (107%)	0.395 (105%)	0.406 (108%)	2.21* (118%)	2.34* (124%)
Spleen, g	0.950	0.985 (104%)	1.06 (112%)	0.981 (103%)	1.07* (113%)	1.08* (114%)	1.12* (118%)
Spleen, %BW	0.191	0.206 (108%)	0.219* (115%)	0.204 (107%)	0.216* (113%)	0.221* (116%)	0.222* (116%)

Histopathology: Histopathological examination of tissues was limited to the testes, epididymides, and gross lesions. Incidences of tubular atrophy in the testes and spermatid debris in the epididymides for formoterol groups displayed no relationship to treatment. The appearance of atrophy was generally unilateral and subcapsular in distinction to the severe bilateral lesions with germ cell depletion (Sertoli cell-only pattern) observed in a previous study with young rats. In the Segment I fertility and reproductive performance study with rats, there was evidence of impaired male fertility.

For toxicokinetic groups sacrificed after dosing for 7 day, all rats had immature testes and epididymides corresponding with age.

Microscopic findings after dosing for 3 months

Organ/Tissue	Vehicle Control	Formoterol, mg/kg/day				Salbutamol, mg/kg BID	
		0.03	0.2	0.8	3	10	25
Testes -tubular atrophy	1/12	1/12	5/12	0/12	1/12	1/12	3/12
Epididymides -spermatid debris	0/12	0/12	1/12	0/12	1/12	0/12	2/12
Liver -congestion	2/2	1/1	-	-	1/1	1/1	3/4

Toxicokinetics: C_{max} and AUC values for formoterol at doses ≥ 0.8 mg/kg/day increased with elevating dose on days 7 and 91. C_{max} and AUC values were approximately proportional to dose on days 7 and 91. Plasma formoterol levels were at or below the limit of detection with a dose of 0.2 mg/kg/day. Plasma formoterol levels were not measured at 0.03 mg/kg/day due to lack of toxicity.

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Toxicokinetic parameters for formoterol and salbutamol on days 7 and 91

Treatment and Dose	C _{max} , nmol/L		AUC, nmol*hr/L			
	Day 7	Day 91	Day 7		Day 91	
			0-6 hr	0-12 hr	0-6 hr	0-12 hr
Formoterol, 0.8 mg/kg/day	0.70	1.35	2.39	-	3.93	-
Formoterol, 3 mg/kg/day	4.4	3.25	12.7	14.1	11.8	19.0
Salbutamol 10 mg/kg BID	Peak 1 57.0 Peak 2 ≥61.7	Peak 1 116 Peak 2 183	AUC ₀₋₉ = 365.4		AUC ₀₋₉ = 650	
Salbutamol 25 mg/kg BID	Peak 1 207 Peak 2 ≥445	Peak 1 530 Peak 2 384	AUC ₀₋₉ = 1720		AUC ₀₋₉ = 2435	

Study title: Formoterol: Three Month Inhalation (HFA pMDI) Toxicity Study in Rats (Study Number 96195).

► In this addendum to the review of the 3-month inhalation toxicology study with formoterol HFA pMDI in rats (IND — Amendment #170; Review #03 dated May 29, 2002), the conclusions regarding histopathological findings in the lung consisting of alveolar histiocytosis, pneumonitis, and congestion induced by the excipients in the vehicle formulation (i.e., PVP K-25 and PEG-600) have been revised.

► Changes were evident in the lungs for vehicle-control group as compared to the air-control group. The incidence and severity of alveolar histiocytosis, pneumonitis, and congestion in the lungs were increased for the vehicle-control group as compared to the air-control group. During final review of this study under the NDA, there was concern that these findings could potentially be indicative of local toxicity induced by the vehicle (PVP K-25 and PEG-1000). However, the sponsor and independent pathologist re-examined these lung tissue slides and results indicated no evidence of local toxicity.

Methods: In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group. Target doses of formoterol fumarate dihydrate for the low, mid, and high dose groups were 0.090, 0.280, and 0.890 mg/kg/day, respectively. Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control). Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively. These doses of excipients were significantly higher than those used in 3-month inhalation bridging toxicology studies with Symbicort HFA pMDI in rats and dogs.

Results: Changes were evident in the lung for the vehicle-control group. Alveolar histiocytosis was observed in 8 of 20 (40%) rats in the vehicle-control group as compared to 4 of 20 (20%) rats in the air-control group. The incidence of alveolar histiocytosis in the vehicle-control group exceeded the published background occurrence in young rats of 16-20% (Handbook of Toxicology, 2nd Edition, CRC Press,

Pages 702-703). Pneumonitis was observed in 9 of 20 (45%) rats in the vehicle-control group as compared to 4 of 20 (20%) rats in the air-control groups. Acute or chronic inflammation consisting of small aggregates of lymphoid cells around bronchioles and small vessels is a relatively common finding in rats observed with a background occurrence of 56%, while more extensive inflammation (e.g., alveolitis, bronchiolitis, pneumonitis) occurs at a lower background incidence of 18-20% (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). Congestion was observed in 4 of 20 (20%) rats in the vehicle-control group as compared to 2 of 20 (10%) rats in the air-control group. It is possible that congestion may have been related to the procedure used to sacrifice animals (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). In the initial review of this study provided in Amendment #170 under IND [redacted] (Review dated May 29, 2002), given that findings were observed in all groups including the air-control and scientific references reporting high incidences of spontaneous inflammation in the lungs of control rats, these findings were not identified as adverse. However, based upon experiences with alveolar histiocytosis in recent years and local toxicity observed with PVP K-25 from another application, these findings are now re-interpreted to potentially be the result of local toxicity induced by excipients in the vehicle formulation (PVP K-25 and PEG-1000).

In the initial submission of IND 63,994, the sponsor provided 3-month inhalation bridging toxicology studies with Symbicort HFA pMDI in rats and dogs that included PVP K-25, PEG-1000, and HFA-227. In addition, the sponsor provided several nonclinical studies, conducted with another inhalation drug product [redacted]

[redacted] that contained relatively similar excipients, PVP K-30, PEG-600, and HFA-227. These studies consisted of a 6-month inhalation toxicity with rats, 6- and 12-month inhalation toxicity studies with dogs, and a 24-month inhalation carcinogenicity study with rats. In our review of the initial submission for IND 63,394, we agreed that PVP K-30 and PEG-600 appeared to be closely related to PVP K-25 and PEG-1000, respectively. Further, PVP K-30 and PEG-600 could be used for the safety assessment of PVP K-25 and PEG-1000, respectively. In all of these studies, there was no evidence of local toxicity induced by the vehicle.

The 3-month toxicity study with Symbicort in rats used very low doses of the vehicle (i.e., 2-fold of human doses, see Table 1). The 3-month toxicity study with Symbicort in dogs (Table 2) as well as the 6-month toxicity with rats, 6- and 12-month toxicity studies with dogs, and a 24-month carcinogenicity study with rats that used closely related excipients, PVP K-30 and PEG-600 (Tables 3 and 4), provided sufficient dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product.

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Table 1: Rat to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation bridging toxicology study with Symbicort in rats.

Compound	Rats		Clinical doses in Symbicort $\mu\text{g/g LW/day}$	Rat to human dose ratio
	Deposited dose $\mu\text{g/kg/day}$	Deposited dose $\mu\text{g/g LW/day}$		
PVP K-25	0.03	0.006	0.0028	2.1
PEG-1000	8.6	1.72	0.8492	2.0

Notes: The deposition factor for rats was approximately 8.6 to 9.4%. Lung weight values for rats and humans used in calculations were 1.5 g and 1000 g, respectively.

Table 2: Dog to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation bridging toxicology study with Symbicort in dogs.

Compound	Dogs		Clinical doses in Symbicort $\mu\text{g/g LW/day}$	Dog to human dose ratio
	Deposited dose $\mu\text{g/kg/day}$	Deposited dose $\mu\text{g/g LW/day}$		
PVP K-25	0.42	0.038	0.0028	13.6
PEG-1000	126	11.455	0.8492	13.5

Notes: The deposition factor for dogs was 100%. Lung weight values for dogs and humans used in calculations were 110 g and 1000 g, respectively.

Table 3: Inhaled dose ($\mu\text{g/kg/day}$) and deposited doses ($\mu\text{g/g lung weight/day}$) of excipients, PVP K-30 and PEG-600, in the vehicle-control group from inhalation toxicology studies

Excipient	6-month rat study		24-month rat study		6-month dog study		12-month dog study	
	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$
PVP K-30	18.4	0.36	110	1.12	2.9	0.26	1.9	0.17
PEG-600	2209.6	44.2	13000	131.9	344.2	31.3	222.2	20.2

a. The deposition factor for rats was 10%.

b. A close-fitting face mask and aerosol delivery mouth tube system was used to deliver aerosols directly to the buccal cavity of the dog. Deposition factors for dogs in the original reviews have been revised to 100%.

Table 4: Rat to human and dog to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of similar excipients, povidone K-30 and PEG-600, in vehicle-control groups of studies

Clinical Doses of Excipients, PVP K-25 and PEG-1000		Dose ratios based upon nonclinical studies with PVP K-30 and PEG 600			
Excipients	Clinical doses in Symbicort, $\mu\text{g/g LW/day}$	6-month	24-month	6-month	12-month
		Rat to Human ratio	Rat to Human ratio	Dog to Human ratio	Dog to Human ratio
Povidone K25	0.0028	128.6	400	94.2	61.7
PEG 1000	0.8492	52	155.4	36.8	23.8

Doses of PVP K-25 and PEG-1000 in the 3-month study with Formoterol HFA pMDI (Table 5) were generally lower than those used with PVP K-30 and PEG-600 (Tables 3 and 4), respectively; however, no toxicity was evident in studies with PVP K-30 and PEG-600 following 1 or 2 years of treatment.

Table 5: Rat to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation toxicology study with Formoterol HFA pMDI in rats.

Excipients	3-month rat study		Clinical doses in Symbicort, $\mu\text{g/g}$ LW/day	Rat to Human Ratios
	Deposited dose, $\mu\text{g/kg/day}$	Deposited dose, $\mu\text{g/g}$ LW/day		
PVP K-25	1.14	0.228	0.0028	81.4
PEG-1000	211.3	42.26	0.8492	49.8

Notes: Rat body and lung weights were estimated to be 300 g and 1.5 g, respectively. Human lung weight was estimated to be 1000 g.

Based upon re-evaluation of histopathological findings in the lung from the 13-week inhalation toxicology study with rats that received formoterol HFA pMDI, it appears that PVP K-25 and PEG-1000 were potentially more toxic than PVP K-30 and PEG-600. This data leads to the question of whether studies with PVP K-30 and PEG-600 can be used to bridge PVP K-25 and PEG-1000, respectively.

In Information Requests dated March 3, 2006 and April 4, 2006, the sponsor was asked to explain the differences in histopathological findings in the lungs between the air-control and vehicle-control groups of the present study (Study Number 96195).

Sponsor's March 17, 2006 and April 26, 2006 Responses:

Review of Lung Changes in Studies No. 96195 and 96010 (AstraZeneca Reference Numbers 0370KY and 0388KY).

- ▶ In the March 17, 2006 Response, the sponsor provided results of their re-examination of lung slides from study numbers 96195 and 96010 (3-month interim sacrifice only).
- ▶ In the re-examination of Study number 96195, the original diagnosis of alveolar histiocytosis was changed to alveolar foamy macrophages and incidences and severity became relatively similar between the air-control and vehicle-control groups. Further, incidences and severity of pneumonitis and congestion were relatively similar between the air-control and vehicle-control groups.
- ▶ In the re-examination of Study 96010, incidences and severity of alveolar foamy macrophages and congestion were increased in the vehicle-control group as compared

to the air-control group. No findings of congestion were reported in the original examination.

► In the April 26, 2006 Response, the sponsor provided results of an analysis of lung slides from Study numbers 96195 and 96010 conducted by an independent pathologist.

► For study number 96195, the independent pathologist found no differences in the incidences or severity for findings of alveolar foamy macrophages, interstitial pneumonitis, congestion, or septal thickening between air-control and vehicle-control groups. For the purposes of safety assessment, results were generally consistent between the March 17, 2006 re-examination by the sponsor and the examination conducted by the independent pathologist.

► For the 3-month interim sacrifice of study number 96010, the independent pathologist found that the incidence of alveolar foamy macrophages was increased for the vehicle-control group. There were no differences in incidences or severity of interstitial pneumonitis, congestion, and septal thickening between the air-control and vehicle-control groups. Results were generally consistent between the March 17, 2006 re-examination and the examination conducted by the independent pathologist. Increased incidence of alveolar histiocytosis and congestion in the vehicle-control group at 3 months were not observed at 6 months.

► For the 6-month terminal sacrifice of study number 96010, the independent pathologist found no differences in incidences or severity for findings of pneumonitis, congestion, or septal thickening between air-control and vehicle-control groups, which were consistent with the original examination.

► Given the general agreement of histopathological diagnoses **between the sponsor's** re-examination and independent pathologist for lung slides from studies 96195 and 96010, it is concluded that neither PVP K-25/PEG-1000 nor PVP K-30/PEG-600 produced local toxicity in the lung. Further, these sets of excipients are considered sufficiently comparable and **the sponsor's bridging program** using PVP K-30 and PEG-600 in chronic inhalation toxicology studies with rats and dogs is acceptable to assess the toxicity profile of PVP K-25 and PEG-1000.

Methods: In the March 17, 2006 Response, lung slide sets for the air-control and vehicle-control groups from Study number 96195 and the 3-month interim sacrifice of Study number 96010 were identified as indicated below. In Study number 96195, the vehicle-control group received PVP K-25, PEG-1000, and HFA-227. In Study number 96010, the vehicle-control group received PVP K-30, PEG-600, and **HFA-227**. **"The 76** lung sections from the two studies were pooled, randomized, and examining blindly by the reviewing pathologist, considering the pathology of the lung as a whole but paying particular attention to the findings highlighted by the FDA reviewer (i.e., alveolar histiocytosis/foamy macrophages, congestion, and interstitial **pneumonitis**)". **"The** reviewing pathologist deliberately suspended any threshold judgments with the aim of capturing a more detailed picture of **pulmonary changes**." **The study pathologist** —

performed both the initial examination and re-examination of Study 96195. This pathologist also conducted the re-examination of the 3-month interim sacrifice data of Study 96010, although a different pathologist conducted the initial examination. Since the term, alveolar foamy macrophages, instead of alveolar histiocytosis, was used in Study number 96010, both terms were used in the re-examination.

