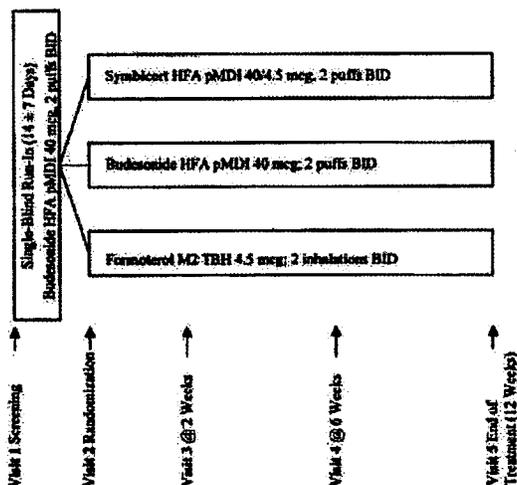


Protocol SD-039-0718: This is a 12-week randomized, double-blind, double-dummy, placebo-controlled trial to compare the safety and efficacy of Symbicort HFA pMDI versus its monoproducts, Budesonide HFA pMDI and Formoterol Turbuhaler, in asthmatic children (6 to 11 years). Symbicort, a fixed combination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

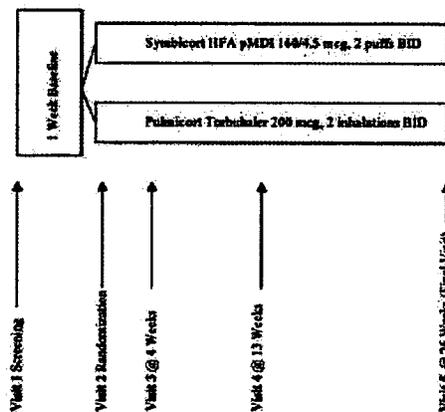
FIGURE 1
Flow Chart of Study Treatments



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

FIGURE 1
Flow Chart of Study Treatments



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

Previous clinical experience:

Budesonide:

Pulmicort Turbuhaler (DPI) is an approved drug product (AstraZeneca). Budesonide HFA pMDI is not an approved drug product. Pulmicort Turbuhaler is indicated for the maintenance treatment of asthma as prophylactic therapy in adult and pediatric patients six years of age or older.

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(pMDI). The aerosol formulation contains excipients, povidone K25 (PVP K25) and polyethylene glycol 1000, and 1,1,1,2,3,3,3-heptafluoropropane (HFA-227) as the propellant. Symbicort® Turbuhaler® is not being developed for the United States, as it is unable to meet current FDA requirements for dry powder inhaler (DPI) drug products.

The sponsor has extensive experience with the mono-products, budesonide and formoterol, that comprise the Symbicort® drug product.

Budesonide is the active ingredient in 5 approved drug products from AstraZeneca that are marketed in the United States. These drug products include Rhinocort (NDA 20-233), Pulmicort Turbuhaler (NDA 20-441), Rhinocort Aqua Nasal Spray (NDA 20-746), Pulmicort Respules (NDA 20-929), and Entocort Capsules (NDA 21-234). These drug products are supported by an extensive array of preclinical studies with budesonide that include carcinogenicity studies. For the Pulmicort Turbuhaler, the approved dose is 200 to 400 µg twice daily (i.e., 400 to 800 µg/day) for adults and children. For a 50-kg person (i.e., ≥12 years of age), this dose is equivalent to 8 to 16 µg/kg/day. For a 20-kg child (i.e., 6-11 years of age), the dose is equivalent to 20-40 µg/kg/day.

Formoterol (Oxis turbuhaler; IND ) is an investigational drug in the United States under development by AstraZeneca. An extensive array of preclinical studies has been conducted under this IND, which include carcinogenicity studies. The human clinical dose of formoterol in Phase III clinical trials is 9 µg twice a day, which is equivalent to 0.36 µg/kg/day.

In the pre-IND meeting package dated February 28, 2001, the sponsor submitted one preclinical question with four parts. The question and Division's responses that were conveyed to the sponsor in a teleconference on May 3, 2001 are listed below.

Does the Agency concur that the presented pharmacology / toxicology / metabolism studies are sufficient to support the described clinical program and registration of Symbicort pMDI, specifically:

- Combination studies of 3-month duration are sufficient to support the proposed clinical program.

The Division stated that completion of 3-month bridging studies with the aerosol inhalation combination drug product in rats and dogs would appear sufficient to support the proposed clinical program. Reports of the completed bridging studies conducted with the dry powder formulation should be submitted along with these studies using the aerosol formulation.

- Juvenile animal studies conducted with the mono-products are sufficient to support pediatric trials (• 6 years of age) with the combination product.

The Division stated that juvenile animal studies are generally required to support clinical trials with inhalation drug products in pediatric patients • 2

years of age. Thus, the 3-month bridging studies described above would appear sufficient to support pediatric trials with children • 6 years of age.

- Genotoxicity and carcinogenicity studies conducted with the mono-products are sufficient to support the combination product.

The Division stated that the genotoxicity and carcinogenicity studies conducted with the mono-products would be sufficient to support the combination product. The excipient Povidone K25 appears to be acceptable based upon its use in the drug product, Trental[®] tablets, which is administered for chronic indications. The excipient polyethylene glycol 1000 appears to be acceptable based upon its use in food (21 CFR 172.820).

- Teratology study in rats conducted with the combination product is sufficient to support women of childbearing potential in phase III trials.

The Division stated that reproductive toxicology studies conducted with individual drug products and the teratology study in rats conducted with the aerosol inhalation combination drug product would appear sufficient to support enrolling women of child bearing potential in the Phase 3 clinical trials.

(Note: The sponsor stated that they would provide this report for a teratology study with the Symbicort HFA pMDI formulation in rats prior to initiating clinical trials in May 2002. It was not provided in the present submission.)

On June 15, 2001, AstraZeneca submitted a second pre-IND meeting package based upon the Division's concerns that the sponsor's proposed plan presented a pharmaceuticals hurdle to show that the relative safety and efficacy of Symbicort HFA pMDI were not confounded by formulation differences between the mono-products and the combination product. The sponsor proposed to utilize a Pulmicort (budesonide) HFA pMDI formulation in clinical studies, having the identical ingredients (except for the absence of formoterol) and device components as Symbicort HFA pMDI, to simplify the interpretation of the comparison of the budesonide component in Symbicort to Pulmicort. The sponsor had one preclinical question in this package. The question and the Division's response are listed below.

At our May 3, 2001 teleconference, the Division stated that completion of 3-month bridging studies in the Symbicort HFA pMDI formulation in rats and dogs and the reproductive studies in rats (see attached Table 3 in the package) would appear sufficient to support the proposed clinical program. Please note that references to Pulmicort HFA pMDI in the toxicology tables 1 and 2 from the February 28, 2001 submission used different formulations. Does the Agency concur that the same information (see attached Table 3, also provided in the February 28, 2001 submission) is adequate to support the use of Pulmicort HFA pMDI formulation

x

(identical formulation as Symbicort HFA pMDI without formoterol) in this revised development program?

Provided that the Symbicort HFA pMDI formulation used in the bridging toxicology studies is identical to the clinical formulation, these studies may also support the use of the Pulmicort HFA pMDI formulation. In addition, preclinical studies conducted with budesonide HFA pMDI may assist in bridging the Pulmicort HFA pMDI, proposed for clinical use, and the approved drug product, Pulmicort Turbuhaler. However, General Correspondence dated June 15, 2001 appears to indicate that the formulation(s) of the budesonide HFA pMDI used in a 3-month inhalation toxicity study with rats (Report No. 96199-1), a 1-month inhalation toxicity study with dogs (Report No. 97119-01), and a teratology study with rats (Report No. T824) may possibly differ from one another as well as the formulation of the Pulmicort HFA pMDI proposed for clinical use. In the IND submission, provide the following: (1) a detailed description of the formulations of the budesonide HFA pMDI used in preclinical studies and the Pulmicort HFA pMDI proposed for clinical use; and (2) reports of preclinical inhalation toxicity studies conducted with the budesonide HFA pMDI (i.e., report numbers 96199-1, 97119-01, and T824).

Excipients, Povidone K25, polyethylene glycol 1000, and HFA-227, are not found in any approved inhalation drug products at present. During the May 3, 2001 teleconference, AstraZeneca agreed to provide references for inhalation toxicology studies conducted with these excipients. These references should be provided in the IND submission. AstraZeneca should ensure that excipients, Povidone K25, polyethylene glycol 1000, and HFA-227, have been included in the 3-month bridging studies with the Symbicort HFA pMDI formulation in rats and dogs as well as the rat teratology study.

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Studies reviewed within this submission:

| STUDY | REPORT NUMBER |
|---|---------------|
| PHARMACOLOGY | |
| PHARMACOKINETICS/TOXICOKINETICS | |
| PK Parameters: | |
| Humans | |
| Comparison of plasma levels of budesonide and formoterol in healthy subjects after Inhalation by HFA-propelled pMDI and turbuhaler. | SD-039-0626 |
| Relative systemic bioavailability of Symbicort pMDI versus Oxis Turbuhaler plus Pulmicort Turbuhaler, administered with and without activated charcoal in healthy subjects. | SD-039-0713 |
| TOXICOLOGY: | |
| Subchronic Toxicity: | |
| Rats | |
| 3-month inhalation (budesonide + formoterol pMDI combination) toxicity study in rats. | SR-00342-02 |
| 3-month inhalation (budesonide + formoterol powder combination) toxicity study in rats. | SR-99022-01 |
| 6-month inhalation (HFA-227 MDI)/subcutaneous toxicity study in rats. | 96010-2 |
| Dogs | |
| 3-month inhalation (budesonide + formoterol pMDI Combination) toxicity study in beagle dogs. | SR-00047-01 |
| 3-month inhalation (budesonide + formoterol powder combination) toxicity study in beagle dogs. | SR-99023-01 |
| 6-month inhalation (HFA-227 MDI)/subcutaneous toxicity study in beagle dogs. | 96012 |
| 12-month inhalation (HFA-227 MDI)/subcutaneous toxicity study in dogs. | 98292 |

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 X Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

Withheld Track Number: Pharm/Tox-

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Mechanism of action:

Effects of Formoterol and budesonide on GM-CSF and IL-8 Secretion in Human Primary Bronchial Epithelial Cells (Report No. 843-RD-0419-01).

Methods: The effect of formoterol alone or in combination with budesonide on tumor necrosis factor α (TNF α)-induced granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-8 (IL-8) production by primary normal human bronchial epithelial cells *in vitro* was examined. Cells were treated with 10 ng/mL human recombinant TNF α with or without racemic formoterol (10^{-11} to 10^{-6} M) and racemic budesonide (10^{-8} M) for 24 hr. In separate experiments, the specificity of formoterol-mediated effects was studied by blocking β_2 -receptors with the nonselective β -antagonist, propranolol, and by measuring cellular cAMP levels. Further, the effect of a glucocorticoid receptor (GR) antagonist, RU486, on the activity of formoterol was assessed. GM-CSF and IL-8 were measured using ELISA methods.

Results: Formoterol decreased TNF α -induced GM-CSF production to a maximum of 60% of the control value (i.e., TNF α alone), with a threshold level observed at 10^{-10} M. Formoterol enhanced TNF α -induced IL-8 production to a maximum of 160% of the control value, with a threshold level observed at 10^{-10} M. Treatment with formoterol for 1 hr produced similar results on TNF α -induced GM-CSF and IL-8 production as observed with 24 hr, although, the threshold level was raised to 10^{-8} M. Budesonide (10^{-8} M) decreased TNF α -induced GM-CSF and IL-8 production by 40 and 45% of control values, respectively. The combination of formoterol and budesonide produced an additive effect on TNF α -induced GM-CSF production, decreasing levels to 75% of the control value. The combination of formoterol and budesonide reduced TNF α -induced IL-8 production as observed with budesonide alone. Treatment with the combination of formoterol and budesonide for 1 hr produced a smaller inhibition of TNF α -induced GM-CSF production (i.e., 45% of the control value); however, there was no effect on TNF α -induced IL-8 production (i.e., essentially identical to TNF α alone). RU486 had no inhibitory activity toward the effects of formoterol (10^{-8} M); however, it abolished the effects of budesonide (10^{-8} M). Formoterol induced a dose-dependent increase of cAMP production. Propranolol at 10^{-9} to 10^{-7} M blocked cAMP production stimulated by formoterol (10^{-7} M). Propranolol at $\geq 10^{-8}$ M blocked effects of formoterol (10^{-9} M) on TNF α -induced GM-CSF and IL-8 production. A 100-fold excess of propranolol was needed to block effects on TNF α -induced GM-CSF and IL-8 production as compared to cAMP production. The inhibitory actions of propranolol on effects of formoterol suggest the involvement of β_2 -receptors. Formoterol had a very low threshold concentration for reducing GM-CSF production by bronchial epithelial cells.

Reviewer: Timothy W. Robison, Ph.D.

IND No. 63,394

Drug activity related to proposed indication:**On the Interaction between formoterol and budesonide in the Guinea Pig: Effects on Airway β -Adrenoceptors after Repeated Inhalation of Powder Aerosols (Report number 850-RD-0474-01).**

Methods: Interaction between the glucocorticoid, budesonide, and the β_2 -adrenoreceptor agonist, formoterol, on airway smooth muscle, with specific reference to the development of tolerance to β_2 -agonists, was assessed in male Dunkin Hartley guinea pigs. Animals were exposed by the nose-only inhalation route to a powder aerosol containing formoterol, budesonide, or the vehicle (micronized lactose only) as listed in the table below. The aerosol, produced by a generator at a flow rate of 10 L/min, was directed into an inhalation chamber. During exposure, animals were restrained in tubes with their snouts directed against the chamber. Aerosol concentrations in the chamber during exposure were measured by filter sampling. The amount of substance Each animal was exposed for 10 min twice daily, with 6 hr between exposures, for a total of 6 days. There were four groups with each group consisting of 6 animals. On day 7, approximately 18 hr after the last exposure, animals were sacrificed and the trachea and lungs were collected for functional and radioligand binding studies. The trachea was cut into sections comprising two cartilage rings with the epithelial layer intact and then cut open ventrally. Cartilage strips were mounted into organ baths and treated with 0.1 or 1 μ mol/L carbachol. After a stable contraction was established, formoterol was added in a cumulative manner. After the final addition of formoterol, maximum relaxation was established by addition of 10 μ mol/L isoprenaline followed by 1 mmol/L theophylline. Lungs were processed and the pellet resulting from centrifugation at 46000 x g was resuspended. The membranes obtained were used for radioligand binding assays with 3 H-labeled dihydroalprenolol (3 H-DHA). Specific binding was defined as the difference between total binding (i.e., 3 H-DHA in the absence of 10 μ mol/L (\pm)-propranolol) and nonspecific binding (i.e., 3 H-DHA in the presence of 10 μ mol/L (\pm)-propranolol).

Vehicle-control and Treatment Groups (target inhaled doses of budesonide and formoterol were 4.5 mg/kg and 75 μ g/kg, respectively).

| Group | Morning Exposure | Afternoon Exposure |
|-------|----------------------|--------------------------|
| 1 | Vehicle | Vehicle |
| 2 | 4.5 mg/kg Budesonide | Vehicle |
| 3 | Vehicle | 75 μ g/kg Formoterol |
| 4 | 4.5 mg/kg Budesonide | 75 μ g/kg Formoterol |

Results:

Based upon analysis of filter samples, inhaled doses of budesonide and formoterol were 4.6 mg/kg and 73 μ g/kg, respectively.

Formoterol caused a concentration-dependent and complete relaxation of tracheal strip preparations from vehicle-control animals. The EC_{50} was 0.3 nmol/L in the presence of 0.1 μ mol/L carbachol. With strip preparations from animals pretreated with formoterol in

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in vivo for 6 days, there was a 6-fold shift to the right of the concentration-effect curve for formoterol with no change in the maximum response. Strip preparations from animals pretreated with budesonide in vivo did not alter the ex vivo response to formoterol. Strip preparations from animals pretreated with the combination of budesonide and formoterol in vivo displayed the same ex vivo response to formoterol as observed with strip preparations obtained from animals pretreated with only formoterol in vivo. Similar results were obtained with tracheal strip preparations incubated in the presence of 1 $\mu\text{mole/L}$ carbachol, although, the EC_{50} for formoterol was increased from 0.3 to 1.6 nmol/L .

Effects of treatment with budesonide and formoterol in vivo, alone or in combination, on the relaxation capacity of formoterol ex vivo.

| Group | Carbachol, 0.1 $\mu\text{mole/L}$ | | Carbachol, 1 $\mu\text{mole/L}$ | |
|-------------------------|-----------------------------------|--------------------|---------------------------------|--------------------|
| | pEC_{50} | $E_{\text{max}}\%$ | pEC_{50} | $E_{\text{max}}\%$ |
| Control | 9.52 ± 0.03 | 100 | 8.80 ± 0.06 | 98 |
| Budesonide | 9.53 ± 0.03 | 100 | 8.80 ± 0.07 | 98 |
| Formoterol | 8.74 ± 0.12 | 99 | 8.27 ± 0.09 | 87 |
| Budesonide + Formoterol | 8.80 ± 0.07 | 100 | 8.45 ± 0.07 | 84 |

β -Adrenoreceptor density, expressed as B_{max} , in lung tissue from animals pretreated with formoterol in vivo for 6 days, with or without budesonide, was reduced by approximately 35% as compared to the vehicle-control or budesonide alone. There were no significant changes in the dissociation constant (K_D) for any treatment group as compared to the vehicle-control.

Effects of treatment with budesonide and formoterol in vivo, alone or in combination, on the density of β -adrenoreceptors in lung membranes.

| Group | K_D , nmol/L | B_{max} , fmole/mg protein |
|-------------------------|-------------------------|--|
| Control | 0.47 ± 0.02 | 1582 ± 209 |
| Budesonide | 0.45 ± 0.02 | 1628 ± 84 |
| Formoterol | 0.59 ± 0.04 | 1053 ± 29 |
| Budesonide + Formoterol | 0.46 ± 0.05 | 1040 ± 111 |

Development of tolerance (i.e., down regulation of β_2 -adrenoreceptor function) was evident following repeated treatment with the β_2 -agonist, formoterol. Tolerance was evident from the rightward shift of the concentration-effect curve of formoterol for the relaxation effect on airway smooth muscle and the reduction of the density of β -adrenoreceptors in the lung. Treatment with budesonide, a glucocorticoid, in combination with formoterol did not alter the development of tolerance to formoterol.

Pharmacology summary: In vitro treatment of normal human bronchial epithelial cells with a combination of formoterol and budesonide produced an additive effect on inhibiting tumor necrosis factor α -induced granulocyte macrophage-colony stimulating factor. Inhibitory actions of propranolol on the effects of formoterol suggest the involvement of β_2 -receptors. Formoterol had a very low threshold concentration (i.e., 10^{-10} M) for

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reducing tumor necrosis factor α -induced GM-CSF production by bronchial epithelial cells. Development of tolerance (i.e., down regulation of β_2 -adrenoreceptor function) was evident following repeated in vivo inhalation treatment of guinea pigs with the β_2 -agonist, formoterol. Tolerance was evident from the rightward shift of the concentration-effect curve of formoterol for the relaxation effect on airway smooth muscle and the reduction of the density of β -adrenoreceptors in the lung. Inhalation treatment with budesonide, a glucocorticoid, in combination with formoterol did not alter the development of tolerance to formoterol (i.e., tachyphylaxis).

Pharmacology conclusions: In vitro treatment of normal human bronchial epithelial cells with a combination of formoterol and budesonide produced an additive effect on inhibiting tumor necrosis factor α -induced granulocyte macrophage-colony stimulating factor. Budesonide had no effect on development of tolerance (i.e., down regulation of β_2 -adrenoreceptor function) following repeated in vivo inhalation treatment of guinea pigs with the β_2 -agonist, formoterol.

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Humans

Comparison of Plasma Levels of Budesonide and Formoterol in Healthy Subjects after Inhalation by HFA-propelled pMDI and Turbuhaler (SD-039-0626).

Methods: Plasma concentrations of budesonide and formoterol were compared following inhalation of a combination of budesonide and formoterol using Symbicort pMDI (Batch number P5994) and Symbicort Turbuhaler (TBH; Batch number ZM16) in healthy human subjects. The study design was an open, randomized, two-way crossover design with fourteen non-smoking healthy Caucasian men or women, between 20 and 33 years of age (7 males and 7 females). Treatments were administered as single doses with a washout period of 3 to 7 days as follows: (1) Symbicort pMDI (budesonide/formoterol), 160/4.5 μg , given as 4 actuations (all at one time) corresponding to a total dose of 640/18 μg ; and (2) Symbicort TBH (budesonide/formoterol), 160/4.5 μg , given as 4 actuations all at once corresponding to a total dose of 640/18 μg . Blood samples for measurement of plasma concentrations of budesonide and formoterol were collected at time points over a 12-hr period after drug administration. Quantities of budesonide and formoterol were measured with liquid chromatography-mass spectrometry methods. The limits of quantitation for budesonide and formoterol in human plasma were 0.10 nmol/L and 5 pmol/L, respectively.

Results: The sponsor reported that both Symbicort treatments were safe and well tolerated. Both Symbicort treatments increased the maximal heart rate by 5-10 bpm as compared to the reference period, although, there were no differences between the two treatments. Maximal QTc was significantly prolonged by 10-20 msec at 0.5, 1, 2, and 4 hr after administration of the Symbicort TBH compared with the reference period or

administration of the Symbicort HFA pMDI. Systemic exposure (i.e., AUC) to budesonide and formoterol was higher with the TBH as compared to the pMDI. AUC values for budesonide and formoterol resulting from administration of the Symbicort HFA pMDI were 45 and 44%, respectively, of those resulting from administration of the turbuhaler. Plasma AUC values for budesonide and formoterol with the pMDI were 4.83 nmolhr/L and 145 pmolhr/L, respectively. Plasma C_{max} values for budesonide and formoterol with the pMDI were 1.42 nmol/L and 52.0 pmol/L, respectively. It should be noted that in the present study, Symbicort pMDI (budesonide/formoterol), 160/4.5 µg, was given as 4 actuations all at one time corresponding to a total dose of 640/18 µg. While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID.

