CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-077

PHARMACOLOGY REVIEW(S)

Jani

MAR 8 2000

REVIEW AND EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
Chemistry Consult

Reviewer: Lawrence F. Sancilio, Ph.D.

Division: PULMONARY DRUG PRODUCTS, HFD-570

Reviewer Completion Date: 3/8/00

NDA No. 21-077

Date of Consult Request: 3/3/00

Information to Sponsor: Yes ( ), No (X)

Sponsor: Glaxo Inc.
5 Moore Drive
Research Triangle Park, NC 27709

Drug: Salmeterol xinafoate (Serevent ™) and fluticasone propionate (Flovent ™) Combination

Trade Name: Advair Diskus

Drug Class: Salmeterol xinafoate, β_2 receptor antagonist
Fluticasone propionate, glucocorticoid steroid

Indication: Maintenance Treatment of asthma [redacted] in patients 12 years of age and older.

Route of Administration: Oral inhalation

Recommended Dose: The proposed maximum daily dose is 50 µg of salmeterol as salmeterol xinafoate and up to 500 µg of fluticasone propionate twice a day. Each Diskus delivers 60 doses (30 daily doses). The mouthpiece weighs 1400 mg.

Dr. D. Koble requested that the safety of the colorant, [redacted] in the mouthpiece of the Diskus be assessed. In the 12/5/99 consult, this chemical was not judged safe due to lack of toxicology data. The sponsor recently sent extractable data using different solvents. From this data, the daily dose was determined by dividing from the amount extracted by 30 (the number of daily doses/Diskus) and again by 50 (the average weight of an adult).

Review

The following table lists the solvents, the amount extracted and the maximum daily dose of the extracted [redacted] The solvents used were based on the guidance of USP<87>,<88>,<661> and 21 CFR 177.1520. The extractions were conducted for 2-24 h at 70°C using various solvents. The limit of detection was [redacted] for the solvents, [redacted]

[redacted] For each solvent, three to five mouthpieces were used.

Solvent	Average Amount Extracted [redacted]	Maximum Daily Dose, [redacted]

<LOD, Below the Limit of detection

Discussion

[redacted] is a colorant that has carcinogenicity alert based on its structure. It is acutely non-toxic as its p.o. LD50 in rats was > 23 g/kg. As a colorant in the mouthpiece of the metered dose inhaler, attention for safety was focused on the amount extracted from aqueous solvents.

The mouthpiece contained [redacted] From the above table, the maximum amount of compound extracted from the best solvent, [redacted] was very low, [redacted] The maximum amount extracted from the various aqueous media

simulating the clinical scenario was [redacted] and below the detection level of [redacted] The daily exposure ranges from [redacted] this would place the inhalation dose of [redacted] below or slightly higher than the daily safe [redacted] risk dose of [redacted], a potent carcinogen. At this range of exposures, [redacted] is considered safe.

Recommendation

[redacted] at a concentration of [redacted] in the mouthpiece is considered safe.

/S/

3/8/00

Lawrence F. Sancilio. Ph.D.

March 8, 2000

cc. /Division File, NDA 21-077
/MPurucker, HFD-570
/C.S.O., HFD-570
/LFSancilio, HFD-570
/DKoble, HFD-570

/S/

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ON ORIGINAL

REVIEW AND EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
Original Review

Reviewer: Lawrence F. Sancilio, Ph.D.

Division: PULMONARY DRUG PRODUCTS, HFD-570

Reviewer Completion Date: 1/24/00

NDA No. 21-077

Serial No. /Date/ Type of Submission: Original, 3/25/98

Information to Sponsor: Yes ( ), No (X)

Sponsor: Glaxo Inc.
5 Moore Drive
Research Triangle Park, NC 27709

Drug: Salmeterol xinafoate (Serevent ™) and fluticasone propionate (Flovent ™) Combination

Trade Name: Advair Diskus

Chemical Names: Salmeterol xinafoate: 4-Hydroxy- ^{1}-[[[6-(4-phenylbutoxy)hexyl) amino]-methyl]-1,3-benzenedimethanol, 1-hydroxy-2-naphthalenecarboxylate
Fluticasone propionate: S-fluoromethyl 6, 9 -difluoro-11 -hydroxy-16 - methyl-3-oxo- 17-propionyloxyandrosta-1, 4-diene-17 -carbothioate

CAS Registry No.: Salmeterol xinafoate, 89767-59-9
Fluticasone propionate, 80474-14-2

Molecular Formula: Salmeterol xinafoate, $C_{25}H_{37}NO_4.C_{11}H_8O_3$,
Fluticasone propionate, $C_{25}H_{31}F_3O_5S$

Molecular Weight: Salmeterol xinafoate, 603.8
Fluticasone propionate, 500.6

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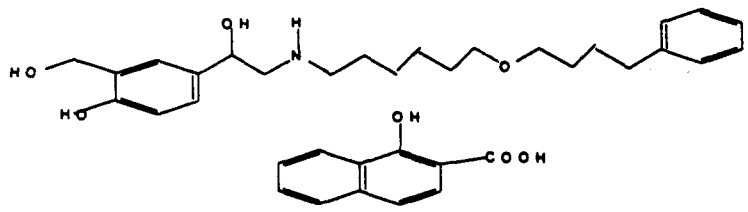
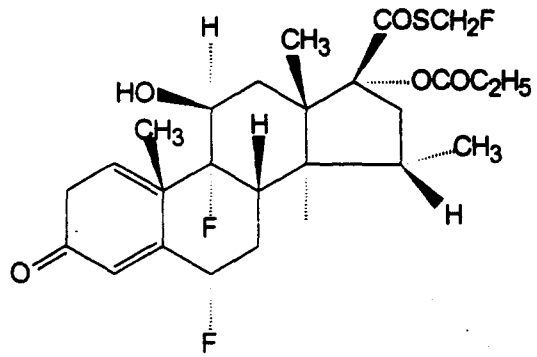
Structure:

Salmeterol xinafoate



Fluticasone propionate



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Relevant IND/NDAs

IND —— Salmeterol xinafoate/Fluticasone propionate Diskus dry powder inhaler
IND ——————————————————————
NDA20-236, Salmeterol xinafoate Inhalation aerosol
NDA 20-692, Salmeterol xinafoate Diskus dry powder inhaler
NDA 20-121, Fluticasone propionate Nasal Spray
NDA 20-548, Fluticasone propionate Inhalation aerosol
NDA 20-549, Fluticasone propionate Rotadisk dry powder inhaler
NDA 20-770, Fluticasone propionate dry powder inhaler

Drug Class: Salmeterol xinafoate, β_2 receptor antagonist
Fluticasone propionate, glucocorticoid steroid

Indication: Maintenance Treatment of asthma ———————— in patients 12 years of age and older.

Clinical Formulation Components: Each blisterpack contains one of the following combinations:

Salmeterol µg	+	Fluticasone propionate µg
50		100
50		250
50		500
Lactose		12.5 mg

Route of Administration: Oral inhalation

Recommended Dose: The proposed maximum daily dose is 50 µg of salmeterol as salmeterol xinafoate and up to 500 µg of fluticasone propionate twice a day. Advair Diskus delivers per dose 45 µg of salmeterol and 466 µg of fluticasone propionate

Previous Clinical Experience

Salmeterol and/or fluticasone propionate alone have been used in the treatment of various upper respiratory diseases. These conditions include: asthma, nocturnal asthma, exercise-induced bronchospasm, and bronchospasm associated with chronic obstructive pulmonary disease (COPD).

Disclaimer: Some of the material was taken directly from the Sponsor's submission.

Introduction and Drug History

Salmeterol xinafoate and fluticasone propionate separately have been approved as a dry powder inhaler for the treatment of asthma as Serevent Diskus (NDA) and Flovent Diskus (NDA 20-833). This NDA is for salmeterol xinafoate and fluticasone propionate as a combination inhalation powder to be used for the maintenance treatment of asthma. Currently, both drugs are being used together in the treatment of asthma.

Studies Reviewed in this Submission: None

Studies Not Reviewed in this Submission

The following studies were not reviewed herewith since the salmeterol xinafoate and fluticasone propionate were delivered by the formulation and not by the powder formulation, the mechanism for delivering these compounds in this NDA. They were, however, reviewed in IND

Pilot 35-Day inhalation study formulation) with salmeterol xinafoate and fluticasone propionate in rats, WPT/93/176, vol. 21.

13-Week inhalation study — formulation) with salmeterol xinafoate and fluticasone propionate in rats, WPT/93/176, vol. 23.

Pilot 14-Day inhalation — formulation) study with salmeterol xinafoate and fluticasone propionate in dogs, WPT/93/189, vol. 19.

The following studies were already reviewed in IND — IND — ;IND — and NDA20-549. Their reports are attached.

Safety Pharmacology

Cardiovascular/Respiratory

Determination of the cardiovascular and respiratory effects of an i.v. administration of salmeterol xinafoate in anesthetized guinea pigs pretreated with fluticasone propionate, WPT/95/140, vol. 10.

Other

14-Day study in mice to determine if pretreatment with fluticasone propionate affects the pharmacological sensitivity of the uterus to salmeterol xinafoate, WPT/93/377, vol. 13.

Pharmacokinetics/Toxicokinetics

Absorption

Pilot absorption study in dogs following inhalation of a single dose of salmeterol xinafoate and fluticasone propionate, GDM/p93/058, vol. 28.

Distribution

Determination of placental transfer of radioactivity (day 12 and day 18) in pregnant female mice after p.o. administration of 10 mg of base/kg of salmeterol xinafoate and s.c. administration of 100 mg/kg of fluticasone propionate, GDM/92/040, vol. 28.

Toxicology

Acute

Acute inhalation toxicity of salmeterol xinafoate and fluticasone propionate in Wistar rats, WPT/93/395, vol. 11.

Acute inhalation toxicity of salmeterol xinafoate and fluticasone propionate in Wistar rats, WPT/93/397, vol. 11.

Acute inhalation toxicity of salmeterol xinafoate and fluticasone propionate in Charles River Wistar rats: Assessment of cardiovascular function using remote telemetry, WPT/95/253, vol. 12.

Multi-Dose

Preliminary 15-Day inhalation (powder formulation) study with salmeterol xinafoate and fluticasone propionate in rats, WPT/92/182, vol. 13.

Pilot 15-Day inhalation study (powder formulation) with salmeterol xinafoate and fluticasone propionate in Sprague-Dawley and Wistar rats, WPT/92/318, vol. 14.

Pilot 35-Day inhalation study (powder formulation) with salmeterol xinafoate and fluticasone propionate in rats, WPT/93/175, vol. 15.

13-Week inhalation study (powder formulation) with salmeterol xinafoate and fluticasone propionate in rats, WPT/95/011, vol. 17.

Preliminary 15-Day inhalation (powder formulation) study with salmeterol xinafoate and fluticasone propionate in dogs, WPT/92/178, vol. 19.

Pilot 14-Day inhalation (powder formulation) study with salmeterol xinafoate and fluticasone propionate in dogs, WPT/93/089, vol. 19.

5-Week inhalation (powder formulation) study with salmeterol xinafoate and fluticasone propionate in dogs, WPT/95/233, vol. 20.

Reproductive Toxicity

Organogenesis dose-ranging study with salmeterol xinafoate and fluticasone propionate in mice, WPT/92/179, vol. 25.

Organogenesis dose-ranging study by oral/ subcutaneous routes with salmeterol xinafoate and fluticasone propionate in mice, WPT/92/196, vol. 26.

A preliminary study to determine in pregnant AHA rats the effect of co-administration of p.o. salmeterol xinafoate and s.c. fluticasone propionate, WPT/92/125, vol. 27.

Pregnant AHA rats and their progeny: The effect of co-administration of p.o. salmeterol xinafoate and s.c. fluticasone propionate, WPT/92/371, vol. 27.

OVERALL SUMMARY AND EVALUATION

Salmeterol xinafoate

This NDA consists of a combination inhalation formulation of salmeterol xinafoate, a β_2 receptor agonist, and fluticasone propionate, a glucocorticoid steroid. Both drugs are currently being prescribed alone and together for the treatment of asthma. They are administered by inhalation as an aerosol (NDA 20-236 and NDA 20-548) or as a dry powder (NDA 20-692 and NDA 20-549). Since these drugs are being prescribed together, it seems reasonable to combine these 2 drugs as a combination product, the subject of this NDA. The combination product in this NDA is a dry powder.

Salmeterol xinafoate (salmeterol hydroxynaphthoate) is a β_2 receptor antagonist resulting in bronchodilating properties. The hydroxynaphthoate salt was developed, since by the inhaled route in rats, it was less irritating to the respiratory tract than the ——— Salmeterol is structurally related to albuterol, The alteration of the albuterol molecule resulted in increased bronchodilating potency and both a longer onset and duration of action. Salmeterol xinafoate is a racemate whose bronchodilating activity is due to the R+ enantiomer. Salmeterol xinafoate blocked the eosinophilia induced by platelet activating factor or by egg albumin in sensitized guinea pigs, inhibited the release of mediators from antigen challenged passively sensitized human lung tissues, prevented histamine-induced bronchoconstriction and increased in plasma protein extravasation from guinea pig lungs and inhibited mouse and human T-cell proliferation. In some of these models, the activity was blocked by propranolol, a β receptor antagonist, confirming its β_2 receptor agonist properties.

In humans, one of metabolites identified was the aliphatic hydroxylated salmeterol. This metabolite showed bronchodilating properties that was twice as potent and shorter acting than the parent compound. This metabolite strongly binds to guinea pig tracheal tissue.

In unanesthetized dogs, salmeterol xinafoate produced tachycardia of long duration, vasodilation and emesis. In anesthetized cats, tachycardia was accompanied by hypotension. The R-enantiomer produced in monkeys and cats greater cardiovascular effects than the S-enantiomer. Salmeterol xinafoate was not an enzyme inducer. Using radioligand techniques, salmeterol unlike salbutamol interacted with β-adrenergic receptors in a non-competitive manner. Salmeterol had modest affinity for the sigma, $5HT_1$, α_1 and b_1 adrenergic receptors and dopamine uptake sites. It was not irritating when applied to the intact or abraded skin of guinea pigs.