Table 2: Identification of lung slides sets from Studies Nos. 96195 and 96010 for inclusion in the slide review

Sex	Study No. 96195 ^a (Formoterol 3-month)				Study No. 96010 ^b (3-month/Interim)			
	Male		Female		Male		Female	
Dose Group	1	2	1	2	1	2	1	2
Treatment	Air	Veh	Air	Veh	Air	Veh	Air	Veh
Rat Nos.	1-10	21-30	11-20	31-40	13-21	67-75	40-48	94-102

a: Vehicle contained PVP K-25 and PEG-1000

b: Vehicle contained PVP K-30 and PEG 600

In the April 26, 2006 Response, the lungs from a 3-month inhalation study (Study number 96195) and a 6-month study with a 3-month interim sacrifice (Study number 96010) were reviewed by an independent pathologist for histopathological changes likely to indicate local pulmonary toxicity. Randomly color-coded slides of hematoxylin and eosin stained sections of lung from two inhalation toxicity studies in rats were provided for review by AstraZeneca (Charnwood). Normally, sections of 5 lobes of lung were present on each slide. The slides from each study were shuffled, placed in a container and mixed further. To grade intra-alveolar cells, the slides were removed once at a time from the container and allocated to categorical sets for Grade "-" and Grade 1-3 foamy alveolar macrophages and alveolar histiocytes. The grade score for each animal number was recorded in the random order into which they were allocated to each set. To grade septal parameters, the slides were remixed and the assignment repeated for interstitial pneumonitis and septal thickening. Grade scores for various parameters assessed for each animal were entered into a computerized database and each animal number was allocated to the correct group and sex so that data could be sorted and group summary tables printed.

Examination of lung histopathology slides	
Study number 96195: 3-month inhalation study	40 slides from the Air-control and Vehicle-control groups
Study number 96010: 6 month inhalation study	36 slides from the Air-control and Vehicle-control groups (3-month interim sacrifice)
	48 slides from the Air-control and Vehicle-control groups (6-month terminal sacrifice)

Results: In the March 17, 2006 Response, the sponsor provided results of their re-examination of lung slides from study numbers 96195 and 96010 (3-month interim sacrifice only). Results of the original examination and subsequent re-examination are shown in the tables below (see columns dated March 17, 2006). In the re-examination

of Study number 96195, the original diagnosis of alveolar histiocytosis was changed to alveolar foamy macrophages and incidences and severity became relatively similar between the air-control and vehicle-control groups. Further, incidences and severity of pneumonitis and congestion were relatively similar between the air-control and vehicle-control groups.

In the re-examination of Study 96010, incidences and severity of alveolar foamy macrophages and congestion were increased in the vehicle-control group as compared to the air-control group. No findings of congestion were reported in the original examination.

The sponsor contended that the vehicle in Study number 96195 did not exacerbate (in terms of either incidence or severity), the spontaneous pathology reported in air-controls. Congestion was considered to be a post-mortem finding, which may or may not be reported by a pathologist depending on their threshold for reporting events. Historical control incidences of foamy macrophages and pneumonitis in 3-month inhalation toxicology studies conducted at AstraZeneca Charnwood were reported to be 35% (3/10 males + 4/10 females) and 10% (1/10 males and 1/10 females), respectively.

To further support the contention that the vehicles in Study numbers 96195 and 96010 did not exacerbate the spontaneous pathology reported in air-controls, the sponsor noted that lung weights and laryngeal histopathology were unchanged. Increased lung weights and/or changes of laryngeal histopathology might be expected with an irritating/toxic agent; however, the absence of these findings does not exclude potential lung toxicity. It was also noted in original examination of Study number 96195 that differences were mainly apparent in male rats whereas findings were relatively similar between the female air-control and vehicle-control groups, which would not be generally expected for an irritant/toxic agent.

Both Study numbers 96195 and 96010 were reported to be conducted in compliance with GLP regulations. There are concerns about the significance changes of incidences and severity of reported findings between the initial examination and subsequent re-examination, particularly for Study number 96195 that was conducted by the same pathologist. These changes have the appearance of attempting to remove potential adverse findings. A reporting bias cannot be excluded. It was recommended that the sponsor obtain an independent pathologist to examine lung slides from Study number 96195 as well as the 6-month terminal sacrifice of Study number 96010. The 6-month data is pivotal for bridging to the inhalation route.

In the April 26, 2006 Response, the sponsor provided results of an analysis of lung slides from Study numbers 96195 and 96010 conducted by an independent pathologist. Results are shown in the tables below (see columns dated April 26, 2006). The independent pathologist added the diagnosis of septal thickening, not reported in the original examination or re-examination, which referred to foci or areas with apparent thickening of alveolar septa, but with no obvious perivascular inflammatory cell infiltration. Septal thickening was generally considered an artifact of fixation. All septal

lesions in the re-examination reported in the March 17, 2006 submission were classified under the term of pneumonitis; however, the independent pathologist divided findings into changes with significant inflammatory cell infiltration (i.e., pneumonitis) and without significant inflammatory cell infiltration (i.e., septal thickening). The incidence of pneumonitis was generally decreased for the April 26, 2006 examination as compared to the March 17, 2006 examination. For the term of congestion, the independent pathologist elected to restrict recording to extensive cases only.

For study number 96195, the independent pathologist found no differences in the incidences or severity for findings of alveolar foamy macrophages, interstitial pneumonitis, congestion, or septal thickening between air-control and vehicle-control groups. For the purposes of safety assessment, results were generally consistent between the March 17, 2006 re-examination by the sponsor and the examination conducted by the independent pathologist.

For the 3-month interim sacrifice of study number 96010, the independent pathologist found that the incidence of alveolar foamy macrophages was increased for the vehicle-control group. There were no differences in incidences or severity of interstitial pneumonitis, congestion, and septal thickening between the air-control and vehicle-control groups. Results were generally consistent between the March 17, 2006 re-examination and the examination conducted by the independent pathologist. Increased incidence of alveolar histiocytosis and congestion in the vehicle-control group at 3 months were not observed at 6 months.

For the 6-month terminal sacrifice of study number 96010, the independent pathologist found that the incidence of pneumonitis was increased to 30% for female rats in the vehicle-control group, which contrasts to no differences between the air-control and vehicle-control groups in the original examination. The incidence of pneumonitis in young rats is 18-20%, although the incidence increases with age (Handbook of Toxicology 2nd Edition, Page 703). Small aggregates of lymphoid cells around bronchioles and small vessels occur with an incidence up to 56% in young rats (Handbook of Toxicology 2nd Edition, Page 703). The incidence of pneumonitis observed by the independent pathologist appears to be within the historical control range for rats at approximately 6 months of age or less. There were no differences in incidences or severity for findings of pneumonitis, congestion, or septal thickening between air-control and vehicle-control groups, which were consistent with the original examination.

The independent pathologist contended that lungs from rats in study numbers 96195 and 96010 (3-month interim sacrifice and 6-month terminal sacrifice) were within the normally expected range for animals <1 year of age. Given the general agreement of histopathological diagnoses **between the sponsor's re-examination** and independent pathologist for lung slides from studies 96195 and 96010, it is concluded that neither PVP K-25/PEG-1000 nor PVP K-30/PEG-600 produced local toxicity in the lung. Further, these sets of excipients are considered sufficiently comparable and the **sponsor's bridging program** using PVP K-30 and PEG-600 in chronic inhalation

toxicology studies with rats and dogs is acceptable to assess the toxicity profile of PVP K-25 and PEG-1000.

**Appears This Way
On Original**

Study 96195: 3-month study (PVP K-25 and PEG-1000)

Organ/Tissue	Original report				March 17, 2006				April 26, 2006					
	Air-Control		Vehicle-Control		Air-Control		Vehicle-Control		Air-Control		Vehicle-Control			
	M	F	M	F	M	F	M	F	M	F	M	F		
Lungs														
-number examined	10	10	10	10	10	10	10	10	10	10	10	10		
-alveolar histiocytosis														
Total	1	3	5	3	1	0	0	1	0	0	0	0		
Grade 1	0	3	3	3	0			1						
Grade 2	1	0	1	0	1			0						
Grade 3	0	0	1	0	0			0						
-alveolar foamy macrophages														
Total					2	5	3	2	4	4	6	3		
Grade 1	0	0	0	0	1	4	3	1	3	4	6	3		
Grade 2					0	1	0	1	1	0	0	0		
Grade 3					1	0	0	0	0	0	0	0		
-(interstitial) pneumonitis														
Total	3	1	6	3	9	8	9	6	6	2	5	0		
Grade 1	3	1	3	3	3	3	2	4	6	2	4			
Grade 2	0	0	3	0	6	4	5	2	0	0	1			
Grade 3	0	0	0	0	0	1	2	0	0	0	0			
-congestion														
Total					2	1	3	0	0	0	0	0		
Grade 1	1	1	4	0	1	1	1							
Grade 2	0	1	0		1	0	2							
-septal thickening														
Total	0	0	0	0	0	0	0	0	6	8	8	5		
Grade 1									3	4	3	2		
Grade 2									3	4	4	3		
Grade 3									0	0	1	0		

Appears This Way
On Original

Study 96010: 3-month interim sacrifice (PVP K-30 and PEG-600)

Organ/Tissue	Original report				March 17, 2006				April 26, 2006				
	Air-Control		Vehicle-Control		Air-Control		Vehicle-Control		Air-Control		Vehicle-Control		
	M	F	M	F	M	F	M	F	M	F	M	F	
Lungs													
-number examined	9	9	9	9	9	9	9	9	9	9	9	9	9
-alveolar histiocytosis													
Total	0	0	0	0	1	0	0	0	0	0	0	0	0
Grade 1					1								
Grade 2													
Grade 3													
-alveolar foamy macrophages													
Total	1	0	1	3	2	1	5	6	3	4	5	7	7
Grade 1	1		1	3	1	1	2	3	2	4	4	7	7
Grade 2					0	0	3	3	1	0	1	0	0
Grade 3					1	0	0	0	0	0	0	0	0
-(interstitial) pneumonitis													
Total	0	1	0	1	6	8	7	8	4	4	2	3	3
Grade 1		1		1	3	7	4	5	2	3	2	3	3
Grade 2					1	1	3	3	2	1	0	0	0
Grade 3					2	0	0	0	0	0	0	0	0
-congestion													
Total	0	0	0	0	2	3	6	5	0	0	0	0	0
Grade 1					2	3	3	3					
Grade 2					0	0	3	2					
-septal thickening													
Total	0	0	0	0	0	0	0	0	3	5	3	4	4
Grade 1									2	4	3	4	4
Grade 2									1	1	0	0	0
Grade 3									0	0	0	0	0

Appears This Way
On Original

Study 96010: 6-month terminal sacrifice (PVP K-30 and PEG-600)

Organ/Tissue	Original report				April 26, 2006			
	Air-Control		Vehicle-Control		Air-Control		Vehicle-Control	
	M	F	M	F	M	F	M	F
Lungs								
-number examined	9	12	12	12	12	12	12	12
-alveolar histiocytosis								
Total	0	0	0	0	0	0	0	0
Grade 1								
Grade 2								
Grade 3								
-alveolar foamy macrophages								
Total	2	3	2	3	6	5	5	5
Grade 1	2	3	1	0	4	0	3	3
Grade 2	0	0	1	0	2	0	1	2
Grade 3					0	0	1	0
-(interstitial) pneumonitis								
Total	0	2	0	1	1	0	0	4
Grade 1		2		1	1			4
Grade 2								
Grade 3								
-congestion								
Total	0	0	0	0	0	0	0	0
Grade 1								
Grade 2								
-septal thickening								
Total	0	0	0	0	1	2	1	1
Grade 1					1	2	1	1
Grade 2								
Grade 3								

Dogs

Study title: Symbicort (Budesonide + Formoterol): 3-Month Inhalation (pMDI) Toxicity Study in the Dog.

In this addendum to the review of the 13-week inhalation toxicology study with dogs (IND 63,394 Reviews #01 dated January 23, 2002), the deposition factor is revised to 100% from the earlier review, based upon the use of a close-fitting face mask and aerosol delivery tube system to deliver the aerosol directly into the buccal cavity of the dog.

Deposited doses ($\mu\text{g}/\text{kg}/\text{day}$): should be revised to total inhaled doses based upon a deposition factor of 100%.

Group	Measured		Estimated		
	Budesonide	Formoterol	PVP K-25	PEG-1000	HFA-227
1 (Air-Control)	0	0	0	0	0
2 (Vehicle-Control)	0	0	0.420	126	41835
3 (LD)	1.96	0.117	0.0158	4.74	1574
4 (MD)	10.3	0.62	0.0831	24.9	8271
5 (HD)	52.1	3.11	0.420	126	41835

Safety margins for clinical doses of budesonide and formoterol in the Symbicort HFA drug product should be revised as shown in the table below.

Clinical Doses of Symbicort				Dose Ratios for Clinical Doses
80/4.5	$\mu\text{g}/\text{actuation}$	4 actuations/day	$\mu\text{g}/\text{kg}/\text{day}$	Dog to Human Ratio ^a
Budesonide	80	320	6.4	0.31
Formoterol	4.5	18	0.36	0.31
160/4.5				
160/4.5	$\mu\text{g}/\text{actuation}$	4 actuations/day	$\mu\text{g}/\text{kg}/\text{day}$	Dog to Human Ratio ^a
Budesonide	160	640	12.8	0.16
Formoterol	4.5	18	0.36	0.31

a. Deposited doses at the NOAEL for dogs in the 3-month bridging study were 2.0 $\mu\text{g}/\text{kg}/\text{day}$ budesonide and 0.11 $\mu\text{g}/\text{kg}/\text{day}$ formoterol assuming 100% deposition.

Safety margins for clinical doses of PVP K-25 and PEG-1000 in the Symbicort HFA pMDI drug product should be revised as shown in the table below.

Compound	Dogs		Clinical dose $\mu\text{g}/\text{g}$ LW/day	Safety margins
	Deposited dose $\mu\text{g}/\text{kg}/\text{day}$	Deposited dose $\mu\text{g}/\text{g}$ LW/day		
PVP K-25	0.42	0.038	0.0028	13.6
PEG-1000	126	11.455	0.8492	13.5

Notes: The deposition factor for dogs was 100%. Lung weight values for dogs and humans used in calculations were 110 g and 1000 g, respectively.

2.6.6.4 Genetic toxicology

See attached reviews of genetic toxicology studies conducted with formoterol under IND . See NDA 20-441 for genetic toxicology studies conducted with budesonide.

Study title: Mutagenicity Evaluation of _____ in the Ames Salmonella/Mammalian Microsome Mutagenicity Test.

Key findings:

► The mutagenic potential of _____ was assessed in the in vitro bacterial reverse mutation assay.