SD-039-0626 - Pharmacokinetic parameters for budesonide.

| Parameter | Symbicort pMDI | | Symbicort Turbuhaler | | pMDI vs. Turbuhaler ¹ | | |
|-------------------------------------|----------------|-------------|----------------------|--------------|----------------------------------|--------------|--------------|
| | mean | 95% CI | mean | 95% CI | mean | 95% CI | 90% CI |
| AUC (nmol/L·h) | 4.83 | 3.84 - 6.07 | 10.62 | 8.45 - 13.35 | 45 | 33 - 63 | 35 - 59 |
| AUC ₀₋₄ (nmol/L·h) | 4.51 | 3.55 - 5.74 | 10.00 | 7.85 - 12.72 | 45 | 32 - 63 | 34 - 60 |
| C _{max} (nmol/L) | 1.42 | 1.10 - 1.83 | 3.43 | 2.65 - 4.43 | 41 | 29 - 59 | 31 - 56 |
| T _{1/2} (h) | 3.20 | 2.81 - 3.64 | 3.17 | 2.79 - 3.60 | 101 | 84 - 121 | 87 - 117 |
| MRT (h) | 4.07 | 3.74 - 4.40 | 3.85 | 3.52 - 4.19 | 0.22 | -0.25 - 0.69 | -0.17 - 0.60 |
| T _{max} (min) ² | 18 | 10 - 60 | 13 | 5 - 60 | 3 | -13 - 10 | -10 - 9 |

¹Ratios (%) for T_{1/2}, AUC₀₋₄, AUC and C_{max}, differences for MRT and T_{max}.

²Treatment median and range for T_{max}, Hodge-Lehmann estimate for treatment comparison.

SD-039-0626 - Pharmacokinetic parameters for formoterol.

| parameter | Symbicort pMDI | | Symbicort Turbuhaler | | pMDI vs. Turbuhaler ¹ | | |
|-------------------------------------|----------------|-------------|----------------------|--------------|----------------------------------|--------------|--------------|
| | mean | 95% CI | mean | 95% CI | mean | 95% CI | 90% CI |
| AUC (pmol/L·h) | 145 | 90 - 232 | 329 | 205 - 528 | 44 | 23 - 86 | 25 - 76 |
| AUC ₀₋₄ (pmol/L·h) | 91 | 50 - 164 | 254 | 141 - 459 | 36 | 15 - 82 | 18 - 71 |
| C _{max} (pmol/L) | 52.0 | 38.0 - 71.2 | 98.8 | 72.2 - 135.3 | 53 | 34 - 82 | 37 - 76 |
| T _{1/2} (h) | 4.25 | 3.05 - 5.92 | 5.63 | 4.04 - 7.85 | 75 | 47 - 120 | 51 - 111 |
| MRT (h) | 7.05 | 6.15 - 7.95 | 8.01 | 7.11 - 8.91 | -0.96 | -2.24 - 0.31 | -2.01 - 0.08 |
| T _{max} (min) ² | 6 | 5 - 10 | 5 | 5 - 10 | 1 | 0 - 2 | 0 - 2 |

¹Ratios (%) for T_{1/2}, AUC₀₋₄, AUC and C_{max}, differences for MRT and T_{max}.

²Treatment median and range for T_{max}, Hodge-Lehmann estimate for treatment comparison.

An Open-Label, Randomized, Four-Way Crossover Study of the Relative Systemic Bioavailability of Symbicort pMDI versus Oxis Turbuhaler plus Pulmicort Turbuhaler, Administered With and Without Activated Charcoal in Healthy Subjects (SD-039-0713).

Methods: Systemic bioavailability of budesonide and formoterol from the Symbicort pMDI was compared with monoproducs, budesonide and formoterol, administered by Turbuhaler (TBH) at the same time. The study design was an open-label, randomized, four-way crossover study in twenty-eight healthy subjects, 19 to 34 years of age. There were 4 single-dose treatment periods, two with Symbicort pMDI and two with budesonide TBH + formoterol TBH given at the same time. Each treatment was given with and without

activated charcoal. The activated charcoal was used to block gastrointestinal absorption of drugs. There was a washout period of at least 4 days between treatments. The following treatments \pm activated charcoal were administered: (1) Symbicort pMDI (budesonide/formoterol; Batch number P6041), 160/4.5 μ g (delivered dose), 12 inhalations corresponding to a total dose of 1920/54 μ g; and (2) budesonide TBH, 200 μ g (metered dose yielding a delivered dose of 160 μ g), 6 inhalations corresponding to a total dose of 1200 μ g (metered dose yielding a delivered dose of 960 μ g) plus formoterol TBH, 4.5 μ g (delivered dose), 6 inhalations corresponding to a total dose of 27 μ g. Blood samples to measure plasma concentrations of budesonide and formoterol were collected at time points over a 12-hr period after the last dose. Quantities of budesonide and formoterol were measured using liquid chromatography - mass spectrometry methods.

Results: Twenty-eight healthy subjects were randomized to treatment and 26 completed the study. Plasma AUC and C_{max} values for budesonide and formoterol obtained with pMDI or TBH are shown in the table below, although, the values have not been normalized for differences in doses. Systemic bioavailability of budesonide and formoterol were greater following administration by TBH as compared with pMDI (with or without charcoal). The relative bioavailability of budesonide and formoterol following administration of the Symbicort HFA pMDI were 72 and 82%, respectively, as compared to Pulmicort TBH + Oxis TBH (without charcoal). Assuming complete charcoal block of gastrointestinal absorption of drugs for the Symbicort pMDI or Pulmicort TBH + Oxis TBH treatments, systemic absorption of drugs (i.e., budesonide + formoterol) through the lungs was 72 and 78%, respectively.

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Table 2. Pharmacokinetic parameters for budesonide, administration without charcoal (dose correction only in comparison between treatments).

| parameter | Symbicort pMDI | | Pulmicort Turbuhaler + Oxis Turbuhaler | | Symbicort vs. Pulmicort + Oxis ¹ | |
|-------------------------------------|----------------|----------------|--|----------------|---|----------------|
| | mean | 95% conf. lim. | mean | 95% conf. lim. | mean | 95% conf. lim. |
| AUC (nmol/L-h) | 19.0 | 17.3 - 20.9 | 13.1 | 11.9 - 14.5 | 72 | 63 - 82 |
| AUC ₀₋₁₂ (nmol/L-h) | 17.7 | 16.1 - 19.5 | 12.4 | 11.2 - 13.7 | 71 | 63 - 81 |
| C _{max} (nmol/L) | 6.05 | 5.47 - 6.69 | 4.68 | 4.22 - 5.19 | 65 | 56 - 74 |
| T _{1/2} (h) | 3.38 | 3.15 - 3.62 | 3.13 | 2.91 - 3.35 | 108 | 98 - 119 |
| MRT (h) | 4.02 | 3.88 - 4.16 | 3.75 | 3.61 - 3.90 | 0.27 | 0.07 - 0.47 |
| T _{max} (min) ² | 20 | 10 - 60 | 10 | 10 - 42 | 5 | 0 - 10 |

¹Ratios (%) for T_{1/2}, AUC₀₋₁₂, AUC and C_{max}, differences for MRT and T_{max}. ²Treatment median and range for T_{max}, Hodges-Lehmann estimate for treatment comparison.

Table 3. Pharmacokinetic parameters for budesonide, administration with charcoal (dose correction only in comparison between treatments).

| parameter | Symbicort pMDI | | Pulmicort Turbuhaler + Oxis Turbuhaler | | Symbicort vs. Pulmicort + Oxis ¹ | |
|-------------------------------------|----------------|----------------|--|----------------|---|----------------|
| | mean | 95% conf. lim. | mean | 95% conf. lim. | mean | 95% conf. lim. |
| AUC (nmol/L-h) | 15.9 | 14.5 - 17.4 | 12.5 | 11.2 - 13.4 | 65 | 57 - 74 |
| AUC ₀₋₁₂ (nmol/L-h) | 13.1 | 13.8 - 16.5 | 11.6 | 10.6 - 12.8 | 65 | 57 - 74 |
| C _{max} (nmol/L) | 6.09 | 5.33 - 6.71 | 4.45 | 4.04 - 4.91 | 68 | 60 - 78 |
| T _{1/2} (h) | 3.13 | 2.93 - 3.35 | 3.00 | 2.81 - 3.20 | 104 | 95 - 115 |
| MRE (h) | 3.47 | 3.33 - 3.61 | 3.52 | 3.38 - 3.66 | -0.05 | -0.25 - 0.15 |
| T _{max} (min) ² | 20 | 10 - 40 | 20 | 10 - 60 | 0 | -2 - 4 |

¹Ratios (%) for T_{1/2}, AUC₀₋₁₂, AUC and C_{max}, differences for MRT and T_{max}. ²Treatment median and range for T_{max}, Hodges-Lehmann estimate for treatment comparison.

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Table 4. Pharmacokinetic parameters for formoterol, administration without charcoal (dose correction only in comparison between treatments).

| parameter | Symbicort pMDI | | Pulmicort Turbuhaler + OxisTurbuhaler | | Symbicort vs. Pulmicort + Oxis ¹ | |
|-------------------------------------|----------------|----------------|---------------------------------------|----------------|---|----------------|
| | mean | 95% conf. lim. | mean | 95% conf. lim. | mean | 95% conf. lim. |
| AUC (pmol/L·h) | 731 | 677 - 833 | 456 | 412 - 506 | 82 | 71 - 95 |
| AUC ₀₋₁₂ (pmol/L·h) | 557 | 503 - 616 | 335 | 303 - 371 | 83 | 72 - 95 |
| C _{max} (pmol/L) | 227 | 201 - 256 | 119 | 105 - 134 | 95 | 81 - 113 |
| T _{1/2} (h) | 6.37 | 5.61 - 7.24 | 6.34 | 5.18 - 7.21 | 100 | 84 - 120 |
| MRT (h) | 9.56 | 8.28 - 10.83 | 9.40 | 8.11 - 10.68 | 0.17 | -1.38 - 1.92 |
| T _{max} (min) ² | 10 | 9 - 11 | 10 | 10 - 10 | 0 | 0 - 0 |

¹Ratios (%) for T_{1/2}, AUC₀₋₁₂, AUC and C_{max}, differences for MRT and T_{max}. ²Treatment median and range for T_{max}. Hodge-Lehmann estimate for treatment comparison.

Table 5. Pharmacokinetic parameters for formoterol, administration with charcoal (dose correction only in comparison between treatments).

| parameter | Symbicort pMDI | | Pulmicort Turbuhaler + Oxis Turbuhaler | | Symbicort vs. Pulmicort + Oxis ¹ | |
|-------------------------------------|----------------|----------------|--|----------------|---|----------------|
| | mean | 95% conf. lim. | mean | 95% conf. lim. | mean | 95% conf. lim. |
| AUC (pmol/L·h) | 538 | 487 - 594 | 356 | 322 - 393 | 76 | 66 - 87 |
| AUC ₀₋₁₂ (pmol/L·h) | 402 | 364 - 443 | 263 | 238 - 290 | 76 | 67 - 88 |
| C _{max} (pmol/L) | 219 | 195 - 246 | 114 | 101 - 128 | 96 | 82 - 114 |
| T _{1/2} (h) | 6.38 | 5.82 - 7.45 | 6.03 | 5.33 - 6.82 | 109 | 92 - 130 |
| MRT (h) | 9.08 | 7.84 - 10.31 | 9.32 | 8.08 - 10.55 | -0.24 | -1.99 - 1.50 |
| T _{max} (min) ² | 10 | 10 - 12 | 10 | 10 - 12 | 0 | 0 - 0 |

¹Ratios (%) for T_{1/2}, AUC₀₋₁₂, AUC and C_{max}, differences for MRT and T_{max}. ²Treatment median and range for T_{max}. Hodge-Lehmann estimate for treatment comparison.

PK/TK summary: Plasma concentrations of budesonide and formoterol were compared following inhalation of a combination of budesonide and formoterol using Symbicort pMDI and Symbicort Turbuhaler (TBH) in healthy human subjects. Symbicort pMDI or Turbuhaler (budesonide/formoterol), 160/4.5 µg, was given as 4 actuations all at one time corresponding to a total dose of 640/18 µg. While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID. Systemic exposure (i.e., AUC) to budesonide and formoterol was higher with the TBH as compared to the pMDI. Plasma AUC values for budesonide and formoterol with the pMDI were 4.83 nmolhr/L and 145 pmolhr/L, respectively.

PK/TK conclusions: Symbicort pMDI (budesonide/formoterol), 160/4.5 µg, was given as 4 actuations all at one time corresponding to a total dose of 640/18 µg to healthy human volunteers. While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID. Plasma AUC values for budesonide and formoterol with the pMDI were 4.83 nmolhr/L and 145 pmolhr/L, respectively.

IV. GENERAL TOXICOLOGY:

Rats

Study title: Symbicort (Budesonide + Formoterol): General toxicity Study after Daily Administration via the Inhalation (pMDI) Route to Rats for 3 Months.

Key study findings:

◆ In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, povidone K-25 (PVP K-25), polyethylene glycol 1000 (PEG-1000), and HFA-227. Total doses of budesonide and formoterol in the low, mid, and high dose groups were 2 + 0.11, 10 + 0.56, and 51 + 2.9 µg/kg/day, respectively. Using a deposition factor of 0.086 to 0.094, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were 0.2 + 0.010, 0.8 + 0.048, and 4.8 + 0.27 µg/kg/day, respectively. Two additional groups were exposed daily to either an excipient-only HFA pMDI aerosol formulation (vehicle control) or to air only (air control).

◆ The NOAEL was identified as the mid dose based on decreases in body weight gain and thymus weights observed at the high dose. There were no target organs of toxicity.

◆ Body weight gains for male and female rats in the high dose group were reduced to 67 and 56% of the air-control, respectively. Thymus weights were decreased for male and female rats at the high dose, although, there were no corresponding histopathological findings. Decreases of body weight gains and thymus weights could be attributed to the effects of budesonide. There was no evidence of additive or synergistic toxic effects between formoterol and budesonide.

◆ There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to PVP K-25, PEG-1000, and HFA-227 at deposited doses of 0.03, 8.6, and 30300 µg/kg/day, respectively.

Study no: 00342

Volume #, and page #: Volume 5 (of 25), pages 5 to 360

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

Date of study initiation: September 1, 2000

GLP compliance: Yes

QA report: yes (X) no ()

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Drug, lot #, radiolabel, and % purity: Budesonide/Formoterol fumarate dihydrate, Batch No. P5992

Formulation/vehicle: See table.

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

| Formulation | Used in group | Material | Percent, w/w |
|-------------|---------------|---|--------------|
| I | 1 | Air | |
| II | 2 | PVP K-25 PEG 1000 HFA-227 | |
| III | 3, 4, and 5 | Budesonide Formoterol PVP K-25 PEG 1000 HFA-227 | |

Methods (unique aspects): A Symbicort pMDI formulation containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227 was administered once daily by nose-only inhalation to 3 groups of rats for 3 months. Two similarly sized groups were exposed daily to either an excipient-only HFA pMDI aerosol formulation (vehicle) or to air only (air control).

Dosing:

Species/strain: Wistar () rats were obtained from

#/sex/group or time point (main study): 10 rats/sex/group

Satellite groups used for toxicokinetics or recovery: Satellite animals were included for toxicokinetic measurements. Groups 6 and 10 consisted of 2 rats/sex/group. Groups 7 and 11 consisted of 18 rats/sex/group. Groups 8, 9, 12, and 13 consisted of 6 rats/sex/group.

Age: Rats were approximately 2 months old at the start of dosing.

Weight: Body weight ranges were 240-300 g for male rats and 160-210 g for female rats.

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Doses in administered units: pMDI canisters for the mid dose clinical formulation were used in preclinical studies.

| Group | Compound | Inhaled target dose, µg/kg/day | Target concentration µg/L | Exposure Duration, min | MMAD, µm (B/F) | Deposited Dose, µg/kg/day | % of Inhaled Dose |
|-------------|-------------------------|--------------------------------|---------------------------|------------------------|----------------|---------------------------|-------------------|
| 1 | Air Control | 0 | 0 | 20 | - | 0 | - |
| 2 6 & 10 | Vehicle | 0 | 0 | 20 | - | 0 | - |
| 3 7 & 11 | Budesonide + Formoterol | 2.0 + 0.11 | 0.15 + 0.081 | 8 | | 0.2 + 0.010 | 9.1 |
| 4 8 & 12 | Budesonide + Formoterol | 10.0 + 0.56 | 0.74 + 0.041 | 5 | | 0.8 + 0.048 | 8.6 |
| 5 9 & 13 | Budesonide + Formoterol | 50.0 + 2.80 | 3.7 + 0.21 | 28 | | 4.8 + 0.27 | 9.4 |

Summary of exposure generating conditions.

| Group | Air | Vehicle | Low Dose | Mid Dose | High Dose |
|--|-----|----------------|------------|----------|-----------|
| Duration of exposure, minutes | 20 | 20 | 8 | 5 | 27 |
| Generator air flow (L/min) | 30 | 30 | 30 | 30 | 30 |
| Total chamber air supply (L/min) | 50 | 50 | 50 | 50 | 50 |
| Aerosol flow (L/min) | 0 | 25 | 3 | 7 | 26 |
| Dilution air flow (L/min) | 20 | 25 | 47 | 43 | 24 |
| Extraction air flow (L/min) | 49 | 49 | 49 | 49 | 49 |
| Number of pMDI canisters | - | 3 | 1 | 2 | 3 |
| Puff frequency (/min) | - | 5 | 5 | 5 | 5 |
| Mass per actuated canister Budesonide/Formoterol (µg) | 0 | 0 | 88/4.8 | 88/4.8 | 88/4.8 |
| Nominal Output Budesonide/Formoterol (µg/min) | 0 | 0 | 440/24 | 880/48 | 1320/72 |
| Nominal Concentration Budesonide/Formoterol (µg/L) | 0 | 0 | 15/0.8 | 29/1.6 | 44/2.4 |
| Reduction of aerosol mass dilution | - | 1:2 | 1:17 | 1:7 | 1:1.9 |
| Mass flow to chamber Budesonide/Formoterol µg/L | - | 0 ^a | 0.9/0.012 | 4.1/0.2 | 23/1.3 |
| Actual Concentration Budesonide/Formoterol ^b µg/L | - | 0 | 0.48/0.026 | 3.6/0.2 | 3.5/0.19 |

a. Vehicle generating condition was similar to that of the high dose.

b. Formoterol values are based on analytical data from budesonide assuming same composition in the aerosol as in the compound mixture.

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Group mean daily doses

| Group | Body weight, g | Exposure duration, min | Actual aerosol concentration µg/L | Inhaled Dose µg/kg/day | |
|-------------|------------------------|------------------------|-------------------------------------|------------------------|--|
| | | | | Target | Actual |
| 2 - Males | 348.5 (271.2-400.9) | 20.04 (12.24-24.72) | 289.324 (120.331-561.467) | | 2830.666 (1211.243-5283.269) |
| 2 - Females | 218.7 (184.8-239.0) | 20.04 (12.42-24.72) | 289.324 (120.331-561.467) | | 3177.151 (1349.408-6008.846) |
| 3 - Males | 358.7 (267.1-411.2) | 8.33 (6.67-11.74) | 0.476 ^a (0.237-0.565) | 2.00 ^b | 1.894 ^b (1.873-2.002) |
| 3 - Females | 215.5 (182.0-237.6) | 8.33 (6.67-11.74) | 0.476 ^a (0.237-0.565) | 2.00 ^b | 2.149 ^b (2.116-2.241) |
| 4 - Males | 342.8 (266.2-392.1) | 5.44 (4.63-11.37) | 3.647 ^a (1.773-4.036) | 10 ^b | 9.586 ^b (9.511-10.281) |
| 4 - Females | 207.5 (179.3-225.9) | 5.44 (4.63-11.37) | 3.647 ^a (1.773-4.036) | 10 ^b | 10.857 ^b (10.660-11.763) |
| 5 - Males | 332.1 (272.5-371.9) | 27.71 (19.77-42.63) | 3.508 ^a (2.033-4.962) | 50 ^b | 47.158 ^b (41.726-47.926) |
| 5 - Females | 194.2 (179.5-208.8) | 27.71 (19.77-42.63) | 3.508 ^a (2.033-4.962) | 50 ^b | 53.883 ^b (48.211-54.872) |

a. Budesonide concentration measured by HPLC.

b. Dose of budesonide, µg/kg/day

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

The exposure by the inhalation route was effected using nose-only exposure chambers. A separate 10-level chamber with 160 animal ports was used for each target concentration. Animals allocated to the same aerosol formulation were exposed together (both sexes) in the same chamber. During exposure, the rats were restrained in polycarbonate tubes with their snouts protruding into the chamber and their tails sticking out into the ambient air. Each exposure chamber was operated in a separate ventilated cabinet to avoid cross-contamination between groups.

The actual aerosol concentration was measured on-line during each exposure by the use of a light-scatter monitor. Prior to animal exposures, separate tests were performed to establish a relation between the actual aerosol mass and recordings from the light-scatter device (i.e., substance correlation factor). Tests were performed with Formulation III and for each target aerosol concentration at the nominal target aerosol generating conditions for Groups 3-5. For each test, a set of five filters, was mounted in the animal breathing position and exposed for an appropriate time and at a filter sampling flow rate of 0.25 mL/min. The particulate mass of aerosol (i.e., budesonide) collected from the formulation used in dose groups 3-5 was determined by HPLC. The vehicle group was exposed at the same aerosol generating conditions as that used for the high dose group. Particle size distribution of the test aerosol was determined using a cascade impactor, followed by measurement of the amount of test compound (i.e.,

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budesonide) on the impactor stages by HPLC. It was estimated that _____ by mass of the particles, were less than ____

Observations and times:

Clinical signs: Animals were checked twice daily for moribundity or death. All animals were observed daily for clinical signs of toxicity.