Salmeterol xinafoate was bioavailable approximately 10 % in the rat and 70% in the dog. The low bioavailability in the rat was due to high pre-systemic metabolism, since the bioavailability of the hydroxynapthoic acid portion of the salmeterol salt was 100% bioavailable. Dogs show a higher $AUC_{0\text{-}inf}$ than rats due to the higher bioavailability, lower t1/2 life (2 vs 5 hr), a lower clearance (32 vs 97 ml/kg/min) and a lower volume of distribution (5.5 vs 42.5 l/kg). Salmeterol did not accumulate upon repeated dosing.

[redacted] studies in M rats following p.o. administration show radioactivity in the stomach, liver, cecum, intestines, bile duct and kidneys; little radioactivity was found in the lungs and central nervous system. In dogs following p.o. administration, radioactivity was found in the liver, gall bladder, small intestine and large intestine. In mice, salmeterol was predominantly not metabolized, although there were some unidentified metabolites. In the rat, salmeterol was extensively metabolized. At least 15 metabolites were found; one was identified as the hydroxylated salmeterol. Unchanged salmeterol accounted for only 14% of the dose. Most of the metabolites were glucuronides. In pregnant rabbits, there were 7 radioactive components in the urine that were primarily glucuronides. In dogs, there were 9 metabolites; two were identified. The major metabolite was the sulfate conjugate, and the other, a minor metabolite, was the hydroxylated salmeterol. In humans, hydroxylated salmeterol is a major metabolite and the O-dealkylated salmeterol, a minor metabolite. Aliphatic oxidation to the major metabolite is predominantly due to the P450 isoenzyme CYP3A4 and to a lesser degree to the P450 CYP2E1 enzyme. This indicates that adverse effect may occur when salmeterol is given with a drug like ketoconazole that inhibits these P450 enzymes.

In mice, rats, pregnant rabbits and dogs, excretion was predominantly by the fecal route. In the rats and dogs a major portion of the fecal excretion was by way of the biliary tract. Urinary excretion played a minor role in all 4 species. In rats and dogs, excretion was also in the expired air.

Inhalation studies conducted in rats and dogs show a higher concentration of salmeterol in lung tissue than that for hydroxynaphthoic acid. This indicates that salmeterol was not rapidly cleared from the lungs.

In pregnant rats and rabbits, following p.o. administration of radioactive salmeterol xinafoate, radioactivity was present in the dam's liver, the placenta and fetal liver. This indicates that salmeterol and/or its metabolites cross the placental barrier and are distributed in the fetus. In lactating rats, radioactivity was present in the milk.

The acid portion of salmeterol xinafoate, hydroxynaphthoic acid, possesses a long half-life in mice, rats and dogs. Several metabolites in addition to unchanged hydroxynaphthoic acid as the glucuronide were found in the urine, the major excretory route. In pregnant rabbits, there were at least 3 urinary metabolites; the major one was the glucuronide of hydroxynaphthoic acid. The acid/metabolites were also excreted in the feces and to a smaller extent in the expired air of rats. In pregnant rabbits, hydroxynaphthoic acid crosses the placenta, and at 48 hr, radioactivity was still present indicating a long half-life.

Binding studies show that salmeterol binds to plasma proteins (92% to 97%) of mice, rats, rabbits, dogs and humans. In humans, albumin and α_1 acid glycoprotein are the major binding proteins. Hydroxynaphthoic acid binds primarily to albumin.

Acute toxicities of salmeterol xinafoate were conducted in mice, juvenile and adult rats and dogs. In mice, 150 mg/kg p.o. was relatively not toxic producing decreased body weight gained and

hypothermia. In juvenile rats, 300 mg/kg p.o. produced a decrease in body weight gained. By the i.p. route, doses ≥ 23 mg/kg caused peritonitis apparently due to local irritation; 90 mg/kg i.p. was lethal. In adult rats, 600 mg/kg p.o. caused anal staining, ptosis, rough coat and discolored feces. 1000 mg/kg p.o. produced heavy rapid breathing, lethargy, ptosis, watery eyes and decreased body weight gained. By the i.p. route, doses ≥ 75 mg/kg caused writhing (a response to pain) and peritonitis; 150 mg/kg i.p. was lethal. By the inhalation route, rats at 2.9 mg/kg showed a slight increase in absolute and relative liver weight. In dogs, salmeterol xinafoate at 0.7 mg/kg in dogs was relatively toxic. There were tachycardia, vasodilation, and trembling and mild lung inflammation. The lung inflammation indicated a local inflammatory effect, while the tachycardia, vasodilation, and trembling and cardiac toxicity were an extension of β_2 agonism. Kidneys also manifested inflammatory lesions.

——— is a degradant formed by the interaction between salmeterol and lactose in the formulation of the salmeterol xinafoate dry powder inhalation product. In single dose studies in rats, the NOAEL for ——— was 768 mg/kg, p.o. and 37 mg/kg by the inhalation route.

Multidose studies were conducted in mice, rats and dogs. In a 90-day p.o. study in mice, whereby emphasis was focused on the on the uterus, hypertrophy, β_2 agonist effect, was seen at 1.4 and 10 mg/kg.

In toxicity studies in rats, rabbits and dogs, salmeterol xinafoate was administered by the oral route or in combination with the inhalation and oral routes.

In a 2-week oral study in rats, 2 mg/kg produced no significant toxic effects. Plasma levels were transient, since no salmeterol was detected at 24 h. In a 39-day p.o. study in 3-day old rats, doses of 1 and 10 mg/kg p.o. were lethal. Deaths occurred in the HD within the first 10 days. The survivors showed increased body weight gained and acceleration in the rate of eye opening. These changes were associated with β_2 agonism. Juvenile rats may be more sensitive than adult animals.

In a 13-week inhalation/p.o. study in rats with salmeterol xinafoate at doses up to 0.7 mg/kg by inhalation and 2.0 mg/kg p.o, no significant toxicity was seen. Hypoglycemia, slight increases in cardiac weight and increases in serum enzymes were observed although no histopathology was noted. Extending a similar study to 26-weeks, the observed changes were similar to those seen in the 13-week study.

A 13-week inhalation study was conducted in rats and dogs with the degradant, ———. This was administered in the salmeterol xinafoate with aged lactose powder mixture. In both species, no toxicity was seen that could be associated with the degradant.

In a 78-week study in rats, doses of 0.06, 0.18 and 0.63 mg/kg were administered by inhalation. Some of the changes seen were related to β_2 agonism, i.e., hypoglycemia and hypokalemia. Increased food consumption was seen at all doses in both sexes. The M showed a decrease in vacuolated hepatocytes and an increase in alveolar macrophages. In the F_1 there were ovarian

follicular cysts, bilateral mesovarian leiomyoma and mammary lobular hyperplasia. Both sexes showed laryngeal epithelial hyperplasia accompanied by squamous metaplasia at the MD and HD. The latter indicated an inflammatory response attributed to the aerosolized salmeterol xinafoate. There was a 50% decrease in the Cmax from week 1 to week 75 suggesting enzyme induction.

In the 13-, 28- and 52- week toxicity studies in dogs, salmeterol xinafoate was administered p.o. and by inhalation using daily doses of 0.15, 0.5 and 2 mg/kg p.o. and twice daily inhalation doses of 5 x 50 μg bursts, 10 x 50 μg bursts and 20 x 50 μg bursts for the LD, MD and HD, respectively. In one or all the studies, the animals showed transient tachycardia, vasodilation, increased muscle development and hypokalemia. At the HD in the 28-and 52-week studies, the dogs showed seizures and prostration. One animal died following seizures in the 52-week study. Myocardial papillary fibrosis with and without calcification was seen in all three studies. The incidences were increased at the MD in the 13-week study, at all doses in the 28- week study and at the MD and HD in the 52-week study.

Long-term carcinogenicity assays with salmeterol xinafoate were conducted in rats and mice. In the rat carcinogenicity assay, salmeterol xinafoate was administered by inhalation followed by gavage. The doses were: LD (0.06 mg/kg by inhalation + 0.15 mg/kg p.o.), MD (0.18 mg/kg by inhalation + 0.5 mg/kg p.o.) and HD (0.58 mg/kg by inhalation + 2.0 mg/kg p.o.). In M, non-neoplastic lesions seen involved the reproductive system [testes (HD), epididymis (HD) and seminal vesicles (HD)], thyroid gland (HD), liver (MD, HD) and larynx (all doses). The lesions (epithelial hyperplasia and squamous hyperplasia) seen in the larynx were indicative of inflammation apparently resulting from the irritant properties of the salmeterol xinafoate formulation. There was a dose related increase in the incidence of mesovarian leiomyomas and ovarian cysts at the MD and HD.

In the mouse carcinogenicity assay, salmeterol xinafoate was administered by the p.o. route at doses of 0.2, 1 and 10 mg/kg. Salmeterol xinafoate was not carcinogenic in M; it was lethal at the HD. Myocardial fibrosis was seen at all doses although increased incidence was only significant at the HD. In F, salmeterol was neoplastic; uterine leiomyomas were seen at the MD and HD and lymphosarcomas at the HD. The leiomyomas were due to β_2 agonism. Non-neoplastic lesions seen at the MD and HD were uterine smooth muscle hyperplasia, cystic glandular hyperplasia and ovarian follicular cysts.

In the reproductive toxicology studies, salmeterol xinafoate did not affect fertility (0.15, 0.5 and 2 mg/kg p.o.) and was not teratogenic in rats (0.1, 1 and 10 mg/kg p.o.). However, in rabbits (0.1- 10 mg/kg p.o.), teratogenicity (e.g., open eyelids and/or cleft palates) were seen at doses ≥ 1 mg/kg p.o. These results were characteristic of β_2 agonism. In the peri- and postnatal development stage in rats, 10 mg/kg p.o. was fetotoxic and decreased the fertility of the survivors.

In the Genetic Toxicology studies, salmeterol xinafoate was inactive in the Microbial Mutagenicity (Ames), Chinese hamster V79/HGPRT and the Mouse Micronucleus assays. The degradant, ———— , was also inactive in the Microbial Mutagenicity assay.

Fluticasone propionate

Fluticasone propionate is a fluorinated corticosteroid that is being used alone for the maintenance treatment of asthma. It is administered by inhalation as a metered dose or as dry powder inhaler. In human glucocorticoid receptor binding studies, fluticasone propionate was 4- 18 X more potent than dexamethasone and in inhalation studies in guinea pigs, fluticasone propionate was 100 X more potent than dexamethasone in blocking eosinophilia induced by platelet activating factor. In the human dermal vasoconstriction model, fluticasone propionate applied topically was 9 times more potent than fluocinolone acetonide. By the p.o. route, fluticasone propionate possesses low bioavailability due to first pass metabolism. Fluticasone propionate possesses 1/3 the binding properties of progesterone and has antiandrogenic and antianabolic properties. Unlike other corticosteroids, fluticasone propionate increases the excretion of sodium and potassium. Its principal metabolite, GR36264, in humans and other species possesses little or no glucocorticoid activity.

Safety pharmacology studies indicate that fluticasone propionate possesses no cardiovascular or central nervous system properties, is not an enzyme inducer and does not affect the hepatic P450 enzyme system.

In p.o. studies in mice, fluticasone propionate was not detectable (limits of detection: ———— in plasma at 1 mg/kg. Following s.c., i.v. and intranasal administration to rats, the respective half lives were approximately 12 h, 17 h and 12 h . In a single p.o. dose study in rats using radioactive fluticasone propionate, radioactivity was detected in the stomach and its contents, the jejunum and large intestine. Other organs showing significant levels of radioactivity were the liver and brown fat and to a lesser degree, the adrenals, pancreas and skin. Following i.v. administration, the kidneys also showed significant levels of radioactivity. Radioactivity in the plasma following p.o. or i.v. administration at its peak level was < 1% of that found in the liver. In 6-h disposition studies in rats and dogs following inhalation with radioactive fluticasone propionate, the lungs showed the highest levels of radioactivity. The bioavailability of fluticasone propionate was 15% by the intranasal route in rats; this was comparable to that seen in humans by the inhaled route. Distribution studies of aerosolized fluticasone propionate in rats and dogs indicate that the highest levels were found in the lungs of both species; in contrast to the rat, very little ~~fluticasone~~ propionate was ingested orally by the dog.

Fluticasone propionate binds to the same high degree (94.6-96.5%) to plasma proteins of rats, dogs and humans. In addition, fluticasone propionate also binds to a lower degree (17-31%) to the red blood cells of rats, dogs and humans.

In mice, rats, dogs and humans, fluticasone propionate partially undergoes hydrolysis of the –$COSCH_2F$ substitution at the 17-position to the –COOH derivative (GR36264). In dogs,

fluticasone propionate also undergoes defluorination at the 6 position (GR100151). Both metabolites are excreted as the glucuronide. The predominant route of excretion for the metabolites and unchanged fluticasone propionate was fecal.

In acute toxicity studies, fluticasone propionate was not toxic by the p.o. and s.c. routes in adult animals. In rats and mice, the LD_{50}s were > 1000 mg/kg. By the inhalation route, the LD_{50}s were >1.6 mg/kg in the rat and >0.82 mg/kg in the dog. In all studies, the effects of glucocorticoids were seen, i.e., weight loss, decreased thymus weight and/or decreased cortisol levels. Mice manifested cardiac inflammation by the s.c. route. Three-day old rats were very sensitive to fluticasone propionate administered s.c. as their LD_{50} was 10 mg/kg. These animals showed marked signs of hyperglucocorticoidism, i.e., decreased body weight gained, emaciation and atrophy of the thymus. By the p.o. route, these rats were less sensitive as the LD_{50} was approximately 1500 mg/kg. These animals also showed signs of hyperglucocorticoidism. The marked difference in the LD50s by the p.o. and s.c. routes suggests that there was high first pass metabolism to the acid metabolite, GR36246, which was not toxic.

Inhalation studies using aerosolized fluticasone propionate were conducted in the rat for 1-month (15, 30 and 60 μ/kg), 6-months (5, 24 and 80 μ/kg) and 78-weeks (4, 15 and 57 μ/kg). Changes were seen at the MD and HD. These doses were not lethal, and the effects observed were characteristic of glucocorticoids. These effects included hair loss, decreased body weight gained, food consumption and lymphocyte levels, hyperglycemia, increased liver and kidney weight, decreased adrenal, splenic and thymus weight and histological changes in the liver, thymus, adrenal glands and spleen. Keratitis occurred after 52 weeks. Plasma levels were consistently detected at the HD.