► The study was inadequate with regard to selection of tester strains. The standard set of strains used in bacterial mutation assays should include strains that will detect point mutations at A-T sites, such as TA102 or *Escherichia coli*/WP2 uvrA. Neither strain was

included in the present study. Further, the number of plates per dose \pm S9 was inadequate (i.e., only 1 plate per dose \pm S9).

at high doses up 12300 or 11600 $\mu\text{g}/\text{plate} \pm$ S9 produced no increases of revertant colony counts for tester strains TA1535, TA100, TA1538, TA98, or TA1537.

contains a structural alert (i.e., aromatic amine) that is similar to the structural alert in the parent compound, formoterol (i.e., aromatic amine with a formyl group). Genetic toxicity testing with the parent compound, formoterol, is considered sufficient.

Study no.: 91117

Conducting laboratory and location: AB Astra
Laboratory of Safety Assessment
S-151 85 Sodertalje
Sweden

Date of study initiation: September 27, 1991

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: _____, batch number op.2 (Purity, _____)

Methods

Strains: *Salmonella typhimurium* tester strains, TA1535, TA100, TA1538, TA98, and TA1537, were used in the present study.

Doses used in definitive study: In the first mutagenicity study, _____ was tested at doses of 0, 3.87, 11.6, 38.7, 116, 387, 1160, 3870, and 11600 $\mu\text{g}/\text{plate}$ in the absence and presence of S9. In the second mutagenicity study, _____ was tested with TA100 only at doses of 0, 3.87, 11.6, 38.7, 116, 387, 1160, 3870, and 11600 $\mu\text{g}/\text{plate}$ in the absence and presence of S9.

Basis of dose selection: In a preliminary study, the toxicity of _____ was assessed with tester strains at doses of 0, 4.08, 12.3, 40.8, 123, 408, 1230, 4080, and 12300 $\mu\text{g}/\text{plate}$ in the absence and presence of S9. In the absence of S9, reduced colony counts were observed for TA98 at 4080 and 12300 $\mu\text{g}/\text{plate}$ and TA1535, TA100, TA1538, and TA1537 at 12300 $\mu\text{g}/\text{plate}$. In the presence of S9, there was no evidence of toxicity (i.e., reduced colony counts) for all tester strains at doses up to 12300 $\mu\text{g}/\text{plate}$.

Negative controls: DMSO

Positive controls: Positive controls in the absence of metabolic activation were sodium azide at 0.50 $\mu\text{g}/\text{plate}$ for TA1535 and TA100, 2-nitrofluorene at 0.50 $\mu\text{g}/\text{plate}$ for TA1538 and TA98, and 9-aminoacridine at 75 $\mu\text{g}/\text{plate}$ for TA1537. The positive control

in the presence of metabolic activation was 2-aminoanthracene at 5 µg/plate for TA1535, TA100, TA1538, TA98, and TA1537.

Metabolic activation: Rat liver homogenate (S9 fraction) was purchased from ———. It was prepared from the liver of male Sprague-Dawley rats treated with Aroclor 1254. Metabolic activity was assessed by monitoring the mutagenicity of 2-aminoanthracene, benzo(a)pyrene, and cyclophosphamide with appropriate *Salmonella typhimurium* tester strains. The concentration of S9 in S9 mix was 10%.

Incubation and sampling times: It appeared that only one plate per dose ± S9 was used. Colonies were counted manually or with an automatic colony counter after incubation at 37°C

Results

Study validity: The study was inadequate with regard to selection of tester strains. The standard set of strains used in bacterial mutation assays should include strains that will detect point mutations at A-T sites, such as TA102 or *Escherichia coli* WP2 uvrA. Neither strain was included in the present study. Further, the number of plates per dose ± S9 was inadequate (i.e., only 1 plate per dose ± S9). There should have been at least 3 plates per dose ± S9. Dose selection was considered adequate. The high doses of 12300 and 11600 µg/plate used in the present study exceeded the limit dose of 5000 µg/plate (ICH S2A). Positive controls produced expected increases of revertant colony counts in the presence and absence of metabolic activation.

Study outcome: In the first mutagenicity study in the absence of S9, decreased colony counts were observed for TA98 and TA100 at 3870 and 11600 µg/plate and TA1535, TA1538, and TA1537 at 11600 µg/plate. In the presence of S9, there was no evidence of toxicity (i.e., reduced colony counts) for all tester strains at doses up to 11600 µg/plate. Revertant colony counts for TA100 in vehicle-control and treated plates ± S9 exceeded the normal background round so the sponsor elected to conduct a second mutagenicity assay with TA100 only. In second mutagenicity study in the absence of S9, decreased colony counts were observed for TA100 at 11600 µg/plate. In the presence of S9, there was no evidence of toxicity. ——— at high doses up 12300 or 11600 µg/plate ± S9 produced no increases of revertant colony counts for tester strains TA1535, TA100, TA1538, TA98, or TA1537. As noted above, the assay was inadequate with regard to selection of tester strains and number of plates per dose ± S9.

2.6.6.5 Carcinogenicity

See attached reviews of 2-year carcinogenicity studies conducted with formoterol in mice and rats under IND ———. See NDA 20-441 for carcinogenicity studies conducted with budesonide.

2.6.6.6 Reproductive and developmental toxicology

See attached reviews of reproductive toxicology studies conducted with formoterol (i.e., a fertility and reproductive performance study with rats, teratology studies with rats and rabbits, and a perinatal and postnatal development study with rats) under IND —
See NDA 20-441 for reproductive toxicology studies conducted with budesonide.

Embryofetal development**Study title: Symbicort (Budesonide-Formoterol): Embryofetal Development Study After Administration to Pregnant Rats Via the Inhalation Route.****Key study findings:**

- ▶ In embryofetal development study, three groups of 24 mated female rats were exposed to a combination of budesonide and formoterol in a Symbicort HFA pMDI formulation by nose-only inhalation at actual doses of 2.5 + 0.14, 12 + 0.66, and 80.0 + 4.4 µg/kg/day from days 6 to 16 of gestation. Deposited doses of budesonide and formoterol for low, mid, and high dose groups were 0.24 + 0.014, 1.01 + 0.057, and 6.8 + 0.39 µg/kg/day, respectively. Target doses selected were identical to those used in the 3-month inhalation toxicology study with the Symbicort HFA pMDI formulation in rats. The pMDI formulation contained excipients, PVP K-25 and PEG 1000, and the propellant, HFA-227. The pMDIs used in this study were approximately 4 months beyond their documented shelf life. A control group was exposed to air only.
- ▶ Body weight gains for female rats in the low, mid, and high dose groups from days 6 to 16 of gestation were decreased to 87.6, 85.5, and 33% of the control, respectively. Maternal toxicity was evident for the high dose group.
- ▶ Post-implantation losses were slightly increased for dams in mid and high dose groups, although there were no treatment-related effects on numbers of live fetuses per dams.
- ▶ A major external malformation, umbilical hernia, was observed for 1 fetus (0.4%) at the mid dose and 2 fetuses at the high dose (0.9%). These incidences exceeded the mean historical control incidence of 0.01%.
- ▶ A major visceral malformation, aortic arch: right sided, was observed for 1 fetus in the high dose group. For sternebra, a major malformation, one or more fused, was observed for 1 fetus in the high dose group. These findings were each confined to one fetus and their relationship to treatment was unclear given that maternal toxicity was evident in the high dose group.
- ▶ The Symbicort HFA pMDI formulation was found to be teratogenic for mid and high dose groups. There were no teratogenic findings for the low dose group.

Study no.: SR00581-01

Volume #, and page #: Volume 1, Pages 1 to 216

Conducting laboratory and location: AstraZeneca R&D Sodertalje
S-151 85 Sodertalje
Sweden

Date of study initiation: February 2, 2001

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Formulations of budesonide (Batch number 4285J (120-01)) and formoterol fumarate dihydrate (Batch number 4259J (1743-98)) in a HFA-227 pMDI (Batch number P5993) were supplied by AstraZeneca R&D Charnwood. The pMDIs were stored at room temperature in the valve down orientation.

Formulation I was filtered air and was used as the control. Formulation II was the active pMDI formulation containing micronized budesonide and formoterol, hydro-fluoroalkane (HFA-227) propellant, povidone (PVP K-25), and polyethylene glycol (PEG 1000).

Formulation	Used in Group	Material	Percent, w/w
I	1	Air	
II	2, 3, 4	Budesonide	
		Formoterol	
		PVP K-25	
		PEG 1000	
		HFA-227	

The pMDIs were sealed delivery systems and stored under controlled conditions. However, these pMDIs were approximately 4 months beyond their documented shelf life, post-study analyses were performed to confirm the stability and identity. Analyses found that the used substances were stable and compliant with the release specification.

Formulation/vehicle: The control group was exposed to air only. A vehicle-control group was not included in this study.

Methods:

Species/strain: Female Sprague-Dawley rats were obtained from _____ Female were approximately 9-10 weeks old at the start of mating. During the mating period, 3 or 4 female rats and 2 male rats were housed together and allowed to mate overnight only. Detection of a vaginal plug and/or sperm in the vaginal smear was designated as day 0 of gestation. Each day, mated females were evenly distributed to different groups. The body weight range of mated female rats on day 0 of gestation was 200-270 g.

Doses employed: Target doses were identical to those used in the 3-month inhalation toxicology study with Symbicort in rats. Target, actual, and deposited doses for this study are shown in the table below. Deposition factors for budesonide/formoterol in the low, mid, and high dose groups were 0.095/0.098, 0.084/0.086, and 0.086/0.086, respectively.

Dose groups

Group	Compound		Target Dose µg/kg/day	Inhaled Actual Dose µg/kg/day	Inhaled Deposited Dose µg/kg/day
1	Air-control		0	0	0
2	Budesonide + Formoterol		2.0 + 0.11	2.5 + 0.14	0.24 + 0.014
3	Budesonide + Formoterol		10.0 + 0.56	12 + 0.66	1.01 + 0.057
4	Budesonide + Formoterol		50.0 + 2.80	80 + 4.4	6.8 + 0.39

Exposure Conditions.

Group	Body weight, g	Target Concentration µg/L	Actual Concentration ^a µg/L	Exposure Duration min
1 and 5	264.3 (248.0-279.7)	0	0	20.07
2 and 6	259.7 (239.2-277.1)	0.15 + 0.0081	0.251 (0.230-0.272) + 0.014	18.81 (17.37-20.48)
3 and 7	263.2 (248.3-294.2)	0.74 + 0.041	1.230 (1.054-1.331) + 0.065	19.10 (17.72-20.82)
4 and 8	245.3 (240.0-257.2)	3.7 + 0.21	7.667 (4.484-9.750) + 0.42	20.05 (17.77-26.17)

a. The budesonide concentration was measured by HPLC.

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Table 1:1 Summary of exposure generating conditions

Generating conditions	Chamber concentration			
	Air control	Low dose	Intermediate dose	High dose
Duration of exposure (min)	20	19	19	20
Generator air flow (L/min)	30	30	30	20
Tot chamber air supply (L/min)	50	50	50	50
Aerosol flow (L/min)	0	3	7	16
Dilution air flow (L/min)	25	47	43	34
Extraction air flow (L/min)	49	49	49	49
Number of pMDI canisters	-	1	1	4
Puff frequency (/min)	-	3	4	5
Mass per actuated canister				
- total mass (mg)	0	71	71	71
- budesonide/formoterol (µg)	0	88/4.8	88/4.8	88/4.8
Nominal output				
- total mass (mg/min)	0	213	284	1420
- budes./formot. (µg/min)	0	264/14.5	352/19.3	1760/97
Nominal concentration				
- total mass (mg/L)	0	7.1	9.5	71
- budes./formot. (µg/L)	0	8.8/0.48	12/0.65	88/4.8
Reduction of aerosol mass				
- fraction used ¹⁾	-	3/30	7/30	16/20
- dilution ²⁾	-	1:17	1:7	1:3
Mass flow to chamber				
- total mass (mg/L)	-	0.42	1.4	24
- budes./formot. (µg/L)	-	0.52/0.028	1.7/0.091	29/1.6
Actual concentration				
- budes./formot. ³⁾ (µg/L)	-	0.25/0.014	1.2/0.065	7.7/0.42

- 1) Aerosol flow/generator air flow
- 2) Aerosol flow/total chamber air supply
- 3) Formoterol values are based on analytical data from budesonide assuming same composition in the aerosol as in the compound mixture.

The particle size distribution of the test aerosol was determined for Groups 2, 3, and 4 using a cascade impactor. The amounts of test compounds on the impactor stages were determined by HPLC.

Particle size, MMAD ± GSD (µm)

Compound	Low dose	Mid dose	High dose
Budesonide			
Formoterol			

Route of administration: Nose-only inhalation exposure.

Nose-only inhalation exposure was achieved with "flow-past" exposure chambers. A separate 10-level chamber with 160 animal ports was used for each target concentration. Animals from the main study and toxicokinetic groups allocated to the same aerosol formulation were exposed together in the same chamber. During exposure, rats were restrained in polycarbonate tubes with their snouts protruding into the chamber. The tubes were attached to the chamber by means of push-fits into O-ring seals located in each animal exposure port. Unused exposure ports were closed.

The actual aerosol concentration was measured on-line during each exposure by the use of a light-scatter monitor. Measurements from the light-scatter monitor were converted to actual aerosol masses through use of substance correction factors (SCFs). For determination of SCFs, actual aerosol concentrations were measured under nominal aerosol generating conditions for Groups 2, 3, and 4. For each test, a set of five filters was mounted in the animal breathing position and exposed for an appropriate time interval with a filter sampling rate of 0.25 mL/min. The particulate masses of aerosol collected from the formulation used in Groups 2, 3, and 4 were measured by HPLC. The relation between the actual aerosol mass and the respective light scatter signal was calculated and the mean value from a set of 5-10 filters was considered as the SCF for the formulation and target concentration.

Study design:

Three groups of 24 mated female rats were exposed to a combination of budesonide and formoterol in a Symbicort HFA pMDI formulation by nose-only inhalation at target doses of 2.0 + 0.11, 10 + 0.56, and 50.0 + 2.80 µg/kg/day from days 6 to 16 of gestation. Target doses selected were identical to those used in the 3-month inhalation toxicology study with the Symbicort HFA pMDI formulation in rats. A control group was exposed to air only. Animals were checked twice daily for mortality/morbidity. All animals were observed daily for clinical signs of toxicity related to dosing. Clinical examinations of each animal were performed on days 0, 7, 14, and 21 of gestation. Body weights were measured on days 0, 3, 6, 9, 12, 16, 18, and 21 of gestation. For toxicokinetic groups, body weights were measured on days 0, 3, 6, 9, 12, and 15 of gestation. Individual food consumption was measured on days 0, 3, 6, 9, 12, 16, 18, and 21 of gestation. On day 21 of gestation, mated female rats were sacrificed. The uterine contents and ovaries were examined with respect to gravid uterus weight, number of corpora lutea, number of implantation sites, sex and number of viable fetuses, number of dead fetuses, number of early and late intrauterine deaths, gross examination of placentas, individual fetal weights (and litter weights), and external abnormalities. Approximately one-half of the fetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning in order to detect visceral anomalies using the modified Wilson's technique. The remaining fetuses were preserved in 70% alcohol before evisceration and transferring to 95% alcohol. Skeletons were stained with alizarin and examined for anomalies. Necropsy examinations of dams was limited to the thoracic and abdominal cavities. The sponsor defined major defects as rare and/or probably lethal. Minor defects were defined as minor differences from normal that are

detected relatively frequently, either at visceral examination (e.g., displaced kidney) or at skeletal examination (e.g., misaligned sternbrae). Variants were defined as alternative structures that occur regularly in the control population. These may be permanent structures (e.g., an extra pair of ribs) or they may be transient stages of development (e.g., incomplete ossified sternbrae).

