CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 21-254

PHARMACOLOGY REVIEW(S)

EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
LABEL REVIEW

NDA No.: 21-254
Division: PULMONARY AND ALLERGY PRODUCTS
HFD: 570
Reviewer Completion Date: 5/17/06
Reviewer: Lawrence F. Sancilio, Ph.D.
Serial No./Types of Submission: Amendment
Date of Submission: 4/14/06
Information to Sponsor: Yes ( ), No (X)
Sponsor: GlaxoSmithKline Inc.
5 Moore Drive
Research Triangle Park, NC 27709

Drugs:

Trade Name: Advair HFA
Generic Names: Salmeterol xinafoate (Serevent ^{TM}) and fluticasone propionate (FLovent^{TM})
Code Names: Unknown.
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/ Molecular Weight: Salmeterol xinafoate, $C_{25}H_{37}NO_4.C_{11}H_8O_3$, 603.8
Fluticasone propionate, $C_{25}H_{31}F_3O_5S$, 500.6

Drug Class: Salmeterol xinafoate, B_2 Receptor agonist.
Fluticasone propionate, Glucocorticoid.

Indication: ———— maintenance treatment of asthma.

Formulation: Suspension of various concentrations fluticasone propionate and a fixed concentration of salmeterol xinafoate in a hydrofluoroalkane propellant (HFA-134a, 1,1,1,2-tetrafluoroethane).

Maximum Daily Inhalation Dose: Salmeterol, 84 mcg and
Fluticasone propionate, 920 mcg
Advair Products: 45 mcg fluticasone propionate/ 21 mcg salmeterol /actuation;
115 mcg fluticasone propionate/ 21 mcg salmeterol /actuation;

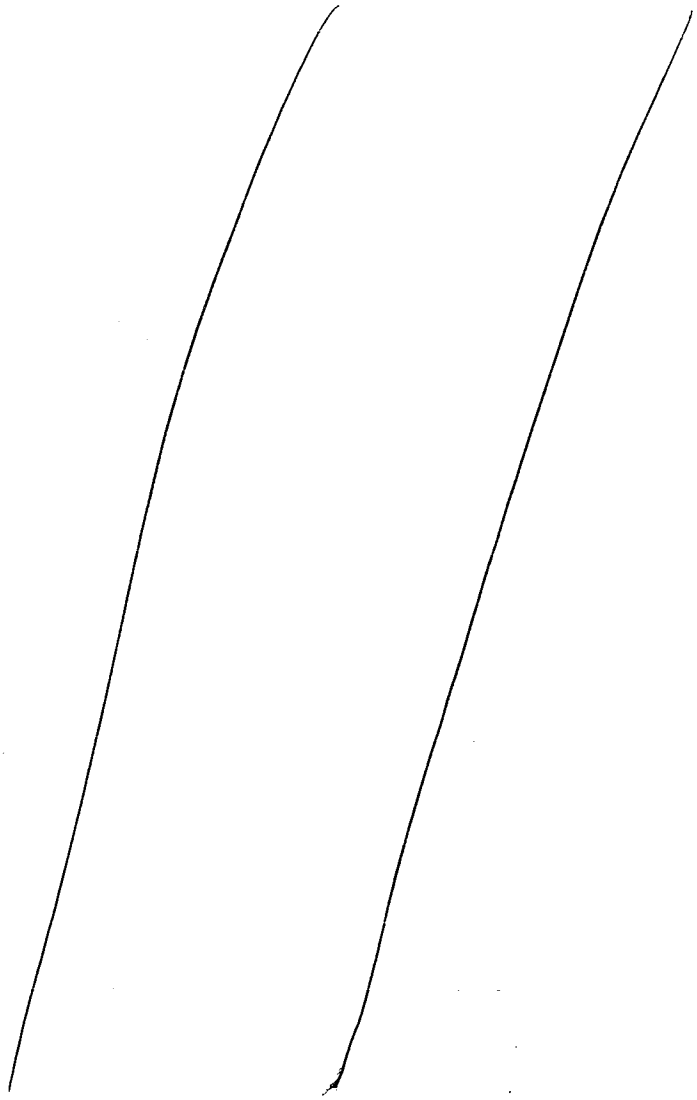
230 mcg fluticasone propionate/ 21 mcg salmeterol /actuation

Proposed Use: Maintenance Treatment of asthma in adults and children ≥ 12 years old.