In the adult dog, inhalation studies were conducted for 1- (40, 80, 150 mcg /kg) month, 6- (6, 15 and 45 μg/kg) and 12- (8, 18 and 51 μg/kg) months. The effects seen were characteristic of the glucocorticoids. In the 1-year study, the lungs showed inflammation and infection, the latter probably due immune suppression. In the 6- and 12- month studies, the cortisol response to ACTH was suppressed at all doses despite showing detectable levels of fluticasone propionate only at the HD. In a 1-year inhalation study in juvenile dogs, the initial dose was 133 μg/kg; due to toxic effects related to glucocorticoid activity, some animals were killed for humane reasons. The dose was decreased twice, and by week 8, the daily dose was 26 μg/kg. In addition to the expected glucocorticoid effects, many of these animals showed respiratory tract changes and abdominal distension resulting in abdominal hernias. These results indicate that the juvenile dogs are more susceptible than the adult dogs to the effect of inhaled fluticasone propionate. Similar findings were seen in the acute s.c. studies in 3-day old rats.

Long-term carcinogenicity studies were conducted in mice by the p.o. route and in rats by inhalation. In the mouse, the daily doses were 0.1, 0.3 and 1 mg/kg and in the rat, 3.7, 14.3 and 57 μg/kg. No neoplasms were seen in the mouse and rat. In mice, necrosis in the renal cortical tubules and centrilobular vacuolation of the hepatocytes were seen at the HD. In the rat, plasma levels were only detected at the HD. At the MD/or HD, there were keratitis, panophthalmitis and gastric lesions.

In the reproductive toxicity studies in rats, doses up to 50 μg/kg s.c. did not affect fertility, although decreased body weight gained was seen at 50 μg/kg. At this dose, prostate weight was significantly decreased. Fluticasone propionate was teratogenic in mice and rats. In rats, cleft palate and delayed ossification of the sternebrae and skull were seen at 100 μg/kg s.c. In mice cleft palate occurred at 45 μg/kg s.c. At 150 μg/kg s.c. in mice, decreased body weight and delayed ossification were also seen. In rabbits, fluticasone propionate was not teratogenic at p.o. doses up to 300 μg/kg. However, at 4 μg/kg s.c., the fetuses showed decreased weight, decreased ossification and cleft palate, while the dams showed fatty livers. At the MD, 0.57 μg/kg s.c., the fetuses also showed decreased body weight. In peri- and postnatal studies in rats, only decrease in body weight gained was observed in the dams at 15 and 50 μg/kg s.c. No changes were seen in the fetuses.

In distribution studies, tritiated fluticasone propionate was used. Fluticasone propionate crosses the placenta. In rats, radioactivity was found in the fetuses from dams receiving 100 μg/kg p.o. or s.c. and in the milk of lactating rats receiving 10 μg/kg s.c. The levels of radioactivity in the fetuses receiving fluticasone propionate p.o. was less than the levels of radioactivity in fetuses from animals receiving the steroid by the s.c. route. This was attributed to low oral bioavailability. In rabbits, radioactivity was also seen in the fetuses from dams receiving 100 μg/kg p.o.

Fluticasone propionate was not a skin sensistizer in guinea pigs nor did it irritate the skin of cynomolgus monkeys; it was slightly irritating when instilled as a suspension in the eyes of rabbits.

Fluticasone propionate was not mutagenic or genotoxic in the in vitro bacterial (Ames) and mammalian (Fluctuation, Gene Conversion, Chinese Hamster V79, HGPRT, Human Peripheral Lymphocyte) assays and in the vivo Mouse Micronucleus test given p.o. or s.c.

Salmeterol Xinafoate and Fluticasone Propionate

In anesthetized guinea pigs, there was no marked effect on the cardiovascular system when 2 doses of 10 mg/kg s.c. of fluticasone propionate was administered prior to a cumulative i.v. dose of 100 μg/kg of salmeterol xinafoate. Fluticasone propionate administered 30 μg/kg s.c. daily for 14 to mice did not affect the contraction of isolated uteri to salmeterol xinafoate showing no drug interaction.

In a single dose inhalation study in dogs, fluticasone propionate and salmeterol xinafoate were administered as a powder in a 1:1 combination using doses ranging from 6 to 180 μg/kg. Salmeterol xinafoate was absorbed faster and to a greater degree than fluticasone propionate.

A placental study in mice shows that both fluticasone propionate at 100 μg/kg s.c. and salmeterol xinafoate at 10 mg/kg p.o. when administered to different animals crossed the placenta. Based on radioactivity, fluticasone propionate crosses the placenta to greater degree than salmeterol

xinafoate. Their levels in the fetuses were low as the maximum percent of the dose was 0.2% for fluticasone propionate and 0.043% for salmeterol xinafoate.

Inhalation toxicity studies were conducted in rats with salmeterol xinafoate and fluticasone propionate administered together in a powder mix. In a single dose inhalation-toxicity study, in a 2:1 combination (3.63 mg/kg of salmeterol xinafoate: 1.93 mg/kg of fluticasone propionate) glucocorticoid (decreased body weight gained and lymphoid depletion) and β_2 agonists effects (cardiotoxicity) were seen. This combination showed signs of irritation to the larynx and nasal cavity. In the 1:2 combination [(0.25 (LD) or 0.49 (HD) mg/kg of salmeterol xinafoate and 0.46 (LD) and 0.91 (HD) mg/kg of fluticasone propionate)], similar findings although to a lesser degree were observed at the HD. The lower dose combination was not irritating to the larynx and nasal cavity. A second inhalation study was conducted in rats in which the salmeterol xinafoate-fluticasone propionate combinations were 1:2 [(salmeterol xinafoate, 0.56 (LD) and 2.8 (HD) mg/kg and fluticasone propionate, 1.1 (LD) and 5.4 (HD) mg/kg)]. The only toxicity noted was atrial myocarditis characteristic for β_2 agonism. In a third single dose inhalation study in rats, salmeterol xinafoate alone at 5.2 mg/kg produced ventricular degeneration; however, combining 3.3 mg/kg of salmeterol xinafoate with 1.9 mg/kg of fluticasone propionate produced atrial myocarditis (control, 0/5; salmeterol xinafoate-fluticasone propionate, 3/5) in addition to the ventricular degeneration suggesting that fluticasone propionate enhanced the cardiotoxicity of salmeterol xinafoate. Tachycardia occurred in both treated groups.

In dogs, a 1:1 combination single dose (salmeterol xinafoate, 0.017 (LD) and 0.178 (HD) mg/kg and fluticasone propionate, 0.016 (LD) and 0.164 (HD) mg/kg) inhalation study was investigated. At the LD, there was a 50 % decrease in body weight gained. The HD showed a 33% increase in body weight gained suggesting that the β_2 agonist's pharmacological effect (increased body weight gained) offset the decreased body weight gained effect of the glucocorticoid. Both groups manifested other effects characteristic of β_2 agonism and glucocorticoid.

In a 15-day preliminary multidose inhalation study in Wistar rats, the combination doses in a ration of 1:10 were salmeterol xinafoate, 0.069 (LD) and 0.90 (HD) mg/kg and fluticasone propionate, 0.0072 (LD) and 0.071 (HD) mg/kg. The principal changes seen in both sexes at one or both doses were thymus atrophy increased urine volume resulting in decreased urinary specific gravity, increased hematocrit and changes in the nasal turbinates and larynx indicative of irritation. Distention of the uterus was seen at both doses. A low incident of cardiac toxicity was present although not significant. Both compounds were absorbed into the systemic circulation; for each compound, plasma levels were dose related but not dose proportional.

In a 5-week pilot study in Wistar rats, the combination (salmeterol xinafoate:fluticasone propionate) doses in a ratio of 1:2 and 1:4 are shown in the following table.

Salmeterol xinafoate (µg/kg)	100	100	200	400
Fluticasone propionate (µg/kg)	200	400	800	800

The changes seen were characteristic of the effects of β_2 agonism and glucocorticoids
The effects seen were more than those observed in the 15-day study. They were decreased body weight gained, increased lymphocyte and neutrophil count, increased ALT and fibrinogen levels, decreased corticosterone levels, decreased spleen and adrenal weight, adrenal atrophy and increased alveolar macrophage aggregates. Myocarditis, an effect of β_2 agonism, was only seen in the M showing greater sensitivity. Plasma levels were dose related but not dose proportional; they were higher in the F. Accumulation was seen with fluticasone propionate.

In a 13-week inhalation study in Wistar rats, salmeterol xinafoate and fluticasone propionate was given alone and in combination (salmeterol xinafoate:fluticasone propionate) in a ratio of 1:9 and 1:1 as shown in the following table.

Salmeterol xinafoate (µg/kg)	36	0	8	72
Fluticasone propionate (µg/kg)	0	71	72	71

In both sexes, salmeterol xinafoate alone produced decreased platelets, hypoglycemia, increased serum potassium levels and epithelial hyperplasia in the larynx. The latter indicates an inflammatory response indicating that the drug was irritating. Fluticasone propionate alone produced increased hematocrit, decreased lymphocytes, increased fibrinogen levels, increased urinary pH, decreased thymus and adrenal weight, alopecia, decreased adipose tissue, decreased nasal lymphoid cellularity, macrophage aggregates around the bronchioles, thymus and adrenocortical atrophy and atrophy of several lymph glands. Vacuolation was seen in the centrilobular hepatocytes of the M and in the periportal hepatocytes of the F differences between sexes in the location of the vacuolation. Myocarditis was unexpectedly found in the F receiving fluticasone propionate and to a low incidence in the salmeterol xinafoate treated animals. Animals receiving the two compounds manifested the changes caused by the compounds when given alone. No drug interaction was indicated. A slight trend toward accumulation with fluticasone propionate occurred in the HD group receiving the combination drugs.

Two 14-day preliminary/pilot inhalation studies were conducted in dogs using the dry powder. In the prelinminary study, salmeterol xinafoate and fluticasone propionate was given in a 1:1 combination as shown in the following table.

Salmeterol xinafoate (µg/kg)	17	178
Fluticasone propionate (µg/kg)	16	164

At the LD, there were, decreased body weight gained, tachycardia, lymphocytosis, increased urinary pH, increased AST levels, myocardial necrosis, thymus atrophy, decreased adrenal weight with decreased width of the zona fasiculata and retularis and swelling and rarefaction of the periportal hepatocytes. At the HD, there was in addition to the effects seen at the LD an increase in body weight gained, anemia, increased monocyte levels, increased BUN, increased liver weight, myocardial fibrosis and reduced cellularity of the tracheobronchial lymph nodes. No kidney toxicity was seen histologically to support the increased BUN levels. The increase in

body weight gained apparently was due to the increased body weight gained effect of β_2 agonism overcoming the glucocorticoid-induced decreased body weight. The C_{max}s were dose related.

In the second 14-day pilot inhalation study in dogs, salmeterol xinafoate and fluticasone propionate was given alone and in combination in a ratio (salmeterol xinafoate:fluticasone propionate) of 1:2 and 1:4 in the powder formulation as shown in the following table.

Salmeterol xinafoate (µg/kg)	41	164	49	52	138	140
Fluticasone propionate (µg/kg)	0	0	82	197	220	537

Animals receiving the salmeterol xinafoate alone showed decreased body weight gained at the LD and a slight increase in body weight gained at the HD; at both doses, there were pigmented alveolar macrophages, tachycardia which was partially reversible and dystrophic mineralization in the renal medulla. In animals receiving the combination, the lowest dose group showed decreased serum phosphate levels, decreased thymus weight, thymus atrophy, decreased width of the zona fasiculata and reticularis of the adrenals and dystrophic mineralization in the renal medulla. The 3 higher dosed groups in addition manifested anemia, and swelling and rarefaction of the periportal hepatocytes. The highest dosed group also showed decreased lymphocyte and increased neutrophil and potassium levels and increased absolute and relative liver weight and decreased ovarian weight. Plasma levels were dose related, and there was no drug interaction.

In the 13-week inhalation study in dogs, salmeterol xinafoate and fluticasone propionate were given alone and in combination in a ratio (salmeterol xinafoate:fluticasone propionate) of 1:10 and 1:2 in the powder formulation as shown in the following table.

Salmeterol xinafoate (µg/kg)	15	0	3	16
Fluticasone propionate (µg/kg)	0	30	31	30

No toxicity was seen in the animals treated with salmeterol xinafoate alone. The changes seen in the fluticasone propionate-treated animals alone were similar in the animals receiving the combination of fluticasone propionate and salmeterol xinafoate indicating glucocorticoid-related effects in the groups receiving both drugs. They were decreased body weight gained, decreased lymphocyte, fibrinogen, glucose, creatinine, eosinophil and cortisol levels, increased urinary pH, increased alkaline phosphatase and cholesterol levels, decreased thymus, lung, and adrenal weights, increased liver weight, thymus involution, decreased germinal centers in the spleen, tonsils, and ~~cervical,~~ mesenteric lymph nodes, adrenal cortical atrophy and swelling and rarefaction of the centrilobular hepatocytes. The C_{max}s were dose related and there were no differences between sexes. There was no drug interaction. The C_{max} in the HD salmeterol xinafoate group decreased upon repeated administration.

In a dose range organogenesis study in mice, salmeterol xinafoate was administered p.o. in combination with s.c. fluticasone propionate. The ratios (salmeterol xinafoate:fluticasone propionate) ranged from 1:1.5 to 1:750, and are shown in the following table.

Salmeterol xinafoate (mg/kg) p.o.	0.2	0.2	10	10
Fluticasone propionate (µg/kg) s.c.	15	150	15	150

Significant changes were seen in the mice receiving the high dose of fluticasone propionate. decreased body weights gained, and cleft palate (1 in each group receiving the HD of fluticasone propionate) was seen. There was also a decrease in fetal weight in the group receiving the high dose of salmeterol xinafoate and fluticasone propionate.

In the definitive organogenesis study in mice, salmeterol xinafoate and fluticasone propionate were administered alone and in combination in a ratio (salmeterol xinafoate:fluticasone propionate) of 1:15 to 1:50 as shown in the following table.