Blood samples for measurement of plasma concentrations of budesonide and formoterol were collected on day 15 from animals in toxicokinetic groups (i.e., Groups 5-8). Blood samples were collected from 2-3 animals per time point by cardiac puncture. The three animals in the control group were sampled at 30 min after completion of dosing on day 15 of pregnancy. The three animals in each of the low and mid dose groups were sampled at 10 min after completion of dosing on day 15 of pregnancy. For the high dose group, blood samples were collected before dosing and at 0.17, 0.5, 1, 2, 4, and 6 hr after completion of dosing on day 15. These animals were used only for measurements of plasma drug concentrations and were killed without necropsy after completion of sampling. The uterus of each animal was examined and numbers of implantations were recorded. Plasma concentrations of budesonide were measured by liquid chromatography ~~_____~~ tandem mass spectrometry (LC- ~~_____~~ MS/MS). Formoterol determinations were performed using liquid chromatography ~~_____~~ tandem mass spectrometry (LC ~~_____~~ MS/MS). Limits of quantification for budesonide and formoterol were 5.00 pM and 0.025 nM, respectively.

Number/sex/group: The four main study groups each consisted of 24 mated female rats. In the toxicokinetics portion of study, the control, low dose, and mid dose groups each consisted of 3 male female rats. The high dose group consisted of 15 mated female rats.

Parameters and endpoints evaluated: Effects of Symbicort upon organogenesis and early fetal development in rats were assessed.

Results:

Mortality: None.

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

Body weight: Body weight gains for all treatment groups were impaired by >10% as compared to the control. In particular, body weight gain for the high dose group was only 33% of the control. Maternal toxicity was evident for the high dose group. These impairments of body weight gain can most likely be attributed to the pharmacological effects of budesonide.

Body weights for female controls on days 6 and 16 of gestation were 251.11 and 296.57 g, respectively. Body weight gains for female rats in the low, mid, and high dose groups from days 6 to 16 of gestation were 87.6, 85.5, and 33% of the control, respectively.

Food consumption: Food consumption for female rats in the high dose group was reduced to 85.3% of the control (21.77 g/animal/day).

Toxicokinetics: At 10 min after dosing ($C_{0.17hr}$), plasma concentrations of budesonide and formoterol increased with elevating dose. Plasma concentrations of formoterol increased in an approximate dose-related manner; however, this was observed with budesonide concentrations. Toxicokinetic parameters for budesonide and formoterol were determined for the high dose group. Absorption of budesonide and formoterol was relatively rapid with T_{max} values of 0.90 and 0.48 hr, respectively. The half-lives of budesonide and formoterol were relatively short with values of 2.5 and 1.8 hr, respectively. AUC values for budesonide and formoterol at steady state were 22.6 nM·hr and 471 pM·hr, respectively.

Group	Animal ID	Budesonide		Formoterol	
		Dose ($\mu\text{g}/\text{kg}/\text{day}$)			
		Nominal	Actual	Nominal	Actual
5	97-99	0	0	0	0
6	100-102	2.0	2.4	0.11	0.13
7	103-105	10	12	0.56	0.66
8	106-120	50	88	2.8	4.8

Animal Nos. 107, 109 and 111 were proved not to be pregnant.

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Table 7: Plasma concentrations of budesonide and formoterol obtained on Day 15 at 10 minutes after completed Symbicort inhalat groups of pregnant rats

Group	actual time Mean (h)	Budesonide				Formoterol			
		actual dose Mean (µg/kg)	C _{0.17h} Mean (nM)	C _{0.17h} S.D. (nM)	C _{0.17h} - Norm Mean (kg/L)	actual dose Mean (µg/kg)	C _{0.17h} Mean (pM)	C _{0.17h} S.D. (pM)	C _{0.17h} - Norm Mean (kg/L)
6	0.48	2.4	0.384	0.094	0.069	0.13	10.2	0.54	0.033
7	0.49	12	2.43	0.89	0.087	0.66	37.5	10.8	0.024
8	0.48	88	9.88 a	N.C.	0.048	4.8	284	N.C.	0.025

a: concentration from single animal, others in group not pregnant. N.C.: not calculated

Table 8: Toxicokinetic parameters for budesonide on Day 15 obtained in group 8 after daily Symbicort inhalation during Days 6-15

Nominal (actual) Dose	Actual Dose	T _{max} a	C _{max}	t _{1/2}	AUC ₀₋₆	AUC _{0-∞}	C _{max} norm	AUC _{inf} norm
µg/kg	(nmol/kg)	h	nM	h	nM*h	nM*h	kg/L	kg*h/L
50(88)	204	0.90	13.3	2.5	21.0	22.6	0.065	0.110

a: T_{max} calculated from time of start dosing.

Table 9: Toxicokinetic parameters for formoterol on Day 15 obtained in group 8 after daily Symbicort inhalation during Days 6-15

Nominal (actual) Dose	Actual Dose	T _{max} a	C _{max}	t _{1/2}	AUC ₀₋₆	AUC _{0-∞}	C _{max} norm	AUC _{inf} norm
µg/kg	(nmol/kg)	h	pM	h	pM*h	pM*h	kg/L	kg*h/L
2.8(4.8)	11.5	0.48	284	1.8	440	471	0.025	0.041

a: T_{max} calculated from time of start dosing.

14 December, 2001

17(19)

Embryofetal development studies:

Terminal and necroscopic evaluations:

Dams: Post-implantation losses were slightly increased for dams in mid and high dose groups; however, there were no treatment-related effects on numbers of live fetuses per dams. Fetal body weights for males and females in the high dose group were slightly decreased to 92.5 and 93.9% of control values (5.23 and 4.95 g), respectively. Litter weight for the high dose group was decreased to 88.9% of the control. Mean gravid uterus weight for the high dose group was decreased to 87.3% of the control. Gross examinations of thoracic and abdominal cavities of F₀ dams did not reveal any abnormalities.

Litter data for female rats that received a Symbicort HFA pMDI formulation from days 6 to 16 of gestation.

Parameter	Control	Low Dose	Mid Dose	High Dose
Number of animals	24	24	24	24
Not pregnant	1	1	4	4
Pregnant	23	23	20	20
Number of corpora lutea/dam	13.8 (318/23)	13.7 (315/23)	13.9 (278/20)	13.8 (276/20)
Number of implantations/dam	12.1 (278/23)	12.5 (288/23)	13.1 (261/20)	12.0 (240/20)
Pre-implantation loss, %	12.6 [12.7]	8.6 [8.5]	5.6 [6.1]	13.0 [12.3]

	(40/318)	(27/315)	(17/278)	(36/276)
Live fetuses/dam	11.8 (271/23)	12.3 (284/23)	12.0 (240/20)	11.4 (227/20)
Early embryofetal deaths	7	4	19	12
Late embryofetal deaths	0	0	2	1
Number of dead fetuses	0	0	0	0
Total	7	4	21	13
Post-implantation loss, %	2.5 [3.4] (7/278)	1.4 (4/288)	8.1 (21/261)	5.4 [6.2] (13/240)
Number of male: female fetuses	131: 140	147: 137	118: 122	114: 113
Fetal weight, g				
-males	5.23	5.23	5.21	4.84
-females	4.95	5.00	4.94	4.65
Litter weight, g	60.1	62.7	60.7	53.4
Mean gravid uterus weight, g	79.3	82.0	79.6	69.2

Offspring: A major external malformation, umbilical hernia, was observed for 1 fetus at the mid dose and 2 fetuses at the high dose. This one fetus (Litter #71) in the mid dose group had additional findings of agnathia, cleft palate, microglossia, and misshapen kidney. The two fetuses in the high dose group were from the same litter (Litter #91). These incidences exceeded the mean historical control incidence of 0.01%. Historical control incidences of external, visceral, and skeletal abnormalities were obtained from the MARTA Historical Control Project in the Handbook of Developmental Toxicology, Edited by R.D. Hood, CRC Press, Inc., 1997.

External examination of F₁ fetuses obtained from dams exposed to Symbicort by inhalation from days 6 to 16 of gestation.

Observation	Control	Low Dose	Mid Dose	High Dose
Fetuses/Litters examined	271/23	284/23	240/20	227/20
Umbilicus				
Umbilicus: umbilical hernia (Major malformation)	0	0	1 (0.4%)	2 (0.9%)

A major visceral malformation, aortic arch: right sided, was observed for 1 fetus in the high dose group (Litter #79). This finding was confined to one fetus and its relationship to treatment is unclear. This fetus in the high dose group was also observed with a minor malformation, subclavian artery - unilateral or bilateral: common origin on aortic arch. A variant, thymus - right lobe: partially undescended, was increased in incidence for treatment groups. Although, there were no differences in the incidences of thymus - left lobe: partially undescended between control and treatment groups. The incidence of the variant, thymus - both lobes: partially undescended, was increased for the high dose group.

Visceral examination of F₁ fetuses obtained from dams exposed to Symbicort by inhalation from days 6 to 16 of gestation.

Observation	Control	Low Dose	Mid Dose	High Dose
Fetuses/Litters examined	141/23	147/23	124/20	119/20
Thoracic cavity				
Subclavian artery – unilateral or bilateral: common origin on aortic arch (Minor)	0	0	0	1 (0.8%)
Aortic arch: right sided (Major malformation)	0	0	0	1 (0.8%)
Thymus-right lobe: partially undescended (Variant)	2 (1.4%)	6 (4.1%)	7 (5.7%)	8 (6.7%)
Thymus-left lobe: partially undescended (Variant)	12 (8.5%)	12 (8.2%)	12 (9.7%)	8 (6.7%)
Thymus-both lobes: partially undescended (Variant)	4 (2.8%)	2 (1.4%)	4 (3.2%)	8 (6.7%)

Skeletal examinations of fetuses revealed a malformation for one fetus in the high dose group and a number of findings of minor malformations or variations for treatment groups in which incidences were increased as compare to the air-control. The incidence of incomplete ossification and not ossified was increased for the high dose group and possibly for lower dose groups.

For the skull, the incidences of incomplete ossification of the parietal and occipital bones were increased for the high dose group. An additional suture line in the left parietal bone was observed for 1 fetus in the high dose group.

For thoracic vertebrae, the incidences of one or more centra dumbbell-shaped, were slightly increased for treatment groups. These incidences were well within the historical control fetal incidence, which ranged up to 70.0%. The incidence of one or more centra asymmetrically ossified was also slightly increased for treatment groups. The incidence of one or more centra: bipartite was increased for treatment groups. The historical control incidence of centra split ranged up to 34.78%.

For lumbar vertebra, the incidence of one or more centra: dumbbell-shaped was increased for the high dose group. These incidences were within or comparable to the historical control fetal incidence, which ranged up to 7.14%.

For the ribs, wavy 3rd or 4th right rib was each observed for one fetus in the high dose group. These incidences were within the historical control fetal incidence, which ranged up to 20.00%. The incidences of a rudimentary 14th right rib were increased for treatment groups.

For sternebra, a major malformation, one or more fused, was observed for 1 fetus in the high dose group (Litter #90). This finding was confined to one fetus and its relationship to treatment is unclear. The incidence of incomplete ossification of the 1st sternebra was increased for mid and high dose groups. The incidence of incomplete ossification of the

2nd sternebra was increased for treatment groups. A 2nd sternebra not ossified was observed for 1 fetus in the high dose group. The incidence of one or more: bilobed bipartite, misshapen or misaligned sternebra was slightly increased for the high dose group. These incidences were within historical control fetal incidences for misshapen (27.78%), misaligned (47.37%), and split (21.74%). Incomplete ossification of the 3rd and 6th sternebra was observed for a small number of fetuses in mid and high dose groups.

For the forelimb, the incidence of one or more metatarsals not ossified was increased for the high dose group.

Skeletal examination of F₁ fetuses obtained from dams exposed to Symbicort by inhalation from days 6 to 16 of gestation.

Observation	Control	Low Dose	Mid Dose	High Dose
Fetuses/ Litters examined	130/23	137/23	116/20	108/20
Skull				
Parietal-left additional suture line (minor)	0	0	0	1 (0.9%)
Parietal-bilateral incomplete ossification (minor)	1 (0.8%)	0	1 (0.9%)	14 (13%)
Occipital: incomplete ossification (minor)	4 (3.0%)	3 (2.2%)	3 (2.6%)	17 (15.7%)
Thoracic vertebra				
One or more centra dumbbell-shaped (variant)	39 (30%)	47 (34.3%)	44 (37.9%)	47 (43.5%)
One or more centra asymmetrically ossified (minor)	6 (4.6%)	10 (7.3%)	8 (6.9%)	10 (9.3%)
One or more centra: bipartite (minor)	8 (6.2%)	17 (12.4%)	12 (10.3%)	17 (15.7%)
Lumbar vertebra				
One or more centra: ell-shaped (minor)	4 (3.1%)	6 (4.4%)	4 (3.5%)	8 (7.4%)
Rib				
3 rd rib – right: wavy (minor)	0	0	0	1 (0.9%)
4 th rib – right: wavy (minor)	0	0	0	1 (0.9%)
14 th rib – right: rudimentary (variant)	7 (5.4%)	19 (13.5%)	12 (10.6%)	14 (13.5%)
Sternum				
1 st sternebra: incomplete ossification (minor)	7 (5.4%) [9.3%]	9 (6.6%)	11 (9.5%)	11 (10.2%)
One or more: fused (major)	0	0	0	1 (0.9%)
2 nd sternebra: not ossified (minor)	0	0	0	1 (0.9%)
2 nd Sternebra: incomplete ossification (minor)	3 (2.3%)	7 (5.1%)	9 (7.8%)	9 (8.3%)
One or more: bilobed bipartite, misshapen or misaligned (minor)	4 (3.1%) [6.5%]	5 (3.65%)	5 (4.3%)	6 (5.6%)
3 rd Sternebra: incomplete ossification (minor)	0	0	1 (0.9%)	1 (0.9%)
6 th Sternebra: incomplete	0	0	2 (1.7%)	2 (1.85%)

ossification (minor)				
Forelimb				
1-8 Phalanges-right: not ossified (variant)	0	1 (0.7%)	0	1 (0.9%)
One or more metatarsals: not ossified (variant)	17 (13.1%) [16.2%]	21 (15.3%)	14 (12.1%)	31 (28.7%)

The reviewer calculated the percentages shown in parentheses. The sponsor's calculations of percentages are shown in brackets where there was a significant difference with the reviewer's calculation.