Body weights: Body weights were measured weekly.

Food and Water consumption: Food consumption was recorded weekly. Water consumption was assessed by daily inspection of water bottles.

Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of treatment and at the end of study period.

EKG: Not performed.

Hematology: Blood for measurement of hematology parameters was assessed after approximately 1 month of treatment and during the last week of dosing.

Clinical chemistry: Blood for measurement of blood chemistry parameters was assessed after approximately 1 month of treatment and during the last week of dosing.

Urinalysis: Urinalysis was conducted after approximately 1 month of treatment and during the last week of dosing. Animals were placed in special cages over a time period of 6 hr.

Gross pathology: At the end of the treatment period, animals were sacrificed and submitted to necropsy examination.

Organs weighed: Absolute and relative organ weights were determined for the brain, lungs, liver, heart, thymus, spleen, adrenal glands, testes, prostate, ovaries, and uterus.

Histopathology: Tissues were embedded in Histowax, sectioned at 4-6 μm , and stained with hematoxylin and eosin. Tissues were examined for all animals in the air-control, vehicle-control, and high dose groups. Tissues were examined for any animals found dead or sacrificed in a moribund condition.

Toxicokinetics: Blood for measurement of plasma budesonide and formoterol levels was collected on days 7 and 89 from satellite animals (Groups 6-13). Blood samples were collected at predose and at 10, 30, 120, 240, and 360 min after completion of dosing. Animals were sampled only once (i.e., 1 animal/sex) at each sampling time except for Groups 7 and 11, which consisted of 3 animals/sex/sampling time. Clinical observations were conducted to ascertain that these animals were representative of those in the main study. Satellite animals were killed without necropsy after completed blood sampling. Plasma concentrations of budesonide were measured using liquid chromatography \pm _____ tandem mass spectrometry (LC — MS/MS). Plasma concentrations of formoterol were measured using _____ tandem mass spectrometry (LC — MS/MS). The limit of quantification was 5.00 pmol/L for formoterol and 0.025 nmol/L for budesonide.

Results:

Mortality: None.

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

Body weights: Body weight gains for male and female rats in the high dose group were severely depressed.

Body weights for males in the air-control group during weeks 1 and 14 were 272.57 and 421.49 g, respectively. Body weight gains for males in the vehicle-control, low dose, mid dose, and high dose groups were 87.6, 89.6, 86.6, and 66.8% of the air-control, respectively. Body weights for females in the air-control group during weeks 1 and 14 were 180.93 and 233.86 g, respectively. Body weight gains for females in the vehicle-control, low dose, mid dose, and high dose groups were 95.5, 101.3, 88.8, and 55.9% of the air-control, respectively.

Food consumption: There were no treatment-related effects on food consumption. Water consumption for female vehicle-control and treatment groups during weeks 1-2, 6-7, and 11-12 were slightly increased to 107.4-116.7, 106.5-118.5, and 106.25-121.4% of the air-control (21.6-22.4 mL/animal/day), respectively.

Ophthalmoscopy: Ophthalmic examinations did not find any treatment-related effects.

Hematology: Alterations of white cell counts were evident for the male mid and high dose groups.

Week 5: Neutrophil ratios for the male mid and high dose groups were elevated to 165.8 and 155.3% of the air-control (0.114), respectively. Neutrophil ratios for the female mid and high dose groups were both elevated to 122.4% of the air-control (0.138), respectively. Platelet counts for female rats in mid and high dose groups were decreased to 90.4 and 93.1% of the air-control ($991.0 \times 10^9/L$), respectively.

Week 13: White blood cell counts for male rats in mid and high dose groups were decreased to 88.6 and 82.7% of the air-control ($7.99 \times 10^9/L$), respectively. Neutrophil ratios for the male mid and high dose groups were elevated to 117.75 and 137.9% of the air-control (0.169), respectively. The neutrophil ratio for female rats in the high dose group was elevated to 133.7% of the air-control (0.184), respectively. Platelet counts for male rats in mid and high dose groups were decreased to 92.2 and 90.3% of the air-control ($1169.7 \times 10^9/L$), respectively. Platelet counts for the female mid and high dose groups were decreased to 90.1 and 91.9% of the air-control ($1014.4 \times 10^9/L$), respectively.

Clinical chemistry: Slight elevations of urea levels were evident for male and female treatment groups, although, a treatment relationship was unclear.

Week 5: Urea levels for male rats in the low, mid, and high dose groups were elevated to 118.2-129% of the air-control (4.67 mmol/L). Urea levels for female rats in mid and high dose groups were elevated to 112.5 and 115% of the air-control (6.01 mmol/L), respectively. Creatinine levels for male rats in the vehicle-control and treatment groups were elevated to 107-111% of the air-control (40.7 $\mu\text{mol/L}$), respectively. Glucose levels for female rats in mid and high dose groups were elevated to 111.6-112% of the air-control

(5.77 mmol/L). Potassium levels for female rats in the low, mid, and high dose groups were increased to 108-111.8% of the air-control (3.39 mmol/L).

Week 13: Urea levels for male rats in mid and high dose groups were elevated to 115.6 and 125% of the air-control (5.83 mmol/L), respectively. Urea levels for female rats in mid and high dose groups were increased to 109 and 116% of the air-control (7.07 mmol/L), respectively. Bilirubin levels for male rats in the high dose group were elevated to 122% of the air-control (11.8 µmol/L), respectively.

Urinalysis: There were no treatment-related urinalysis findings.

Organ weights: Absolute and relative thymus weights were decreased for male and female rats in the high dose group; however, there were no corresponding histopathological findings. Absolute and relative liver weights were decreased for the male mid and high dose groups, which may correlate with histopathological findings of inflammatory foci.

Thymus: Absolute thymus weights for male vehicle-control, low dose, mid dose, and high dose groups were decreased to 89, 94.6, 85.8, and 65.2% of the air-control (0.34540 g), respectively. Relative thymus weights for the male vehicle-control, low dose, mid dose, and high dose groups were decreased to 88.8, 94.9, 86.7, and 66.3% of the air-control (16.58% Br.W.), respectively. Absolute thymus weights for the female vehicle-control, low dose, mid dose, and high dose groups were decreased to 94.8, 89.7, 91.8, and 69% of the air-control (0.28240 g), respectively. Relative thymus weights for the female vehicle-control, low dose, mid dose, and high dose groups were decreased to 92.3, 88.1, 91.5, and 70.6% of the control (15.14% Br.W.), respectively.

Liver: Absolute liver weights for the male mid and high dose groups were decreased to 89.7 and 85.1% of the air-control (13.09 g), respectively. Relative liver weights for the male mid and high dose groups were decreased to 90.1 and 86.1% of the air-control (630.3% Br.W.), respectively.

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

Histopathology: There were no treatment-related histopathological changes.

Histopathological findings for rats treated with a budesonide/formoterol HFA pMDI for 3 months.

| Tissue | Sex | Air-Control | Vehicle-Control | Low Dose | Mid Dose | High Dose |
|---------------------------------------|-----|-------------|-----------------|----------|----------|-----------|
| Liver -inflammatory foci (Grade I) | M | 0/10 | 0/10 | - | - | 2/10 |
| | F | 0/10 | 1/10 | - | - | 1/10 |

Toxicokinetics: Using a deposition factor of 0.086 to 0.094, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were 0.2 + 0.010, 0.8 +

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0.048, and 4.8 + 0.27 µg/kg/day, respectively. AUC and C_{max} values for budesonide and formoterol on days 7 and 89 increased in an approximately proportional manner with dose.

Toxicokinetic parameters for budesonide on days 7 and 89 in rats that received budesonide + formoterol by nose-only inhalation exposure.

| Day | Low Dose | | Mid Dose | | High Dose | |
|--------|-----------------------|-----------|-----------------------|-----------|-----------------------|-----------|
| | C _{max} , nM | AUC, nMhr | C _{max} , nM | AUC, nMhr | C _{max} , nM | AUC, nMhr |
| Day 7 | 0.271 | 0.313 | 1.42 | 2.01 | 4.96 | 6.87 |
| Day 89 | 0.318 | 0.349 | 1.77 | 1.95 | 8.65 | 10.3 |

Toxicokinetic parameters for formoterol on days 7 and 89 in rats that received budesonide + formoterol by nose-only inhalation exposure.

| Day | Low Dose | | Mid Dose | | High Dose | |
|--------|-----------------------|-----------|-----------------------|-----------|-----------------------|-----------|
| | C _{max} , pM | AUC, pMhr | C _{max} , pM | AUC, pMhr | C _{max} , pM | AUC, pMhr |
| Day 7 | 7.97 | 16.8 | 37.1 | 44.7 | 161 | 252 |
| Day 89 | 9.55 | 16.1 | 58.1 | 67.0 | 293 | 392 |

Summary of Individual study findings:

In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, povidone K-25 (PVP K-25), polyethylene glycol 1000 (PEG-1000), and HFA-227. Total doses of budesonide and formoterol in the low, mid, and high dose groups were 2 + 0.11, 10 + 0.56, and 51 + 2.9 µg/kg/day, respectively. Using a deposition factor of 0.086 to 0.094, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were 0.2 + 0.010, 0.8 + 0.048, and 4.8 + 0.27 µg/kg/day, respectively. Two additional groups were exposed daily to either an excipient-only HFA pMDI aerosol formulation (vehicle control) or to air only (air control). The NOAEL was identified as the mid dose. Body weight gains for male and female rats in the high dose group were reduced to 67 and 56% of the air-control, respectively. Thymus weights were decreased for male and female rats at the high dose, although, there were no corresponding histopathological findings. There were no target organs of toxicity (histopathological analysis of tissues was confined to air control, vehicle control, and high dose groups).

Decreases of body weight gains and thymus weights could be attributed to the effects of budesonide. There was no evidence of additive or synergistic toxic effects between formoterol and budesonide.

There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to excipients, PVP K-25 and PEG-1000, or the propellant, HFA-227. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were estimated to be 0.03, 8.6, and 30300 µg/kg/day, respectively. The deposition factor for budesonide, formoterol, PVP K-25, and PEG-1000 was estimated to approximately 8.6 to 9.4%. Given that HFA-227 is a gas, deposition was assumed to be 100%. The aerosol concentrations of excipients were not measured. The PVP K-25, PEG-1000, and HFA-227 doses were estimated from the measured dose of budesonide using the ratio of the four materials in the pMDI formulation on the assumption that the aerosol generation efficiency was similar. However, the aerosol generation

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efficiency of HFA-227 would have been 100% (gas). Concentrations of excipients in the vehicle-control were identical to those in high dose formulation; however, exposure times for the vehicle-control and high dose groups were 20 and 27 min, respectively, requiring that excipient doses for the vehicle-control group be adjusted by a factor of 20/27.

Study title: Symbicort (Budesonide + Formoterol): 3-Month Inhalation Study In Rats Using a Dry Powder Formulation.

Key study findings:

◆ In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort formulation containing budesonide and formoterol as a micronized dry powder blend. Total target doses of budesonide and formoterol in the low, mid, and high dose groups were 2 + 0.12, 10 + 0.60, and 50 + 3.0 µg/kg/day, respectively. Using a deposition factor of approximately 10%, actual deposited doses for budesonide and formoterol in the low, mid, and high dose combination groups were 0.30 + 0.018, 1.3 + 0.073, and 4.8 + 0.25 µg/kg/day, respectively. Positive control groups were treated with target doses of 50 µg/kg/day budesonide alone or 3.0 µg/kg/day formoterol alone (actual deposited doses of 7.2 and 0.24 µg/kg/day, respectively). A control group received air only.

◆ The NOAEL was identified as the mid dose combination. Target organs of toxicity were the thymus and spleen. Decreased body weight gains were observed for groups receiving budesonide only and the high dose of budesonide + formoterol. Observed changes can be primarily attributed to the effects of budesonide.

◆ The incidence of lymphocytolysis in the thymus was increased for male and female rats in the budesonide only group and male and female rats in the high dose budesonide + formoterol group.

◆ The incidence and magnitude of extramedullary hematopoiesis was decreased for male rats in the budesonide only group and male rats in the high dose budesonide + formoterol group.

◆ Systemic exposures to budesonide and formoterol were generally greater with the dry powder formulation as compared to the HFA pMDI formulation.

Study no: SR99022-01

Volume #, and page #: Volume 7 of 25, pages 5 to 383

Conducting laboratory and location: _____

Date of study initiation: April 12, 1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity:

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Budesonide + Formoterol, Batch number 1111/98 – supplied as a micronized dry powder consisting of — (w/w) budesonide and — (w/w) formoterol in lactose (— w/w).
 Budesonide, Batch number 1849/98 – supplied as a micronized dry powder mix consisting of — (w/w) budesonide in lactose (— w/w).
 Formoterol, Batch number ZB 202 – supplied as a micronized dry powder mix consisting of — (w/w) formoterol in lactose — w/w).
Formulation/vehicle: Control animals were exposed to air only. Test articles were supplied as micronized dry powders in lactose.

Methods (unique aspects): The toxicity of budesonide and formoterol, from a dry powder formulation, was assessed after inhalation administration for 3 months.

Dosing:

Species/strain: Wistar rats

#/sex/group or time point (main study): In the main toxicology study, there were 10 rats/sex/group.

Satellite groups used for toxicokinetics or recovery: There were 12 rats/sex/group for Group 1 (air control) and 15 rats/sex/group for Groups 2, 3, 5, and 6. There were no animals for toxicokinetic assessments of Group 4 (low dose of budesonide + formoterol).

Age: At the start of treatment, animals were approximately 10-12 weeks old.

Weight: Body weight ranges were 288-390 g for male rats and 191-270 g for female rats at the start of treatment.

Doses in administered units: Inhaled doses were estimated as follows

$$\text{Dose } (\mu\text{g/kg}) = \frac{\text{MV} \times \text{T} \times \text{CC}}{\text{BW}}$$

MV = respiratory volume/minute (liters)

T = duration of exposure (min)

CC = chamber concentration of active drug (μg/L)

BW = body weight (kg)

Target doses (μg/kg/day)

| Dose Group/Treatment | Target Dose Level, μg/kg | | |
|----------------------|--------------------------|------------|---------|
| | Budesonide | Formoterol | Aerosol |
| 1 – Air Control | 0 | 0 | 0 |
| 2 – Budesonide | 50 | 0 | 600 |
| 3 – Formoterol | 0 | 3.0 | 150 |
| 4 – B + F | 2 | 0.12 | 20 |
| 5 – B + F | 10 | 0.60 | 100 |
| 6 – B + F | 50 | 3.0 | 500 |

Gravimetric concentrations of the test aerosols were measured in the animals' breathing zone for Groups 2-6, throughout each exposure period. Gravimetric filter samples were collected daily. During each exposure period, chamber air was sampled continuously through pressed glass fiber filters placed in an open faced conical filter holder in-line with a sampling system comprising a vacuum pump, flow meter, and gas meter.

Budesonide and formoterol on gravimetric filters was determined by chemical analysis. An aerosol monitor was also used to assess total concentrations of particulate matter present in the test aerosol during each exposure.

The aerodynamic particle size distribution of the test aerosols was assessed from each treatment group during weeks 1, 3, 6, 9, and 12 using a cascade impactor. Substrate collection plates were weighed to determine the amount of test material in each particle size range. Amounts of budesonide and formoterol were assessed by HPLC analysis.

Inhaled and deposited doses (µg/kg/day)

| Group | Concentration, µg/L | | Time, min | Inhaled Dose, µg/kg/day | | MMAD, µm | Deposited Dose, µg/kg/day | |
|-----------|---------------------|-------|-----------|-------------------------|------|----------|---------------------------|-------|
| | B | F | | B | F | | B | F |
| 1 - Air | | | 20 | 0 | 0 | | 0 | 0 |
| 2 - B | 7.05 | 0 | 20 | 73 | - | | 7.2 | - |
| 3 - F | - | 0.231 | 20 | - | 2.3 | | - | 0.24 |
| 4 - B + F | 0.234 | 0.014 | 20 | 2.4 | 0.14 | | 0.30 | 0.018 |
| 5 - B + F | 1.17 | 0.064 | 20 | 11 | 0.61 | | 1.3 | 0.073 |
| 6 - B + F | 5.16 | 0.270 | 20 | 51 | 2.7 | | 4.8 | 0.25 |

Aerosol concentrations

| Group | Target Concentration, µg/L | Aerosol Concentration, µg/L | Inhaled Concentration, µg/L | Aerosol Target Aerosol Dose, µg/kg/day | Aerosol Mass MMAD (GSD), µm |
|-----------|----------------------------|-----------------------------|-----------------------------|--|-----------------------------|
| 1 - Air | 0 | | 0 | 0 | |
| 2 - B | 55 | | 70.82 | 600 | |
| 3 - F | 14 | | 13.26 | 150 | |
| 4 - B + F | 1.9 | | 2.30 | 20 | |
| 5 - B + F | 9.4 | | 14.12 | 100 | |
| 6 - B + F | 47 | | 59.72 | 500 | |

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

Animals were dosed by nose-only inhalation for 20 min daily, for at least 91 consecutive days, using a modular stainless steel chamber system. Separate exposure chambers were used for the control and test groups. For inhalation exposure, the rats were restrained in clear, tapered, polycarbonate tubes. Animals' snouts protruded through the anterior end of the restraint tubes. Test atmospheres were generated using a rotating brush generator device.

Observations and times:

Clinical signs: Animals were monitored for moribundity/mortality twice per day. Animals were monitored for clinical signs of toxicity during exposure, 1-2 hr after exposure, and at the end of each working day.

Body weights: Body weights were measured weekly.

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Food and Water consumption: Food consumption was measured weekly. Water consumption was monitored qualitatively by visual inspection of water bottles throughout the treatment period.

Ophthalmoscopy: Ophthalmic examinations were conducted prior the start of treatment and during week 13.

EKG: Not performed.

Hematology: Blood for measurement of hematology parameters was collected on day 91 prior to exposure.

Clinical chemistry: Blood for measurement of clinical chemistry parameters was collected on day 91 prior to exposure.

Urinalysis: Urine for analysis was collected overnight on days 88/89 or 89/90 of treatment.

Gross pathology: After completion of the 91-day treatment period (days 92/93), all animals were sacrificed and submitted to necropsy examination.

Organs weighed: Absolute organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, ovaries, parathyroids, pituitary gland, prostate gland, seminal vesicles, spleen, submaxillary salivary glands, testes plus epididymides, thymus, thyroid gland, uterus, and lung.

Histopathology: Histopathological evaluation of all tissues was conducted for main study animals in Groups 1 (air-control), 2 (budesonide only), 3 (formoterol only), and 6 (high dose, budesonide + formoterol). Tissues were processed, sectioned at 4-6 μm thick, stained with hematoxylin and eosin, and submitted to microscopic examination.

Toxicokinetics: Blood samples for measurement of plasma drug levels were collected from rats in Groups 2-6 on days 7/8 and during week 13 at predose and at 0.18, 0.5, 2, 4, and 6 hr after the completion of dosing. Blood was collected from rats in Group 1 (Air-control) on days 7/8 and during week 13 at predose and 45 min after completion of dosing. The plasma concentrations of budesonide were measured using liquid chromatography tandem mass spectrometry (LC — MS/MS).

The plasma concentrations of formoterol were measured using liquid chromatography tandem mass spectrometry (LC — MS/MS). Limits of quantitation for budesonide and formoterol were 0.025 nmol/L and 5.0 pmol/L, respectively.

Results:

Mortality: None.

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

Body weights: Decreased body weight gains were observed for male and female rats in Group 2 (budesonide only), Group 5 (mid dose of budesonide + formoterol), and Group 6 (high dose of budesonide + formoterol). Decreased body weight gains were also observed for female rats in Group 4 (low dose of budesonide + formoterol). Impaired body weight gains appear to be attributable to the effects of budesonide.

Body weights for male rats in the air-control group at weeks 0 and 13 were 341 and 506 g, respectively. Body weight gains for male rats in Groups 2, 3, 4, 5, and 6 were 54.6, 106.8, 103.3, 85.8, and 46.9 of the air-control, respectively.

Body weights for female rats in the air-control group at weeks 0 and 13 were 231 and 276 g, respectively. Body weight gains for female rats in Groups 2, 3, 4, 5, and 6 were 20.3, 131.1, 76.9, 65, and 27.1% of the air-control, respectively.

Food consumption: Food consumption from weeks 0 to 13 for male rats in Group 6 (High dose of budesonide + formoterol) was decreased to 90.1% of the air-control (30.59 g/animal/day), respectively.

Ophthalmoscopy: Ophthalmic examinations revealed no treatment-related findings.

Hematology: White blood cell (i.e., total, lymphocytes, and eosinophils) counts were decreased for Group 2 (budesonide only), Group 5 (mid dose of budesonide + formoterol), and Group 6 (high dose of budesonide + formoterol). These changes can appear to be attributable to the effects of budesonide.

White blood cell counts for male rats in Groups 2, 5, and 6 were decreased to 82.4, 89.8, and 71.9% of the air-control ($7.16 \times 10^9/L$), respectively. White blood cell counts for female rats in Groups 2, 3, 4, 5, and 6 were decreased to 59.9, 81.3, 80.3, 70.9, and 57.6% of the air-control ($5.09 \times 10^9/L$), respectively. Lymphocyte counts for male rats in Groups 2, 5, and 6 were decreased to 71.3, 93.8, and 61.6% of the air-control ($5.02 \times 10^9/L$), respectively. Lymphocyte counts for female rats in Groups 2, 5, and 6 were decreased to 49.1, 72.4, and 50.3% of the air-control ($3.30 \times 10^9/L$), respectively. Neutrophil counts for female rats in Groups 2, 3, 4, 5, and 6 were decreased to 81.6, 65.3, 63.9, 67.3, and 70.7% of the air-control ($1.47 \times 10^9/L$), respectively. Eosinophil counts for male rats in Groups 2, 5, and 6 were decreased to 66.7, 72.2, and 72.2% of the air-control ($0.18 \times 10^9/L$), respectively. Eosinophil counts for female treatment groups were decreased to 62.5-75% of the air-control ($0.16 \times 10^9/L$).