Salmeterol xinafoate (mg/kg) p.o.	1.4	0	0.2	1.4	10
Fluticasone propionate (µg/kg) s.c.	0	40	10	40	150

All the fluticasone-treated animals showed decreased body weight gained. Changes seen occurred in the group receiving the highest dose of salmeterol xinafoate and fluticasone propionate. These were: a decrease in the number of fetuses, increased implantation loss, and an increase in delayed ossification. There was a 2.1% incidence (5 in 5 litter) of cleft palate as compared to 1% (3 in 2 litters) in the control group. This incidence was above the 0-0.3% incidence seen in 2 previous control groups. Despite this, the sponsor stated that this incidence was not treatment related since cleft palate was seen in 3 control fetuses. This reviewer disagrees since there was one fetus with cleft palate in each of the 5 litters in the HD treated group as compared to 3 fetuses in 2 litters in the control group. Further, the incidence in the control group was higher than the incidence seen in the control of two previous studies. C_{max}s were seen only in the HD salmeterol xinafoate treated animals while a dose related increase occurred with fluticasone propionate. Neither drug showed accumulation. The HD combination was teratogenic; The cleft palate characteristic of glucocorticoids was most likely attributed to the fluticasone propionate. The NOAEL was 1.4 mg/kg p.o. of salmeterol xinafoate and 40 µg/kg oxandrolone fluticasone propionate s.c.

In a dose range organogenesis study in AHA rats, salmeterol xinafoate was administered p.o. in combination with s.c. fluticasone propionate in a ratio (salmeterol xinafoate:fluticasone propionate) ranging from 1:1 to 1:1000. The doses administered are shown in the following table.

Salmeterol xinafoate (mg/kg) p.o.	0.1	0.1	10	10
Fluticasone propionate (µg/kg) s.c.	10	100	10	100

Severe maternal toxicity, marked decreased body weight gained (-99% and –74%) and decreased food consumption (-14% and –14%), occurred in the 2 HD fluticasone propionate-salmeterol xinafoate -treated animals. Decreased fetal weight and delayed skeletal development were seen

in their offsprings. In the group receiving the HD of salmeterol xinafoate and fluticasone propionate, there were left sided umbilical artery and changes in the occipital bone. The HD salmeterol xinafoate-LD fluticasone propionate-treated animals showed increased body weight gained indicating a β_2 agonist effect. Placental weights were decreased in all treated animals. The combination was not teratogenic, but fetotoxic and delayed development.

In the definitive organogenesis study in rats, salmeterol xinafoate and fluticasone propionate were administered alone and in combination in a ratio (salmeterol xinafoate:fluticasone propionate) ranging from 1:10 to 1:100 as shown in the following table.

Salmeterol xinafoate (mg/kg) p.o.	1	0	0.1	1	10
Fluticasone propionate (µg/kg) s.c.	0	30	10	30	100

Salmeterol xinafoate alone increased body weight gained while all the animals receiving fluticasone propionate alone and with salmeterol xinafoate manifested decreased body weight gained. In the fluticasone propionate-treated animals alone, fetal weight was decreased. All other significant changes occurred in the HD combination group. These included: decreased body weight gained (-83%), decreased food consumption (-15%), decreased placenta and fetal weight, increased incidence of left sided umbilical artery (C, 0%; T, 16.1%), umbilical hernia (C, 0%; T, 2.9%), and delayed ossification and changes in the occipital bone. Plasma levels were detected in the HD salmeterol xinafoate group and in all the fluticasone propionate groups. Both drugs showed accumulation at the HD. The umbilical hernia is a known teratogenic effect of glucocorticoids (Sponsor).

Conclusion

There was no drug interaction in the pharmacological and toxicity studies between salmeterol xinafoate and fluticasone propionate. In the toxicity studies, the results were indicative of the effects of β_2 agonism and those due to glucocorticoids. No unexpected clinical adverse effects are anticipated from the combination studies in animals.

Labeling Review

The animal to human dose ratios on a mg/m^2 basis is based on the delivered dose from the Diskus: salmeterol xinafoate: 45 µg and fluticasone propionate: 466 µg. The changes are in **BOLD.** Calculations of these ratios are shown in the tables at the end of the report. The changes made in the animal to human dose ratios on a mg/m^2 basis were slightly different from the labels of the respective products for salmeterol and fluticasone propionate alone. They were attributed to the amount of salmeterol and fluticasone propionate delivered was slightly less than the amount delivered from the respective products.

Carcinogenesis, Mutagenesis, Impairment of Fertility: *Salmeterol:* In an 18-month carcinogenicity study in CD-mice, salmeterol at oral doses of 1.4 mg/kg and above

(approximately 20 times the maximum recommended daily inhalation dose in adults based on comparison of the **plasma area- under the curves** [AUCs]) caused a dose-related increase in the incidence of smooth muscle hyperplasia, cystic glandular hyperplasia, leiomyomas of the uterus, and cysts in the ovaries. The incidence of leiomyosarcomas was not statistically significant. No tumors were seen at 0.2 mg/kg (approximately 3 times the maximum recommended daily inhalation doses in adults based on comparison of the AUCs).

In a 24-month oral and inhalation carcinogenicity study in Sprague Dawley rats, salmeterol caused a dose-related increase in the incidence of mesovarian leiomyomas and ovarian cysts at doses of 0.68 mg/kg and above (approximately 60 times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). No tumors were seen at 0.21 mg/kg (approximately 20 times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). These findings in rodents are similar to those reported previously for other beta-adrenergic agonist drugs. The relevance of these findings to human use is unknown.

Salmeterol produced no detectable or reproducible increases in microbial and mammalian gene mutation in vitro. No clastogenic activity occurred in human lymphocytes in vitro or in the in vivo rat micronucleus test.

No effects on fertility were identified in male and female rats treated with salmeterol at oral doses up to 2 mg/kg (approximately **180** times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis).

Fluticasone Propionate: Fluticasone propionate demonstrated no tumorigenic potential in mice at oral doses up to 1000 mcg/kg (approximately 4 times the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis) for 78 weeks or in rats at inhalation doses up to 57 mcg/kg (**less than** the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis) for 104 weeks.

Fluticasone propionate did not induce gene mutation in prokaryotic or eukaryotic cells in vitro. No significant clastogenic effect was seen in cultured human peripheral lymphocytes in vitro or in the in vivo mouse micronucleus test.

No evidence of impairment of fertility was observed in reproductive studies conducted in male and female rats at subcutaneous doses up to 50 mcg/kg (**less than** the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis). Prostate weight was significantly reduced at a subcutaneous dose of 50 mcg/kg.

Pregnancy: *Teratogenic Effects:* Pregnancy Category C. *Salmeterol:* No teratogenic effects occurred in rats at oral doses up to 2 mg/kg (approximately **180** times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). In pregnant Dutch rabbits administered oral doses of 1 mg/kg and above (approximately 50 times the maximum recommended daily inhalation dose in adults based on comparison of the AUCs), salmeterol exhibited fetal toxic effects characteristically resulting from beta-adrenoceptor stimulation. These included precocious eyelid openings, cleft palate, sternebral fusion, limb and paw flexures,

and delayed ossification of the frontal cranial bones. No significant effects occurred at an oral dose of 0.6 mg/kg (approximately 20 times the maximum recommended daily inhalation dose in adults based on comparison of the AUCs).

New Zealand White rabbits were less sensitive since only delayed ossification of the frontal bones was seen at an oral dose of 10 mg/kg (approximately **1800** times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). Extensive use of other beta-agonists has provided no evidence that these class effects in animals are relevant to their use in humans. There are no adequate and well-controlled studies with salmeterol in pregnant women. Salmeterol should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Salmeterol xinafoate crossed the placenta following oral administration of 10 mg/kg to mice (900 times, respectively, the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis).

Fluticasone Propionate: Subcutaneous studies in the mouse and rat at 45 and 100 mcg/kg, respectively, (**less than and equivalent to,** the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis) revealed fetal toxicity characteristic of potent corticosteroid compounds, including embryonic growth retardation, omphalocele, cleft palate, and retarded cranial ossification.

In the rabbit, fetal weight reduction and cleft palate were observed at a subcutaneous dose of 4 mcg/kg (**less than** the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis). However, no teratogenic effects were reported at oral doses up to 300 mcg/kg (approximately 5 times the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis) of fluticasone propionate.

Fluticasone propionate crossed the placenta following the subcutaneous administration of 100 mcg/kg to mice and rats (less than and equivalent to, respectively, the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis) and following the oral administration of 300 mcg/kg to rabbits (5 times the maximum recommended daily inhalation dose in adults on a mcg/m^2 basis).

ADVAIR DISKUS: ——————— **10 mg/kg, orally of salmeterol (approximately 450 times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) → 150 mcg/kg, subcutaneously of fluticasone propionate (less than the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) was teratogenic. Cleft palate, fetal death, increased implantation loss and delayed ossification was seen. These observations are characteristic of glucocorticoids. No developmental toxicity was observed**

at combination doses up to 1.4 mg/kg orally of salmeterol (approximately 65 times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) and up to 40 mcg/kg subcutaneously of fluticasone propionate (less than the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). In rats, no teratogenicity was observed at combination doses up to 1 mg/kg orally of salmeterol (approximately 90 times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) and up to 30 mcg/kg subcutaneously of fluticasone propionate (less than the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). Combining 10 mg/kg, orally of salmeterol (approximately — times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) with 100 mcg/kg, subcutaneously of fluticasone propionate (less than the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) produced maternal toxicity, decreased placenta weight, decreased fetal weight, umbilical hernia, delayed ossification and changes in the occipital bone.

OVERDOSAGE:

No deaths were seen in rats given salmeterol at an inhalation dose of 2.9 mg/kg (approximately — times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) and in dogs at an inhalation dose of 0.7 mg/kg (approximately — times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). By the oral route, no deaths occurred in mice at 150 mg/kg (approximately — times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis) and in rats at 1000 mg/kg (approximately — times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis). Fluticasone Propionate: The oral and subcutaneous median lethal doses in mice and rats were >1000 mg/kg (>4300 and >8700 times, respectively, the maximum recommended daily inhalation dose in adults on a mg/m^2 basis).

ADVAIR DISKUS: No deaths occurred in rats given combinations of salmeterol and fluticasone propionate at acute inhalation doses of 3.6 and 1.9 mg/kg, respectively (approximately 320 and 15 times the maximum recommended daily inhalation dose in adults on a mg/m^2 basis).

RECOMMENDATIONS

This NDA is approvable with the labeling revision as indicated above.

/S/ 1/24/00

Lawrence F. Sancilio, Ph.D.

/S/ Jan. 24, 2000

cc: Orig. NDA21-077
HFD-570/Division File
HFD-570/LSancilio
HFD-570/SJohnson
HFD-570/CSO

Amendments (4)

Approved by Dr. J. Sun

APPEARS THIS WAY
ON ORIGINAL

Drug: **Fluticasone propionate**
ADVAIR_

	age	Mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric	[redacted]			0	25	0.8000	25	20.00
Adult	>12	[redacted]		0.932	50	0.0186	37	0.69

	route	Mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
Mouse	[redacted]		3	3	4.35		4	
rat			6	0.342	0.50		1/2	
Hamster			4	0	---		---	
rat			6	0	---		---	
rat			6	0	---		---	
Reproduction and Fertility:								
rat	[redacted]		6	0.3	0.43		1/2	
rat			6	0.06	0.09		1/11	
rat			6	12	17.40		15	
rat			6	0.18	0.26		1/4	
Teratogenicity:								
Mouse	[redacted]		3	0.135	0.20		1/5	
rat			6	0.6	0.87		1/1	
Rabbit			12	0.048	0.07		1/14	
Rabbit			12	3.6	5.22		5	
rat			6	0.3	0.43		1/2	
Overdosage:								
Mouse	[redacted]		3	3000	4349.84		4300	
Mouse			3	0	---		---	
rat			6	6000	8699.69		8700	
rat			6	11.4	16.53		15	---
Other:	(Describe studies here)							
Mouse	[redacted]		3	0.12	0.17	0.01	1/6	1/167
Mouse			3	0.45	0.65	0.02	1/2	1/44
Mouse			3	0.3	0.43	0.02	1/2	1/67
rat			6	60	87.00	3.00	85	3
Extra			---	---	---	---	---	---

Drug: **Salmeterol Advair**

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric dose	[redacted]			0	3	0.00	25	0.00
Adult dose	>12	[redacted]	2	0.09	50	0.00	37	0.07

	route	mg/kg/day	factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
Mouse	[redacted]		3	0	---		---	
Rat			6	4.08	61.26		60	
Hamster			4	0	---		---	
Rat			6	1.26	18.92		20	
Extra			---	---	---		---	
Reproduction and Fertility:								
Rat	[redacted]		6	12	180.18		180	
Rat			6	0	---		---	
Mouse			3	0	---		---	
Rat			6	60	900.90		900	
Teratogenicity:								
Mouse	[redacted]		3	4.2	63.06		65	
Mouse			3	30	450.45		450	
Rat			6	6	90.09		90	
Rabbit			12	120	1801.80		1800	
Rat			6	0	---		---	
Overdosage:								
Mouse	[redacted]		3	450	6756.76		6800	
Rat			6	21.6	324.32		320	
Rat			6	6000	#######		90000	
Rat			6	17.4	261.26		260	
Other:	Additional Acute tox for Overdosage							
Dog	[redacted]		20	14	210.21	---	210	---
Monkey			12	0	---	---	---	---
Rabbit			12	0	---	---	---	---
Extra			---	---	---	---	---	---
Extra			---	---	---	---	---	---

Jani

JAN 18 2000

REVIEW AND EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
Chemistry Consult

Reviewer: Lawrence F. Sancilio, Ph.D.

Division: PULMONARY DRUG PRODUCTS, HFD-570

Reviewer Completion Date: 1/18/00

NDA No. 21-077

Date of Consult Request: 12/15/99

Information to Sponsor: Yes (X), No ( )

Sponsor: Glaxo Inc.
5 Moore Drive
Research Triangle Park, NC 27709

Drug: Salmeterol xinafoate (Serevent ™) and fluticasone propionate (Flovent ™) Combination

Trade Name: Advair Diskus

Drug Class: Salmeterol xinafoate, β_2 receptor antagonist
Fluticasone propionate, glucocorticoid steroid

Indication: Maintenance Treatment of asthma [redacted] in patients 12 years of age and older.

Route of Administration: Oral inhalation

Recommended Dose: The proposed maximum daily dose is 50 μg of salmeterol as salmeterol-xinafoate and up to 500 μg of fluticasone propionate twice a day. Advair Diskus delivers per dose 45 μg of salmeterol and 466 μg of fluticasone propionate

Dr. D. Koble requested that the safety of the colorants in the mouthpiece of the Diskus be assessed.