2.6.6.7 Local tolerance

See attached reviews of studies conducted with formoterol. See NDA 20-441 for studies conducted with budesonide.

2.6.6.8 Special toxicology studies

See attached reviews of studies conducted with formoterol. See NDA 20-441 for studies conducted with budesonide.

2.6.6.9 Discussion and Conclusions

Toxicities observed in 3-month bridging toxicology studies with Symbicort HFA pMDI in rats and dogs appear to be primarily attributable to the pharmacological effects of budesonide. In addition, tachycardia, attributable to formoterol, was observed in dogs. There was no evidence in these studies of additive or synergistic toxic effects between budesonide and formoterol.

The HFA pMDI formulation contains excipients, PVP K-25 and PEG-1000, and propellant, HFA-227, which are not found in any approved inhalation drug products. Extensive preclinical toxicology studies have been conducted with HFA-227 by IPACT-II, for which the sponsor has rights of reference. Both PVP K-25 and PEG-1000 are found in several approved oral drug products for chronic use. Further, PEG-1000 has been classified as GRAS for use in foods. A 6-month inhalation bridging toxicology study for PVP K-25 and PEG-1000 in the most appropriate species is required for bridging from the oral to inhalation route. Due to similarities between PVP K-25 and PVP K-30 as well as PEG-600 and **PEG-1000**, the sponsor's bridging program involved chronic inhalation toxicology studies conducted with similar excipients, povidone K-30 (PVP K-30) and polyethylene glycol 600 (PEG-600). The sponsor did conduct 3-month inhalation toxicology studies in rats and dogs exposed to Symbicort HFA containing excipients, PVP K-25 and PEG-1000. A 3-month inhalation toxicology study (Study 96195) conducted with rats that received Formoterol HFA pMDI containing excipients, PVP K-25 and PEG-1000, as found in the Symbicort drug product, was also considered. There was no evidence of local or systemic toxicity attributable to these excipients following administration by the inhalation route.

2.6.6.10 Tables and Figures

Table 1 Bridging toxicology studies in support of SYMBICORT pMDI clinical development

Study type and duration	Route of administration	Species	Compounds administered
Single dose toxicity	Inhalation	Rat and dog	Budesonide/formoterol in micronized powder
Repeat dose toxicity	3 months	Rat and dog	Budesonide/formoterol in pMDI formulation
	3 months	Rat and dog	Budesonide/formoterol in lactose powder
	6 months	Rat and dog	pMDI excipients
	13 months	Inhalation	Dog
Carcinogenicity	24 months	Rat	pMDI excipients
	Embryofetal toxicity	Inhalation	Rat

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not provided.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Symbicort® is a combination inhalation drug product consisting of a glucocorticoid (budesonide) and a long acting β₂ agonist (formoterol). Symbicort pMDI is intended for the long-term maintenance treatment of asthma (160/9 µg or 320/9 µg twice daily) in adult and adolescent subjects 12 years of age and older. 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 the propellant, 1,1,1,2,3,3,3-heptafluoropropane (HFA-227). There are no approved inhalation drug products that contain PVP K-25, PEG-1000, or HFA-227. The sponsor is attempting to prove that the combination of budesonide and formoterol in asthma therapy delivers a greater benefit than either drug alone.

AstraZeneca has several approved drug products containing budesonide in the U.S. (i.e., Pulmicort, Rhinocort, Pulmicort Respules, and Entocort). AstraZeneca has conducted extensive nonclinical pharmacology and toxicology studies with formoterol, although they have no approved formoterol drug products in the U.S.

Budesonide: Pulmicort Turbuhaler (DPI) is an approved drug product (AstraZeneca). Pulmicort Turbuhaler is indicated for the maintenance treatment of asthma as prophylactic therapy in adult and pediatric patients six years of age or older. The recommended starting dose and the highest recommended dose of Pulmicort Turbuhaler, based on prior asthma therapy, are listed in the table below. Doses of

budesonide in the Symbicort drug product are within those approved for Pulmicort Turbuhaler.

	Previous Therapy	Recommended Starting Dose	Highest Recommended Dose
Adults:	Bronchodilators alone	200 to 400 mcg twice daily	400 mcg twice daily
	Inhaled Corticosteroids*	200 to 400 mcg twice daily	800 mcg twice daily
	Oral Corticosteroids	400 to 800 mcg twice daily	800 mcg twice daily
Children:	Bronchodilators alone	200 mcg twice daily	400 mcg twice daily
	Inhaled Corticosteroids*	200 mcg twice daily	400 mcg twice daily
	Oral Corticosteroids	The highest recommended dose in children is 400 mcg twice daily	

* In patients with mild to moderate asthma who are well controlled on inhaled corticosteroids, dosing with PULMICORT TURBUHALER 200 mcg or 400 mcg once daily may be considered. PULMICORT TURBUHALER can be administered once daily either in the morning or in the evening.

Formoterol:

For the Symbicort HFA pMDI drug product, formoterol will be administered at a maximum dose of 18 µg/day, which is equivalent to 0.36 µg/kg/day for a 50-kg patient.

The sponsor has conducted subchronic and chronic toxicology studies with formoterol in both rats and dogs under IND — . 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 studies 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.

In toxicology studies with dogs, the target organ of toxicity as the heart. NOAELs were not established for formoterol-induced cardiac effects in dogs; however, these effects were attributed to increased heart rate, which is monitorable in a clinical setting.

In toxicology studies with rats, target organs of toxicity were the heart, testes, and epididymides. NOAELs were identified for these organs and tissues. Safety margins for the clinical dose of formoterol at 18 µg/day based upon deposited dose or systemic exposure at the NOAEL identified in the 6-month inhalation toxicology study with rats are 6.4- and 20.9-fold, respectively.

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Safety margin for clinical dose of formoterol at 18 µg/day in the Symbicort drug product based upon the NOAEL in the 6-month rat study.

6-month inhalation toxicology study with rats, NOAEL = Low dose of 2.3 µg/kg/day (deposited dose)	Clinical dose of formoterol at 18 µg/day	Safety margin
NOAEL = 2.3 µg/kg/day	18 µg/50 kg = 0.36 µg/kg/day	6.4
AUC = 3.3 nmole·hr/L (estimated from AUC at mid dose assuming dose proportionally) ^a	0.158 nmole·hr/L	20.9

a. The reliability of analytical measurements of plasma formoterol concentrations in animals was questionable.

Formoterol was negative in the in vitro bacterial reverse mutation assay, in vitro human lymphocytes chromosomal aberration assay, in vitro L5178Y mouse lymphoma cell thymidine kinase forward mutation assay, and in the in vivo rat micronucleus assay. In the in vitro bacterial reverse mutation assay, increased revertant colony counts were observed for TA1538 in the presence of S9 liver fraction; however, a 3-fold elevation over the vehicle-control was not achieved. Statistical analysis of the in vitro bacterial reverse mutation assay is generally not considered appropriate. Further, the significance of increased revertant colony counts for TA1538 was questionable given that TA98 was negative. The only difference between strains TA1538 and TA98 is that TA98 possesses an error-prone repair system (i.e., TA98 should be more sensitive). Strain selection for the bacterial reverse mutation assay was inadequate. The standard set of strains used in bacterial mutation assays should include strains that will detect point mutations at A-T sites, such as TA102 or *Escherichia coli* WP2 uvrA. Neither strain was included in the present study. It is noted that racemic formoterol was negative in in vitro bacterial reverse mutation assays reported in NDA 20-831 (Novartis, Foradil®).

In 2-year carcinogenicity studies with mice and rats treated with formoterol, there were findings of ovary and/or uterine leiomyomas. Increases in leiomyomas of the rodent female genital tract have been similarly demonstrated with other beta-agonist drugs.

In a combined fertility, reproductive performance, and embryofetal development study with rats, formoterol was administered at oral doses of 0, 0.2, 3, and 15 mg/kg/day. Decreased fertility and/or reproductive performance were observed for males in the 15 mg/kg/day group. The duration of pregnancy was prolonged at 15 mg/kg/day, which might be attributed to relaxation of the uterine musculature, a known class effect of β₂-agonists. Formoterol produced teratogenic effects with findings of umbilical hernia in the 3 and 15 mg/kg/day groups and brachygnathia in the 15 mg/kg/day group. Embryoletality was observed at doses of 3 and 15 mg/kg/day. Placental weights were increased in the 15 mg/kg/day group. Embryoletality and pup loss at birth and during lactation were observed at 3 and 15 mg/kg/day. Pup body weights were decreased at 15 mg/kg/day.

In a 3-month oral toxicology study with young rats that received formoterol at doses of 0.2, 0.8, and 3.0 mg/kg/day, there were dose-related findings of testicular tubular

atrophy, and spermatic debris, and oligospermia in the epididymides in all treatment groups. A NOAEL was not identified based upon these histopathological findings in the testes and epididymides.

In an inhalation teratology study, pregnant female rats received formoterol by nose-only exposure at doses of 0, 3.9, 86, and 1200 µg/kg/day from gestation days 6 to 15. Deposited doses were estimated to be 0, 0.37, 7.7, and 91 µg/kg/day, respectively. Formoterol was not teratogenic at inhaled deposited doses up to 91 µg/kg/day.

In an oral teratology study, pregnant female rabbits received formoterol at doses of 0, 0.2, 3.5, and 60 mg/kg/day from gestation days 6 to 18. Late embryonic deaths were increased in the 3.5 and 60 mg/kg/day groups. Incidence of anomalous cervicothoracic arteries were increased for all treatment groups, although a dose-response relationship was not present. The incidence of subcapsular cysts on the liver was increased at 60 mg/kg/day (23/115 = 20%). Incidences of satural cranial bones were increased at 3.5 and 60 mg/kg/day. Incidences of asymmetric bipartite sternebrae for the low, mid, and high dose groups were increased to 4.1, 4.2, and 9.4%, respectively, as compared to 1.1% for the control. Formoterol was teratogenic at 60 mg/kg/day in rabbits based upon findings of subcapsular cysts on the liver. There were no teratogenic findings in rabbits at doses of 0.2 and 3.5 mg/kg/day.

In a pre- and postnatal development study, pregnant female rats received formoterol at oral doses of 0, 0.21, 0.84, and 3.4 mg/kg/day from gestation day 6 through the lactation period. Pregnant dams were allowed to deliver their pups naturally. Development of the F₁ generation was followed through reproduction. Cumulative litter loss (i.e., neonatal mortality) from birth to postpartum day 26 for the low, mid, and high dose groups was increased to 21.6, 28.8, and 27.8%, respectively, as compared to 11.0% for the control group, although there was no evidence of a dose-response relationship. There were no treatment-related effects on the physical, functional, and behavioral development of the F₁ generation. There were no treatment-related effects on the reproductive capacity of the F₁ generation. Neonatal mortality was increased for all formoterol treatment groups.

Symbicort HFA pMDI: For the Symbicort HFA pMDI drug product (i.e., combination of budesonide and formoterol), 3-month inhalation bridging toxicology studies with rats and dogs were provided. For comparison, 3-month inhalation toxicology studies with the Symbicort dry powder in rats and dogs were also provided. Excipients in the Symbicort HFA pMDI are discussed below.

Toxicities observed in 3-month bridging studies with Symbicort HFA pMDI in rats and dogs appear to be primarily attributable to the pharmacological effects of budesonide. In addition, tachycardia, attributable to formoterol, was observed in dogs. There was no evidence in these studies of additive or synergistic toxic effects between budesonide and formoterol. Similar results were obtained with 3-month inhalation toxicology studies with the Symbicort dry powder in rats and dogs. For the 3-month rat study with the Symbicort HFA pMDI formulation, the NOAEL was identified as the mid dose. Deposited

doses of budesonide and formoterol for mid dose treatment group were 0.8 and 0.048 µg/kg/day, respectively. For the 3-month dog study with the Symbicort HFA pMDI formulation, the NOAEL was identified as the low dose. Doses of budesonide and formoterol for low dose treatment group were 2.0 and 0.11 µg/kg/day, respectively. Safety margins derived from NOAELs of preclinical studies with Symbicort compared to clinical doses were significantly less than 1 (see table below), given that rats and dogs are known to be significantly more sensitive to the toxic effects of corticosteroids as compared to humans. These findings are in concordance with previous inhalation toxicology studies conducted with budesonide in rats and dogs that were reviewed under NDA 20-441. Previous clinical experience with the budesonide supports clinical doses of budesonide in the combination (i.e., Symbicort).

Rat to human and dog to human dose ratios for clinical doses budesonide and formoterol in the Symbicort HFA pMDI formulation in humans with a body weight of 50 kg (based upon comparisons to NOAELs from 3-month inhalation bridging toxicology studies with rats and dogs).

Clinical Doses of Budesonide/Formoterol in the Symbicort drug product				Dose Ratios for Clinical Doses of Budesonide and Formoterol in the Symbicort HFA pMDI	
80/4.5	µg/actuation	4 actuations/day	µg/kg/day	Rat to Human ratio ^a	Dog to Human Ratio ^b
Budesonide	80	320	6.4	0.13	0.31
Formoterol	4.5	18	0.36	0.13	0.31
160/4.5	µg/actuation	4 actuations/day	µg/kg/day	Rat to Human ratio ^a	Dog to Human Ratio ^b
Budesonide	160	640	12.8	0.06	0.16
Formoterol	4.5	18	0.36	0.13	0.31

a. Deposited doses at the NOAEL for rats in the 3-month bridging study were 0.8 µg/kg/day budesonide and 0.048 µg/kg/day formoterol assuming a deposition factor of 8.6 to 9.4%.

b. Deposited doses at the NOAEL for dogs in the 3-month bridging study were 2.0 µg/kg/day budesonide and 0.11 µg/kg/day formoterol assuming 100% deposition.

AUC values for budesonide and formoterol at NOAELs in 3-month inhalation toxicology studies with the Symbicort HFA pMDI formulation in rats and dogs were compared to corresponding AUC values obtained in asthmatic and healthy volunteers that received the Symbicort HFA pMDI drug product. Symbicort HFA pMDI (budesonide/formoterol), 160/4.5 µg, was administered as 2 actuations BID corresponding to a total daily dose of 640/18 µg. Similarly as observed with deposited doses above, safety margins for budesonide and formoterol derived from comparison of AUC values at NOAELs of nonclinical studies compared to corresponding values from the maximum recommended clinical dose of the Symbicort HFA pMDI drug product were significantly less than 1. These findings are in concordance with previous inhalation toxicology studies conducted with budesonide in rats and dogs that were reviewed under NDA 20-441, and can be attributed to the sensitivity of animals to corticosteroids.