Clinical chemistry: Small alterations of a few clinical chemistry parameters (i.e., urea, alkaline phosphatase activity, potassium, inorganic phosphate) were observed; however, their toxicological significance appears minimal.

Potassium levels for male rats in Group 6 were increased to 107.8% of the air-control (5.1 mmol/L). Potassium levels for female rats in Group 2 were decreased to 91.3% of the air-control (4.6 mmol/L). Urea levels for male rats in Groups 2, 4, 5, and 6 were increased to 110.7, 109.2, 110.7, and 120% of the air-control (6.5 mmol/L), respectively. Alkaline phosphatase activities for male rats in Groups 2, 3, and 6 were slightly elevated to 121.3, 118.5, and 129.9% of the air-control (211 IU/L), respectively. Inorganic phosphate levels for male rats in Groups 3, 4, 5, and 6 were increased to 111.4, 117.1, 117.1, and 115.5% of the air-control (1.93 mmol/L), respectively. Inorganic phosphate levels for female rats in Groups 3, 4, 5, and 6 were increased to 121.4, 121.4, 120.1, and 111% of the air-control (1.54 mmol/L), respectively.

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

Organ weights: Decreased thymus weights were observed, which may be attributed to the effects of budesonide. Organ weights were normalized to body weights of 464 and 262 g for male and female rats, respectively.

Thymus: Thymus weights for male rats in Groups 2 and 6 were decreased to 69.2 and 76% of the air-control (0.334), respectively. Thymus weights for female rats in Groups 2, 5, and 6 were decreased to 59.7, 73.1, and 58% of the air-control (0.238), respectively.

Lungs: Lung weights for male rats in Groups 3 and 6 were increased to 115.8 and 116.4% of the air-control (1.71), respectively.

Adrenal glands: Adrenal gland weights for female rats in Group 2 were decreased to 89.9% of the air-control (0.0915).

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

Histopathology: Treatment-related changes were observed in the thymus and spleen.

Thymus: The incidence and severity of lymphocytolysis was increased in Group 2 (budesonide only) and Group 6 (high dose of budesonide + formoterol) as compared to the air-control.

Spleen: The incidence and magnitude of extramedullary hematopoiesis was decreased for male rats in Group 2 (budesonide only) and Group 6 (high dose of budesonide + formoterol).

Other: A malignant astrocytoma in the brain was observed for 1 female rat in Group 2 (budesonide only). Focal transitional cell hyperplasia in the kidney was observed for 1 female rat in Group 6 (budesonide + formoterol). Focal ductal hyperplasia was observed in the salivary gland for 1 male rat in Group 3 (formoterol only). Stomach ulcers were observed for 2 male rats in Group 2 (budesonide only) and 1 female rat in Group 6 (high dose of budesonide + formoterol).

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Reviewer: Timothy W. Robison, Ph.D.

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Histopathological changes for rats in Group 1 (air only), Group 2 (budesonide only), Group 3 (formoterol only), and Group 6 (high dose of budesonide + formoterol) following 91 days of consecutive treatment.

| Tissue | Sex | Group 1 | Group 2 | Group 3 | Group 6 |
|--|-----|---------|---------|---------|---------|
| Thymus -lymphocytolysis, minimal to mild | M | 0/10 | 6/10* | 0/10 | 5/9* |
| | F | 0/10 | 5/10* | 0/10 | 10/10* |
| Spleen -extramedullary hematopoiesis, minimal to moderate | M | 8/10 | 4/10 | 6/10 | 2/10* |
| | F | 8/10 | 8/10 | 9/10 | 7/10 |
| Bronchial lymph node -erythrophagocytosis | M | 0/9 | 0/8 | 0/9 | 0/7 |
| | F | 0/7 | 0/9 | 1/10 | 2/8 |
| Kidneys -basophilic tubules | M | 5/10 | 1/10 | 0/10* | 1/10 |
| | F | 0/10 | 0/10 | 1/10 | 1/10 |
| -mineral deposits, corticomedullary minimal to mild | M | 0/10 | 0/10 | 0/10 | 0/10 |
| | F | 2/10 | 1/10 | 3/10 | 5/10 |
| -focal transitional cell hyperplasia | M | 0/10 | 0/10 | 0/10 | 0/10 |
| | F | 0/10 | 0/10 | 0/10 | 1/10 |
| Stomach -ulcer, mild to moderate | M | 0/10 | 2/10 | 0/10 | 0/10 |
| | F | 0/10 | 0/10 | 0/10 | 1/10 |
| Brain -malignant astrocytoma | M | 0/10 | 0/10 | 0/10 | 0/10 |
| | F | 0/10 | 1/10 | 0/10 | 0/10 |
| Salivary gland -hyperplasia, ductal, focal, minimal | M | 0/10 | 0/10 | 1/10 | 0/10 |
| | F | 0/10 | 0/10 | 0/10 | 0/10 |

Toxicokinetics: Toxicokinetic parameters were not determined for Group 4 (low dose of budesonide + formoterol). There were significant variations in the estimated inhaled doses for budesonide and formoterol between weeks 1 and 13 rendering it difficult to compare AUC and C_{max} values between these two time points. However, AUC and C_{max} values for groups within each time point can be compared. AUC and C_{max} values for budesonide were generally higher in female rats, due to lower metabolism, as compared to male rats. During weeks 1 and 13, AUC and C_{max} values for budesonide and formoterol in Group 6 were less than proportional to values for Group 5 on the basis of differences in inhaled doses.

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Reviewer: Timothy W. Robison, Ph.D.

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Toxicokinetic parameters for budesonide and formoterol in rats from Group 2 (budesonide only), Group 3 (formoterol only), Group 5 (mid dose of budesonide + formoterol), and Group 6 (high dose of budesonide + formoterol) during week 1.

| Group | Estimated Inhaled Dose | | | | AUC _{0-24hr} | | | | C _{max} | | | |
|-------|-------------------------|------|-------------------------|------|-------------------------|------|-------------------------|-----|----------------------|------|----------------------|-----|
| | Budesonide µg/kg/day | | Formoterol µg/kg/day | | Budesonide nmolehr/L | | Formoterol pmolehr/L | | Budesonide nmol/L | | Formoterol pmol/L | |
| | M | F | M | F | M | F | M | F | M | F | M | F |
| 2 | 23.7 | 26.4 | 0 | 0 | 11.9 | 18.4 | - | - | 11.0 | 20.9 | - | - |
| 3 | 0 | 0 | 0.92 | 1.02 | - | - | 777 | 673 | - | - | 527 | 657 |
| 5 | 4.0 | 4.4 | 0.21 | 0.24 | 4.3 | 8.6 | 196 | 259 | 4.75 | 6.73 | 142 | 194 |
| 6 | 17.7 | 19.7 | 0.80 | 0.89 | 7.5 | 16.1 | 438 | 533 | 8.6 | 14.6 | 266 | 368 |

Toxicokinetic parameters for budesonide and formoterol in rats from Group 2 (budesonide only), Group 3 (formoterol only), Group 5 (mid dose of budesonide + formoterol), and Group 6 (high dose of budesonide + formoterol) during week 13.

| Group | Estimated Inhaled Dose | | | | AUC _{0-24hr} | | | | C _{max} | | | |
|-------|-------------------------|------|-------------------------|------|-------------------------|------|-------------------------|------|----------------------|------|----------------------|------|
| | Budesonide µg/kg/day | | Formoterol µg/kg/day | | Budesonide nmolehr/L | | Formoterol pmolehr/L | | Budesonide nmol/L | | Formoterol pmol/L | |
| | M | F | M | F | M | F | M | F | M | F | M | F |
| 2 | 70.7 | 80.8 | 0 | 0 | 37.4 | 43.0 | - | - | 49.2 | 46.2 | - | - |
| 3 | 0 | 0 | 1.98 | 2.29 | - | - | 1339 | 1368 | - | - | 1050 | 1190 |
| 5 | 19.4 | 22.5 | 1.07 | 1.24 | 14.4 | 29.1 | 772 | 999 | 19.6 | 27.6 | 734 | 817 |
| 6 | 81.8 | 93.7 | 4.45 | 5.10 | 34.3 | 40.8 | 1661 | 1611 | 51.9 | 35.4 | 1380 | 1090 |

Summary of individual study findings: In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort formulation containing budesonide and formoterol as a micronized dry powder blend. Total target doses of budesonide and formoterol in the low, mid, and high dose groups were 2 + 0.12, 10 + 0.60, and 50 + 3.0 µg/kg/day, respectively. Using a deposition factor of approximately 10%, deposited doses for budesonide and formoterol in the low, mid, and high dose combination groups were 0.30 + 0.018, 1.3 + 0.073, and 4.8 + 0.25 µg/kg/day, respectively. Positive control groups were treated with total target doses of 50 µg/kg/day budesonide alone or 3.0 µg/kg/day formoterol alone (actual deposited doses of 7.2 and 0.24 µg/kg/day, respectively). A control group received air only. The NOAEL was identified as the mid dose combination. Target organs of toxicity were the thymus and spleen. Decreased body weight gains were observed for groups receiving budesonide only and the high dose of budesonide + formoterol. Observed changes can be primarily attributed to the effects of budesonide. The incidence of lymphocytolysis in the thymus was increased for male and female rats in the budesonide only group and male and female rats in the high dose budesonide + formoterol group. The incidence and magnitude of extramedullary hematopoiesis was decreased for male rats in the budesonide only group and male rats in the high dose budesonide + formoterol group. Systemic exposures to budesonide and formoterol were generally greater with the dry powder formulation as compared to the HFA pMDI formulation.

Dogs

Reviewer: Timothy W. Robison, Ph.D.

IND No. 63.394

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

- ◆ In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, povidone K-25 (PVP K-25), polyethylene glycol 1000 (PEG-1000), and HFA-227. Target doses of budesonide and formoterol in the low, mid, and high dose groups were 2.0 + 0.11, 10 + 0.56, and 50.0 + 2.80 µg/kg/day, respectively. Using a deposition factor of approximately 25%, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were 0.52 + 0.032, 2.90 + 0.18, and 14.6 + 0.89 µg/kg/day, respectively.
- ◆ The NOAEL was identified as the low dose.
- ◆ Sinus tachycardia (i.e., increased heart rate) was observed in mid and high dose groups on day 1 and during week 2. No changes were evident during week 12.
- ◆ Target organs of the toxicity were the adrenal cortex, thymus, and bronchial lymph nodes. Observed histopathological changes appear to be primarily attributable to the effects of budesonide.
- ◆ Cortical atrophy of the adrenal cortex was observed for male and female mid and high dose groups.
- ◆ The incidence and severity of thymic atrophy was increased for the male and female mid and high dose groups.
- ◆ The incidence and severity of lymphoid depletion in the bronchial lymph nodes was increased for the male and female high dose groups.
- ◆ There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to PVP K-25, PEG-1000, and HFA-227 at deposited doses of 0.10, 31.5, and 42000 µg/kg/day, respectively.

Study no: 00047**Volume #, and page #:** Volume 6 of 25, pages 5 to 336**Conducting laboratory and location:** AstraZeneca R&D Charnwood
Safety Assessment
Bakewell Road
Loughborough
Leicestershire LE11 5RH
United Kingdom**Date of study initiation:** May 15, 2000**GLP compliance:** Yes

Reviewer: Timothy W. Robison, Ph.D.

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QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: The test formulation, Symbicort pMDI (lot number P5993), consisted of pMDIs containing micronized budesonide and formoterol fumarate dihydrate, polyvinylpyrrolidone K-25 (PVP), polyethylene glycol 1000 (PEG 1000), and heptafluoropropane (HFA-227) delivering a theoretical nominal 80 µg of budesonide and 4.5 µg formoterol per actuation from a 50 µL valve. Formoterol fumarate dihydrate (batch number SRS1995) with a purity of _____ and budesonide (batch numbers SRS1999 and SRS1998) with a purity of _____ were used in the preparation of Symbicort.

| Used in Groups | Material | Percent, w/w |
|----------------|---|--------------|
| 3,4,5 | Budesonide Formoterol PVP K-25 PEG-1000 HFA-227 | |

Formulation/vehicle: The vehicle-control formulation (lot number P5992), consisted of pMDIs delivering PVP K-25, PEG-1000, and HFA-227 from a 50 µL valve.

| Used in Groups | Material | Percent, w/w |
|----------------|---------------------------------|--------------|
| 2 | PVP K-25 PEG 1000 HFA-227 | — |

Methods (unique aspects): A Symbicort formulation containing budesonide and formoterol in a pMDI containing polyvinylpyrrolidone K-25 (PVP K-25), polyethylene glycol 1000 (PEG-1000), and heptafluoropropane (HFA-227) was administered daily, by inhalation to 3 groups of 3 beagle dogs/sex/group for 3 months. Another two similarly sized control groups were exposed daily to either an excipients-only HFA pMDI aerosol formulation (vehicle-control) or to air-only (air-control).

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 3 beagle dogs/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: Dogs were approximately 7-9 months old at the start of dosing.

Weight: Body weight ranges were 8.8-11.4 kg for male dogs and 6.9-10.1 kg for female dogs at the start of dosing.

Doses in administered units: Animals were dosed daily for a minimum of 13 consecutive weeks.

| Symbicort | Group 3 (Low) | | Group 4 (Mid) | | Group 5 (High) | |
|-----------------------------|---------------|-------|---------------|------|----------------|------|
| | B | F | B | F | B | F |
| Total Dose µg/kg/day | 2.0 | 0.11 | 10 | 0.56 | 50 | 2.8 |
| Deposited Dose µg/kg/day | 0.52 | 0.032 | 2.90 | 0.18 | 14.6 | 0.89 |

The concentration of the aerosol delivered to dogs was monitored continuously using an aerosol monitor. The mean aerosol concentration delivered to each dog, each day, was determined from the aerosol monitor readings. Substance correction factors required to determine the concentrations of budesonide and formoterol from readings were determined by comparison of the readings with HPLC analyses of concurrent aerosol filter samples. Aerosol samples (5 min, corresponding to 40 L of aerosol) were collected from each dose group (8 samples per group, one device per formulation). Samples were collected onto filters held in open faced filter units, sealed onto the outlet of the dosing system (i.e., in place of the dog mask). All filters were retained for determination of the amount of budesonide and formoterol using an HPLC method.

The particle size distribution of the aerosol (budesonide and formoterol) leaving the dosing device was determined with a cascade impactor. Quantities of budesonide and formoterol deposited on each stage were determined using a HPLC method.

Total inhaled doses were calculated on the basis that all the material present in the aerosol was inhaled, using the following formula:

$$\text{Dose } (\mu\text{g/kg}) = \frac{\text{Minute Volume (L/min)} \times \text{Concentration } (\mu\text{g/L}) \times \text{Time (min)}}{\text{Body weight (kg)}}$$

where minute volume was assumed to be 5 L/min and the concentration equaled the mean reading x relevant substance correction factor. The deposited dose was estimated to be approximately 25% of the total inhaled dose (i.e., deposition factor was approximately 0.25) based upon a particle size (MMAD) of

The aerosol concentrations of excipients (PVP K-25, PEG-1000, and HFA-227) were not measured; however, doses of excipients were estimated from the measured dose of budesonide using the ratio of the 4 materials in the pMDI formulation on the assumption that the aerosol generation efficiency was similar. The aerosol generation efficiency of HFA-227 was assumed to be 100%, given that as a gas, deposition losses were minimal. The excipient doses for the vehicle-control group (Group 2) were assumed to have been the same as for the high dose group, since the vehicle-control formulation and aerosol conditions matched those of the high dose in terms of excipients.

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| Group | Dose | Measured | | Estimated | | |
|-------|---------------|------------|------------|-----------|----------|---------|
| | | Budesonide | Formoterol | PVP K-25 | PEG 1000 | HFA-227 |
| 1 | Total Inhaled | 0 | 0 | 0 | 0 | 0 |
| | Body Burden | 0 | 0 | | | |
| | Lung Burden | 0 | 0 | | | |
| 2 | Total Inhaled | 0 | 0 | 0.420 | 126 | 41835 |
| | Body Burden | 0 | 0 | | | |
| | Lung Burden | 0 | 0 | | | |
| 3 | Total Inhaled | 1.96 | 0.117 | 0.0158 | 4.74 | 1574 |
| | Body Burden | 0.95 | 0.055 | | | |
| | Lung Burden | 0.32 | 0.032 | | | |
| 4 | Total Inhaled | 10.3 | 0.62 | 0.0831 | 24.9 | 8271 |
| | Body Burden | 5.20 | 0.31 | | | |
| | Lung Burden | 2.90 | 0.18 | | | |
| 5 | Total Inhaled | 32.1 | 3.11 | 0.420 | 126 | 41835 |
| | Body Burden | 26.1 | 1.53 | | | |
| | Lung Burden | 14.6 | 0.89 | | | |

Appendix 1:5 Particle size distribution (MMAD and GSD)

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Appendix 1:2 Overall mean doses ($\mu\text{g}/\text{kg}/\text{day}$)

| Dose Group | | Budesonide | | | Formoterol | | |
|------------|---------------|------------|--------|------|------------|--------|-------|
| | | Male | Female | Mean | Male | Female | Mean |
| 3 | Total Inhaled | 1.86 | 2.06 | 1.96 | 0.111 | 0.123 | 0.117 |
| | Body Burden | 0.90 | 1.00 | 0.95 | 0.052 | 0.058 | 0.055 |
| | Lung Burden | 0.49 | 0.54 | 0.52 | 0.030 | 0.033 | 0.032 |
| 4 | Total Inhaled | 9.47 | 11.2 | 10.3 | 0.57 | 0.67 | 0.62 |
| | Body Burden | 4.76 | 5.64 | 5.20 | 0.28 | 0.33 | 0.31 |
| | Lung Burden | 2.65 | 3.14 | 2.90 | 0.16 | 0.19 | 0.18 |
| 5 | Total Inhaled | 49.8 | 54.3 | 52.1 | 2.97 | 3.24 | 3.11 |
| | Body Burden | 24.9 | 27.2 | 26.1 | 1.46 | 1.59 | 1.53 |
| | Lung Burden | 13.9 | 15.2 | 14.6 | 0.85 | 0.92 | 0.89 |

Appendix 1:3 Mean deposition estimates (%)

| Group and Region | | Formoterol | Budesonide |
|------------------|------|------------|------------|
| 3 | Body | 47.2 | 48.4 |
| | Lung | 26.8 | 26.1 |
| 4 | Body | 48.7 | 50.3 |
| | Lung | 28.1 | 28.0 |
| 5 | Body | 49.1 | 50.0 |
| | Lung | 28.5 | 28.0 |

Route, form, volume, and infusion rate: Daily treatment consisted of a single oral inhalation exposure of 5 min.

Test formulations were administered by inhalation, as an aerosol generated from an automated pMDI actuator and shaking device, which actuates a pMDI directly into a spacer. Each dog was lightly restrained by a handler and a close-fitting face mask and aerosol delivery tube system was used to deliver the aerosol directly into the buccal cavity of the dog.

Observations and times:

Clinical signs: Animals were observed daily for clinical signs of toxicity, before, during, and after dosing. Physical examinations were conducted weekly.

Body weights: Body weights were measured weekly.

Food consumption: Food consumption was recorded daily.

Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of treatment and during week 12.

EKG: Electrocardiographic recordings were collected from all animals prior to the start of treatment (week -2), on day 1, and during weeks 2 and 12. During the treatment period, recordings were conducted at 1 hr after exposure. In addition, heart rate (i.e., pulse) was recorded manually on day 1 and during weeks 2 and 12 at predose, immediately postdose, and approximately 4 and 24 hr after dosing.

Hematology: Blood for measurement of hematology parameters was collected during weeks -2, -1, 7, and 13. During the treatment period, blood was collected prior to dosing.

Clinical chemistry: Blood for measurement of clinical chemistry parameters was collected during weeks -2, -1, 7, and 13. During the treatment period, blood was collected prior to dosing.

Urinalysis: Urine samples for analyses were collected during weeks -2, 7, and 13. During the treatment period, urine was collected prior to dosing.

Gross pathology: After a minimum of 13 consecutive weeks of treatment, animals were sacrificed and submitted to necropsy examination.

Organs weighed: Organ weights were determined for the adrenal glands, brain, kidneys, heart, liver, lungs ovaries, prostate, spleen, testes, thymus, thyroid gland, uterus, and pituitary gland.

Histopathology: Samples of all tissues retained at necropsy from all animals were processed into paraffin blocks. Sections were cut at between approximately 4-5 μm , stained with hematoxylin and eosin, and submitted to microscopic examination.

Toxicokinetics: Blood samples for measurement of plasma concentrations of budesonide and formoterol were collected on days 7 and 87 at predose and at 10, 30, 120, 240, and 360 min after the start of dosing. The samples were pooled within each group/sex and time point prior to analysis for budesonide and formoterol. The plasma concentrations of budesonide were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). The plasma concentrations of formoterol were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Other: Basal and adrenocorticotrophic hormone (ACTH)-stimulated cortisol levels were measured in blood samples collected during weeks -1 and 13.

Results:

Mortality: None.

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

Body weights: Body weight gains were lower for male treatment groups; however, there were no effects on female treatment groups.