The following table lists the colorants, their concentration in the mouthpiece and whether the concentrations meet those acceptable as listed in the CFR.

Color Component	% in the Mouthpiece	Do the Proposed Concentrations Meet CFR Acceptable Levels
[redacted]		

Discussion

[redacted] components of the colorant at the proposed levels meet the acceptable concentrations cited in the CFR. No information was given for [redacted] A literature search was unsuccessful. Consequently, assessment of its safety at the proposed concentration cannot be made.

Recommendations

The following components of the colorant are acceptable in the mouthpiece at the concentrations proposed: [redacted]

[redacted] is not acceptable due to lack of safety data. It is recommended that the sponsor submit the quantitative extractable profile of the mouthpiece to enable us to determine whether the maximum daily exposure of this compound is safe.

Letter to the Sponsor

[redacted] one of the components of the colorant is not acceptable due to lack of toxicology data to assess a safe dose. It is recommended that you submit a quantitative extractable profile of this compound to enable us to determine whether its maximum daily exposure is safe.

/S/

1/18/00

Lawrence F. Sancilio. Ph.D.

cc. /Division File, NDA 20-833, HFD-570
/MPurucker, HFD-570
/C.S.O., HFD-570
/LFSancilio, HFD-570
/DKoble, HFD-570

/S/

Jan. 18, 2000

[redacted]

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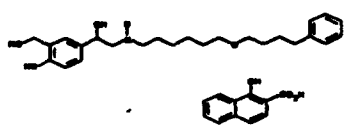
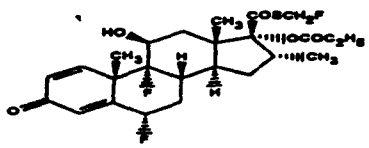
DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 1

Reviewer Name:	Luqi Pei, D.V.M., Ph.D.
Division Name:	HFD-570
Review Completion Date	January 11, 1999
IND No.	———
Serial Nos., Contents and Dates of Submission:	000, Original submission, Oct. 23, 1998
Information to be conveyed to Sponsor:	Yes (x), No ( ).
Sponsor:	Glaxo Wellcome, Research Triangle Park, NC (919-483-2100)
Supplier:	
Drug:	
Code Name:	GR33343G, Salmeterol CCI18781, Fluticasone
Generic Name:	Salmeterol/fluticasone/ ——— inhalation Aerosol
Trade Name:	None
Structure:	



Salmeterol



Fluticasone

Relevant INDs/NDAs/DMFs	NDA 20-121 Flonase Nasal Spray NDA 20-548 Flovent Inhalation Aerosol NDA 20-549 Flovent Rotodisk NDA 20-833 Flovent Diskus NDA 20-236 Serevent Inhalation Aerosol —— Serevent Diskus IND — Salmeterol/fluticasone DPI IND —— DMF ——
Class:	Beta 2 adrenergic bronchodilator/corticosteroid
Indication:	Asthma in 12 years and older
Route of Administration:	Topical (skin test)
Previous clinical experience:	Both drugs are on the market individually. A combination product (DPI) is in clinical trial

Clinical Formulation:

Formulation	Strength — µg	Strength — µg	Strength — µg	Function
Salmeterol xinafoate (—— , mg)	—	—	—	Active ingredient
Fluticasone propionate (—— , mg)	—	—	—	Active ingredient
——	——	——	——	——

Document submitted and reviewed in this IND:

Study	Report #	Vol.	Page
Rat, acute IH toxicity – effect of and combination on cardiovascular function	WPT/95/253	3	62
Rat, 5-wk IH toxicity*	WPT/93/176	4	2
Rat, 13-wk IH toxicity*	WPT/96/076	6	1
Mouse, Effect of pre-treatment on the sensitivity of uterus	WPT/93/377	8	262
Dog (beagle), pilot 14-day IH toxicity study*	WPT/93/189	8	67

* Studies were done with the combination of GR33343G (salmeterol xinafoate) and CCI18781 (fluticasone propionate) in —— ——.

Document not reviewed in this IND:

A 5-week subcutaneous toxicity study of fluticasone in RH rats. Reference Report No. WPT/84/088. Study No. R10490.

Background Information

Currently, there are several nasal and/or inhalation salmeterol or fluticasone products on the market. Salmeterol (xinafoate) products include Serevent inhalation aerosol (NDA 20-236) and Serevent diskus (NDA 20-692). Both products were approved on September 8, 1997. Fluticasone (propionate) products include Flonase Nasal Spray (DNA 20-121), Flovent inhalation aerosol (NDA 20548), Flovent Rotodisk (NDA 20549), and Flovent Diskus (NDA 20833). These fluticasone products were approved between October 14, 1994 and November 7, 1997. A combination product of salmeterol and fluticasone (salmeterol/fluticasone DPI) is in clinical trial (IND ———

[ ]

In the current submission, Glaxo Wellcome submitted 3 protocols (Protocols SAS30001, SAS30003, and SAS30004) to study the safety and efficacy of salmeterol/fluticasone combination in asthmatic patients. The proposed dose levels of salmeterol/fluticaosne were (in μg): ——— or 50/500. Treatment duration would be 12 weeks. A total of 1295 patients would be involved in these trials. Trials with two low dose groups would be conducted here in the US and the high dose trial will be conducted in Canada or Europe.

REVIEW

I. Safety Pharmacology

Effect of fluticasone pretreatment on uterine sensitivity to salmeterol in mouse. Report No. WPT/93/377. Vol. 8, page 262.

Testing laboratory:	Glaxo Wellcome Research and Development, Ware, Hertfordshire, UK
Study dates:	Report date: 1/21/97; (Study No. R13256)
GLP:	Yes

Sexually mature female mice (CR/H, n = 7-8/group) were given fluticasone (SC, 30 μg/kg/day) for 14 days and sacrificed at the end of treatment period. Their uteri were removed and then tested for the sensitivity to salmeterol *in vitro*. This sensitivity was determined by the dose-response relationship of uterine contractility to various concentrations of salmeterol (10^{-4} to 10^{-9} M). Uterine contractility was measured using an ——— Plasma drug levels were assayed to determine systemic exposure. Results showed that the dose-response curves of the treated and control groups overlapped each other. Thus, fluticasone pretreatment did not affect the uterine sensitivity to salmeterol in mouse.

II. Toxicology

1. An acute inhalation toxicity study of Salmeterol and fluticasone in Wistar Rats – assessment of cardiovascular function using remote telemetry. WG Report No. WPT/95/253. Vol. 3, p 62.

Testing laboratory:	Glaxo Wellcome Research and Development, Ware, Hertfordshire, UK; and
Study dates:	5/18/95 – 8/30/95, report date: 6/27/97; (Study No. R20927)
GLP:	Yes
Batch Information:	U962/018, U962/009

Method:

Male Wistar Rats (, 5/group), weighing between 416 – 587 g and implanted with radiotransmeters, were exposed to a single dose (1 hr inhalation) of either salmeterol or salmeterol/fluticasone in lactose aerosol to study their effects on cardiovascular function. The study design and dose estimation are summarized in Table 1 (below). These animals (group 1 – 3) were sacrificed on day 14 for histologic examinations. Additional animals that had sham surgery operation were exposed to the drug aerosols to serve as the controls. They (Group 4 – 6) were sacrificed after an observation period of 48 hours (day 2).

Table 1. Study Design and Dosage of the Acute Toxicity Study in Male Rats (n = 5/group)

Group #	Treatment	Dose (mg/kg)	Aerosol conc. (µg/L)	Time of Sacrifice	
				Day 2	Day 14
1 & 4	Control	0*	0	Group 4	Group 1
2 & 5	Salmeterol	5.2	165.5	Group 5	Group 2
3 & 6	Salmeterol/ fluticasone	3.3/1.9	107/60.1	Group 6	Group 3

* The control group received vehicle (lactose) alone.

The following parameters were monitored during the experiment.

Clinical signs:	At least daily
Body weight:	Days -2, -1, 0, 1, 2, 7, 14
Food consumption:	Days -2, -1, 0, 1; 2
Telemtery:	Group 1, 2, 3, measured blood pressure, heart rate, and ECG for 10 seconds prior to exposure, 15 minutes after dosing, then every 15 minutes for the first 2 hours, and hourly to 48 hours.

Plasma drug level: Immediately after the exposure, assayed with [redacted]

Gross pathology: At sacrifice

Histopathology: Heart and tissues with macroscopic abnormalities in group 4, 5, and 6.

Results:

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Mortality: None.

Clinical signs: Noisy respiration was seen in 2 Group 2 rats on day 1. Salivation was seen in one Group 3 rat immediately after dosing on day 1. One rat in group 6 had cold extremities on day 2.

Body weight: The combination treatment groups (Group 3 & 6) showed a statistically significant decrease in body weight on day 1 and 2. But it returned to normal by day 7 after the treatment (Group 3).

Plasma drug concentration: The concentrations of salmeterol and fluticasone are presented in Table 2 (below). The both salmeterol and fluticasone were also detected in control animals that were not exposed to drugs. The sponsor stated that it was probably from contamination during sampling. The cross contamination during sample analysis should affect the overall interpretation of the study results.

Table 2. Plasma Drug Concentration of the Acute Inhalation Study in Rats

Group	4	5	6
Exposure (mg/kg)			
Salmeterol	-	5.2	3.3
Fluticasone	-	-	1.9
Plasma (ng/ml)			
Salmeterol [1]	0.8 – 2.1[2]	16.4 – 31.8[3]	16.9 - > 50.0[4]
Fluticasone	0.37 – 1.29	0.33 – 0.95	0.89 – 3.72

[1] The lower limit of detection were [redacted] for salmeterol and [redacted] for fluticasone.

[2] Drug levels in 3/5 control animals. Possible cross contamination may exist.

[3] Individual salmeterol plasma concentrations for Group 5 were [redacted], and [redacted] (mean = 21.6).

[4] Individual salmeterol plasma concentrations for Group 6 were no sample, [redacted] (mean = 28.5).

Food consumption: A dose-related decrease in food consumption was seen in Groups 5 & 6 only.

Telemetry: Heart rate was elevated (↑ about 50%) in both group 2 and 3.

ECG: An increase in the total incidences of cardiac arrhythmia occurred in the combination treatment group (Table 3, below). There was no change in wave configuration.

Table 3. ECG Findings of the Acute Inhalation Study in Rats (n = 5/group)

Group	1	2	3
Dose (mg/kg): Salmeterol	-	5.2	3.3
Fluticasone	-	-	1.9
Ventricular premature contraction	1 (5[1])	0	0
Sinus arrest	0	1 (7[2])	1 (12[2])
2° Atrioventricular blockage	0	0	2 (11[3], 15[4])
Atrial premature complex	0	0	1 (11)
Supraventricular premature complex	0	0	1 (15)
Total: Rat# w/ abnormal findings	1/5	1/5	3/5

[1] Rat No. 5 showed ventricular premature contraction 41.3 hours post dosing.
[2] Rat No. 7 showed sinus arrest at 0.75, 1.5, and 1.75 hrs after dosing while Rat No. 12 showed it at 48.3 hours after dosing.
[3] Rat No. 11 showed 2° atrioventricular block at 1.0 hour post dosing and atrial premature complex at 23.3 hours post dosing.
[4] Supraventricular premature complex and second degree atrioventricular block occurred in this rat at 15 minutes post dosing.

Histopathology: Slight myocardial and submyocardial degeneration was seen in the ventricles of the salmeterol treated groups (Table 4). Atrial myocarditis was seen in the salmeterol/ fluticasone treatment group only. Note: Because histological examination was not done at the terminal sacrifice in Groups 1, 2 and 3, direct associations between pathology and functional changes can not be made.

Table 4. Microscopic Findings of the Acute Inhalation Study in Rats

Group	4	5	6
Dose (mg/kg): Salmeterol	-	5.2	3.3
Fluticasone	-	-	1.9
Myocardial degeneration, ventricular	0/5	3/5	3/5
Subendocardial generation, ventricular,	0/5	3/5	2/5
Myocarditis, atrial	0/5	0/5	3/5

Summary: Rats treated with both salmeterol and fluticasone showed atrial myocardis and CKG abnormalities that were absent in the concurrent salmeterol controls.

2. A pilot 35-day inhalation toxicity study in rats. Report No. WPT/93/176. Vol. 4, p 2.

Testing laboratory: [redacted]
Study dates: 5/18/95 – 8/30/95, report date: 6/27/97; (Study No. R20927)
GLP: Yes, but parts of the report were not audited.
Batch Information: U92/112A, U92/113A

Method:

Wistar Rats ([redacted] 10/sex/dose) were exposed by nose-only inhalation to salmeterol/fluticasone/ [redacted] mixture for 35 days to study their toxicity. The daily exposure duration was one hour. Additional animals (5-6/sex/dose) were used for interim analysis (2 week-exposure) and recovery evaluation (2 weeks of recovery after 35-day exposure). The study design is summarized in Table 5 (below).

Table 5. Study Design of the 5 -Week Inhalation Toxicity Study in Rats

Group	No. of animals per group per sex				Delivered dose (µg/kg)[3]	
	Interim[1]	Main	Recovery	Satellite[2]	Salmeterol	Fluticasone
1	5	10	5	6	0	0
2	5	10	-	6	74	140
3	5	10	-	6	75	370
4	5	10	5	6	140	710
5	5	10	5	6	380	760

1. Sacrifice time were Days 14 and 35 of exposure and 14 days after the 5-week exposure for interim, main and recovery groups respectively.
2. For toxicokinetic study to determine plasma drug levels
3. Dose estimation was based on: Dose (mg/kg) = {[C (µg/l) x RMV (ml/min) x D (min) x F]/[W (g) x 10^{-3}]}, where RMV = 4.19 x $W^{0.66}$).

[redacted]

The following parameters were observed during the study:

Clinical signs: Twice daily
Body weight: Weekly
Food consumption: Weekly
Ophthalmology: Prior to treatment, week 4
Clinical pathology: Weeks 2, 5 and 7
EKG: 5 rats after exposure on day 1, week 2 and 5
Plasma drug level: 7 – 26 minutes after exposure on day 1, weeks 2 and 5 (n = 2/sex/dose), but samples were discarded.