Rat to human and dog to human exposure (AUC) ratios for clinical exposures to budesonide and formoterol obtained with the maximum recommended dose of the Symbicort HFA pMDI drug product (based upon comparison to systemic exposures at NOAELs in 3-month inhalation bridging toxicology studies with rats and dogs)

Symbicort pMDI	Human ^a – AUC _{0-12hr} 4 x 160/4.5 µg	Rat		Dog	
		NOAEL AUC	Rat to Human ratio	NOAEL AUC	Dog to Human ratio
Budesonide	4.91 nmol/hr/L	1.95 nmol/hr/L	0.4	0.630 nmol/hr/L	0.13
Formoterol	158 pmol/hr/L	67.0 pmol/hr/L	0.4	24.5 pmol/hr/L	0.15

a. Values for humans were obtained from Study D-5896C00011 in which Symbicort pMDI (160/4.5 µg budesonide/formoterol) was administered two actuations twice daily (for a daily dose of 640/18 µg) for 6 and a half days to asthma patients and healthy adults. In asthma patients, AUC₀₋₁₂ was 4.91 nmol/hr/L for budesonide and 158 pmol.hr/L for formoterol. The human clinical dose (based upon 50 kg) is equivalent to 12.8/0.36 µg/kg of budesonide/formoterol.

Excipients in Symbicort pMDI:

The Symbicort HFA pMDI drug product contains excipients, PVP K-25 and PEG-1000, and the propellant, HFA-227. There are no approved inhalation drug products that contain PVP K-25, PEG-1000, or HFA-227. Extensive preclinical toxicology studies have been conducted with HFA-227 by IPACT-II, for which the sponsor has rights of reference. These studies were reviewed under DMF 10378 (See reviews dated October 2, 1996 and January 28, 1997) In general, there were no toxicologically significant findings with HFA-227 and it is considered safe for use in the Symbicort HFA pMDI drug product. PVP K25 and PEG-1000 are found in several drug products approved for chronic use that are administered by the oral route. Further, PEG-1000 has been **classified as “generally recognized as safe”** (GRAS) by the oral route and is used in food (21 CFR 172.820). A 6-month inhalation toxicology study is required to bridge excipients, PVP K25 and PEG-1000, from the oral to inhalation route.

In the initial submission of IND 63,394, the sponsor provided 3-month inhalation bridging toxicology studies with Symbicort HFA pMDI in rats and dogs that included PVP K-25, PEG-1000, and HFA-227. In addition, the sponsor provided chronic inhalation toxicology studies with rats and dogs, conducted with another inhalation drug product _____ studied _____ by AstraZeneca) that contained relatively similar excipients, povidone K-30 (PVP K-30) and polyethylene glycol 600 (PEG-600), and the propellant, HFA-227.

The various pharmaceutical grades of PVPs are defined by the average polymer length, given by molecular weight. PVP K-25 and PVP K-30 have mean molecular weights of 26000 and 42000 daltons, respectively. K-25 and K-30 both consist of a broad range of polymer sizes with molecular weights from 1000 to 1000000 (see Figure below) with K-25 containing slightly less of the high MW material than K-30. Therefore, the only

difference between PVP K-25 and PVP K-30 is a small shift in the average polymer length.

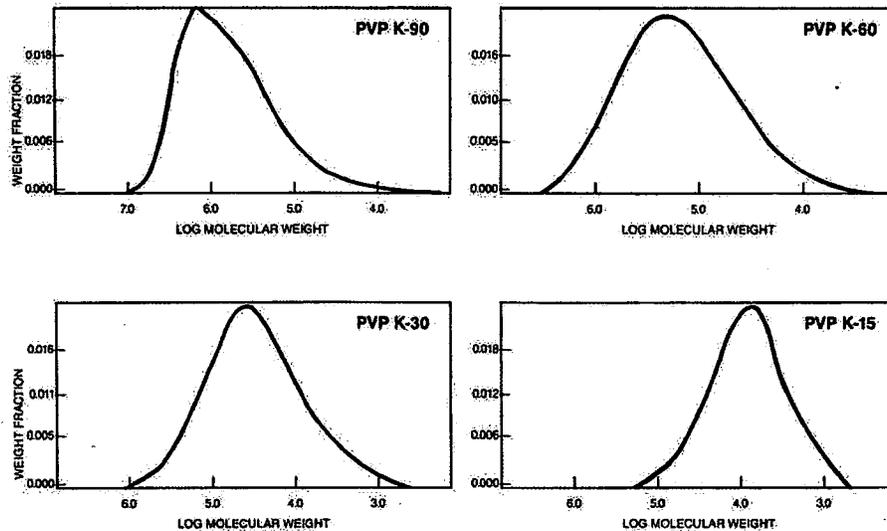


Figure 2. Gel permeation chromatography of PVP. Absolute differential molecular weight distributions for PVP K-90, K-60, K-30, and K-15 grades.

SYNTHESIS AND PROPERTIES OF PVP 15

Polyethylene glycols are polymers of the general formula, $H(OCH_2CH_2)_nOH$, where n is greater than 4. PEG 600 has an average n of 12.5-13.9 with a MW range of 570-630. PEG 1000 is estimated to have an average n of 20.8-23.2 with a MW range of 950-1050.

It was expected that differences between PVP K-25 and PVP K-30 as well as PEG-600 and PEG-1000 would have minimal or no toxicological impact. Therefore, PVP K-30 and PEG-600 could be used for the safety assessment of PVP K-25 and PEG-1000, respectively. Based upon reviews of the 6-month study with rats, 6- and 12-month studies with dogs, and the 2-year inhalation carcinogenicity study with rats conducted with the _____

(see reviews dated September 17, 1998, December 2, 1998, February 21, 2001, and July 31, 2003), no apparent local or systemic toxic effects attributable to PVP K-30, and PEG 600 were observed.

The 3-month toxicity study with Symbicort HFA in rats used very low doses of the vehicle and provides inadequate safety margins for clinical doses of PVP K-25 and PEG-1000 (i.e., 2-fold, see Table A). The 3-month toxicity study with Symbicort in dogs provides adequate safety margins for PVP K-25 and PEG-1000 (Table B), although duration was only 3 months. The 6-month toxicity with rats, 6- and 12-month toxicity studies with dogs, and a 24-month carcinogenicity study with rats that used closely related excipients, PVP K-30 and PEG-600 (Tables C and D), provided sufficient dose

ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product.

Table A: Rat to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation bridging toxicology study with Symbicort in rats.

Compound	Rats		Clinical doses in Symbicort $\mu\text{g/g LW/day}$	Rat to human dose ratio
	Deposited dose $\mu\text{g/kg/day}$	Deposited dose $\mu\text{g/g LW/day}$		
PVP K-25	0.03	0.006	0.0028	2.1
PEG-1000	8.6	1.72	0.8492	2.0

Notes: The deposition factor for rats was approximately 8.6 to 9.4%. Lung weight values for rats and humans used in calculations were 1.5 g and 1000 g, respectively.

Table B: Dog to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation bridging toxicology study with Symbicort in dogs.

Compound	Dogs		Clinical doses in Symbicort $\mu\text{g/g LW/day}$	Dog to human dose ratio
	Deposited dose $\mu\text{g/kg/day}$	Deposited dose $\mu\text{g/g LW/day}$		
PVP K-25	0.42	0.038	0.0028	13.6
PEG-1000	126	11.455	0.8492	13.5

Notes: The deposition factor for dogs was 100%. Lung weight values for dogs and humans used in calculations were 110 g and 1000 g, respectively.

Table C: Inhaled dose ($\mu\text{g/kg/day}$) and deposited doses ($\mu\text{g/g lung weight/day}$) of excipients, PVP K-30 and PEG-600, in the vehicle-control group from inhalation toxicology studies

Excipient	6-month rat study		24-month rat study		6-month dog study		12-month dog study	
	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$	Inhaled dose $\mu\text{g/kg}$	Deposited dose $\mu\text{g/g LW}$
PVP K-30	18.4	0.36	110	1.12	2.9	0.26	1.9	0.17
PEG-600	2209.6	44.2	13000	131.9	344.2	31.3	222.2	20.2

a. The deposition factor for rats was 10%.

b. A close-fitting face mask and aerosol delivery mouth tube system was used to deliver aerosols directly to the buccal cavity of the dog. Deposition factors for dogs in the original reviews have been revised to 100%.

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Table D: Rat to human and dog to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of similar excipients, povidone K-30 and PEG-600, in vehicle-control groups of studies

Clinical Doses of Excipients, PVP K-25 and PEG-1000		Dose ratios based upon nonclinical studies with PVP K-30 and PEG 600			
Excipients	Clinical doses in Symbicort, µg/g LW/day	6-month	24-month	6-month	12-month
		Rat to Human ratio	Rat to Human ratio	Dog to Human ratio	Dog to Human ratio
Povidone K25	0.0028	128.6	400	94.2	61.7
PEG 1000	0.8492	52	155.4	36.8	23.8

The sponsor provided a 3-month inhalation toxicology study with rats that received Formoterol HFA pMDI containing excipients, PVP K-25 and PEG-1000, and propellant, HFA-227, as found in the Symbicort HFA pMDI drug product. The study included air-control and vehicle-control groups. The vehicle-control group received PVP K-25, PEG-1000, and HFA-227. Changes were evident in the lung for the vehicle-control group as compared to the air-control group. Alveolar histiocytosis was observed in 8 of 20 (40%) rats in the vehicle-control group as compared to 4 of 20 (20%) rats in the air-control group. The incidence of alveolar histiocytosis in the vehicle-control group exceeded the published background occurrence in young rats of 16-20% (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). Pneumonitis was observed in 9 of 20 (45%) rats in the vehicle-control group as compared to 4 of 20 (20%) rats in the air-control groups. Acute or chronic inflammation consisting of small aggregates of lymphoid cells around bronchioles and small vessels is a relatively common finding in rats observed with a background occurrence of 56%, while more extensive inflammation (e.g., alveolitis, bronchiolitis, pneumonitis) occurs at a lower background incidence of 18-20% (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). Congestion was observed in 4 of 20 (20%) rats in the vehicle-control group as compared to 2 of 20 (10%) rats in the air-control group. It is possible that congestion may have been related to the procedure used to sacrifice animals (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703).

In the initial review of this study provided in Amendment #170 under IND (Review dated May 29, 2002), given that findings were observed in all groups including the air-control and scientific references reporting high incidences of spontaneous inflammation in the lungs of control rats, these findings were not identified as adverse. However, based upon experiences with alveolar histiocytosis in recent years and local toxicity observed with PVP K-25 from another application, these findings were subsequently re-interpreted to potentially be the result of local toxicity induced by excipients in the vehicle formulation (PVP K-25 and PEG-1000).

Doses of PVP K-25 and PEG-1000 in the 3-month study with Formoterol HFA pMDI (Table E) were higher than doses of PVP K-25 and PEG-1000 in 3-month studies with Symbicort HFA in rats and dogs (Tables A and B), but generally lower than doses of

PVP K-30 and PEG-600 used in chronic toxicity studies with rats and dogs (Tables C and D).

Table E: Rat to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation toxicology study with Formoterol HFA pMDI in rats.

Excipients	3-month rat study		Clinical doses in Symbicort, $\mu\text{g/g LW/day}$	Rat to Human Ratios
	Deposited dose, $\mu\text{g/kg/day}$	Deposited dose, $\mu\text{g/g LW/day}$		
PVP K-25	1.14	0.228	0.0028	81.4
PEG-1000	211.3	42.26	0.8492	49.8

Notes: Rat body and lung weights were estimated to be 300 g and 1.5 g, respectively. Human lung weight was estimated to be 1000 g.

The comparability of PVP K-25 and PEG-1000 to PVP K-30 and PEG-600, respectively, was called into question.

In an information request dated March 3, 2006, the sponsor was offered the options of explaining the lung histopathology findings in study 96195 and/or conducting a 6-month inhalation bridging toxicology study with PVP K-25 and PEG-1000 in rats.

In a submission dated March 17, 2006, the sponsor reported results of their re-examination of lung histopathology slides from study 96195. In the re-examination, the original diagnosis of alveolar histiocytosis was changed to alveolar foamy macrophages and incidences and severity became relatively similar between the air-control and vehicle-control groups. Further, incidences and severity of pneumonitis and congestion were relatively similar between the air-control and vehicle-control groups. For reference, the sponsor also examined lung slides from the 3-month interim sacrifice of the 6-month inhalation toxicology study with PVP K-30 and PEG-600 in rats (Study 96010).

Both study numbers 96195 and 96010 were reported to be conducted in compliance with GLP regulations. There are concerns about the significance changes of incidences and severity of reported findings between the initial examination and subsequent re-examination, particularly for Study number 96195 that was conducted by the same pathologist.

In an information request dated April 4, 2006, the sponsor was requested to have an independent pathologist examined lung histopathology slides from study 96195 and the 6-month terminal sacrifice of Study 96010.

In a submission dated April 26, 2006, the sponsor provided the results of independent **pathologist's analysis of studies 96195 and 96010**. Essentially, the analysis of the independent histopathologist confirmed the March 17, 2006 re-examination of study 96195 and the 3-month interim sacrifice of study 96010 as well as the original examination of the 6-month terminal sacrifice of study 96010.

The independent pathologist contended that lungs from rats in study numbers 96195 and 96010 (3-month interim sacrifice and 6-month terminal sacrifice) were within the normally expected range for animals <1 year of age. Given the general agreement of histopathological diagnoses **between the sponsor's re-examination** and independent pathologist for lung slides from studies 96195 and 96010, it is concluded that neither PVP K-25/PEG-1000 nor PVP K-30/PEG-600 produced local toxicity in the lung. Further, these sets of excipients are considered sufficiently comparable and the bridging program using PVP K-30 and PEG-600 in chronic inhalation toxicology studies with rats and dogs is acceptable to assess the toxicity profile of PVP K-25 and PEG-1000.

Conclusions: The sponsor has complete nonclinical pharmacology and toxicology programs for the mono-products, budesonide and formoterol. For the combination Symbicort pMDI HFA drug product, 3-month inhalation bridging toxicology studies with rats and dogs were provided. Toxicities observed in 3-month bridging studies with Symbicort HFA pMDI in rats and dogs appear to be primarily attributable to the pharmacological effects of budesonide. In addition, tachycardia, attributable to formoterol, was observed in dogs. There was no evidence in these studies of additive or synergistic effects between budesonide and formoterol.