This is a resubmission. All preclinical reviews were completed without any issues.

Label Review

The recommended changes are in **BOLD;** deletions are ~~strikeout.~~



4 Page(s) Withheld

1 Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

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

/s/

Lawrence Sancilio
5/17/2006 01:56:34 PM
PHARMACOLOGIST

Joseph Sun
5/17/2006 02:27:49 PM
PHARMACOLOGIST
I concur.

PHARMACOLOGY/TOXICOLOGY COVER SHEET
CHEMISTRY CONSULT

NDA No.: 21-254
Date of Consult Request: 2/22/06
Reviewer: Lawrence F. Sancilio, Ph.D.
Reviewer Completion Date: 3/27/06
Date of Submission: 12/07/05
Information to Sponsor: Yes ( ), No (X)
Sponsor: GlaxoSmith Kline
Drug Product: Advair-HFA
Drugs: Salmeterol xinafoate and fluticasone propionate
Drug Class: Salmeterol xinafoate, B_2 Receptor agonist.
Fluticasone propionate, Glucocorticoid.
Indication ——— maintenance treatment of asthma.

Consult request by Craig Bertha, Ph.D. on the safety of the extractable/leachable, 1, 3, 5 trioxane.

From the submission, Dr. Bertha indicated that from the — stability studies, the levels of the non-structure alert leachable, ———, ranged from — mcg/inhaler. Dr. Bertha indicated that this leachable is not structurally related to known irritants and that it breaks down to ——— a structure alert. The report indicated that the leachable, ———, was negative in the Ames test assayed in the National Toxicology Programme. Based on —actuations /inhaler and the maximum daily administrations of 4 inhalations, the maximum daily intake of the leachable or its breakdown product, ——— is — mcg — ng/kg).

Review

No acceptance criterion was proposed for ——— by the sponsor. The consult was to determine whether the daily exposure of — ng/kg of the leachable, ——— or its breakdown product, ———, was safe. For the non-structure alert and non-irritant leachable, ———, with no animal or human data, the acceptable safe daily inhalation exposure is 100 ng/kg. For ——— the safe daily inhalation exposure is — ng/kg/day. The latter is based on the human TLV (ACGIH) for — to be — ng/kg/day divided by a safety factor of 14 which incorporates the differences in population sensitivity (10) and the exposure frequency (4). Both safety values are well above the proposed - ng/kg/day of the leachable, ——— and its breakdown product.

Recommendation

The daily exposure of — ng/kg of the leachable, ———, and its breakdown product, ——— is safe.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

BLANK
PAGE

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

/s/

Lawrence Sancilio
3/27/2006 03:50:41 PM
PHARMACOLOGIST

Joseph Sun
3/27/2006 05:31:11 PM
PHARMACOLOGIST
I concur.

REVIEW AND EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
Chemistry Consult

Division: PULMONARY DRUG PRODUCTS, HFD-570

Reviewer: Lawrence F. Sancilio, Ph.D.
Reviewer Completion Date: 6/20/01
NDA No.: 21-254
Date of Submission: 12/20/00
Date of Consult Request: 5/18/01
Information to Sponsor: Yes ( ), No (X)
Sponsor: Glaxo Inc.
5 Moore Drive
Research Triangle Park, NC 27709
Drugs:
Trade Name: Advair-HFA
Generic Names: Salmeterol xinafoate (Serevent ™) and fluticasone propionate (FLovent™)
Chemical Names: Salmeterol xinafoate and fluticasone propionate

Drug Class: Salmeterol xinafoate, β_2 Receptor antagonist.
Fluticasone propionate, Glucocorticoid.