Pathology:	Days 15, 36, and 50
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus
Necropsy:	All animals
Hisotology:	Main and interim groups: Adrenals, heart, kidneys, larygnx, liver, lungs, lymph nodes (treacheobronchial), ovaries, nasal passages, pharynx, thymus, and gross abnormalities. Recovery groups: Adrenals, larygnx, liver, lungs, lymph nodes (treacheobronchial), ovaries, nasal chambers, thymus, and tracheal bification.

Results:

Mortality: No treatment mortality was observed.

Clinical signs: A slight increase in the incidences of hair loss was seen (Table 6, below).

Table 6. Percentage of Hair Loss of the 5-Week Inhalation Toxicity Study in Rats

	Male					Female				
Dose Salm./ Flut. (mg/kg)	0 /0	74 /140	75 /370	140 /710	380 /760	0 /0	74 /140	75 /370	140 /710	380 /760
Wk 1	0	0	0	0	0	0	0	0	0	0
5	7	10	0	21	33	7	10	60	27	33
7	80	-	-	100	100	0	-	-	60	100

Body weight: Dose-related decreases in body weight gain were seen in during the treatment (Table 7, below). Body weight tended to recover when the treatment was withdrawn (Week 5 – 7), but failed to catch up with the controls. There was a 12% decrease in absolute body weight even after two weeks of recovery.

Table 7. Body Weight in the 5-Week Inhalation Toxicity Study in Rats

Dose (Salm./Flut. in µg/kg)	0/0	74/140	75/370	140/710	380/760
BW gain (%), Wk_{0-5}, Male	66 (g)	7**.[1]	-13**	-52**	-53**
Female	25 (g)	-22**	-37**	-50**	-46**
Wk_{5-7} Male	38 (g)	-	-	79**	77**
Female	12 (g)	-	-	49**	47**
Absolute BW at wk 7					
Male	428 g	-	-	339	348
Female	256 g	-	-	224	227

** *Dunnett's test: P < 0.01.*

[1] Changes (%) compared to the control.

Food consumption: There were no significant differences in food consumption. (Table 8, below).

Table 8. Food Consumption (%) in the 5-Week Rat Inhalation Toxicity Study

Dose (Salm/Flut in mg/kg)	0/0	74/140	75/370	140/710	380/760
Wk 0 – 5, Male	-	102	102	93	93
Female	-	94	99	101	102
Wk 5 – 7, Male	-			93	97
Female	-			107	104

Heart rate: Increases in heart rate were seen in the treated groups, but lacked apparent dose-response relationship (Table 9, below). Group 5 received the highest doses of salmeterol and fluticasone, but the heart rate was similar to the control. Note that heart rate in the control group was about 50% higher than that of the previous study.

Table 9. Heart Rate (beat/min) in the 5-Week Rat Inhalation Toxicity Study

	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose Sal/ Fl (mg/kg)	0 /0	74 /140	75 /370	140 /710	380 /760	0 /0	74 /140	75 /370	140 /710	380 /760
Wk 1	531	563*	552	542	540	520	555	559	550	523
2	539	566	541	538	534	525	577*	607**	563	545
5	535	581*	594**	603**	579*	566	605	613*	606	579
7	518			512	552	534			571	548

** P<0.05, ** P < 0.01 in Dunnett's test.*

EKG: No data was submitted, but the report indicated: 1) an increase in the amplitude of the T wave in animals from Groups 3, 4 and 5 during weeks 2 and/or 5 of dosing. 2) this finding was considered possibly related and expected to the treatment of beta agonists.

Clinical pathology: A number of changes in clinical pathology parameters were observed during the treatment (Table 10, next page). These changes are typical effects of beta 2 agonists and glucocorticosteroids. Changes in hematology parameters included mild but statistically significant increases in packed cell volume, hemoglobin, red blood cell count, and neutrophil counts; and decreases in mean corpusular hemoglobin concentration, and in the counts of total white cell, lymphocyte and eosinophil in treated groups. Lower platelet and thromboplastin values were also seen in the treated groups. Biochemistry changes included slight elevations in glucose, proteins, urea nitrogen, AST and cholesterol levels. Changes in urine parameters included the increases in pH and sodium concentration, and decreases in phosphorus and potassium concentrations in the urine of the treated groups. These changes were detected by the week 2 of the exposure and persisted during continuing exposure, but lacked an increase in severity. All parameters returned to the normal range after a recovery period of 2 weeks.

Table 10. Clinical Pathology (Group Means) in the 5-Week Rat Inhalation Toxicity Study, Week 5

	Male					Female				
Salmeterol (mg/kg)	0	74	75	140	380	0	74	75	140	380
Fluticasona (mg/kg)	/0	/140	/370	/710	/760	/0	/140	/370	/710	/760
Hematology										
RBC: PVC (%)	56	**62**	**64**	**60**	**61**	54	**59**	**59**	**56**	**58**
Hemoglobin (g/dl)	15.6	**16.8**	**17.5**	**16.5**	**16.6**	15.3	**16.3**	**16.5**	**15.7**	**16.2**
RBC (x 10^6/mm^3)	7.6	**8.6**	**8.7**	**8.1**	**8.3**	7.1	**7.9**	**7.8**	7.3	**7.6**
WBC: Total (x 10^3/mm^3)	7.4	**5.3**	**4.5**	**5.2**	**4.9**	4.1	**3.0**	**2.8**	3.8	3.8
Neutrophil	1.83	2.33	2.49	**3.93**	**3.7**	1.09	1.08	1.52	**2.83**	**2.88**
Lymphocyte	5.37	**2.86**	**1.90**	**1.17**	**1.11**	2.92	**1.81**	**1.23**	**0.89**	**0.88**
Clot.: platelet (x 10^3/mm^3)	897	870	853	845	**784**	935	830	794	736	725
TT (s)	23	24	**21**	**21**	**21**	21	22	22	21	21
APTT (s)	18.3	19.9	**20.9**	**19.6**	**20.2**	18.2	**19.8**	**21.5**	**21.2**	**21.0**
Fibrin. (mg/dl)	218	**264**	**269**	**278**	**274**	191	212	**241**	**227**	**246**
Blood chemistry										
Glucose (mg/dl)	92	81	100	**131**	**123**	90	100	**129**	**129**	**128**
Protein (total, g/dl)	6.6	**7.1**	**7.3**	**7.2**	**7.3**	6.8	6.6	**7.2**	6.9	7.0
Urea nitrog. (mg/dl)	22	**29**	**27**	**26**	24	25	27	25	23	24
ALT (mU/ml)	28	34	37	**53**	**50**	26	32	**41**	**52**	**57**
AST (mU/ml)	60	60	69	**78**	**83**	**56**	**56**	59	**68**	**81**
K (mEq/l)	3.4	**3.9**	**4.0**	**4.4**	**4.3**	3.1	**3.7**	**4.0**	**4.3**	**4.2**
Ca (mEq/l)	5.4	**5.6**	**5.6**	5.5	**5.6**	5.5	5.5	**5.8**	**5.6**	**5.7**
P (mEq/l)	4.4	4.7	**5.3**	**4.8**	**5.1**	3.5	**4.6**	**4.2**	**4.2**	**4.8**
Cl (mEq/l)	100	**99**	**97**	**99**	**97**	99	100	98	99	98
Cholesterol	70	**91**	**103**	**132**	**122**	73	82	97	**102**	**101**
Cortisone (µg/dl)	53	**38**	**31**	**17**	**19**	77	79	**36**	**25**	**20**
Uralysis										
pH	6.4	**7.0**	**7.2**	**7.4**	**7.7**	5.7	**6.4**	**6.7**	**6.8**	**6.8**
Na (mEq/ml)	0.32	**0.65**	**0.80**	**0.59**	**0.53**	0.29	0.41	**0.51**	**0.45**	0.41
P (mEq/vol)	594	**277**	**258**	**264**	**181**	521	**243**	**282**	**268**	**219**
K (mEq/vol)	0.95	**0.65**	**0.69**	**0.71**	**0.62**	0.56	0.45	0.57	0.59	0.58

Bold indicates statistically significant.

Organ weights: Noticeable and/or relevant changes in organ weights are summarized in Table 11 (next page). The high dose animals showed smaller adrenal glands, thymus and spleen, and larger liver. Small thymus was evident even in the low dose females. Changes in liver (increase) and spleen (decrease) weights were dose-related, but none reached the statistical significance.

Table 11. Organ Weight (Group Means) in the 5-Week Rat Inhalation Toxicity Study, Week 5

	Male					Female				
Salmeterol/ Fluticasone (mg/kg)	0/ 0	74/ /40	75/ 370	140/ 710	380/ 760	0/ 0	74/ 140	75/ 370	140/ 710	380/ 760
Body weight (g)	374	331	309	267	269	255	201	187	175	176
Adrenals, (mg)	31.6	**23.6**	**14.3**	**10.3**	**11.4**	38.6	25.4	**11.0**	**11.0**	**11.3**
Thymus (g)	0.29 4	**0.08 7**	**0.038**	**0.033**	**0.034**	0.30	**0.06 2**	**0.03 3**	**0.02 6**	**0.02 5**
Spleen (g)	0.89	**0.64**	**0.52**	**0.45**	**0.45**	0.67	0.47	**0.40**	0.37	0.37
Heart, (g)	1.13	1.20	1.26	1.17	1.15	0.89	0.89	**0.92**	**0.91**	**0.91**

Gross pathology: Small thymus, spleen and adrenals were observed in all treated groups (Table 12, below). These observations correlate well with their organ weights (Table 11). Changes in organ weights were also already evident at week 2. All these changes except small adrenals disappeared after a recovery period of 2 weeks.

Table 12. Gross pathological Findings in the 5-Week Rat Inhalation Toxicity Study, Week 5

	Male					Female				
Salmeterol (mg/kg)/ Fluticasone (mg/kg)	0 /0	74 /140	75 /370	140 /710	380 /760	0 /0	74 /140	75 /370	140 /710	380 /760
Number examined	10	10	10	9	10	10	10	10	10	10
Skin, scab	0	2	0	1	4	0	0	0	1	0
, alopecia	0	3	0	5	6	0	2	9	4	6
Thymus, small	0	10	10	9	10	0	10	10	10	10
Lungs, pale ubpleural fucus/i	1	6	7	8	6	2	8	9	7	9
, congested	1	2	1	0	3	0	0	0	3	1
Forestomach, white raised area	0	0	1	4	3	0	0	2	1	1
Adrenals, small	0	0	10	9	10	0	3	10	9	10

Histopathology: Microscopic findings related to the treatment were seen both in the respiratory and other systems (Table 13, next page). Locally, the following changes were observed in the treatment groups: minimal epithelial hyperplasia in the nasal cavity; squamous epithelial hyperplasia and metaplasia, and cartilage necrosis in the ventral region of the larynx; aggregation of macrophages in the lung; and a decrease in cellularity in the tracheabrochial lymph node. These changes, with the exception of the decrease in lymph node cellularity, are probably a response to the irritant effect of salmeterol. Changes in other systems were typical steroid effects: atrophy of thymus, adrenals and reduced cellularity in the spleen.

Table 13. Microscopic Findings in the 5-Week Rat Inhalation Toxicity Study, Week 5

	Male					Female				
Salmeterol (mg/kg)/	0	74	75	140	380	0	74	75	140	380
Fluticasone (mg/kg)	/0	/140	/370	/710	/760	/0	/140	/370	/710	/760
No. of animal examined	10	10	10	9	10	10	10	10	10	10
Nasal, hypersplasia/ epithelial	0	0	2	5	10	1	1	1	4	5
Larynx, squam. epi. hyperplasia	0	10	8	8	10	0	7	10	9	10
Squamous metaplasia/vent	0	0	0	4	6	0	0	0	1	4
Necrosis of ventral cartilage	0	0	2	4	7	0	0	0	3	7
Lung, MΦ aggregation (minim)	1	6	3	8	7	2	6	9	7	9
Thymus, atrophy	0	10	9	9	9	0	10	8	9	8
Lymph node (TB), ↓ cellularity	0	6	7	9	9	0	6	9	9	10
Liver, periportal hepatocyte vacuolation	0	0	1	1	0	0	2	7	8	9
Kidney, glomerulonephrosis	0	1	1	1	4	0	0	1	0	0
Adrenals, atrophy	0	4	10	9	10	0	8	10	10	10
Skin, scab(s)		2/2		1/1	3/4					

Most changes listed in Table 13 were already evident even after 2 weeks of treatment (Table 14, below). In addition, the local irritant effect of salmeterol seemed to be treatment-duration dependent. At the same dose level (0.07/0.14 μg/kg/day), animals treated for 5 weeks showed a higher incidence (10/10) of squamous hyperplasia in the ventral region of larynx than ones treated for 2 weeks (3/5).

Table 14. Microscopic Findings in the 5-Week Rat Inhalation Toxicity Study at Week 2

	Male					Female				
Salmeterol (mg/kg)/	0	74	75	140	380	0	74	75	140	380
Fluticasone (mg/kg)	/0	/140	/370	/710	/760	/0	/140	/370	/710	/760
No. of animal examined	5	5	5	5	5	5	5	5	5	5
Nasal, epit. hypersplasia (mini)	0	0	0	1	4	0	0	0	0	0
Larynx (ventral regions),										
Squamous epi. hyperplasia	0	3	3	2	4	0	2	2	3	4
Squamous epi. metaplasia	0	0	0	1	4	0	0	1	2	3
Necrosis of ventral cartilage	0	0	0	1	3	0	0	1	2	3
Lung, MΦ aggregation (minim)	2	1	1	4	3	1	3	5	4	5
Thymus, atrophy	0	5	5	5	5	0	5	5	5	5
Lymph node (TB), ↓ cellularity	0	0	2	5	4	0	1	2	4	5
Spleen, reduced cellularity	0	0	0	1	2	0	0	5	5	5
Liver, peroportal hepatocyte vacuolation	0	0	0	0	0	0	0	2	5	5
Adrenals, atrophy	0	4	5	5	5	0	4	5	5	5

Upon the withdrawal of the treatment, all treatment related changes gradually retreated. Rats with a recovery period of 2 weeks showed lower incidences of local and systemic lesions than rats sacrificed immediately after treatment. However, abnormalities were still evident (Table 15, next page). They included squamous epithelial hyperplasia and cartilage necrosis of the ventral region in the larynx, aggregation of macrophages in the lung, and adrenal atrophy.