As communicated during the IND development phase, excipients, povidone K-25 (PVP K-25), polyethylene glycol 1000 (PEG-1000), and HFA-227, in the Symbicort HFA pMDI drug product are not found in any approved inhalation drug products. HFA-227 is considered safe for use in the Symbicort HFA pMDI drug product based upon extensive preclinical toxicology studies conducted by IPACT-II, for which the sponsor has rights of reference. A 6-month inhalation toxicology study was needed to bridge excipient, PVP K-25 and PEG-1000, from the oral to inhalation route. The sponsor conducted 13-week inhalation toxicity studies with rats and beagle dogs that were treated daily with a Symbicort HFA pMDI formulation (batch P5993) containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227. **The sponsor's bridging program involved chronic studies** conducted with similar excipients, povidone K-30 (PVP K-30) and polyethylene glycol 600 (PEG-600). The results of a 3-month inhalation toxicology study (Study 96195) conducted with rats that received Formoterol HFA pMDI containing excipients, PVP K-25 and PEG-1000, as found in the Symbicort drug product, were also considered. There was no evidence of local or systemic toxicity attributable to these excipients following administration by the inhalation route.

Unresolved toxicology issues (if any): None.

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

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Evaluation of labeling:

1. Exposure margins for formoterol are based upon mcg/m² comparisons rather than AUC comparisons due to concerns regarding **the sponsor's methods for measuring** plasma concentrations of formoterol. As noted in the review and Executive CAC discussion of the mouse carcinogenicity study with formoterol, the sponsor stated that the inability to calculate AUC data for mice was due to the use of different analytical methods for rodents and humans. The assays for mouse plasma samples, conducted in 1992, had a lower sensitivity and selectivity than that improved assay for human samples (1995), which had a 30-fold lower detection limit.
2. In the sections for budesonide, the reviewer attempted to retain the labeling used for Pulmicort Respules.
4. In the Overdosage section, single dose inhalation toxicity studies were conducted with Symbicort dry powder in rats and dogs. This differs from the HFA formulation under review.

Suggested labeling:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Budesonide

Long-term studies were conducted in rats and mice using oral administration to evaluate the carcinogenic potential of budesonide.

In a two-year study in Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of gliomas in male rats at an oral dose of 50 mcg/kg (2/3 of the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No tumorigenicity was seen in male and female rats at respective oral doses up to 25 and 50 mcg/kg (1/3 and 2/3 maximum recommended daily inhalation dose in adults on a mcg/m² basis). In two additional two-year studies in male Fischer and Sprague-Dawley rats, budesonide caused no gliomas at an oral dose of 50 mcg/kg (2/3 of the maximum recommended daily inhalation dose in adults on a mcg/m² basis). However, in the male Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of hepatocellular tumors at an oral dose of 50 mcg/kg (2/3 of the maximum recommended daily inhalation dose in adults on a mcg/m² basis). The concurrent reference corticosteroids (prednisolone and triamcinolone acetonide) in these two studies showed similar findings.

In a 91-week study in mice, budesonide caused no treatment-related carcinogenicity at oral doses up to 200 mcg/kg (approximately equal to the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Budesonide was not mutagenic or clastogenic in six different test systems: Ames Salmonella /microsome plate test, mouse micronucleus test, mouse lymphoma test,

chromosome aberration test in human lymphocytes, sex-linked recessive lethal test in *Drosophila melanogaster*, and DNA repair analysis in rat hepatocyte culture.

In rats, budesonide had no effect on fertility at subcutaneous doses up to 80 mcg/kg (approximately equal to the maximum recommended daily inhalation dose in adults on a mcg/m² basis). However, it caused a decrease in prenatal viability and viability in the pups at birth and during lactation, along with a decrease in maternal body-weight gain, at subcutaneous doses of 20 mcg/kg and above (1/4 of the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No such effects were noted at 5 mcg/kg (1/16 of the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Formoterol

Long-term studies were conducted in mice using oral administration and rats using inhalation administration to evaluate the carcinogenic potential of formoterol fumarate.

In a 24-month carcinogenicity study in CD-1 mice, formoterol at oral doses of 0.1 mg/kg and above (approximately 20 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis) caused a dose-related increase in the incidence of uterine leiomyomas. Increases in leiomyomas of rodent female genital tract have been similarly demonstrated with other beta-agonist drugs.

In a 24-month carcinogenicity study in Sprague-Dawley rats, an increased incidence of mesovarian leiomyoma and uterine leiomyosarcoma were observed at the inhaled dose of 130 mcg/kg (approximately 60 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No tumors were seen at 22 mcg/kg (approximately 10 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Formoterol was not mutagenic or clastogenic in the Ames Salmonella /microsome plate test, mouse lymphoma test, chromosome aberration test in human lymphocytes, and rat micronucleus test.

A reduction in fertility and/or reproductive performance was identified in male rats treated with formoterol at an oral dose of 15 mg/kg (approximately 7000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In a separate study with male rats treated with an oral dose of 15 mg/kg, there were findings of testicular tubular atrophy and spermatic debris in the testes and oligospermia in the epididymides. No such effect was seen at 3 mg/kg (approximately 1400 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No effect on fertility was detected in female rats at oral doses up to 15 mg/kg (approximately 7000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Pregnancy

Symbicort

Teratogenic Effects: Pregnancy Category C

SYMBICORT has been shown to be teratogenic and embryocidal in rats when given at inhalation doses of 12/0.66 mcg/kg (budesonide/formoterol) and above (less than the maximum recommended daily inhaled dose in adults on a mcg/m² basis). Umbilical hernia, a malformation, was observed for fetuses at doses of 12/0.66 mcg/kg group and above (less than the maximum recommended daily inhaled dose in adults on a mcg/m² basis). No teratogenic or embryocidal effects were detected at 2.5/0.14 mcg/kg (less than the maximum recommended daily inhaled dose in adults on a mcg/m² basis). There are no adequate and well-controlled studies in pregnant women. SYMBICORT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Budesonide

Teratogenic Effects:

As with other corticosteroids, budesonide has been shown to be teratogenic and embryocidal in rabbits and rats. Budesonide produced fetal loss, decreased pup weight, and skeletal abnormalities at subcutaneous doses of 25 mcg/kg/day in rabbits (approximately 2/3 the maximum recommended daily inhalation dose in adults on a mcg/m² basis) and 500 mcg/kg/day in rats (approximately 6 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In another study in rats, no teratogenic or embryocidal effects were seen at inhalation doses up to 250 mcg/kg/day (approximately 3 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Experience with oral corticosteroids since their introduction in pharmacologic, as opposed to physiologic, doses suggests that rodents are more prone to teratogenic effects from corticosteroids than humans.

Studies in pregnant women, however, have not shown that Budesonide increases the risk of abnormalities when administered during the first trimester of pregnancy. Despite the animal findings, it would appear that the possibility of fetal harm is remote, if the drug is used during pregnancy. Nevertheless, because the studies in humans cannot rule out the possibility of harm, budesonide should be used during pregnancy only if clearly needed.

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On Original**

Formoterol

Teratogenic Effects:

Formoterol fumarate has been shown to be teratogenic, embryocidal, increase pup loss at birth and during lactation, and decreased pup weights in rats when given at oral doses of 3 mg/kg/day and above (approximately 1400 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). Umbilical hernia, a malformation, was observed in rat fetuses at oral doses of 3 mg/kg/day and above (approximately 1400 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). Brachygnathia, a skeletal malformation, was observed for rat fetuses at an oral dose of 15 mg/kg/day (approximately 7000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). Pregnancy was prolonged at an oral dose of 15 mg/kg/day (approximately 7000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In another study in rats, no teratogenic effects were seen at inhalation doses up to 1.2 mg/kg/day (approximately 500 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Formoterol fumarate has been shown to be teratogenic in rabbits when given at an oral dose of 60 mg/kg (approximately 54000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). Subcapsular cysts on the liver were observed for rabbit fetuses at an oral dose of 60 mg/kg (approximately 54000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No teratogenic effects were observed at oral doses up to 3.5 mg/kg (approximately 3200 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

There are no adequate and well-controlled studies with formoterol in pregnant women.

Nursing Mothers

Since there are no data from controlled trials on the use of SYMBICORT by nursing mothers, a decision should be made whether to discontinue nursing or to discontinue SYMBICORT, taking into account the importance of SYMBICORT to the mother.

It is not known whether budesonide, one of the main components of SYMBICORT, is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised if budesonide is administered to nursing women.

In reproductive studies in rats, formoterol was excreted in the milk. It is not known whether formoterol is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when formoterol is administered to nursing women.

OVERDOSAGE

Symbicort: SYMBICORT contains both budesonide and formoterol; therefore, the risks associated with overdosage for the individual components described below apply to

SYMBICORT. In pharmacokinetic studies, a total of 1920/54 mcg (12 actuations of SYMBICORT 160/4.5) was administered as a single dose to both healthy subjects and patients with asthma and was well tolerated.

Clinical signs in rats and dogs that received single inhalation doses of SYMICORT (a combination of budesonide and formoterol) included tremor, mucosal redness, nasal catarrh, redness of intact skin, increased respiratory rate, abdominal respiration, vomiting, and salivation. No deaths occurred in rats given a combination of budesonide and formoterol at acute inhalation dose of 97 and 3 mg/kg, respectively (approximately 1200 and 1350 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No deaths occurred in dogs given a combination of budesonide and formoterol at the acute inhalation dose of 732 and 22 mcg/kg, respectively (approximately 30 and 30 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Budesonide: The potential for acute toxic effects following overdose of budesonide is low. If used at excessive doses for prolonged periods, systemic corticosteroid effects such as hypercorticism may occur (see **PRECAUTIONS**). Budesonide at five times the highest recommended dose (3200 mcg daily) administered to humans for 6 weeks caused a significant reduction (27%) in the plasma cortisol response to a 6-hour infusion of ACTH compared with placebo (+1%). The corresponding effect of 10 mg prednisone daily was a 35% reduction in the plasma cortisol response to ACTH.

In mice the minimal lethal inhalation dose was 100 mg/kg (approximately 600 times the maximum recommended daily inhalation dose in adults a mcg/m² basis). In rats there were no deaths at an inhalation dose of 68 mg/kg (approximately 900 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In mice the minimal oral lethal dose was 200 mg/kg (approximately 1300 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In rats, the minimal oral lethal dose was less than 100 mg/kg (approximately 1300 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

Formoterol: An overdose of formoterol would likely lead to an exaggeration of effects that are typical for beta₂-agonists; therefore, the following adverse experiences may occur: tremor, headache, palpitations, tachycardia hypotension, metabolic acidosis, hypokalemia, hyperglycemia, prolonged QTc-interval, arrhythmia, nausea and vomiting. Formoterol was well tolerated at a delivered dose of 90 mcg/day over 3 hours in adult patients with acute bronchoconstriction and when given three times daily for a total dose of 54 mcg/day for 3 days to stable asthmatics.

Treatment of formoterol overdosage consists of discontinuation of the medication together with institution of appropriate symptomatic and/or supportive therapy. The judicious use of a cardioselective beta-receptor blocker may be considered, bearing in mind that such medication can produce bronchospasm. There is insufficient evidence to determine if dialysis is beneficial for overdosage of formoterol. Cardiac monitoring is recommended in cases of overdosage.

No deaths were seen in mice given formoterol at an inhalation dose of 276 mg/kg (approximately 62000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In rats the minimum lethal inhalation dose was 40 mg/kg (approximately 18000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No deaths were seen in mice that received an oral dose of 2000 mg/kg (approximately 450000 times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). Maximum non-lethal oral doses were 600 mg/kg in young rats and 1500 mg/kg in adult rats (approximately 270000 and 675000 times the maximum recommended inhalation dose in adults, on a mcg/m² basis).

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

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

cc: list:

NDA 21-929, HFD-570
JacksonC, HFD-570
StarkeP, HFD-570
SunC, HFD-570
RobisonT, HFD-570

APPENDIX/ATTACHMENTS

- Appendix 1: Addendum to Review of IND 63,394 Initial Submission dated January 23, 2002
- Appendix 2: Review of IND 63,394 Initial Submission dated January 23, 2002
- Appendix 3: Review of IND 63,394 Amendment #017 dated April 4, 2002
- Appendix 4: Review of IND _____ dated March 5, 1997
- Appendix 5: Review of IND _____ dated December 2, 1997
- Appendix 6: Reviews of IND _____ dated March 2, 1998 and April 6, 1998
- Appendix 7: Minutes of Executive CAC Meeting dated April 14, 1998
- Appendix 8: IND _____ - Miscellaneous telephone conversations between the sponsor and FDA reviewer.
- Appendix 9: Addendum to Review of IND _____ Amendment #170 dated May 29, 2002
- Appendix 10: Review of IND _____ Amendment #170 dated May 29, 2002
- Appendix 11: Reviews of 6-month toxicology studies with _____, Povidone K30, Polyethylene glycol 600, and HFA 227 in rats and dogs _____ dated December 2, 1998
- Appendix 12: Review of 12-month toxicology study with _____ Povidone K30, Polyethylene glycol 600, and HFA 227 in dogs _____
- Appendix 13: Review of 2-year inhalation carcinogenicity study \ _____

Povidone K30, Polyethylene glycol 600, and HFA 227 in rats under IND -
 dated July 31, 2003

Drug: **Formoterol**

		# daily			
	age	µg/dose	doses	µg/day	nmole*hr/L
Adult	>12	4.5	4	18	0.158
Study	route	µg/kg/d	nmole*hr/L	Exposure margin	
				Adults	
Carcinogenicity:					
mouse	Oral/F	100	-	-	
mouse	Oral/F	500	-	-	
mouse	Oral/F	2500	20.5	129.75	
rat	IH/F	4.7	-	-	
rat	IH/F	22	1.79		
rat	IH/F	130	15.5	98.10	
Reproduction and Fertility:					
rat	Oral/F	200	-	-	
rat	Oral/F	3000	38	240.51	
rat	Oral/F	15000	68.2	431.65	
Teratogenicity:					
rat	Oral/F	200	-	-	
rat	Oral/F	3000	24.1	152.53	
rat	Oral/F	15000	435	2753.16	
rabbit	Oral/F	200	11.2	70.89	
rabbit	Oral/F	3500	125.6	794.94	
rabbit	Oral/F	60000	3576	22632.91	
6-month inhalation study:					
rat	IH/F	32	-	-	
rat	IH/F	260	17.25	109.18	
rat	IH/F	870	66.9	423.42	
12-month oral study:					
dog	Oral/F	0.72	-	-	
dog	Oral/F	8.6	6.68	42.28	
dog	Oral/F	92	78.4	496.20	