Body weights for male controls during weeks 1 and 14 were 9.63 and 11.70 kg, respectively, yielding a 21.5% increase of initial weight. Body weights during week 14 for male dogs in the vehicle-control, low dose, mid dose, and high dose groups were increased by 18.2, 14.3, 13.2, and 10.0% of body weights during week 1, respectively. Body weights for female controls during weeks 1 and 14 were 8.23 and 9.50 kg, respectively, yielding a 15.4% increase of initial weight. Body weights during week 14 for female dogs in the vehicle-control, low dose, mid dose, and high dose groups were increased by 21.2, 19, 15.9, and 15.8% of body weights during week 1, respectively.

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

Ophthalmoscopy: Ophthalmic examinations found no treatment-related effects.

Electrocardiography: Sinus tachycardia (i.e., increased heart rate) was observed in mid and high dose groups on day 1 and during week 2. No changes in heart rate were evident during week 12.

Day 1: Sinus tachycardia (i.e., increased heart rate) was evident in the high dose group immediately after dosing and in mid and high dose groups at 1 hr after dosing. QRS intervals for male dogs in the vehicle-control and treatment groups were increased to 128.4-134% of the air-control (39.1 msec). QRS intervals for female dogs in mid and high dose groups were increased to 110.8 and 115.8% of the air-control (42.4 msec), respectively. The QTc interval for female dogs was increased to 105.5% of the air-control (217 msec).

Week 2: Sinus tachycardia (i.e., increased heart rate) was evident in mid and high groups at 1 and 4 hr after dosing. Heart rate was elevated for male and female dogs in the mid dose group (Group 4) at 4 hr after dosing, although, no changes were evident immediately and 1 hr after dosing. Heart rate was elevated for male and female dogs in the high dose group (Group5) immediately and at 1 and 4 hr after dosing.

Week 12: Heart changes in treatment groups were comparable to the air-control. The QRS interval for male dogs in mid and high dose group were increased to 115.4 and 123.3% of the control (48.1 msec), respectively. QRS intervals for female dogs in the vehicle-control and treatment groups were increased to 108.7-116.9% of the air-control (47.3 msec). QT intervals for male dogs in the vehicle-control and treatment groups were increased to 103.7-108.1% of the air-control (185 msec). QTc intervals for male dogs in mid and high dose treatment groups were increased to 103.6 and 105.4% of the air-control (222 msec), respectively.

Heart rates on day 1 and during weeks 2 and 12 (Notable changes have been marked in bold).

| Period | Group | Measurement Time | | | | |
|---------|-----------|------------------|-----------------|-----------------|-----------------|-----------------|
| | | Pre | Post | +1 hr | + 4 hr | +24 hr |
| Day 1 | 1-Males | 76 | 75 (98.7%) | 109 (143.4%) | 74 (97.4%) | 92 (121%) |
| | 1-Females | 84 | 93 (110.7%) | 112 (133.3%) | 98 (116.7%) | 92 (109.5%) |
| | 2-Males | 86 | 96 (111.6%) | 83 (96.5%) | 74 (86%) | 105 (122%) |
| | 2-Females | 115 | 117 (101.7%) | 131 (113.9%) | 97 (84.3%) | 113 (98.3%) |
| | 3-Males | 89 | 86 (96.6%) | 97 (109%) | 108 (121.3%) | 109 (122.5%) |
| | 3-Females | 91 | 101 (111%) | 120 (131.9%) | 91 (100%) | 92 (101.1%) |
| | 4-Males | 92 | 92 (100%) | 113 (122.8%) | 105 (114.1%) | 93 (101.1%) |
| | 4-Females | 100 | 103 (103%) | 138 (138%) | 111 (111%) | 95 (95%) |
| | 5-Males | 93 | 117 (125.8%) | 137 (147.3%) | 106 (114%) | 106 (114%) |
| | 5-Females | 86 | 127 (147.7%) | 174 (202.3%) | 112 (130.2%) | 102 (118.6%) |
| Week 2 | 1-Males | 98 | 96 (98%) | 115 (117.3%) | 97 (99%) | 96 (98%) |
| | 1-Females | 90 | 94 (104.4%) | 111 (123.3%) | 93 (103.3%) | 110 (122.2%) |
| | 2-Males | 91 | 94 (103.3%) | 92 (101%) | 94 (103.3%) | 114 (125.3%) |
| | 2-Females | 106 | 98 (92.5%) | 118 (111.3%) | 92 (86.8%) | 114 (107.5%) |
| | 3-Males | 105 | 103 (98.1%) | 92 (87.6%) | 93 (88.6%) | 113 (107.6%) |
| | 3-Females | 105 | 107 (101.9%) | 105 (100%) | 114 (108.6%) | 102 (97.1%) |
| | 4-Males | 75 | 87 (116%) | 94 (125.3) | 112 (149.3%) | 96 (128%) |
| | 4-Females | 88 | 92 (104.5%) | 114 (129.5%) | 102 (115.9%) | 103 (117%) |
| | 5-Males | 83 | 93 (112.0%) | 106 (127.5%) | 119 (143.4%) | 100 (120.5%) |
| | 5-Females | 85 | 97 (114%) | 130 (152.9%) | 131 (154%) | 84 (98.8%) |
| Week 12 | 1-Males | 86 | 99 (115.1%) | 106 (123.3%) | 96 (111.6%) | 102 (118.6%) |
| | 1-Females | 107 | 92 (86%) | 110 (102.8%) | 103 (96.3%) | 97 (90.7%) |
| | 2-Males | 76 | 85 (111.8%) | 83 (109.2%) | 120 (157.9%) | 91 (119.7%) |
| | 2-Females | 103 | 92 (89.3%) | 105 (101.9%) | 104 (101%) | 92 (89.3%) |

| | | | | | |
|-----------|-----|-----------------|-----------------|-----------------|-----------------|
| 3-Males | 93 | 95 (102.2%) | 95 (102.2%) | 101 (108.6%) | 90 (96.8%) |
| 3-Females | 100 | 88 (88%) | 97 (97%) | 101 (101%) | 92 (92%) |
| 4-Males | 94 | 90 (95.7%) | 101 (107.4%) | 103 (109.6%) | 107 (113.8%) |
| 4-Females | 85 | 82 (96.5%) | 103 (121.2%) | 96 (112.9%) | 90 (105.9%) |
| 5-Males | 99 | 102 (103%) | 106 (107.1%) | 100 (101%) | 100 (101%) |
| 5-Females | 96 | 101 (105.2%) | 112 (116.7%) | 117 (121.9%) | 105 (109.4%) |

Hematology: Decreased white cell counts were observed for male dogs in mid and high dose groups during weeks 7 and 13. Increased red blood cell counts, hemoglobin levels, and hematocrits were observed in female dogs in mid and high dose groups during weeks 7 and 13. These changes were relatively small and their toxicological significance is unclear. Changes in red blood cell counts, hemoglobin levels, and hematocrit in female dogs in mid and high dose groups might be indicative of dehydration.

Week 7: White blood cell counts for male dogs in mid and high dose groups were decreased to 78.7 and 81.6% of the air-control ($11.693 \times 10^9/L$), respectively. Neutrophil counts for male dogs in mid and high dose groups were decreased to 78.4 and 84% of the air-control ($6.863 \times 10^9/L$), respectively. Lymphocyte counts for male dogs in mid and high dose groups were decreased to 74.1 and 75% of the air-control ($3.983 \times 10^9/L$), respectively. Red blood cell counts for female dogs in mid and high dose groups were increased to 111.4 and 110.35% of the air-control ($6.376 \times 10^{12}/L$), respectively. Hemoglobin levels for female dogs in the high dose group were increased to 109.2% of the air-control (14.80 g/dL). Hematocrits for female dogs in mid and high dose groups were increased to 106.5 and 109.7% of the air-control (0.4380), respectively. Eosinophil counts for female dogs in the high dose group were decreased to 34.8% of the air-control ($0.316 \times 10^9/L$).

Week 13: White blood cell counts for male dogs in mid and high dose groups were decreased to 69.4 and 72.3% of the air-control ($12.440 \times 10^9/L$), respectively. Neutrophil counts for male dogs in mid and high dose groups were decreased to 65 and 71.3% of the air-control ($7.793 \times 10^9/L$), respectively. Lymphocyte counts for male dogs in the high dose groups were decreased to 65.7% of the air-control ($3.806 \times 10^9/L$). Monocyte counts for male dogs in the high dose group were increased to 157.4% of the air-control ($0.383 \times 10^9/L$). Red blood cell counts for female dogs in mid and high dose groups were increased to 116.2 and 111.7% of the air-control ($6.000 \times 10^{12}/L$), respectively. Hemoglobin levels for female dogs in mid and high dose groups were increased to 110 and 110.2% of the air-control (14.03 g/dL), respectively. Hematocrits for female dogs in mid and high dose groups were both increased to 110.4% of the air-control (0.4150). Eosinophil counts for female dogs in the high dose groups were decreased to 56.9% of the air-control ($0.316 \times 10^9/L$).

Clinical chemistry: Potassium levels were elevated in male and female dogs in mid and/or high dose groups. A number of other changes (i.e., ALT, AST, ALP, cholesterol, triglyceride, creatinine, phosphate) were also observed for treatment groups, although, their toxicological significance was unclear. Mean cortisol values during week 13, pre- and post-stimulation with ACTH, for female dogs in the low dose group and for male and female dogs in mid and high dose group were markedly lower as compared to the air-control.

Week 7: Potassium levels for male dogs in the high dose group were increased to 112.3% of the air-control (4.130 mmol/L). Potassium levels for female dogs in mid and high dose groups were increased to 110 and 111% of the air-control (4.160 mmol/L), respectively. Alanine aminotransferase (ALT) activity for male dogs in the high dose group was increased to 142.9% (52, 37, and 101 IU/L) of the air-control (44.3 IU/L). Alkaline phosphatase (ALP) activity for female dogs in the high dose group was increased to 116.7% (103, 191, and 210 IU/L) of the air-control (144.0 IU/L). Cholesterol levels for male dogs in vehicle-control and treatment groups were increased to 135.3-139.1% of the air-control (2.266 mmol/L). Cholesterol levels for female treatment groups were increased to 114.7-138.7% of the air-control (2.296 mmol/L; vehicle-control was 2.476 mmol/L), respectively

Week 13: Mean cortisol values during week 13, pre- and post-stimulation with ACTH, for female dogs in the low dose group and for male and female dogs in mid and high dose group were markedly lower as compared to the air-control (see table below). There was some evidence of an effect of the vehicle-control on cortisol levels in female dogs, although, this was not observed with male dogs. Potassium levels for male dogs in the high dose group were increased to 108.9% of the air-control (4.196 mmol/L). Potassium levels for female dogs in mid and high dose groups were increased to 106.7 and 109.5% of the air-control (4.150 mmol/L). Phosphate levels for female vehicle-control and treatment groups were increased to 124.3-144.9% of the air-control (1.343 mmol/L). ALT activity for male dogs in the high dose group was increased to 142.6% (91, 30, and 87 IU/L) of the air-control (48.6 IU/L). Aspartate aminotransferase activities for male treatment groups were increased to 129.4-153.6% of the air-control (36.0 IU/L). ALP activity for female dogs in the high dose group was increased to 156% (98, 173, and 208 IU/L) of the air-control (102.3 IU/L). Cholesterol levels for male dogs in vehicle-control and treatment groups were increased to 122.8-139.8% of the air-control (2.453 mmol/L). Cholesterol levels for female dogs in mid and high dose groups were increased to 109.9 and 119.65% of the air-control (3.053 mmol/L), respectively. Triglyceride levels for male treatment groups were elevated to 114.7-136.9% of the air-control (0.360 mmol/L). Triglyceride levels for female dogs in the high dose group were increased to 129.6% of the air-control (0.496 mmol/L). Creatinine levels for female dogs in the high dose group were elevated to 115.9% of the air-control (79.0 μ mol/L).

Reviewer: Timothy W. Robison, Ph.D.

IND No. 63.394

Cortisol levels (nmole/L) during week 13, pre- and post-stimulation with ACTH. Values in parentheses are percent of air-control.

| Group | Pre-stimulation | | Post-stimulation | |
|-----------------|-----------------|--------------|------------------|----------------|
| | Male | Female | Male | Female |
| Air-control | 53.7 | 86.3 | 421.7 | 456.7 |
| Vehicle-control | 49 | 48.7 (56.4) | 443 | 348.7 (76.35%) |
| Low dose | 61 | 33.3 (38.6%) | 353 (83.7%) | 276.3 (60.5%) |
| Mid dose | 13.3 (24.7%) | 34 (39.4%) | 194.3 (46%) | 251.7 (55.1%) |
| High dose | 12 (22.3%) | 10.3 (11.9%) | 30.3 (7.2%) | 73.7 (16.1) |

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

Organ weights: Decreased adrenal gland and thymus gland weights were observed for mid and high dose groups.

Thymus: Thymus weights for male dogs in mid and high dose groups were decreased to 35.1 and 26.8% of the air-control (14.6846% Br.W.), respectively. Thymus weights for female dogs in mid and high dose groups were decreased to 86.5 and 64.3% of the air-control (10.1487% Br.W.), respectively.

Adrenal gland: Adrenal gland weights for male dogs in mid and high dose groups were decreased to 85.2 and 61.1% of the air-control (1.6344% Br.W.), respectively. Adrenal gland weights for female dogs in mid and high dose groups were decreased to 67.5 and 56.6% of the air-control (1.8981% Br.W.), respectively.

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

Histopathology: Target organs of toxicity were the thymus, adrenal cortex, and bronchial lymph nodes. An increased incidence and severity of thymic atrophy was observed for mid and high dose groups. Cortical atrophy of the adrenal gland was evident for mid and high dose groups. An increased incidence and severity of lymphoid depletion in the bronchial lymph nodes was evident for the high dose group.

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Histopathological changes for dogs that were treated with a Symbicort HFA pMDI for 3 months.

| Tissue | Air-Control | | Vehicle-Control | | Low Dose | | Mid Dose | | High Dose | |
|--|-------------|---|-----------------|---|----------|---|----------|---|-----------|---|
| | M | F | M | F | M | F | M | F | M | F |
| Thymus (n = 3/group) | | | | | | | | | | |
| -atrophy (total) | 1 | 1 | 2 | 1 | 2 | 0 | 3 | 2 | 3 | 3 |
| -Grade 1 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| -Grade 2 | 1 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | 0 |
| -Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| -Grade 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 |
| Adrenal gland (n = 3 group) | | | | | | | | | | |
| -cortical atrophy (total) | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 3 |
| -Grade 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 |
| -Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| -Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 |
| -Grade 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -vacuolation of zona glomerulosa (total) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Bronchial LN (n = 3/group) | | | | | | | | | | |
| -lymphoid depletion (total) | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 3 |
| -Grade 1 | 0 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 0 |
| -Grade 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| -Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -Grade 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Mesenteric LN (n = 3/group) | | | | | | | | | | |
| -lymphoid depletion (total) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Prostate gland (n = 3/group) | | | | | | | | | | |
| -focal lymphoid hyperplasia (total) | 0 | - | 0 | - | 0 | - | 0 | - | 1 | - |
| -Grade 4 | 0 | | 0 | | 0 | | 0 | | 1 | |
| Mandibular glands (n = 3/group) | | | | | | | | | | |
| -focal atrophy (total) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| -Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

Toxicokinetics: Using a deposition factor of approximately 25%, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were 0.52 + 0.032, 2.90 + 0.18, and 14.6 + 0.89 µg/kg/day, respectively. C_{max} and AUC values for budesonide were approximately proportional to dose. The AUC for formoterol with the low dose was smaller than expected based upon values at mid and high doses. AUC values for formoterol at mid and high doses were approximately proportional to dose. C_{max} values for formoterol were approximately proportional to dose.

Reviewer: Timothy W. Robison, Ph.D.

IND No. 63.394

Toxicokinetic parameters for budesonide in dogs that were treated with a Symbicort HFA pMDI for 3 months.

| Day | Low Dose | | Mid Dose | | High Dose | |
|-----|----------|-----------|----------|-----------|-----------|-----------|
| | Cmax, pM | AUC, pMhr | Cmax, pM | AUC, pMhr | Cmax, pM | AUC, pMhr |
| 7 | 0.244 | N.C. | 1.64 | 2.56 | 7.71 | 8.86 |
| 87 | 0.448 | 0.630 | 2.56 | 3.25 | 11.7 | 13.0 |

Toxicokinetic parameters for formoterol in dogs that were treated with a Symbicort HFA pMDI for 3 months.

| Day | Low Dose | | Mid Dose | | High Dose | |
|-----|----------|-----------|----------|-----------|-----------|-----------|
| | Cmax, pM | AUC, pMhr | Cmax, pM | AUC, pMhr | Cmax, pM | AUC, pMhr |
| 7 | 9.22 | N.C. | 69.9 | 295 | 254 | 1380 |
| 87 | 14.6 | 24.5 | 95.5 | 301 | 300 | 1110 |

Summary of individual study findings:

In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort pMDI formulation containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227. Target doses of budesonide and formoterol in the low, mid, and high dose groups were $2.0 + 0.11$, $10 + 0.56$, and $50.0 + 2.80$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Using a deposition factor of approximately 25%, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were $0.52 + 0.032$, $2.90 + 0.18$, and $14.6 + 0.89$ $\mu\text{g}/\text{kg}/\text{day}$, respectively.

The NOAEL was identified as the low dose.

Sinus tachycardia (i.e., increased heart rate) was observed in mid and high dose groups on day 1 and during week 2. No changes were evident during week 12. Target organs of toxicity were the adrenal cortex, thymus, and bronchial lymph nodes. Observed histopathological changes appear to be primarily attributable to the effects of budesonide. Cortical atrophy of the adrenal cortex was observed for male and female mid and high dose groups. The incidence and severity of thymic atrophy was increased for the male and female mid and high dose groups. The incidence and severity of lymphoid depletion in the bronchial lymph nodes was increased for the male and female high dose groups.

There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to excipients, PVP K-25 and PEG-1000, or the propellant, HFA-227. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control were approximately 0.10, 31.5, and 42000 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The deposition factor for budesonide, formoterol, PVP K-25, and PEG-1000 was estimated to approximately 25%. Given that HFA-227 is a gas, deposition was assumed to be 100%. The aerosol concentrations of excipients were not measured. The PVP K-25, PEG-1000, and HFA-227 doses were estimated from the measured dose of budesonide using the ratio of the four materials in the pMDI formulation on the assumption that the aerosol generation efficiency was similar. However, the aerosol generation efficiency of HFA-227 would have been 100% (gas). The excipient doses for the vehicle-control group (Group 2) are assumed to have been the same as those for the high dose group.

Study title: Symbicort (Budesonide + Formoterol): 3-Month Inhalation Study in Dogs Using a Dry Powder Formulation.

Key study findings:

- ◆ In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort formulation containing budesonide and formoterol as a dry micronized powder blend with lactose. Target doses of budesonide and formoterol for the low, mid, and high dose groups were 2.0 + 0.11, 10.0 + 0.56, and 50 + 2.8 µg/kg/day, respectively. Using a deposition factor of approximately 25%, deposited doses of budesonide and formoterol for low, mid, and high dose group were 0.44 + 0.022, 2.19 + 0.11, and 10.9 + 0.57 µg/kg/day, respectively. Comparator groups were treated with 50 µg/kg/day budesonide alone or 3.0 µg/kg/day formoterol alone (deposited doses of 10.3 and 0.56 µg/kg/day, respectively). A control group received air only.
- ◆ The NOAEL was identified as the low dose combination for male dogs.
- ◆ Body weight gain was reduced for the male and female dogs that received budesonide only and mid and high doses of budesonide + formoterol.
- ◆ On day 1, elevated heart rates were evident for male and female dogs that received formoterol only or the high dose of budesonide + formoterol. During week 2, elevated heart rates were evident in male and female dogs that received formoterol only and low, mid, and high doses of budesonide + formoterol. No changes in heart rate were evident during week 12.
- ◆ Target organs of toxicity were the thymus, adrenal gland, and spleen, and toxicities could be primarily attributed to the effects of budesonide.
- ◆ Thymic atrophy was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide + formoterol.
- ◆ Atrophy of the zona fasciculata of the adrenal gland was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide and formoterol.
- ◆ Vacuolation of the zona glomerulosa of the adrenal gland was observed for the male and female dogs that received budesonide only and male dogs that received the high dose of budesonide + formoterol.
- ◆ Lymphoid depletion in the spleen was observed for female dogs that received budesonide and male dogs that received mid and high doses of budesonide + formoterol.

Study no: 99023-01

Volume #, and page #: Volume 8 of 25, Pages 5 to 436

Conducting laboratory and location: Safety Assessment
 AstraZeneca R&D
 Bakewell Road
 Loughborough
 Leicestershire LE11 5RH
 United Kingdom

Date of study initiation:

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity:

| Formulation Batch Number | Used in Groups | Material | % w/w | Test Batch Number |
|--------------------------|----------------|-------------------------------------|-------|-------------------|
| II | 2 | Budesonide Lactose | | 1849/98 |
| III | 3 | Formoterol Lactose | | ZB 202 |
| IV | 4, 5, and 6 | Budesonide Formoterol Lactose | | 1111/98 |

Formulation/vehicle: Symbicort, budesonide, and formoterol were formulated as micronized dry powder blends with lactose. The formulations were lightly packed by hand into powder reservoirs each day, for use in aerosol generators. The control group was exposed to air only.

Methods (unique aspects): A Symbicort formulation, containing budesonide and formoterol as a dry micronized powder blend with lactose, was administered daily, by inhalation, to 3 groups of 3 beagle dogs/sex/group for 3 months (Groups 4 to 6). Another two groups were exposed daily to either budesonide (Group 2) or formoterol (Group 3), as micronized dry powder blends with lactose, and served as reference-controls. A control group was exposed daily to air only (Group 1).

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 3 beagle dogs/sex/group

Satellite groups used for toxicokinetics or recovery: None.

Age: Animals were 6-7 months of age at the start of dosing.