Table 15. Microscopic Findings in the 5-Week Rat IH Toxicity Study, After 2-week recovery

Salmeterol (μg/kg)/ Fluticasone (μg/kg)	Male					Female				
	0 /0	74 /140	75 /370	140 /710	380 /760	0 /0	74 /140	75 /370	140 /710	380 /760
No. of animal examined	5	-	-	5	5	5	-	-	5	5
Larynx (ventral region),										
Squamous epith. hyperplasia	0	-	-	2	2	0	-	-	2	2
Cartilage necrosis	0	-	-	1	3	0	-	-	1	3
Lung, MΦ aggregation	2	-	-	3	3	2	-	-	5	5
Thymus, atrophy	1	-	-	2	3	0	-	-	1	4
Lymph node (TB), ↓ cellularity	0	-	-	5	5	0	-	-	2	3
Adrenals, atrophy	0	-	-	5	3	0	-	-	2	3

Toxicokinetics:

Table 16. Mean Plasma Drug Concentration in the 5-Week Rat Inhalation Toxicity Study

Drug	Delivered dose: salmeterol / Fluticasona (μg/kg) 0/0	74/140	75/370	140/710	380/760
Salmeterol (ng/ml)					
Day 1	< 1.0	2.20	1.90	2.15	8.10
15	< 1.0	2.60	2.50	6.60	>20.9
35	< 1.0	3.10	2.90	7.40	>14.7
Mean	< 1.0	2.63	2.43	5.38	> 8.10
Fluticasone (ng/ml),					
Day 1	0.11	3.27	7.53	6.90	9.18
15	0.10	4.04	6.01	6.45	6.38
35	0.09	3.45	9.20	8.63	12.18
Mean	0.10	3.59	7.58	7.33	9.25

Summary of the 5-week inhalation study in rats: Rats treated with both fluticasone and salmeterol showed a typical effect of fluticasone and a minimal effect of salmeterol. Rats treated with salmeterol only also showed a minimal effect of salmeterol. The absence of salmeterol toxicity in both control and the combination groups suggests that salmeterol exposure level may be too low.

3. A 13-Week inhalation toxicity study in rats. Report No. WPT/96/075. Vol. 6, p 1.

Testing laboratory:

Study dates: 1/12/96 – 6/28/96, report date:26/27/97; (Study No. R13225)

GLP: Yes

Batch Information: Salmeterol: U95/167A; fluticasone: U95/059A; Combination of salmeterol and fluticasone: U95/168A and

U95/169A.

Method:

Wistar Rats (Crl:WI BR, 10 weeks of age) were exposed by nose-only inhalation to salmeterol/fluticasone/ ——— mixture for 13 weeks to study their toxicity. Animals were sacrificed at the end of the exposure. Additional animals were used for recovery evaluation. They were terminated after a recovery period of 4 weeks following a 13-wk treatment period. The study design is summarized in Table 17 (below). The daily inhalation exposure was 20 minutes.

Table 17. Study Design of the 5=Week Rat Inhalation Toxicity Study (n/sex)

Group	No. of animals per group per sex			Delivered dose (µg/kg/day)[4]	
	Main[1]	Recovery[2]	Satellite[3]	Salmeterol	Fluticasone
1	15	10	16	0	0
2	15	-	16	37.4	-
3	15	-	16	-	75.1
4	15	-	16	7.2	71.4
5	15	10	16	40.5	75.1

1. Sacrificed after 13 weeks of exposure.
2. Sacrificed after a 28-day recovery period following 13 weeks of exposure.
3. For toxicokinetic study to determine plasma drug levels
4. Dose estimation was based on the same formula used in the 5-week rat study.

The following parameters were observed during the study:

Clinical signs:	Daily, + weekly examinations
Body weight:	Weekly
Food consumption:	Weekly
Ophthalmology:	Prior to treatment, week 12
Clinicalpathology:	Weeks 4 & 12
Plasma drug level:	Pre-dose, immediately and 1 hr after exposure on day 1, weeks 5 and 13 (n = 4/sex/dose)
Pathology:	Weeks 13 & 17
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus
Necropsy:	All animals
Hisotology:	A complete panel for Groups 1, 4 and 5; animals died early in Groups 2 and 3; and selected tissues in recovery groups (1 and 5): larynx, lungs, thymus, lymph nodes,

spleen, nasal cambers, liver, adrenals and stomach.

Results:

Mortality: No treatment mortality was observed.

Clinical signs: A slight increase in the incidences of hair loss was seen (Table 17, below).

Table 18. Percentage of Hair Loss in 13-Week Rat Inhalation Toxicity Study

	Male					Female				
Dose Salm/ Flut (µg/kg)	0 /0	37.4 /0	0/ 75.1	7.2/ 71.4	40.5/ 75.1	0 /0	37.4 /0	0/ 75.1	7.2/ 71.4	40.5/ 75.1
Wk 1	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	16	0	0	0	0	12
8	0	0	7	14	20	4	0	14	33	12
13	0	0	60	36	64	20	0	86	67	76
17	0	-	-	-	80	0	0	-	-	100

Body weight: Dose-related decreases in body weight gain were seen in all fluticasone treated groups in both sexes (Table 19, below). Males treated with salmeterol alone also showed a decrease in body weight. Body weight tended to recover when the treatment was withdrawn, but failed to catch up with the controls. There was a 9 - 15% decrease in body weight even after four weeks of recovery.

Table 19. Body Weight (Group Means) in the 13-Week Rat Inhalation Toxicity Study (g)

	Male					Female				
Dose Salm./ Flut (µg/kg)	0 /0	37.4 /0	0/ 75.1	7.2/ 71.4	40.5/ 75.1	0 /0	37.4 /0	0/ 75.1	7.2/ 71.4	40.5/ 75.1
Week 0	296	290	289	291	296	212	211	215	214	212
4	368	366	323	329	335	236	244	203	209	208
8	441	425	365	369	377	258	270	211	218	217
13	484	457	385	397	402	274	289	215	227	226
17	537	-	-	-	457	297	-	-	-	271

Food consumption: No treatment-related effects were observed.

Ophthalmic examinations: No treatment-related effects were observed.

Clinical pathology: A number of changes in clinical pathology parameters were observed during the treatment (Table 20, next page). These changes are typical effects of beta 2 agonists and glucocorticosteroids. Changes in hematology parameters included mild but statistically significant increases in packed cell volume, hemoglobin, red blood cell count, and neutrophil

counts; and decreases in the counts of lymphocyte in treated groups. Biochemistry changes included slight elevations in ALT levels. Changes in urinalysis parameters included the increase in pH and sodium concentrations. All parameters returned to the normal range after a recovery period of 4 weeks.

Table 20. Clinical Pathology (Group Means) in the 13-Week Rat IH Toxicity Study, Week 12

	Male					Female				
Salmeterol (µg/kg)	0	37.4	0/	7.2/	40.5/	0	37.4	0/	7.2/	40.5/
Fluticasona (µg/kg)	/0	/0	75.1	71.4	75.1	/0	/0	75.1	71.4	75.1
Hematology										
RBC: PVC (%)	43.3	43.3	**45.8**	**44.6**	**45.5**	40.7	41.4	**42.7**	**42.6**	**44.0**
Hemoglobin (g/dl)	15.2	15.3	**16.1**	**15.8**	**16.2**	14.3	14.7	**15.2**	**15.1**	**15.5**
RBC (x 10^6/mm^3)	8.64	8.81	**9.49**	**9.12**	**9.44**	7.81	8.02	**8.34**	8.24	**8.57**
WBC: Total (x 10^3/mm^3)	8.01	9.94	6.35	7.19	7.89	5.17	6.05	4.26	5.42	4.27
Neutrophil	2.12	2.48	2.62	**3.09**	**3.10**	1.39	1.63	**2.16**	**2.66**	1.72
Lymphocyte	5.36	**6.85**	**3.17**	**3.57**	**4.07**	3.53	4.09	**1.70**	**2.35**	**2.21**
Clot. Fibrin. (mg/dl)	305	298	298	308	**375**	188	**234**	**240**	**240**	**242**
Blood chemistry										
Glucose (mg/dl)	120	**98**	134	131	116	104	**84**	**133**	**138**	100
Urea nitrog. (mg/dl)	17	19	**22**	**23**	21	21	21	**26**	23	23
ALT (mU/ml)	27	28	**31**	**33**	**31**	27	29	**53**	**37**	**52**
Cortisone (µg/dl)	54	**67**	**41**	**52**	**45**	69	71	55	59	67
Urinalysis,										
Volume (ml)	5.4	**7.7**	**7.6**	**7.8**	**8.0**	4.8	5.6	5.4	6.0	5.0
pH	6.3	**6.6**	**7.0**	**7.1**	**7.1**	6.0	6.1	**6.5**	**6.5**	**6.4**
Na (mEq/ml)	0.33	0.42	**0.59**	**0.68**	**0.75**	0.37	**0.40**	**0.50**	**0.51**	0.47
Cl (mEq/vol)	0.65	**0.91**	0.73	**0.85**	**0.95**	0.55	0.60	0.57	0.60	0.59

Bold indicates statistically significant.

Organ weights: The fluticasone treated animals showed smaller adrenal glands, thymus and spleen, and large liver (Table 21).

Table 21. Organ Weight (Group Means) in the 13-Week Rat Inhalation Toxicity Study, Week 14

Dose (µg/kg)	Male					Female				
Salmeterol/	0	37.4	0/	7.2/	40.5/	0	37.4	0/	7.2/	40.5/
Fluticasona	/0	/0	75.1	71.4	75.1	/0	/0	75.1	71.4	75.1
Body weight (g)	475	455	390	398	408	273	291	217	229	227
Adrenals (mg)	55.6	61.3	46.7	52.6	53.5	75.2	73.7	**51.2**	**52.0**	**57.8**
Thymus (g)	0.244	0.220	**0.123**	**0.149**	**0.150**	0.212	0.249	**0.087**	**0.093**	**0.099**
Spleen (g)	0.95	0.93	0.73	0.75	0.79	0.70	0.69	**0.49**	**0.53**	**0.51**
Heart, (g)	1.44	1.44	1.24	1.29	1.39	0.91	1.00	0.82	0.87	0.92

Bold indicates statistically significant.

Gross pathology: Small thymus, spleen and adrenals were observed in all treated groups (Table 22, below). These observations correlate well with their organ weights (Table 21, p. 16).

Table 22. Gross pathological Findings in the 13-Week Inhalation Toxicity Study in Rats

Dose (μg/kg)	Male					Female				
Salmeterol/	0	37.4	0/	7.2/	40.5/	0	37.4	0/	7.2/	40.5/
Fluticasone	/0	/0	75.1	71.4	75.1	/0	/0	75.1	71.4	75.1
Number examined	15	15	15	15	15	15	15	15	15	15
Thymus, small	0	1	10	6	7	0	0	14	12	13
Lungs, pale focus/i	5	8	11	3	8	2	7	7	8	13
Forestomach, depression	0	1	3	5	4	0	0	4	3	2
Skin, alopecia	0	0	8	8	11	5	0	13	14	13

Histopathology: Microscopic findings related to the treatment were seen both in the respiratory and other systems (Table 23, below). Locally, the following changes were observed in the treatment groups: the decrease in the cellularities was observed in nasal associated lymphnoid tissue. Squamous epithelial hyperplasia in the ventral region of the larynx was seen in high dose salmeterol animals. The aggregation of macrophages in the lung and a decrease in cellularity in the tracheabrochial lymph node were seen in the fluticasone-treated groups. Other changes included atrophy of thymus, adrenals, reduced cellularity in the spleen, hepatocyte vacuolation, and epithelial hyperplasia in the stomach.

Table 23. Microscopic Findings in the 13-Week Inhalation Toxicity Study in Rats

Dose (μg/kg)	Male					Female				
Salmeterol	0	37.4	0/	7.2/	40.5/	0	37.4	0/	7.2/	40.5/
Fluticasone	/0	/0	75.1	71.4	75.1	/0	/0	75.1	71.4	75.1
No. animals examined	14	15	15	14	15	15	15	14	15	15
Heart, myocardial degeneration	0	0	0	0	3	0	0	0	0	0
Nasal, ↓ cellularity of NALT[1]	0	0	8	6	5	1	0	3	5	8
Larynx, squam. epi. hyperplasia	0	3	0	3	7	0	7	0	0	5
Lung, MΦ aggregation around terminal bronchioles	0	0	0	3	4	3	0	1	3	2
Thymus, involution	0	3	**12/14**	**11**	**14**	3	0	**14**	**14**	**14**
Lymph node (TB), ↓ cellularity	0	1/14	**9**	**12**	**12**	1	0	**10**	**11**	**12**
Liver, hepatocyte vacuolation	0	0	**14**	**9**	**9**	0	1	**9**	**6**	**7**
Spleen, ↓ cellularity	0	0	**8**	**6**	**11**	0	1	**13**	**12**	**12**
Stomach, epi. hyperplasia	0	1	1	4	3	0	0	4	1	2
Adrenals, atrophy	0	0	**10**	**5**	**8**	1	0	**9**	**9**	**5**

Bold indicate statistically different from the controls ($P < 0.05$ with Fishers Exact Test.)

[1] NALT = nose associated lymphoid tissue.

After a recovery period of 4 weeks, most treatment-related changes retreated. Only the presence of aggregation of the macrophages at the terminal bronchial area persisted in the treated group (incidences: 0/20-control vs. 7/20-high dose, Group 5). Note that other groups were not examined in the recovery study.

Toxicokinetics: Plasma drug levels are summarized in Table 24.

Table 24. Mean Plasma Drug Concentration in the 13-Week Rat Inhalation Toxicity Study

Plasma drug levels	Delivered dose: salmeterol / Fluticasone (µg/kg)				
	0/0	37.4/0	0/75.1	7.2/71.4	40.5/75.1
Salmeterol (ng/ml)[1]	-	1.93 ± 0.2 (< 0.5 – 4.9)	-	< 0.5 (<0.5 – 1.4)	2.37 (1.1 -5.9)
Fluticasone (ng/ml) [2]	-	-	2.13 ± 0.3 (< 0.125–3.72)	1.56 ± 0.1 (0.76 – 2.09)	1.55 ± 0.1 (0.55 – 2.0)

[1] Immediately after exposure.

[2] 1 hour after exposure.

[3] n = 11 - 16/sex/group, numbers in parenthesis represents the range.

Summary of the 13-week inhalation study in rats: Rats treated with both fluticasone and salmeterol showed a typical effect of fluticasone and salmeterol. However, animals treated with the combination also showed the cardiac effect (dose-dependent myocardial degeneration in the males) that was absent in the salmeterol control group.