Drug: **Formoterol**

			# daily					
	age	µg/dose	doses	µg/day	kg	µg/kg	factor	µg/m ²
Adult	>12	4.5	4	18	50	0.3600	37	13.32
	route	µg/kg/d	conv. factor	µg/m ²	Dose Ratio		Rounded Dose Ratio	
					Adults		Adults	
Carcinogenicity:								
mouse	Oral/F	100	3	300	22.52		20	
mouse	Oral/F	500	3	1500	112.61		110	
mouse	Oral/F	2500	3	7500	563.06		560	
rat	IH/F	4.7	6	28.2	2.12		2	
rat	IH/F	22	6	132	9.91		10	
rat	IH/F	130	6	780	58.56		60	
Reproduction and Fertility:								
rat	Oral/F	200	6	1200	90.09		90	
rat	Oral/F	3000	6	18000	1351.35		1400	
rat	Oral/F	15000	6	90000	6756.76		6800	
Teratogenicity:								
rat	Oral/F	200	6	1200	90.09		90	
rat	Oral/F	3000	6	18000	1351.35		1400	
rat	Oral/F	15000	6	90000	6756.76		7000	
rat	IH/F	3.9	6	23.4	1.76		2	
rat	IH/F	86	6	516	38.74		40	
rat	IH/F	1200	6	7200	540.54		500	
rat	IH/SMB	0.14	6	0.84	0.06		1/16	
rat	IH/SMB	0.66	6	3.96	0.30		1/3	
rat	IH/SMB	4.4	6	26.4	1.98		1	
rabbit	Oral/F	200	12	2400	180.18		180	
rabbit	Oral/F	3500	12	42000	3153.15		3200	
rabbit	Oral/F	60000	12	7E+05	54054.05		54000	
Overdosage:								
mouse	IN/F	276000	3	8E+05	62162.16		62000	
mouse	Oral/F	2E+06	3	6E+06	450450		450000	
rat	IH/F	40000	6	2E+05	18018.02		18000	
rat	Oral/F	600000	6	4E+06	270270		270000	
rat	Oral/F	2E+06	6	9E+06	675675		675000	
rat	IN/SMB	3000	6	18000	1351.35		1350	
Other: (Describe studies here)								
dog	IN/SMB	22	20	440	33.03		30	
rat	Oral/F	210	6	1260	94.59		90	
rat	Oral/F	840	6	5040	378.38		380	
rat	Oral/F	3400	6	20400	1531.53		1500	

Conversion, Correction, and Rounding Factors:

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

Drug: **Budesonide**

	age	$\mu\text{g}/\text{dose}$	# daily doses	mg/day	kg	$\mu\text{g}/\text{kg}$	factor	$\mu\text{g}/\text{m}^2$
Adult	>12	160	4	640	50	12.8000	37	473.60
	route	$\mu\text{g}/\text{kg}/\text{d}$	conv. factor	mg/m ²	Dose Ratio Adults		Rounded Dose Ratio Adults	
Carcinogenicity:								
mouse	Ora/B	200	3	600	1.27		1	
rat	Ora/B	25	6	150	0.32		1/3	
rat	Ora/B	50	6	300	0.63		1/2	
Reproduction and Fertility:								
rat	SC	5	6	30	0.06		1/16	
rat	SC	20	6	120	0.25		1/4	
rat	SC	80	6	480	1.01		1	
Teratogenicity:								
rat	INSYMB	25	6	15	0.03		1/32	
rat	IH/SYMB	12	6	72	0.15		1/7	
rat	INSYMB	80	6	480	1.01		1	
rat	IH/B	250	6	1500	3.17		3	
rat	SC/B	500	6	3000	6.33		6	
rabbit	SC/B	25	12	300	0.63		1/2	
Overdosage:								
mouse	IH/B	100000	3	3E+05	633.45		600	
mouse	Ora/B	200000	3	6E+05	1266.89		1300	
rat	IH/B	68000	6	4E+05	861.49		900	
rat	Ora/B	100000	6	6E+05	1266.89		1300	
rat	IH/SYMB	97000	6	6E+05	1228.89		1200	
Other: (Describe studies here)								
dog	INSYMB	737	20	14740	31.12		30	

Conversion, Correction, and Rounding Factors:

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

Appendix 1: Addendum to Review of IND 63,394 Initial Submission dated January 23, 2002

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW
Addendum to Review #01 of Initial Submission

IND number: 63,394

Review number: #03

Sequence number/date/type of submission: #000/October 5, 2001/Initial Submission

Information to sponsor: Yes () No (X)

Sponsor and/or agent: AstraZeneca LP
725 Chesterbrook Blvd.
Wayne, PA 19087-5677

Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D.

Division name: Pulmonary and Allergy Products

HFD #: 570

Review completion date: March 3, 2006

Drug:

Trade name: Symbicort

Generic name (list alphabetically): Combination drug product of Budesonide and Formoterol fumarate dihydrate

Chemical name:

Budesonide, 16 α ,17 α -butylidenedioxyprogna-1,4-diene-11 β , 21-diol-3,20-dione
Formoterol fumarate dihydrate, (R*,R*)-(\pm)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]phenyl]formamide, (E)-2-butendioate (2:1), dihydrate (asterisks denote asymmetric carbon atoms)

Molecular formula/molecular weight:

Budesonide, C₂₅H₃₄O₆ / 430.5 g/mole

Formoterol fumarate dihydrate, C₄₂H₅₆N₄O₁₄ / 840.9 g/mole

Relevant INDs/NDAs/DMFs:

IND (Formoterol fumarate dihydrate, AstraZeneca)

NDA 21-929 (Symbicort, AstraZeneca)

DMF 10378 (1,1,1,2,3,3,3-heptafluoropropane (HFA-227), IPACT-II)

Drug class: Budesonide, corticosteroid

Formoterol, β_2 -adrenergic agonist

Indication: Asthma

Route of administration: Inhalation

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

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Studies reviewed within this submission:

Symbicort (Budesonide + Formoterol): 3-Month Inhalation (pMDI) Toxicity Study in the Dog

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

Study title: Symbicort (Budesonide + Formoterol): 3-Month Inhalation (pMDI) Toxicity Study in the Dog.

In this addendum to the review of the 13-week inhalation toxicology study with dogs (IND 63,394 Reviews #01 dated January 23, 2002), the deposition factor is revised to 100% from the earlier review, based upon the use of a close-fitting face mask and aerosol delivery tube system to deliver the aerosol directly into the buccal cavity of the dog.

Deposited doses ($\mu\text{g}/\text{kg}/\text{day}$): should be revised to total inhaled doses based upon a deposition factor of 100%.

Group	Measured		Estimated		
	Budesonide	Formoterol	PVP K-25	PEG-1000	HFA-227
1 (Air-Control)	0	0	0	0	0
2 (Vehicle-Control)	0	0	0.420	126	41835
3 (LD)	1.96	0.117	0.0158	4.74	1574
4 (MD)	10.3	0.62	0.0831	24.9	8271
5 (HD)	52.1	3.11	0.420	126	41835

Safety margins for clinical doses of budesonide and formoterol in the Symbicort HFA drug product should be revised as shown in the table below.

Clinical Doses of Symbicort				Dose Ratios for Clinical Doses	
	$\mu\text{g}/\text{actuation}$	4 actuations/day	$\mu\text{g}/\text{kg}/\text{day}$	Dog to Human Ratio ^a	
80/4.5	80	320	6.4	0.31	
Budesonide	80	320	6.4	0.31	
Formoterol	4.5	18	0.36	0.31	
160/4.5	160	640	12.8	0.16	
Budesonide	160	640	12.8	0.16	
Formoterol	4.5	18	0.36	0.31	

a. Deposited doses at the NOAEL for dogs in the 3-month bridging study were 2.0 $\mu\text{g}/\text{kg}/\text{day}$ budesonide and 0.11 $\mu\text{g}/\text{kg}/\text{day}$ formoterol assuming 100% deposition.

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Safety margins for clinical doses of PVP K-25 and PEG-1000 in the Symbicort HFA pMDI drug product should be revised as shown in the table below.

Compound	Rats		Clinical dose µg/g LW/day	Safety margins
	Deposited dose µg/kg/day	Deposited dose µg/g LW/day		
PVP K-25	0.42	0.038	0.0028	13.6
PEG-1000	126	11.455	0.8492	13.5

Notes: The deposition factor for dogs was 100%. Lung weight values for dogs and humans used in calculations were 110 g and 1000 g, respectively.

Recommendation: None.

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

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

cc: list:
NDA 21-929, HFD-570
JacksonC, HFD-570
SunC, HFD-570
RobisonT, HFD-570

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**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
3/3/2006 11:06:13 AM
PHARMACOLOGIST
Revision of deposition factor for dogs

Joseph Sun
3/3/2006 11:11:37 AM
PHARMACOLOGIST
I concur.

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Appendix 2: Review of IND 63,394 Initial Submission dated January 23, 2002

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 63,394

Review number: #01

Sequence number/date/type of submission: #000/October 5, 2001/Initial Submission
 #002/October 23, 2001/Amendment
 #012/December 21, 2001/Amendment

Information to sponsor: Yes () No (X)

Sponsor and/or agent: AstraZeneca LP
 725 Chesterbrook Blvd.
 Wayne, PA 19087-5677

Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D.

Division name: Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: January 23, 2002

Drug:

Trade name: Symbicort

Generic name (list alphabetically): Combination drug product of Budesonide and Formoterol fumarate dihydrate

Code name:

Chemical name:

Budesonide, 16 α ,17 α -butylidenedioxypregna-1,4-diene-11 β , 21-diol-3,20-dione
 Formoterol fumarate dihydrate, (R*,R*)-(\pm)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]phenyl]formamide, (E)-2-butendioate (2:1), dihydrate (asterisks denote asymmetric carbon atoms)

CAS registry number:

Mole file number:

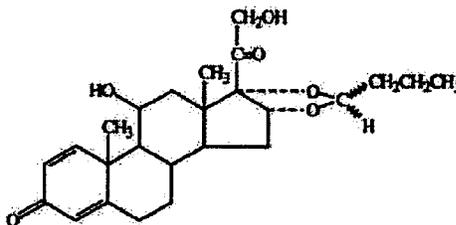
Molecular formula/molecular weight:

Budesonide, C₂₅H₃₄O₆ / 430.5 g/mole

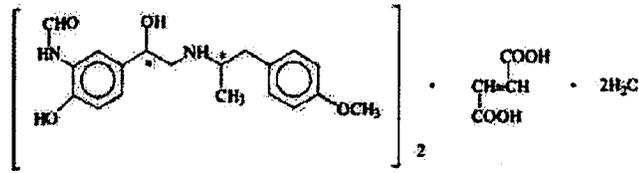
Formoterol fumarate dihydrate, C₄₂H₅₆N₄O₁₄ / 840.9 g/mole

Structure:

Budesonide



Formoterol fumarate dihydrate



Relevant INDs/NDAs/DMFs:

IND (Formoterol fumarate dihydrate, AstraZeneca)

NDA 20,441 (Pulmicort Turbuhaler, AstraZeneca)

NDA 20-831 (Foradil[®], Novartis)

DMF 10378 (1,1,1,2,3,3,3-heptafluoropropane (HFA-227), IPACT-II)

Drug class: Budesonide, corticosteroid
Formoterol, β_2 -adrenergic agonist

Indication: Asthma

Clinical formulations:

TABLE 1
Composition of Symbicort pMDIs

Ingredient	% w/w		Quantity (per 50 μ l)		Function	Standard
	80/4.5	160/4.5	80/4.5	160/4.5		
Budesonide micronised					Active	AstraZeneca
FFD micronised, conditioned					Active	AstraZeneca
Povidone K25					Suspending agent	USP
PEG 1000					Lubricant	NF
HFA-227 (1,1,1,2,3,3,3-heptafluoropropane)					Propellant	AstraZeneca

To facilitate dispensing and dispersion of drug particles, Symbicort pMDI is comprised of three components:

1. A — aluminum canister which contains the propellant and drugs. The drugs are suspended in the propellant.
2. A metering valve, which dispenses the drug and propellant.
3. A — actuator with mouthpiece, which aids the dispersion of the drugs by propellant and allows the patient to conveniently take a dose.

Composition

Budesonide HFA pMDIs are formulated to deliver 120 actuations of either 80 µg or 160 µg budesonide per actuation ex-actuator. The compositions are given in Table 39. These products have not been tested to date therefore these are preliminary formulations based upon the Symbicort pMDI formulations.

Table 39: Composition of budesonide HFA pMDIs

Ingredient	% w/w		Quantity (per 50 µl)		Function	Standard
	80	160	80	160		
Budesonide micronised					Active	AstraZeneca
Povidone K25					Suspending agent	USP
PEG 1000					Lubricant	NF
HFA-227 ^b					Propellant	AstraZeneca

Route of administration: Inhalation

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Proposed clinical protocol:

Protocol SD-039-0716: 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 children (≥6 years) and adults with mild to moderate asthma. Symbicort, a fixed combination product containing budesonide and formoterol, 80/4.5 µg per puff, respectively, will be administered as two inhalations twice daily. Budesonide (80 µg/puff) or formoterol (4.5 µg/inhalation) will be administered as two inhalations, twice daily. Subjects will undergo a 2-week placebo run-in period to washout their current asthma therapy followed by a 12-week randomized double-blind treatment in one of four treatment groups as shown in the table below. Each group will consist of approximately 112 patients. Co-primary efficacy variables will be 12-hr serial FEV₁ and withdrawals due to asthma exacerbation.

TABLE 2
Treatment Dosing Schedule

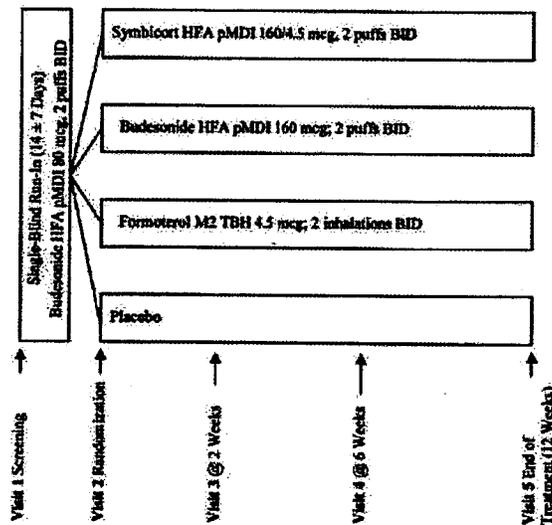
Treatment mg/puff or inhalation	Daily Regimen ¹	
	Morning	Evening
Symbicort pMDI 80/4.5 mcg	2 puffs active pMDI 2 inhalations placebo TBH	2 puffs active pMDI 2 inhalations placebo TBH
Budesonide pMDI 80 mcg	2 puffs active pMDI 2 inhalations placebo TBH	2 puffs active pMDI 2 inhalations placebo TBH
Formoterol TBH 4.5	2 puffs placebo pMDI 2 inhalations active TBH	2 puffs placebo pMDI 2 inhalations active TBH
Placebo	2 puffs placebo pMDI 2 inhalations placebo TBH	2 puffs placebo pMDI 2 inhalations placebo TBH

¹ pMDI should always be administered first, followed by TBH.

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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 FEV₁ and withdrawals due to asthma exacerbation.

FIGURE 1
Flow Chart of Study Treatments



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