Weight: Body weight ranges at the start of dosing were 8.9-10.7 kg for male dogs and 8.7-10.7 kg at the start of dosing.

Doses in administered units:

$$\text{Dose } (\mu\text{g/kg}) = \frac{\text{Minute volume} \times \text{Concentration } (\mu\text{g/L}) \times \text{Time (minutes)}}{\text{BW (kg)}}$$

Minute volume = 5 L/min (respiratory volume/minute in liters)

Concentration = mean Reading x relevant substance correction factor (SCF)

Time = duration of exposure (min)

BW = body weight (kg)

Target doses (µg/kg/day)

| Dose Group/Treatment | Target Dose Level, µg/kg | |
|----------------------|--------------------------|------------|
| | Budesonide | Formoterol |
| 1 - Air Control | 0 | 0 |
| 2 - Budesonide | 50 | 0 |
| 3 - Formoterol | 0 | 2.8 |
| 4 - B + F | 2.0 | 0.11 |
| 5 - B + F | 10.0 | 0.56 |
| 6 - B + F | 50 | 2.8 |

Aerosol concentrations delivered to dogs were monitored continuously using an aerosol monitor. Concentrations of budesonide and formoterol were determined from readings and substance correction factors.

Aerosol samples (5 min, corresponding to 40L of aerosol) were collected from each dosing device (4-8 samples per device, one device per formulation). Samples were collected onto filters that were held in open faced filter units sealed onto the outlet of the dosing system (in place of the dog mask). All filters were retained for determination of amounts of budesonide and formoterol using a HPLC method.

Particle size distribution of the aerosol (budesonide and formoterol) leaving each dosing device was determined at least twice during the study period using a cascade impactor. Quantities of budesonide and formoterol deposited on each stage were determined using a HPLC method.

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Inhaled and deposited doses ($\mu\text{g}/\text{kg}/\text{day}$) for male (M) and female (F) dogs.

| Group | Concentration, $\mu\text{g}/\text{L}$ | | Time, min | Inhaled Dose, $\mu\text{g}/\text{kg}/\text{day}$ | | MMAD, μm | Deposited Dose, $\mu\text{g}/\text{kg}/\text{day}$ | |
|-----------|---------------------------------------|-------------|-----------|--|----------------------|---------------------|--|----------------------|
| | B | F | | B | F | | B/F | B |
| 1 - Air | 0 | 0 | 5 | 0 | 0 | - | 0 | 0 |
| 2 - B | 16.28-22.84 | 0 | 5 | M: 47.6 F: 48.7 | 0 | | M: 10.2 F: 10.4 | 0 |
| 3 - F | 0 | 1.05-1.31 | 5 | 0 | M: 2.56 F: 2.73 | | 0 | M: 0.54 F: 0.57 |
| 4 - B + F | 0.71-1.12 | 0.037-0.058 | 5 | M: 2.01 F: 2.05 | M: 0.104 F: 0.106 | | M: 0.43 F: 0.44 | M: 0.021 F: 0.022 |
| 5 - B + F | 3.84-4.92 | 0.19-0.25 | 5 | M: 9.87 F: 9.66 | M: 0.51 F: 0.50 | | M: 2.21 F: 2.16 | M: 0.11 F: 0.11 |
| 6 - B + F | 18.09-22.72 | 0.98-1.23 | 5 | M: 50.0 F: 49.2 | M: 2.70 F: 2.66 | | M: 11.0 F: 10.8 | M: 0.57 F: 0.56 |

Aerosol concentrations of lactose were not measured during the study; however, doses were estimated from measured doses of budesonide and formoterol in conjunction with ratios of materials in formulations. It was assumed that particle size distributions of each component were similar and therefore, the aerosol generation efficiency was similar.

Inhaled and deposited doses of lactose ($\mu\text{g}/\text{kg}/\text{day}$). Deposition of budesonide and formoterol were estimated to be 21.2-22.4% and 20.6-21.8%, respectively.

| Group | Inhaled Dose, $\mu\text{g}/\text{kg}/\text{day}$ | Deposited Dose, $\mu\text{g}/\text{kg}/\text{day}$ |
|-----------|--|--|
| 1 - Air | 0 | 0 |
| 2 - B | 519 | 111 |
| 3 - F | 130 | 27 |
| 4 - B + F | 20 | 4 |
| 5 - B + F | 95 | 21 |
| 6 - B + F | 492 | 107 |

Route, form, volume, and infusion rate: Oral Inhalation

Test formulations were administered by inhalation as an aerosol generated from a powder aerosol generator. Each dog was restrained by a handler and a close-fitting facemask and aerosol delivery tube system was used to deliver the aerosol directly into the buccal cavity of the dog.

Observations and times:

Clinical signs: Animals were observed daily, before, during, and after dosing, for clinical signs of toxicity. Detailed examinations were conducted once per week.

Body weights: Body weights were measured once per week.

Food consumption: Food consumption was measured daily. Weekly individual and group mean food consumption were calculated and reported.

Ophthalmoscopy: Ophthalmic examinations were performed prior to dosing and during week 13.

EKG: Electrocardiograms (6-Lead) were recorded from each animal prior to dosing (week -2), on day 1, and during weeks 2 and 12. Recordings were conducted 1 hr after dosing. In addition, on day 1 and during weeks 2 and 12, heart rate (pulse) was recorded manually pre-dose, immediately postdose, and at 4 and 24 hr after dosing.

Hematology: Blood for determination of hematology parameters was collected prior to the start of dosing at weeks -2 and -1, and during the treatment period at weeks 7 and 13. During the treatment period, all blood samples were collected prior to dosing.

Clinical chemistry: Blood for determination of clinical chemistry parameters was collected prior to the start of dosing at weeks -2 and -1, and during the treatment period at weeks 7 and 13. During the treatment period, all blood samples were collected prior to dosing.

Urinalysis: Urine samples for analyses were collected prior to the start of dosing at week -2 and during the treatment period at weeks 7 and 13.

Gross pathology: After a minimum of 13 consecutive weeks of treatment, all animals were necropsied according to a predetermined sequence in which males preceded females.

Organs weighed: Absolute and relative organ weights were determined for the adrenal glands, brain, kidneys, heart, liver, lungs, ovaries, prostate, spleen, testes, thymus, thyroid, uterus, and pituitary.

Histopathology: Samples of all tissues from all animals retained at necropsy were processed into paraffin blocks. Sections were cut at between approximately 4-5 μm , stained with hematoxylin and eosin, and examined by light microscopy.

Toxicokinetics: Plasma concentrations of budesonide and/or formoterol were measured in plasma samples collected from all animals in Groups 2-6 on days 7 and 87 at predose and at 10, 30, 120, 240, and 360 min after dosing. The plasma concentrations of budesonide were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). The plasma concentrations of formoterol were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). The limits of quantitation for budesonide and formoterol were 0.025 nmol/L and 5.0 pmol/L, respectively.

Other: Basal and ACTH-stimulated cortisol levels were measured in blood samples collected from all animals during weeks -1 and 13. Cortisol levels were quantified using an immunoassay method with a defined lower limit of quantitation at 10 nmol/L.

Results:

Mortality: None.

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

Body weights: Body weight gains were suppressed for male and female dogs in Group 2 (budesonide only) and Group 6 (high dose of budesonide + formoterol). Body weight gains were slightly suppressed for male and female dogs in Group 5 (mid dose of budesonide + formoterol).

Body weights for male dogs in the air-control group during weeks 1 and 14 were 10266.7 and 12566.7 g, respectively, yielding a 22.4% increase of initial body weight. Body weights at week 14 for male dogs in Groups 2, 3, 4, 5, and 6 were increased by 9.9,

21.6, 22.4, 19.2, and 14.9% of body weights during week 1, respectively. Body weights of female dogs in the air-control group during weeks 1 and 14 were 9066.7 and 10666.7 g, respectively, yielding a 17.6% increase of initial body weight. Body weights at week 14 for female dogs in Groups 2, 3, 4, 5, and 6 were increased by 7.5, 21.2, 20.2, 15.7, and 13.2% of body weights during week 1, respectively.

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

Ophthalmoscopy: Ophthalmic examinations found no treatment-related effects.

Electrocardiography: On day 1, elevated heart rates (i.e., pulse) were evident in Groups 3 (formoterol only) and 6 (high dose of budesonide + formoterol). During week 2, elevated heart rates were evident in Groups 3 (formoterol only), Group 4 (low dose of budesonide + formoterol), Group 5 (mid dose of budesonide + formoterol), and Group 6 (high dose of budesonide + formoterol). The sponsor reported a mild to moderate sinus tachycardia in Groups 3 (formoterol only) and 6 (high dose of budesonide + formoterol) on day 1 at 1 hr after dosing. No changes in heart rate were evident during week 12. There were no treatment-related changes in electrocardiographic parameters (i.e., PR interval, QRS interval, QT interval, and QTc interval).

Day 1: Heart rates (bpm). Notable changes have been marked in bold.

| Group | Pre-Dose | Post-Dose | 1 hr | 4 hr | 24 hr |
|-------------|----------|---------------------|---------------------|---------------------|---------------------|
| 1 – Males | 96 | 88 (91.7%) | 105 (109.4%) | 113 (117.7%) | 89 (92.7%) |
| 1 – Females | 101 | 100 (99%) | 121 (119.8%) | 112 (110.9%) | 93 (92.1%) |
| 3 – Males | 108 | 125 (115.7%) | 148 (137%) | 137 (126.9%) | 96 (88.9%) |
| 3 – Females | 91 | 144 (158.2%) | 164 (180.2%) | 111 (122%) | 115 (126.4%) |
| 6 – Males | 95 | 118 (124.2%) | 168 (176.8%) | 113 (118.9%) | 108 (113.7%) |
| 6 – Females | 99 | 111 (112%) | 144 (145.4%) | 132 (133.3%) | 114 (115.2%) |

Week 2: Heart rates (bpm). Notable changes have been marked in bold.

| Group | Pre-Dose | Post-Dose | 1 hr | 4 hr | 24 hr |
|-------------|----------|---------------------|---------------------|---------------------|---------------------|
| 1 – Males | 97 | 107 (110.3%) | 105 (108.2%) | 92 (94.8%) | 105 (108.2%) |
| 1 – Females | 99 | 84 (84.8%) | 95 (96%) | 112 (113%) | 105 (106.1%) |
| 2 – Males | 88 | 84 (95.5%) | 106 (120.5%) | 127 (144.3%) | 104 (118.2%) |
| 2 – Females | 93 | 76 (81.7%) | 105 (112.9%) | 131 (140.9%) | 101 (108.6%) |
| 3 – Males | 80 | 117 (146.3%) | 127 (158.8%) | 135 (168.8%) | 100 (125%) |
| 3 – Females | 99 | 96 (97%) | 128 (129.3%) | 136 (137.4%) | 127 (128.3%) |
| 4 – Males | 84 | 120 (142.9%) | 123 (146.4%) | 109 (129.7%) | 111 (132.1%) |
| 4 – Females | 88 | 104 (118.2%) | 104 (118.2%) | 131 (148.9%) | 137 (155.7%) |
| 5 – Males | 88 | 99 (112.5%) | 115 (130.7%) | 125 (142%) | 95 (108%) |
| 5 – Females | 85 | 93 (109.4%) | 112 (131.8%) | 125 (147%) | 103 (121.2%) |
| 6 – Males | 100 | 117 (117%) | 130 (130%) | 125 (125%) | 93 (93%) |
| 6 – Females | 96 | 105 (109.4%) | 121 (126%) | 117 (121.9%) | 92 (95.8%) |

Hematology: Decreased white blood cell, neutrophil, lymphocyte, and eosinophil counts were observed for male treatment groups that may correlate, in part, with effects of budesonide (i.e., Groups 2, 4, 5, and 6). However, corresponding changes were not observed for female treatment groups. Further, changes were generally small and high

variability was present in counts for some cell types (i.e., eosinophils) that made treatment relationships unclear.

Week 7:

White blood cell counts for male dogs in Groups 2, 5, and 6 were decreased to 81.6, 80.4, and 83.2% of the air-control ($14.336 \times 10^9/L$), respectively. Neutrophil counts for male dogs in Groups 2, 5, and 6 were decreased to 86.8, 81.4, and 79% of the air-control ($7.776 \times 10^9/L$), respectively. Lymphocyte counts for male dogs in Groups 2, 4, 5, and 6 were decreased to 78.2, 76.5, 82.1, and 81.6% of the air-control ($5.193 \times 10^9/L$), respectively. Monocyte counts for male dogs in Group 2 were decreased to 65.4% of the air-control ($0.973 \times 10^9/L$), respectively. Eosinophil counts for male dogs in Groups 2, 3, 4, 5, and 6 were decreased to 55.4, 76.2, 54.5, 42.6, and 42.6% of the air-control ($0.336 \times 10^9/L$), respectively.

White blood cell and neutrophil counts for female dogs in Group 3 were elevated to 136.5 and 146.4% of the air-control ($12.143 \times 10^9/L$ and $7.010 \times 10^9/L$), respectively. Monocyte counts for female dogs in Group 6 were increased to 154.5% of the air-control ($0.660 \times 10^9/L$). Eosinophil counts for female dogs in Groups 2, 4, 5, and 6 were decreased to 42.9, 66.6, 57.1, and 46.6% of the air-control ($0.350 \times 10^9/L$), respectively.

Week 13: White blood cell counts for male dogs in Groups 2 and 6 were decreased to 84.7 and 88.7% of the air-control ($12.466 \times 10^9/L$), respectively. Lymphocyte counts for male dogs in Groups 2, 4, 5, and 6 were decreased to 68.3, 80.1, 89.8, and 76.4% of the air-control ($4.966 \times 10^9/L$), respectively. Monocyte counts for male dogs in Group 2 were decreased to 76.1% of the air-control ($0.796 \times 10^9/L$), respectively. Eosinophil counts for male dogs in Groups 2, 3, 4, 5, and 6 were decreased to 32.7, 76.9, 48.1, 44, and 18.3% of the air-control ($0.416 \times 10^9/L$), respectively.

Monocyte counts for female dogs in Groups 3 and 6 were increased to 134.15 and 127.6% of the air-control ($0.653 \times 10^9/L$), respectively. Eosinophil counts for female dogs in Groups 5 and 6 were decreased to 63.8 and 57.7% of the air-control ($0.260 \times 10^9/L$), respectively; however, counts for female dogs in Group 3 were increased to 166.5% of the air-control. Activated partial thromboplastin times for female dogs in Groups 2, 3, 4, 5, and 6 were increased to 117.2, 132.9, 113.8, 124, and 128.7% of the air-control (13.36 sec), respectively.

Clinical chemistry: Elevations of triglyceride, cholesterol, total protein, and albumin levels were evident primarily for Groups 2 and 6 during weeks 7 and 13. These changes may be due to budesonide treatment.

Week 7:

Triglyceride levels for male dogs in Groups 2 and 6 were increased to 150 and 146.9% of the air-control (0.320 mmol/L), respectively. Total protein levels for male dogs in Groups 2 and 6 were increased to 111.7 and 111% of the air-control (51.3 g/L), respectively. Albumin levels for male dogs in Groups 2 and 6 were increased to 109.4 and

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112.9% of the air-control (28.6 g/L), respectively. Alkaline phosphatase activities for male dogs in Group 6 were increased to 114.3% of the air-control (95.0 IU/L).

Cholesterol levels for female dogs in Groups 2, 5, and 6 were increased to 154.5, 148.2, and 173.3% of the air-control (2.670 mmol/L), respectively. Triglyceride levels for female dogs in Groups 2, 5, and 6 were increased to 207.7, 157.5, and 200% of the air-control (0.313 mmol/L), respectively. Total protein levels for female dogs in Groups 2 and 6 were increased to 115.9 and 113.9% of the air-control (50.3 g/L), respectively. Albumin levels for female dogs in Groups 2 and 6 were increased to 104.6 and 106.5% of the air-control (30.6 g/L), respectively. Alkaline phosphatase activities for female dogs in Groups 2, 5, and 6 were increased to 152.6, 141.8, and 114.1% of the air-control (78.0 IU/L), respectively.

Week 13:

Triglyceride levels for male dogs in Groups 2 and 6 were increased to 123.5 and 137.4% of the air-control (0.310 mmol/L), respectively. Alkaline phosphatase activities for male dogs in Group 6 were increased to 124.3% of the air-control (76.6 IU/L).

Cholesterol levels for female dogs in Groups 2, 5, and 6 were increased to 172.4, 161.8, and 199.6% of the air-control (2.513 mmol/L), respectively. Triglyceride levels for female dogs in Groups 2, 3, 4, 5, and 6 were increased to 162.5, 176.1, 176.1, 200, and 215% of the air-control (0.293 mmol/L), respectively. Phosphate levels for female dogs in Group 6 were increased to 116% of the air-control (1.726 mmol/L). Total protein levels for female dogs in Groups 2 and 6 were increased to 112.9 and 114.9% of the air-control (51.0 g/L), respectively. Albumin levels for female dogs in Groups 2 and 6 were increased to 104.4 and 108.5% of the air-control (31.6 g/L), respectively. Alkaline phosphatase activities for female dogs in Groups 2, 5, and 6 were increased to 141.1, 157.8, and 144.2% of the air-control (78.0 IU/L), respectively.

Urinalysis: Urinary osmolality during week 13 was decreased for male and female dogs in Group 2 (budesonide only) and Group 6 (high dose of budesonide + formoterol). The toxicological significance of decreased urinary osmolality was unclear, given that these changes were not statistically significant due to high variability within groups. No changes in urinalysis parameters were evident during week 7.

During week 13, urinary osmolalities for male dogs in Groups 2 and 6 were decreased to 47.8 and 36.6% of the air-control (1409.6 mosmol/kg), respectively. During week 13, urinary osmolalities for female dogs in Groups 2 and 6 were decreased to 54.4 and 48.2% of the air-control (876.0 mosmol/kg), respectively.

Organ weights: Absolute and relative adrenal gland and thymus weights were decreased for male and female dogs in Groups 2, 4, 5, and 6, and appeared to correlate with histopathological findings. These changes appeared to be attributable to the effects of budesonide. Changes were observed for a few other organs, although, there were no correlations to histopathological findings.

Thymus: Absolute thymus weights for male dogs in Groups 2, 3, 4, 5, and 6 were decreased to 60.4, 84.7, 71.2, 62.3, and 53.1% of the air-control (11.013 g), respectively. Relative thymus weights for male dogs in Groups 2, 3, 4, 5, and 6 were decreased to 70.4, 92.6, 76.7, 69.4, and 61.8% of the air-control (0.08710%), respectively. Absolute thymus weights for female dogs in Groups 2, 3, 4, 5, and 6 were decreased to 49.2, 84.1, 63.2, 48.9, and 41.5% of the air-control (15.324 g), respectively. Relative thymus weights for female dogs in Groups 2, 3, 4, 5, and 6 were decreased to 51.3, 78.1, 58.6, 44.5, and 40.7% of air-control (0.14359%), respectively. Changes in absolute and relative thymus weights for dogs in Group 3 (formoterol only) appeared to have little or no biological significance.

Adrenal gland: Absolute adrenal gland weights for male dogs in Groups 2, 3, 4, 5, and 6 were decreased to 44, 76.2, 77.4, 62.3, and 39.8% of the air-control (1.466 g), respectively. Relative adrenal gland weights for male dogs in Groups 2, 3, 4, 5, and 6 were decreased to 50.9, 81.6, 82, 69.1, and 45.6% of the air-control (0.01175%), respectively. Absolute adrenal gland weights for female dogs in Groups 2, 4, 5, and 6 were decreased to 46, 79.3, 74, and 50% of the air-control (1.251 g), respectively. Relative adrenal gland weights for female dogs in Groups 2, 3, 4, 5, and 6 were decreased to 47.1, 93.8, 71.3, 67.2, and 47.6% of the air-control (0.01183%), respectively. Changes in absolute and relative adrenal gland weights for dogs in Group 3 (formoterol only) appeared to have little or no biological significance.

Spleen: Absolute spleen weights for female dogs in Groups 2, 3, 4, 5, and 6 were increased to 156.7, 148.6, 150.6, 150.7, and 108.6% of the air-control (65.037 g), respectively. Relative spleen weights for female dogs in Groups 2, 3, 4, 5, and 6 were increased to 159.6, 138.2, 135.1, 137.3, and 105.1% of the air-control (0.61217%), respectively. No substantial changes were evident for male treatment groups. The magnitude of change observed for female dogs in Group 6 was minimal and suggests these observed changes for the spleen may have little or no relationship to treatment.

Thyroid gland: Absolute thyroid gland weights for male dogs in Groups 3, 4, 5, and 6 were decreased to 83.8, 85, 84.8, and 78.2% of the air-control (0.427 g), respectively.

Testes: Absolute testes weights for male dogs in Group 6 were decreased to 80.6% of the air-control (21.183 g).

Liver: Relative liver weights for male dogs in Groups 2, 5, and 6 were increased to 115.9, 119, and 121.2% of the air-control (3.11811%), respectively. Absolute and relative liver weights for female dogs in Group 6 were increased to 139.9 and 133.7% of air-control values (325.277 g and 3.05577%), respectively. Relative liver weights for female dogs in Groups 2 and 3 were increased to 122 and 113.9% of the air-control (3.05577%), respectively.

Lung: Absolute and relative lung weights for male dogs in Group 6 were decreased to 76.3 and 87.8% of air-control values (100.750 g and 0.80626%), respectively.

Pituitary: Relative pituitary gland weights for male dogs in Groups 2, 5, and 6 were increased to 118.6, 113.6, and 110.2% of the air-control (0.00059%), respectively.

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

Histopathology: Target organs of toxicity were the thymus, adrenal glands, and spleen.