Indication: [redacted] maintenance treatment of asthma.

Formulation: Suspension of various concentrations fluticasone propionate and a fixed concentration of salmeterol xinafoate in a hydrofluoroalkane propellant (HFA-134a, (1,1,1,2-tetrafluoroethane). Each canister delivers 120 metered doses although the total fill was equivalent to [redacted] metered doses.

Dose/Actuation:

Product	Fluticasone propionate mcg Ex Valve	Fluticasone propionate mcg Ex Actuator	Salmeterol xinafoate mcg of Salmeterol Base Ex Valve	Salmeterol xinafoate mcg of Salmeterol Base Ex Actuator
Advair HFA 44/21	50	44	25	21
Advair HFA 110/21	125	110	25	21
Advair HFA 220/21	250	220	25	21

Route of administration: Inhalation.

Maximum Daily Dose: Adults, 880 mcg of salmeterol and 84 mcg of salmeterol (4 puffs/day)

Consult request by Dr. Alan Shroeder on the safety of extractables/leachables from the Advair MDI.

The following table lists the extractables/laechables, their source and their detectable levels in the inhaler/actuator. The levels were determined after storing the Advair HFA inhalers at 30°C for 12 months.

Source/Extractable	Detectable Levels mcg/Canister
Valve	
Lower Stem and Body	
Upper Stem	
Valve	
Valve	
Actuator	

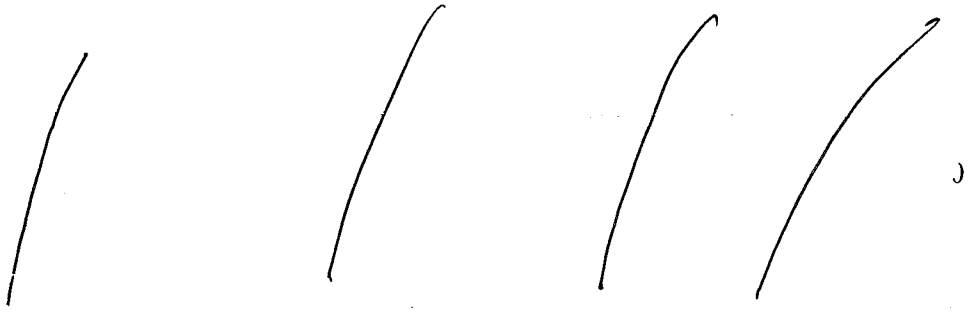
LOQ (limits of quantification)

The daily exposure for each detected extractable based on 50 kg weight and 4 actuations/day is summarized in the following table with their justification for acceptability. A conservative approach was taken in determining the daily exposure, i.e., using the 120 metered doses (four metered doses/day) instead of the total fill equivalent to — metered doses in each canister. At daily inhalation exposures of ≤ 100 ng/kg, extractables/leachables are acceptable provided that they possess no structural alerts for potential genotoxicity or carcinogenicity, or for a highly irritating compound or are structurally similar to chemicals with a known toxicity profile. This amount was determined and accepted by the Division after an extensive analysis of the toxicity of inhaled non-carcinogenic chemicals. The results indicated that at a daily exposure of 100 ng/kg level, there would be a high safety factor of at least 300. Since —— is a carcinogen, its safe daily exposure level was based on the 10 hr TWA from NIOSH of — mcg/kg.

Source/Extractable	Calculated Daily exposure ng/kg/day[a]	Acceptable/Justification
Valve Elastomeric Seals		
ne		Yes, since — /kg/day was approved in Ventolin-HFA.[b]
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
Lower Stem and Body		
——		Yes, since it is structurally related to —— and — ng/kg/day was approved in Ventolin-HFA
——		Yes, since an acceptable daily exposure is — cg/kg based on the ' ——
— *Upper Stem*		
——		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since an acceptable daily exposure is —ncg/kg based on the ——
Valve ——		
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.
Valve ——		
(		Yes, See the text below.
Actuator		
/		Yes, since the daily exposure is < 100 ng/kg.
		Yes, since the daily exposure is < 100 ng/kg.