Summary of the toxicity in rats: The acute inhalation exposure of both fluticasone (1.9 mg/kg) and salmeterol (3.2 mg/kg) causes atrial myocarditis and CCG abnormalities that are absent in the salmeterol control (5.2 mg/kg). The functional ECG abnormalities seem to correlate well with the morphologic abnormalities. No cardiac abnormalities were observed in all treated groups in a 5-week inhalation toxicity study. However, a 13-week inhalation study showed myocardial degeneration that was not present in either salmeterol or fluticasone control groups. Overall, toxicity studies confirm the previous finding that fluticasone enhances cardiac toxicity of salmeterol in rats.

A pilot 14-day inhalation toxicity study in dogs. Report No. WPT/93/189. Vol. 8, page 67.

Testing laboratory:	
Study dates:	3/1/93 – 5/28/93, report date: 4/25/94; (Study No. R13225)
GLP:	Yes
Batch Information:	U92/008A, U92/112A & U92/113A

Method

Beagle dogs were given by oral inhalation via an oropharyngeal tube salmeterol and/or fluticasone ——— once a day for 14 days to study toxicity of the drug combinations. Study design and dose estimates are summarized in Table 25 (below).

Table 25. Design of the pilot 14 day IH toxicity study in Dogs (n = 2/sex)

Group	Formulation (µg/actuation)		Delivered Dose (µg/dog)			Estimated daily Exposure (µg/dog/day) [1]	
	Salmeterol	Fluticasone	Act/ dose	Salm.	Flut.	Salmeterol	Fluticasone
1	0	0	6	0	0	0	0
2	50	0	2	50	0	41	0
3	50	0	6	50	100	48	87
4	50	100	2	50	250	51	207
5	50	250	2	150	0	123	0
6	50	100	6	150	300	153	262
7	50	250	6	150	750	143	622

[1] Based on the deposition factors of approximately 0.45 and 0.39 for salmeterol and fluticasone respectively (Vol. 8, page 115). The recovery study showed that 55% of administered salmeterol and 61% of administered fluticasone were recovered from the dosing apparatus at the end of exposure. Recovery study was conducted from Groups 3, 6, and 7. Values for Groups 2, 4, and 5 were calculated using data obtained from Groups 3, 6, and 7.

The following parameters were monitored during the study:

Clinical signs:	Daily, + weekly examinations
Body weight:	Weekly
Food consumption:	Daily
ECG:	Weeks –8, -4 & -1, and days 1 and 12
Pulse rate:	At pre-dosing, immediately and hours 0.5, 1, 2 and 4 after dosing on days 1, 2, 3, 7 & 14
Clinical pathology:	Week –1, and day 13
Plasma drug level:	Salmeterol: pe-dose, 2 minutes and 6 hours after dosing on days 1 and 14; Fluticasone: pre-dosing, minutes 2, 5, 10, and 40, and hours 2, 4, 6 and 24 after exposure on days 1& 14
Pathology:	Weeks 13 & 17
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid
Necropsy:	All animals
Histopathology:	Selected organs: adrenals, heart, kidneys, larynx, liver, lungs, pituitary, thymus, thymus, trachea and abnormal tissue

Results

A. Mortality: None.
B. Clinical signs: No treatment-related effects were observed.
C. Body weight: All groups treated salmeterol and fluticasone (except Group 4) lost weight during treatment. Group 4 did not show body weight gain (Table 26, below).

Table 26. Body weight gain (kg) in the 2 Week pilot Inhalation Study in Dogs (day 1 – 15)

Group	1	2	3	4	5	6	7
Dose: Salmeterol (µg/kg)	0	41	123	51	48	153	143
Fluticasone (µg/kg)	0	0	0	87	207	262	622
Male	0.4	0.1	0.4	0	-0.1	-0.3	-0.5
Female	0	0.4	0.4	0	-0.1	-0.3	-0.7

D. Food consumption: No treatment-related effects were observed.
E. ECG: No treatment-related effects were observed.
Pulse rate: Salmeterol dose-related increase in pulse rate were observed (Table 27, below). Also seen were the fluticasone dose-dependent increase in pulse rate.

Table 27. Pulse Rate (Adjusted) in the Pilot 14-Day Inhalation Study in Dogs[1]

Group	Dose(S/F, µg/dog)	Time (hours)					
		Pre-dose	0	0.5	1	2	4
1	0/0	115.6	120.2	111.6	110.6	102.6	105.6
2	41/0	109.4	116.2	119	115	119.8	111.8
3	123/0	111.2	124.8	131.8	128.2	119.4	116
4	51/87	109.4	118.8	121.4	119	121	121.6
5	48/204	102.4	117.2	119.2	122.6	122.8	122.4
6	153/262	106.8	124.6	139.6	135.2	131.2	124.4
7	143/622	103.4	128	140.2	140.4	154.4	135

1. Average of the group means (n=4) on days 1, 2, 3, 7 and 14.

Clinical pathology:

Hematology: No significant effects were observed.

Clinical chemistry: Moderate decreases (↓ 43 - 52%) in cortisol levels were seen in all fluticasone treated groups. Moderate increases (↑ 10 – 12%) in serum potassium levels were seen in Groups 5 – 7.

Urinalysis: No significant effects were observed.

Organ weight: Fluticasone dose-dependent and statistically significant decreases (↓ 52 – 72%) in thymus weight were observed in Groups 4 – 7. Slight and statistically non-significant decreases in lung weight were seen in Groups 5 – 7.

Histopathology: Atrophy of the adrenal cortex and thymus, and hepatocyte rarefaction of the in the periportal zone were seen in the fluticasone animals receiving fluticasone (Table 28, below).

Table 28. Histopathology Findings in the 2 Week pilot Inhalation Study in Dogs

Group	1	2	3	4	5	6	7
Dose: Salmeterol (μg/kg)	0	41	123	51	48	153	143
Fluticasone (μg/kg)	0	0	0	87	207	262	622
Adrenal, atrophy	0/4	0/4	0/4	2/4	3/4	4/4	4/4
Thymus, atrophy	0/4	0/4	0/4	2/4	4/4	3/4	2/3
Liver, hepatocyte rarefaction	0/4	0/4	0/4	0/4	2/4	3/4	4/4

Plasma levels: Increases in plasma salmeterol and fluticasone levels were proportional to their individual doses (Table 29, below). There seemed to be a slight accumulation of plasma salmeterol over time. This trend was also observed in humans clinically.

Table 29. Plasma Drug Levels of the pilot 14-day IH toxicity study in Dogs

Group	Delivered dose		Plasma Level			
	Salmeterol	Fluticasone	Salmeterol[1] (ng/ml)			Fluticasone[2]
	(μg/dog)	(μg/dog)	Day 1	Day 14	Mean	(pg/ml)
1	0	0	-	-		-
2	41	0	2.1 ± 0.6	2.3 – 2.6	2.3	-
4	51	87	1.6 ± 0.2	2.3 ± 0.4	1.5	304
5	48	207	1.1 – 1.3	1.1 – 1.4	1.2	423
3	123	0	3.5 ± 0.5	5.6 ± 1.6	4.5	-
6	153	262	4.2 ± 0.9	4.7 ± 1.5	4.5	515
7	143	622	3.1 ± 0.7	3.7 ± 0.7	3.4	1189

[1] Plasma levels at 2 minutes after the exposure (n = 4/group).

[2] Plasma levels at 2 hours after exposure.

Time-course of plasma fluticasone and heart rates are presented in Figure 1. Salmeterol levels are not plotted because only three data points are available (pre-dose, 2 minutes and 6 hours after dose). Time-course of plasma salmeterol level and heart rate can not be readily determined.

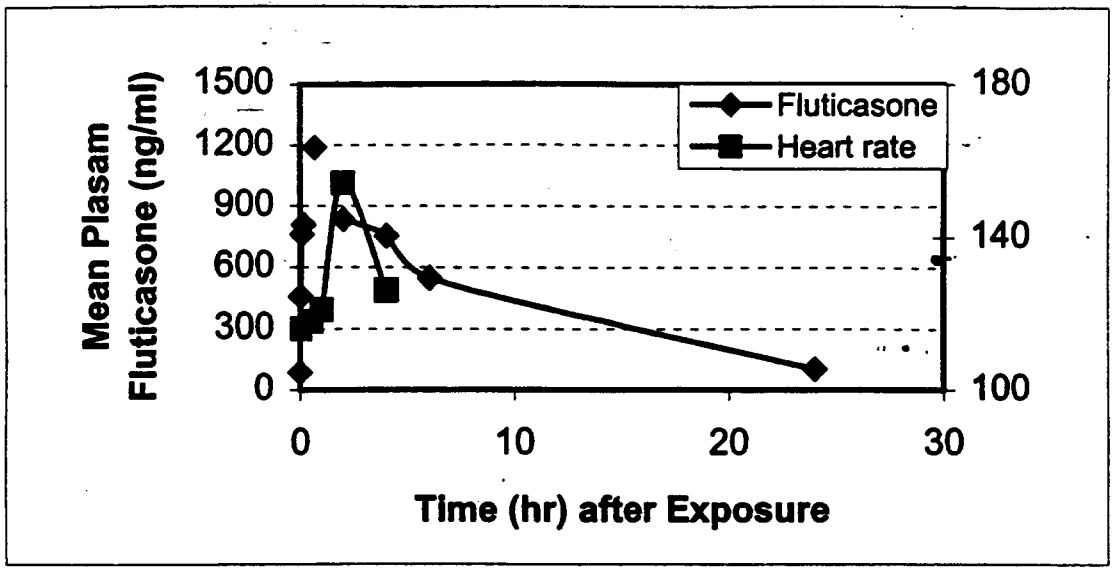

Figure 1. Time course of plasma salmeterol concentration and Heart rate in dogs.

Summary of the 2-week pilot inhalation study in dogs: Dogs treated with both fluticasone and salmeterol showed a typical effect of fluticasone and salmeterol. The cardiac effect of salmeterol was demonstrated by the dose dependent increase in heart rate. Also, the increase in heart rate was also fluticasone dose-dependent at a given salmeterol dose level.

OVERALL SUMMARY & EVALUATION

Summary of Toxicology:

Glaxo Wellcome proposes to study efficacy of salmeterol/fluticasone ——— in asthma. Salmeterol is a beta-adrenergic bronchodilator and fluticasone a corticosteroid. Individual toxicity profiles of both drugs are well known: salmeterol is cardiotoxic while fluticasone causes immune system and growth suppression. Toxicity profile of the two drugs in combination and in the presence of ——— however, is less known.

Glaxo Wellcome conducted bridging toxicity studies to assess the toxicity profile of the newly proposed salmeterol/fluticasone ——— formulation. These studies included a 5-week and a 13-week inhalation toxicity studies in rats, and a 2-week pilot inhalation toxicity study in dogs using the to-be-marketed formulation. The sponsor also performed an acute inhalation toxicity study in dry powder formulation to further evaluate cardiovascular effect of the salmeterol and fluticasone combination. In addition, the sponsor conducted a study on the effect of fluticasone pre-treatment on uterine contractility which showed that fluticasone pretreatment for 14 days did not alter uterine contractility in mouse. The following summary is for toxicity studies only.

Rat:

Previous acute inhalation toxicity studies showed that the combination of salmeterol and fluticasone caused atrial myocarditis in rats that was absent in animals receiving salmeterol only. These studies, however, did not include functional monitoring of the cardiovascular system. Another acute inhalation toxicity study was conducted to evaluate the effect of the combination on cardiovascular function. Histologic examination was also included for the analysis of possible associations between morphologic and functional changes. Wistar male rats (10/group) were given via nose-only exposure either salmeterol (5.2 mg/kg) or salmeterol (3.2 mg/kg) plus fluticasone (1.9 mg/kg). Another group of rats received vehicle (lactose) only for control. Half of the animals in each group were implanted with a telemetry transducer to monitor their cardiac function for fourteen days after the exposure. These animals were sacrificed on day 15 but histologic examinations were not performed. The remaining animals underwent the sham operation and were sacrificed 48 hours after the exposure for histologic examinations. Telemetry monitoring revealed ECK abnormalities [A-V node blockade and (atrial or supraventricular) premature complexes] in the combination group that were absent in the salmeterol control groups. Histologic findings were similar to the previous findings: myocardial degeneration was seen in both salmeterol and salmeterol/fluticasone groups, but atrial myocarditis was present in the salmeterol/fluticasone combination treatment group only.

In a 5-wk pilot inhalation study, Wistar rats (10/sex/group) were exposed by nose-only to aerosol of the clinical formulation of salmeterol/fluticasone ——— formulations 1 hour daily. The estimated dose levels were (salmeterol/fluticasone): 0/0, 74/140, 75/370, 140/710, and 380/760 µg/kg/day (total body deposition). Additional animals (5/sex/group) in the vehicle control, mid high and high dose groups were allowed to recover for 2 weeks before scheduled sacrifice. Findings were mostly typical fluticasone effects and a minimal salmeterol effect. A dose-related decrease in body weight gain was observed in all treated groups during the exposure. Body weight tended to recover upon the cessation of the exposure but failed to catch up with the control group after a recovery period of 2 weeks. At the end of the recovery period, there was a 12% decrease in absolute body weight in the high dose fluticasone groups (MHD and HD). A spectrum of clinical pathology, macroscopic and microscopic findings was also typical fluticasone effects. Noticeable salmeterol effect was mild increase in heart rate that lacked apparent dose-response relationship. No abnormalities, with exception of body weight, were detected in the recovery animals. No NOEL levels were demonstrated.

In a three-month inhalation study, Wistar rats (15/sex/group) were exposed by nose-only to the aerosol of clinical formulation of salmeterol/fluticasone ——— formulations for 20 minutes a day. The estimated dose levels were (salmeterol/fluticasone): 0/0, 37/0, 0/75, 7/71, and 41/75 µg/kg/day (total body deposition). Additional animals (10/sex/group) in the vehicle control and high dose groups were allowed to recover for 4 weeks before scheduled sacrifice. Similar to the previous study, findings were mostly typical fluticasone effects. Decreases in body weight (6% - 20%) were observed in all treated groups, with the exception of the salmeterol control females that showed a slight (5%) increase by the exposure. Body weight also tended to recover upon the cessation of the exposure but failed to catch up with the control group even after a recovery