Thymic atrophy was observed for male and female dogs in Groups 2, 5, and 6 as well as 1 female dog in Group 4. Atrophy of the zona fasciculata in the adrenal gland was observed for male and female dogs in Groups 2, 5, and 6 as well as 2 female dogs in Group 4. Vacuolation of the zona glomerulosa in the adrenal gland was observed for male and female dogs in Group 2 as well as 1 male dog each in Groups 4 and 6. Lymphoid depletion in the spleen was observed for 2 female dogs in Group 2 and 1 male dog each in Groups 5 and 6. Thymic and adrenal cortical atrophy can be attributed to treatment with budesonide and reflect either a direct action of the affected tissue or represent an indirect action due to hormonal perturbation mediated through the hypothalamic-pituitary axis.

Histopathological findings were observed in the skin/subcutis, mammary gland, heart, thyroid gland, and testes for dogs in Group 6 (high dose of budesonide and formoterol), although, the incidence for each finding was 1 of 3 and a treatment relationship was unclear.

Interstitial pneumonia (Groups 1, 2, 4, 5, and 6), alveolitis (Groups 2, 3, 4, 5, and 6), and alveolar casts (Group 6) were observed in the lungs, although, these are common background findings in beagle dogs.

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Histopathological changes for dogs.

| Tissue | Group 1 | | Group 2 | | Group 3 | | Group 4 | | Group 5 | | Group 6 | |
|------------------------------------|---------|---|---------|---|---------|---|---------|---|---------|---|---------|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| Thymus | | | | | | | | | | | | |
| -atrophy (total) | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 1 | 3 | 2 | 3 | 3 |
| Grade 1 | | | | | | | 1 | 1 | 1 | | | |
| Grade 2 | | | | | | | | | 2 | 1 | | |
| Grade 4 | | | 1 | 1 | | | | | | 1 | | |
| Grade 5 | | | 1 | 2 | | | | | | | 3 | 3 |
| Adrenal gland | | | | | | | | | | | | |
| -atrophy, zona fasciculata | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 |
| Grade 1 | | | | | | | 2 | 1 | 1 | | | |
| Grade 2 | | | | | | | | 2 | 2 | | | |
| Grade 4 | | | 2 | 2 | | | | | | | 2 | 1 |
| Grade 5 | | | 1 | 1 | | | | | | | 1 | 2 |
| -vacuolation, zona glom. | 0 | 0 | 3 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Grade 2 | | | 1 | 1 | | | 1 | | | | 1 | |
| Grade 3 | | | 2 | | | | | | | | | |
| Spleen | | | | | | | | | | | | |
| -lymphoid depletion | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| Grade 1 | | | | 1 | | | | | 1 | | 1 | |
| Grade 2 | | | | 1 | | | | | | | | |
| Skin/Subcutis | | | | | | | | | | | | |
| -basal cell tumor (B) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Mammary gland | | | | | | | | | | | | |
| -lobular hyperplasia, Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Lung | | | | | | | | | | | | |
| -alveolar casts, Grade 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| -interstitial pneumonia | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 |
| Grade 1 | | | | 2 | | | 1 | | 1 | | 1 | |
| Grade 2 | | 1 | | 1 | | | | | | 1 | | |
| -alveolitis | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| Grade 1 | | | | 1 | 1 | | 1 | 1 | 1 | | 1 | |
| Grade 2 | | | | | | 1 | | | | | | |
| Grade 3 | | | | | 1 | | | | | | | |
| -granuloma, Grade 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Heart | | | | | | | | | | | | |
| -myocardial fibrosis, Grade 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Thyroid gland | | | | | | | | | | | | |
| -C-cell hyperplasia/focal, Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| -cyst, Grade 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Testes | | | | | | | | | | | | |
| -spermat. giant cells, Grade 1 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 1 | 0 |

Toxicokinetics: For Groups 4, 5, and 6, AUC values for budesonide and formoterol on days 7 and 87 increased with elevating dose, although, increases were generally less than proportional to dose. For Groups 4, 5, and 6, C_{max} and AUC values for budesonide on day 87 were greater than values observed on day 7. For Groups 4, 5, and 6, C_{max} values for formoterol on day 87 were greater than values observed on day 7. For Groups 4 and 5, AUC values for formoterol on day 87 were greater than values observed on day 7; however, for Group 6, AUC values were comparable on days 7 and 87. Higher C_{max} and AUC values for budesonide and formoterol on day 87 as compared to day 7 may be related to attainment of steady state drug levels. There were no sex-related differences in toxicokinetic parameters.

Table 3. Exposure parameters for budesonide. The values given are the mean of the male and female values.

| Group No. | 1 week | | | | 3 months | | | |
|---|-----------|------------|------------|------------|-----------|------------|------------|------------|
| | 2 | 4 | 5 | 6 | 2 | 4 | 5 | 6 |
| Actual dose budesonide/ formoterol, µg/kg | 50.7/ 0.0 | 2.43/ 0.13 | 9.76/ 0.50 | 53.4/ 2.89 | 51.0/ 0.0 | 2.54/ 0.13 | 9.33/ 0.48 | 49.4/ 2.67 |
| AUC _{0-24h} (pmolh/L) | 10.04 | 0.76 | 2.71 | 9.27 | 15.40 | 1.57 | 4.31 | 11.82 |
| AUC/dose ¹ | 0.20 | 0.31 | 0.28 | 0.17 | 0.30 | 0.62 | 0.46 | 0.24 |
| C _{max} (pmol/L) | 9.02 | 0.25 | 1.22 | 5.38 | 7.72 | 0.59 | 1.82 | 10.3 |
| C _{max} /dose ¹ | 0.18 | 0.10 | 0.13 | 0.10 | 0.15 | 0.23 | 0.19 | 0.21 |
| T _{max} (h) | 0.083 | 0.5 | 0.5 | 0.5 | 0.083 | 0.5 | 0.083 | 0.083 |

¹The only purpose of the ratios AUC/dose and C_{max}/dose is to facilitate the comparison of the respective parameter between the groups in this table, and it is therefore not appropriate to use the ratios for comparisons between parameters or between tables.

Table 4. Exposure parameters for formoterol. The values given are mean of the male and female values.

| Group No. | 1 week | | | | 3 months | | | |
|---|-----------|---------------------|------------|------------|-----------|------------|------------|------------|
| | 3 | 4 | 5 | 6 | 3 | 4 | 5 | 6 |
| Actual dose formoterol/ budesonide, µg/kg | 2.89/ 0.0 | 0.13/ 2.43 | 0.50/ 9.75 | 2.89/ 53.4 | 2.87/ 0.0 | 0.13/ 2.54 | 0.48/ 9.35 | 2.67/ 49.5 |
| AUC _{0-24h} (pmolh/L) | 1128 | (14) ² | 223 | 1140 | 996 | 109 | 366 | 1124 |
| AUC/dose ¹ | 0.39 | (0.11) ² | 0.45 | 0.39 | 0.35 | 0.84 | 0.76 | 0.42 |
| C _{max} (pmol/L) | 275 | 8.5 | 32.2 | 155 | 265 | 13.8 | 50.4 | 272 |
| C _{max} /dose ¹ | 0.095 | 0.065 | 0.064 | 0.054 | 0.092 | 0.106 | 0.105 | 0.102 |
| T _{max} (h) | 0.083 | 0.5 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 |

¹The only purpose of the ratios AUC/dose and C_{max}/dose is to facilitate the comparison of the respective parameter between the groups in this table, and it is therefore not appropriate to use the ratios for comparisons between parameters or between tables.

²uncertain value due to small number of datapoints.

Summary of individual study findings: In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort formulation containing budesonide and formoterol as a dry micronized powder blend with lactose. Target doses of budesonide and formoterol for the low, mid, and high dose groups were $2.0 + 0.11$, $10.0 + 0.56$, and $50 + 2.8$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Using a deposition factor of approximately 25%, deposited doses of budesonide and formoterol for low, mid, and high dose group were $0.44 + 0.022$, $2.19 + 0.11$, and $10.9 + 0.57$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Comparator groups were treated with 50 $\mu\text{g}/\text{kg}/\text{day}$ budesonide alone or 3.0 $\mu\text{g}/\text{kg}/\text{day}$ formoterol alone (deposited doses of 10.3 and 0.56 $\mu\text{g}/\text{kg}/\text{day}$, respectively). A control group received air only. The NOAEL was identified as the low dose combination for male dogs. Body weight gain was reduced for the male and female dogs that received budesonide only and mid and high doses of budesonide + formoterol. On day 1, elevated heart rates were evident for male and female dogs that received formoterol only or the high dose of budesonide + formoterol. During week 2, elevated heart rates were evident in male and female dogs that received formoterol only and low, mid, and high doses of budesonide + formoterol. No changes in heart rate were evident during week 12. Target organs of toxicity were the thymus, adrenal gland, and spleen, and toxicities could be primarily attributed to the effects of budesonide. Thymic atrophy was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide + formoterol. Atrophy of the zona fasciculata of the adrenal gland was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide and formoterol. Vacuolation of the zona glomerulosa of the adrenal gland was observed for the male and female dogs that received budesonide only and male dogs that received the high dose of budesonide + formoterol. Lymphoid depletion in the spleen was observed for female dogs that received budesonide and male dogs that received mid and high doses of budesonide + formoterol.

Toxicology summary:

In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227. Total doses of budesonide and formoterol in the low, mid, and high dose groups were $2 + 0.11$, $10 + 0.56$, and $51 + 2.9$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Using a deposition factor of 0.086 to 0.094, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were $0.2 + 0.010$, $0.8 + 0.048$, and $4.8 + 0.27$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Two additional groups were exposed daily to either an excipient-only HFA pMDI aerosol formulation (vehicle control) or to air only (air control). The NOAEL was identified as the mid dose. Body weight gains for male and female rats in the high dose group were reduced to 67 and 56% of the air-control, respectively. Thymus weights were decreased for male and female rats at the high dose, although, there were no corresponding histopathological findings. There were no target organs of toxicity. Decreases of body weight gains and thymus weights could be attributed to the effects of

budesonide. There was no evidence of additive or synergistic toxic effects between formoterol and budesonide. There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to PVP K-25, PEG-1000, and HFA-227 at deposited doses of 0.03, 8.6, and 30300 µg/kg/day, respectively.

In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort formulation containing budesonide and formoterol as a micronized dry powder blend. Total target doses of budesonide and formoterol in the low, mid, and high dose groups were 2 + 0.12, 10 + 0.60, and 50 + 3.0 µg/kg/day, respectively. Using a deposition factor of approximately 10%, deposited doses for budesonide and formoterol in the low, mid, and high dose combination groups were 0.30 + 0.018, 1.3 + 0.073, and 4.8 + 0.25 µg/kg/day, respectively. Positive control groups were treated with target doses of 50 µg/kg/day budesonide alone or 3.0 µg/kg/day formoterol alone (actual deposited doses of 7.2 and 0.24 µg/kg/day, respectively). A control group received air only. The NOAEL was identified as the mid dose combination. Target organs of toxicity were the thymus and spleen. Observed changes can be primarily attributed to the effects of budesonide. The incidence of lymphocytolysis in the thymus was increased for male and female rats in the budesonide only group and male and female rats in the high dose budesonide + formoterol group. The incidence and magnitude of extramedullary hematopoiesis was decreased for male rats in the budesonide only group and male rats in the high dose budesonide + formoterol group. Systemic exposures to budesonide and formoterol were generally greater with the dry powder formulation as compared to the HFA pMDI formulation.

In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227. Target doses of budesonide and formoterol in the low, mid, and high dose groups were 2.0 + 0.11, 10 + 0.56, and 50.0 + 2.80 µg/kg/day, respectively. Using a deposition factor of approximately 25%, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were 0.52 + 0.032, 2.90 + 0.18, and 14.6 + 0.89 µg/kg/day, respectively. The NOAEL was identified as the low dose. Sinus tachycardia (i.e., increased heart rate) was observed in mid and high dose groups on day 1 and during week 2. No changes in heart rate were evident during week 12. Target organs of toxicity were the adrenal cortex, thymus, and bronchial lymph nodes. Observed histopathological changes appear to be primarily attributable to the effects of budesonide. Cortical atrophy of the adrenal cortex was observed for male and female mid and high dose groups. The incidence and severity of thymic atrophy was increased for the male and female mid and high dose groups. The incidence and severity of lymphoid depletion in the bronchial lymph nodes was increased for the male and female high dose groups. There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to PVP K-25, PEG-1000, and HFA-227 at deposited doses of 0.10, 31.5, and 42000 µg/kg/day, respectively.

In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort formulation containing budesonide and formoterol as a dry micronized powder blend with lactose. Target doses of budesonide and formoterol for the low, mid, and high dose groups were 2.0 + 0.11, 10.0 + 0.56, and 50 + 2.8 µg/kg/day, respectively. Using a

deposition factor of approximately 25%, deposited doses of budesonide and formoterol for low, mid, and high dose group were 0.44 ± 0.022 , 2.19 ± 0.11 , and 10.9 ± 0.57 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Comparator groups were treated with 50 $\mu\text{g}/\text{kg}/\text{day}$ budesonide alone or 3.0 $\mu\text{g}/\text{kg}/\text{day}$ formoterol alone (deposited doses of 10.3 and 0.56 $\mu\text{g}/\text{kg}/\text{day}$, respectively). A control group received air only. The NOAEL was identified as the low dose combination for male dogs. Body weight gain was reduced for the male and female dogs that received budesonide only and mid and high doses of budesonide + formoterol. On day 1, elevated heart rates were evident for male and female dogs that received formoterol only or the high dose of budesonide + formoterol. During week 2, elevated heart rates were evident in male and female dogs that received formoterol only and low, mid, and high doses of budesonide + formoterol. No changes in heart rate were evident during week 12. Target organs of toxicity were the thymus, adrenal gland, and spleen, and toxicities could be primarily attributed to the effects of budesonide. Thymic atrophy was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide + formoterol. Atrophy of the zona fasciculata of the adrenal gland was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide and formoterol. Vacuolation of the zona glomerulosa of the adrenal gland was observed for the male and female dogs that received budesonide only and male dogs that received the high dose of budesonide + formoterol. Lymphoid depletion in the spleen was observed for female dogs that received budesonide and male dogs that received mid and high doses of budesonide + formoterol.

Toxicology conclusions: Toxicities observed in 3-month bridging studies with Symbicort 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. Safety margins derived from comparing NOAELs of preclinical studies with Symbicort to clinical doses were significantly less than 1 (see page 62 and 63), given that rats and dogs are known to be more sensitive to the toxic effects of steroids as compared to humans.

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Histopathology Inventory for IND # 63,394

| Study | 3-month rat study with Symbicort HFA pMDI #00342 | 3-month rat study with Symbicort Dry Powder #99022-01 | 3-month dog study with Symbicort HFA pMDI #00047-01 | 3-month dog study with Symbicort Dry Powder #99023-01 |
|----------------------------|--|---|---|---|
| Species | Rat | Rat | Dog | Dog |
| Adrenals | X* | X* | X* | X* |
| Aorta | X(+) | X | X | X |
| Bone Marrow smear | | X (not examined) | X (not examined) | X (not examined) |
| Bone (femur) | X | | X | X |
| Brain | X* | X* | X* | X* |
| Cecum | X (+) | X | X | X |
| Cervix | | | X | X |
| Colon | X | X | X | X |
| Duodenum | X | X | X | X |
| Epididymis | X | X* | | |
| Esophagus | X | X | X | X |
| Eye | X | X | X | X |
| Fallopian tube | | | | |
| Gall bladder | | | X | X |
| Gross lesions | X | | X | X |
| Harderian gland | X (+) | X | | |
| Heart | X* | X* | X* | X* |
| Ileum | X | X | X | X |
| Injection site | | | | |
| Jejunum | X | X | X | X |
| Kidneys | X* | X* | X* (+ ureters) | X* (+ ureters) |
| Lachrymal gland | | | X | X |
| Larynx | X | X | X | X |
| Liver | X* | X* | X* | X* |
| Lungs | X* | X* | X* | X* |
| Lymph nodes, cervical | | X | X | X |
| Lymph nodes mandibular | | | | |
| Lymph nodes, mesenteric | X | X | X | X |
| Lymph nodes, Axillary | X | | | |
| Lymph nodes, Bronchial | | X | X | X |
| Lymph nodes, submandibular | | X | | |
| Mammary Gland | X | X | X | X |
| Nasal cavity | X | X | X (nares, nasopharynx) | X (nares, nasopharynx) |
| Optic nerves | X | | X | X |

| | | | | |
|------------------|------|-----------|--|--|
| Ovaries | X* | X* | X* | X* |
| Pancreas | X | X | X | X |
| Parathyroid | X | X* | X | X |
| Peripheral nerve | | | | |
| Pharynx | | X | X | X |
| Pituitary | X | X* | X* | X* |
| Prostate | X* | X* | X* | X* |
| Rectum | X(+) | X | X | X |
| Salivary gland | X | X* | X (parotid, submandibular, sublingual) | X (parotid, submandibular, & sublingual) |
| Sciatic nerve | X | X | X | X |
| Seminal vesicles | X | X* | | |
| Skeletal muscle | X | X (Thigh) | X | X |
| Skin | X | X | X | X |
| Spinal cord | X(+) | X | X | X |
| Spleen | X* | X* | X* | X* |
| Stemum | X | X | X | X |
| Stomach | X | X | X | X |
| Testes | X* | X* | X* | X* |
| Thymus | X* | X* | X* | X* |
| Thyroid | X | X* | X* | X* |
| Tongue | | X | X | X |
| Trachea | X | X | X (+ carina) | X (+ carina) |
| Urinary bladder | X | X | X | X |
| Uterus | X* | X* | X* | X* |
| Vagina | X | X | X | X |
| Zymbal gland | | | | |
| Preputial gland | X(+) | | | |
| Peyer's patches | | X | | |
| Clitoral gland | X(+) | | | |
| Standard List | | | | |

X, histopathology performed

*, organ weight obtained

(+) tissue collected but not examined

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

Symbicort® is a combination inhalation drug product consisting of a glucocorticoid (budesonide) and a long acting β_2 agonist (formoterol). The sponsor has proposed to develop Symbicort for the treatment of asthma in patients : _____ . Symbicort will available at _____ dosage levels of . _____ 80

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µg budesonide/4.5 µg formoterol per actuation, and 160 µg budesonide/4.5 µg formoterol per actuation. The inhalation device will be a pressurized metered dose inhaler (pMDI). The aerosol formulation contains excipients, povidone K25 (PVP K25) and polyethylene glycol 1000 (PEG-1000), and the propellant, 1,1,1,2,3,3,3-heptafluoropropane (HFA-227).

In the initial IND submission, the sponsor has proposed to conduct 4 clinical trials (i.e., SD-039-0716, SD-039-0717, SD-039-0718, and SD-039-719) with Symbicort HFA pMDI in the United States.

Protocol SD-039-0716 is a 12-week 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. Each group will consist of approximately 112 patients.

Protocol SD-039-0717 is a 12-week 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. Each group will consist of approximately 112 patients.

Protocol SD-039-0718 is a 12-week trial to compare the safety and efficacy of Symbicort HFA pMDI versus its monoproducts, Budesonide HFA pMDI and Formoterol Turbuhaler, in asthmatic children (6 to 11 years). Symbicort, a fixed combination product, containing budesonide and formoterol, 40/4.5 µg, respectively, will be administered as two inhalations twice daily. Budesonide (40 µg/puff) or formoterol (4.5 µg/inhalation) will be administered as two inhalations twice daily.

Protocol SD-039-719 is a 6-month open-label safety study to evaluate and compare the safety of Symbicort HFA pMDI with Pulmicort Turbuhaler in asthmatic children (6-11 years). Symbicort, a fixed combination product of budesonide and formoterol, 160/4.5 µg, respectively, will be administered as two inhalations twice daily. Pulmicort, 200 µg/inhalation, will be administered as 2 inhalations twice daily. The Symbicort and Pulmicort groups will consist of 100 and 50 patients, respectively.

In support of the proposed clinical trials, the sponsor has conducted extensive preclinical pharmacology and toxicology studies with monoproducts, budesonide (Pulmicort Turbuhaler, NDA 20-441) and formoterol (Oxis Turbuhaler,) that include carcinogenicity studies. In the present submission, the sponsor has submitted pharmacology studies, pharmacokinetic studies in healthy human volunteers, and toxicology studies to assess the effects of the combination of budesonide and formoterol as well as excipients, PVP K-25 and PEG-1000, and the propellant, HFA-227. For the

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Symbicort pMDI HFA drug product, 3-month inhalation toxicology studies with rats and dogs were provided. These studies included excipients, PVP K-25 and PEG-1000, and the propellant, HFA-227. For comparison, 3-month inhalation toxicology studies with the Symbicort dry powder in rats and dogs were provided. Three inhalation toxicology studies with budesonide, a 3-month study in rats, a 1-month study in dogs, and a teratology study in rats, were provided in the submission; however, these studies were not reviewed. In support of the excipients, PVP K-25 and PEG-1000, and propellant HFA-227, the sponsor provided several inhalation toxicology studies conducted

where similar excipients, povidone K-30 (PVP K-30) and polyethylene glycol 600 (PEG-600), and the same propellant, HFA-227, were used. These studies, included a 6-month study in rats and 6- and 12-month studies in dogs.

The sponsor conducted two pharmacology studies that examined the interactions of budesonide and formoterol. In vitro treatment of normal human bronchial epithelial cells with a combination of formoterol and budesonide produced an additive effect on inhibiting tumor necrosis factor α -induced granulocyte macrophage-colony stimulating factor. Inhibitory actions of propranolol on the effects of formoterol suggest the involvement of β_2 -receptors. Formoterol had a very low threshold concentration (i.e., 10^{-10} M) for reducing tumor necrosis factor α -induced GM-CSF production by bronchial epithelial cells. Development of tolerance (i.e., down regulation of β_2 -adrenoreceptor function) was evident following repeated in vivo inhalation treatment of guinea pigs with the β_2 -agonist, formoterol. Tolerance was evident from the rightward shift of the concentration-effect curve of formoterol for the relaxation effect on airway smooth muscle and the reduction of the density of β -adrenoreceptors in the lung. Inhalation treatment with budesonide, a glucocorticoid, in combination with formoterol did not alter the development of tolerance to formoterol (i.e., tachyphylaxis).

In a pharmacokinetic study with healthy human volunteers, plasma concentrations of budesonide and formoterol were compared following inhalation of a combination of budesonide and formoterol using Symbicort pMDI or Symbicort Turbuhaler (TBH). Symbicort pMDI or Turbuhaler (budesonide/formoterol), 160/4.5 μg , was given as 4 actuations all at one time corresponding to a total dose of 640/18 μg . While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID. Systemic exposure (i.e., AUC) to budesonide and formoterol was higher with the TBH as compared to the pMDI. Plasma AUC values for budesonide and formoterol delivered as 4 actuations all at one time with the pMDI were 4.83 nmol \cdot hr/L and 145 pmol \cdot hr/L, respectively.

In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227. Total doses of budesonide and formoterol in the low, mid, and high dose groups were $2 + 0.11$, $10 + 0.56$, and $51 + 2.9$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Using a deposition factor of 0.086 to 0.094, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were $0.2 + 0.010$, $0.8 + 0.048$, and $4.8 + 0.27$ $\mu\text{g}/\text{kg}/\text{day}$,

respectively. Two additional groups were exposed daily to either an excipient-only HFA pMDI aerosol formulation (vehicle control) or to air only (air control). The NOAEL was identified as the mid dose. Body weight gains for male and female rats in the high dose group were reduced to 67 and 56% of the air-control, respectively. Thymus weights were decreased for male and female rats at the high dose, although, there were no corresponding histopathological findings. There were no target organs of toxicity. Decreases of body weight gains and thymus weights could be attributed to the effects of budesonide. There was no evidence of additive or synergistic toxic effects between formoterol and budesonide. There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to PVP K-25, PEG-1000, and HFA-227 at deposited doses of 0.03, 8.6, and 30300 µg/kg/day, respectively.

In a 13-week nose-only inhalation toxicology study, Wistar rats were treated daily with a Symbicort formulation containing budesonide and formoterol as a micronized dry powder blend. Total target doses of budesonide and formoterol in the low, mid, and high dose groups were 2 + 0.12, 10 + 0.60, and 50 + 3.0 µg/kg/day, respectively. Using a deposition factor of approximately 10%, deposited doses for budesonide and formoterol in the low, mid, and high dose combination groups were 0.30 + 0.018, 1.3 + 0.073, and 4.8 + 0.25 µg/kg/day, respectively. Positive control groups were treated with total target doses of 50 µg/kg/day budesonide alone or 3.0 µg/kg/day formoterol alone (actual deposited doses of 7.2 and 0.24 µg/kg/day, respectively). A control group received air only. The NOAEL was identified as the mid dose combination. Target organs of toxicity were the thymus and spleen. Decreased body weight gains were observed for groups receiving budesonide only and the high dose of budesonide + formoterol. Observed changes can be primarily attributed to the effects of budesonide. The incidence of lymphocytolysis in the thymus was increased for male and female rats in the budesonide only group and male and female rats in the high dose budesonide + formoterol group. The incidence and magnitude of extramedullary hematopoiesis was decreased for male rats in the budesonide only group and male rats in the high dose budesonide + formoterol group. Systemic exposures to budesonide and formoterol were generally greater with the dry powder formulation as compared to the HFA pMDI formulation.

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In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort HFA pMDI formulation containing budesonide, formoterol, PVP K-25, PEG-1000, and HFA-227. Target doses of budesonide and formoterol in the low, mid, and high dose groups were $2.0 + 0.11$, $10 + 0.56$, and $50.0 + 2.80$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Using a deposition factor of approximately 25%, deposited doses for budesonide and formoterol in the low, mid, and high dose groups were $0.52 + 0.032$, $2.90 + 0.18$, and $14.6 + 0.89$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. The NOAEL was identified as the low dose. Sinus tachycardia (i.e., increased heart rate) was observed in mid and high dose groups on day 1 and during week 2. No changes in heart rate were evident during week 12. Target organs of toxicity were the adrenal cortex, thymus, and bronchial lymph nodes. Observed histopathological changes appear to be primarily attributable to the effects of budesonide. Cortical atrophy of the adrenal cortex was observed for male and female mid and high dose groups. The incidence and severity of thymic atrophy was increased for the male and female mid and high dose groups. The incidence and severity of lymphoid depletion in the bronchial lymph nodes was increased for the male and female high dose groups. There were no significant differences in findings between the air- and vehicle-control groups. Thus, no toxicity can be attributed to PVP K-25, PEG-1000, and HFA-227 at deposited doses of 0.10, 31.5, and 42000 $\mu\text{g}/\text{kg}/\text{day}$, respectively.

In a 13-week inhalation toxicity study, beagle dogs were treated daily with a Symbicort formulation containing budesonide and formoterol as a dry micronized powder blend with lactose. Target doses of budesonide and formoterol for the low, mid, and high dose groups were $2.0 + 0.11$, $10.0 + 0.56$, and $50 + 2.8$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Using a deposition factor of approximately 25%, deposited doses of budesonide and formoterol for low, mid, and high dose group were $0.44 + 0.022$, $2.19 + 0.11$, and $10.9 + 0.57$ $\mu\text{g}/\text{kg}/\text{day}$, respectively. Comparator groups were treated with 50 $\mu\text{g}/\text{kg}/\text{day}$ budesonide alone or 3.0 $\mu\text{g}/\text{kg}/\text{day}$ formoterol alone (deposited doses of 10.3 and 0.56 $\mu\text{g}/\text{kg}/\text{day}$, respectively). A control group received air only. The NOAEL was identified as the low dose combination for male dogs. Body weight gain was reduced for the male and female dogs that received budesonide only and mid and high doses of budesonide + formoterol. On day 1, elevated heart rates were evident for male and female dogs that received formoterol only or the high dose of budesonide + formoterol. During week 2, elevated heart rates were evident in male and female dogs that received formoterol only and low, mid, and high doses of budesonide + formoterol. No changes in heart rate were evident during week 12. Target organs of toxicity were the thymus, adrenal gland, and spleen, and toxicities could be primarily attributed to the effects of budesonide. Thymic atrophy was observed for the male and female dogs that received budesonide only, female dogs that received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide + formoterol. Atrophy of the zona fasciculata of the adrenal gland was observed for the male and female dogs that received budesonide only, female dogs that

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received the low dose of budesonide + formoterol, and male and female dogs that received mid and high doses of budesonide and formoterol. Vacuolation of the zona glomerulosa of the adrenal gland was observed for the male and female dogs that received budesonide only and male dogs that received the high dose of budesonide + formoterol. Lymphoid depletion in the spleen was observed for female dogs that received budesonide and male dogs that received mid and high doses of budesonide + formoterol.

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. PVP K25 is an excipient in the oral drug product, Trental[®] tablets, that is administered for chronic indications. PEG-1000 has been classified as "generally recognized as safe" (GRAS) and is used in food (21 CFR 172.820). Polyethylene glycol 1000 is also an excipient in at least four drug products (i.e., metoclopramide, promethazine, betamethasone) marketed by generic drug manufacturers for chronic indications. 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 August 12, 1996 (Document Room Date of October 2, 1996) and January 28, 1997). These studies, in general, revealed no toxicologically significant findings with HFA-227.

Levels of excipients, PVP K-25 and PEG-1000, in Symbicort were compared with levels of excipients, polyvinylpyrrolidone K-30 (PVP K-30) and polyethylene glycol 600 (PEG-600). Differences between PVP K-25 and PVP K-30 as well as PEG-600 and PEG-1000 would be expected to have no toxicological impact. 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

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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 (1 million) 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. 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. Studies were provided in the initial submission as follows: 6-month inhalation study in rats, 6- and 12-month inhalation studies in dogs, and 24-month inhalation carcinogenicity study in rats. All studies were conducted with the HFA-227 metered dose inhaler (MDI) formulation, PVP K-30, PEG 600, and HFA-227. Based upon reviews of the 6-month study with rats and the 6- and 12-month studies with dogs

no toxicity was associated with the vehicle-control. The carcinogenicity study has not been reviewed, although, the sponsor's summary reported no toxic effects in the two vehicle-control groups. Clinical doses of PVP K-25, PEG-1000, and HFA-227 that will be achieved in the current IND are compared to NOAELs for PVP-30, PEG-600, and HFA-227 in the next section to determine safety margins.

Inhaled and deposited (Dep.) doses (mg/kg/day) of excipients, PVP K-30 and PEG-600, and propellant, HFA-227 in inhalation toxicology studies with the vehicle-control high dose groups.

| Compound | 6-month rat study | | 6-month dog study | | 12-month dog study | | 24-month rat study | |
|----------|-------------------|-----------|-------------------|-----------|--------------------|-----------|--------------------|-----------|
| | Dose | Dep. Dose | Dose | Dep. Dose | Dose | Dep. Dose | Dose | Dep. Dose |
| PVP-K30 | 0.0184 | 0.0018 | 0.0029 | 0.0004 | 0.0019 | 0.0003 | 0.11 | 0.0056 |
| PEG-600 | 2.2096 | 0.221 | 0.3442 | 0.0516 | 0.2222 | 0.0333 | 13 | 0.6597 |
| HFA-227 | 729 | 729 | 113.57 | 113.57 | 73.29 | 73.29 | 4697 | 4697 |

General Toxicology Issues:

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. 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 $\mu\text{g}/\text{kg}/\text{day}$, respectively. For the 3-month dog study with the Symbicort HFA pMDI formulation, the NOAEL was identified as the low dose. Deposited doses of budesonide and formoterol for low dose treatment group were 0.52 and 0.032 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Safety margins derived from NOAELs of

preclinical studies with Symbicort compared to clinical doses were significantly less than 1 (see tables below), given that rats and dogs are known to be significantly more sensitive to the toxic effects of steroids 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 monoproducts (i.e., budesonide and formoterol) appears adequate to support clinical studies with the combination of budesonide and formoterol (i.e., Symbicort); however, adequate safety margins need to be established for PVP K-25, PEG-1000, and HFA-227. For the Pulmicort Turbuhaler, the approved dose is 200 to 400 µg twice daily (i.e., 400 to 800 µg/day) for adults and children. For a 50-kg adult, the approved dose is approximately 8 to 16 µg/kg/day. For a 20-kg child, the approved dose is approximately 20 to 40 µg/kg/day. In Phase III trials with formoterol, the clinical dose is 9 µg twice a day. This dose in adults and children is approximately 0.36 and 0.9 µg/kg/day, respectively. The approved dose of Foradil[®] Aerolizer (Novartis) is 12 µg twice a day.

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Reviewer: Timothy W. Robison, Ph.D.

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Rat to human and dog to human exposure ratios (based upon NOAELs from 3-month inhalation toxicology studies using calculated deposited doses) for clinical doses of the Symbicort HFA pMDI formulation in adults with a body weight of 50 kg.

| Clinical Doses | | | | Rat to Human exposure ratio | Dog to Human exposure ratio |
|----------------|--------------|------------------|-----------|-----------------------------|-----------------------------|
| 40/4.5 | µg/actuation | 4 actuations/day | µg/kg/day | | |
| Budesonide | 43.2 | 172.8 | 3.456 | 0.231 | 0.150 |
| Formoterol | 4.9 | 19.6 | 0.392 | 0.122 | 0.082 |
| | | | | | |
| 80/4.5 | µg/actuation | 4 actuations/day | µg/kg/day | Rat to Human ratio | Dog to Human Ratio |
| Budesonide | 86.3 | 345.2 | 6.904 | 0.116 | 0.075 |
| Formoterol | 4.9 | 19.6 | 0.392 | 0.122 | 0.082 |
| | | | | | |
| 160/4.5 | µg/actuation | 4 actuations/day | µg/kg/day | Rat to Human ratio | Dog to Human Ratio |
| Budesonide | 171.9 | 687.6 | 13.752 | 0.058 | 0.038 |
| Formoterol | 4.9 | 19.6 | 0.392 | 0.122 | 0.082 |

Rat to human and dog to human exposure ratios (based upon NOAELs from 3-month inhalation toxicology studies using calculated deposited doses) for clinical doses of the Symbicort HFA pMDI formulation in children (6-11 years) with a body weight of 20 kg.

| Clinical Doses | | | | Rat to Human exposure ratio | Dog to Human exposure ratio |
|----------------|--------------|------------------|-----------|-----------------------------|-----------------------------|
| 40/4.5 | µg/actuation | 4 actuations/day | µg/kg/day | | |
| Budesonide | 43.2 | 172.8 | 8.64 | 0.093 | 0.060 |
| Formoterol | 4.9 | 19.6 | 0.98 | 0.049 | 0.033 |
| | | | | | |
| 160/4.5 | µg/actuation | 4 actuations/day | µg/kg/day | Rat to Human ratio | Dog to Human Ratio |
| Budesonide | 171.9 | 687.6 | 34.38 | 0.023 | 0.015 |
| Formoterol | 4.9 | 19.6 | 0.98 | 0.049 | 0.033 |

AUC and C_{max} values at NOAELs in 3-month inhalation toxicology studies with the Symbicort HFA pMDI formulation in rats and dogs were compared to AUC and C_{max} values obtained in healthy volunteers that received budesonide and formoterol using the Symbicort pMDI. Symbicort pMDI (budesonide/formoterol), 160/4.5 µg, was given as 4 actuations all at one time corresponding to a total dose of 640/18 µg. While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID. Similarly as observed with deposited doses above, safety margins derived from AUC and C_{max} values at NOAELs of preclinical studies compared to corresponding values at the highest clinical dose 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 steroids.

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AUC Exposure Ratios

| Symbicort pMDI | Human ^a - AUC 4 x 160/4.5 µg | Rat | | Dog | |
|----------------|--|-------------------|--------------|--------------------|--------------|
| | | NOAEL AUC | Rat to Human | NOAEL AUC | Dog to Human |
| Budesonide | 4.83 nmol/hr/L | 1.98 nmol/hr/L | 0.41 | 0.630 nmol/hr/L | 0.13 |
| Formoterol | 145 pmol/hr/L | 55.9 nmol/hr/L | 0.39 | 24.5 pmol/hr/L | 0.17 |

a. Symbicort pMDI (budesonide/formoterol), 160/4.5 µg, was given as 4 actuations all at one time corresponding to a total dose of 640/18 µg. While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID.

C_{max} Exposure Ratios

| Symbicort pMDI | Human ^a - C _{max} 4 x 160/4.5 µg | Rat | | Dog | |
|----------------|---|------------------------|--------------|------------------------|--------------|
| | | NOAEL C _{max} | Rat to Human | NOAEL C _{max} | Dog to Human |
| Budesonide | 1.42 nmol/L | 1.59 nmol/L | 1.12 | 0.448 nmol/L | 0.32 |
| Formoterol | 52.0 pmol/L | 47.6 pmol/L | 0.92 | 14.6 pmol/L | 0.28 |

a. Symbicort pMDI (budesonide/formoterol), 160/4.5 µg, was given as 4 actuations all at one time corresponding to a total dose of 640/18 µg. While the total dose is the same as the maximum proposed clinical dose for trials with the pMDI described in the present review, the drug will be administered as two actuations BID.

In 3-month bridging studies with the Symbicort HFA pMDI formulation in rats and dogs, there were no toxic effects observed in the excipient-only vehicle-control (i.e., PVP K-25, PEG-1000, and HFA-227) groups. However, comparison of excipient levels in vehicle-controls to those in the clinical dose provided inadequate safety margins for povidone K-25 and PEG-1000 as shown in the tables below. It should be noted that levels of excipients and the propellant in the three clinical dosage strengths of the Symbicort HFA pMDI formulation are identical. Safety margins for patients >12 years of age with a body weight of 50 kg ranged from only 0.7 to 1.9. Inadequate safety margins (i.e., <1) were particularly evident for children, 6 to 11 years of age and a body weight of 20 kg.

Rat to human and dog to human exposure ratios for excipients in clinical doses of the Symbicort HFA pMDI formulation in adults with a body weight of 50 kg (based upon excipient levels in vehicle-controls of the 3-month inhalation toxicology studies).

| Clinical Doses | µg/actuation | 4 actuations/day | µg/kg/day | Rat to Human | Dog to Human |
|----------------|--------------|------------------|-----------|----------------|----------------|
| | | | | exposure ratio | exposure ratio |
| Povidone K25 | 0.7 | 2.8 | 0.056 | 0.714 | 1.786 |
| PEG 1000 | 212.3 | 849.2 | 16.984 | 0.724 | 1.855 |
| HFA 227 | 70750 | 283000 | 5660 | 7.244 | 7.391 |

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Rat to human and dog to human exposure ratio for excipients in clinical doses of the Symbicort HFA pMDI formulation in children (6-11 years) with a body weight of 20 kg (based upon excipient levels in vehicle-controls of the 3-month inhalation toxicology studies).

| Clinical Doses | | | | Rat to Human exposure ratio | Dog to Human exposure ratio |
|----------------|--------------|------------------|-----------|-----------------------------|-----------------------------|
| Excipients | µg/actuation | 4 actuations/day | µg/kg/day | | |
| Povidone K25 | 0.7 | 2.8 | 0.14 | 0.286 | 0.714 |
| PEG 1000 | 212.3 | 849.2 | 42.46 | 0.290 | 0.742 |
| HFA 227 | 70750 | 283000 | 14150 | 2.898 | 2.957 |

Adequate safety margins for excipients, povidone K-25 and PEG-1000, in the clinical Symbicort HFA pMDI formulations were obtained through review of inhalation toxicity studies conducted

where similar excipients, povidone K-30 and PEG-600, were used. As noted previously, there are no toxicologically significant differences between PVP K-25 and PVP K-30 as well as PEG-600 and PEG-1000. Vehicle-control levels of PVP K-30 and PEG-600 in the 6-month inhalation toxicity study with rats and 24-month inhalation carcinogenicity study with rats provided sufficient safety margins for PVP K-25 and PEG-1000 as shown in the table below. Both PVP K-25 and PEG-1000 are approved for oral use, and inhalation-bridging studies with one species (i.e., rat) are sufficient to establish safety by the inhalation route.

Rat to human and dog to human exposure ratios for excipients in clinical doses of the Symbicort HFA pMDI formulation in adults with a body weight of 50 kg (based upon levels of excipients, povidone K-30 and PEG-600, in vehicle-controls of studies

| Clinical Doses | | | | 6-month | 24-month | 6-month | 12-month |
|----------------|--------------|------------------|-----------|--------------------|--------------------|--------------------|--------------------|
| Excipients | µg/actuation | 4 actuations/day | µg/kg/day | Rat to Human ratio | Rat to Human ratio | Dog to Human Ratio | Dog to Human Ratio |
| Povidone K25 | 0.7 | 2.8 | 0.056 | 32.857 | 100.000 | 7.682 | 4.955 |
| PEG 1000 | 212.3 | 849.2 | 16.984 | 13.012 | 38.842 | 3.040 | 1.961 |
| HFA 227 | 70750 | 283000 | 5660 | 128.819 | 829.859 | 20.066 | 12.949 |

Rat to human and dog to human exposure ratio for excipients in clinical doses of the Symbicort HFA pMDI formulation in children (6-11 years) with a body weight of 20 kg (based upon levels of excipients, povidone K-30 and PEG-600, in vehicle-controls of studies

| Clinical Doses | | | | 6-month | 24-month | 6-month | 12-month |
|----------------|--------------|------------------|-----------|--------------------|--------------------|--------------------|--------------------|
| Excipients | µg/actuation | 4 actuations/day | µg/kg/day | Rat to Human ratio | Rat to Human ratio | Dog to Human Ratio | Dog to Human Ratio |
| Povidone K25 | 0.7 | 2.8 | 0.14 | 13.143 | 40.000 | 3.073 | 1.982 |
| PEG 1000 | 212.3 | 849.2 | 42.46 | 5.205 | 15.537 | 1.216 | 0.784 |
| HFA 227 | 70750 | 283000 | 14150 | 51.528 | 331.943 | 8.026 | 5.180 |

Recommendations: From a preclinical standpoint, the proposed clinical trials appear to be reasonably safe and should be allowed to proceed.

Reviewer: Timothy W. Robison, Ph.D.

IND No. 63.394

Labeling with basis for findings: None.

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

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

cc: list:
ZeccolaA
AnthraciteR
HuffR
RobisonT

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**This is a representation of an electronic record that was signed electronically and
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/s/

Timothy Robison
1/23/02 06:21:58 PM
PHARMACOLOGIST

Robin Huff
1/24/02 08:49:04 AM
PHARMACOLOGIST
I concur.

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Appendix 3: Review of IND 63,394 Amendment #017 dated April 4, 2002

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 63,394

Review number: #02

Sequence number/date/type of submission: #017/January 16, 2002/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: April 4, 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:

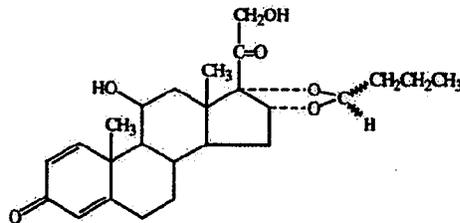
Molecular formula/molecular weight:

Budesonide, C₂₅H₃₄O₈ / 430.5 g/mole

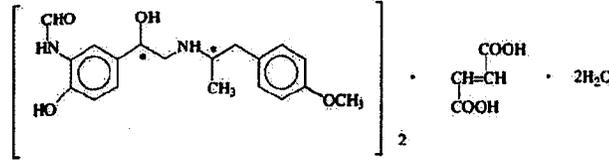
Formoterol fumarate dihydrate, C₄₂H₅₆N₄O₁₄ / 840.9 g/mole

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) ^c | | | | | 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

Proposed clinical protocol:

I. Study Protocols provided in the Initial IND Submission:

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 mcg/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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