[a] Amount present in the device (ng) ÷ No. of daily doses in the inhaler [120/4=30] ÷ 50 kg= ng/kg/day
[b] Ventolin-HFA (NDA 20-983) is a product of the same sponsor, i.e., Glaxo Inc.

Justification for accepting an exposure of [redacted] /kg/day of inhaler [redacted]



The sponsor referred to the DMF, which indicated that, [redacted] were compliant with the USP Biological Reactivity Testing, General Chapter <88>. There is no concern for the lack of data supporting compliance of these components, since they are acceptable at these levels in this NDA and also were acceptable in Ventolin-HFA.

Recommendation

Daily exposure of the extractables/leachables from the drug product is acceptable for one year from the time the drug product was manufactured.

Lawrence F. Sancilio, Ph.D.

Cc ASchroeder

APPEARS THIS WAY
ON ORIGINAL

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

/s/

Lawrence Sancilio
6/20/01 04:08:21 PM
PHARMACOLOGIST

Joseph Sun
6/20/01 04:15:41 PM
PHARMACOLOGIST
I concur.

EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA

Reviewer: Lawrence F. Sancilio, Ph.D.
Division: PULMONARY AND ALLERGY DRUG PRODUCTS
HFD: 570
Reviewer Completion Date: 10/19/01

NDA No.: 21-254
Review No.: 0, **Original**
Serial No./Types of Submission: 0, Original, Amendment
Dates of Submission: 12/20/00, 3/15/01
Information to Sponsor: Yes ( ), No (X)
Sponsor: Glaxo Inc.
5 Moore Drive
Research Triangle Park, NC 27709

Manufacturer for Drug Substance:
Fluticasone propionate: Glaxo Wellcome Operations, Angus, DD10 8EA, United Kingdom
Salmeterol xinafoate: Glaxo Wellcome Manufacturing Pte. Ltd.
Jurong, Singapore

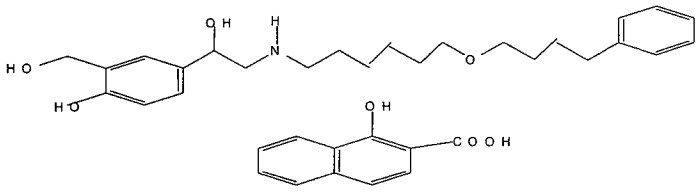
Drugs:

Trade Name: Advair HFA
Generic Names: Salmeterol xinafoate (Serevent ™) and fluticasone propionate (FLovent™)
Code Names: Unknown.
Chemical Names:
Salmeterol xinafoate: 4-Hydroxy-□ [1]-[[[6-(4- phenylbutoxy)hexyl) amino]-methyl]-1,3-benzenedimethanol, 1-hydroxy-2- naphthalenecarboxylate

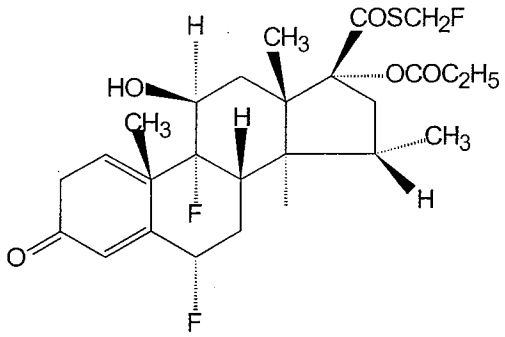
Fluticasone propionate: S-fluoromethyl 6, 9 -difluoro-11 -hydroxy-16 - methyl-3-oxo-17-propionyloxyandrosta-1, 4-diene-17 -carbothioate
CAS Registry No.: Salmeterol xinafoate, 89767-59-9
Fluticasone propionate, 80474-14-2
Mole File Numbers: Unknown.
Molecular Formula/ Molecular Weight: Salmeterol xinafoate, $C_{25}H_{37}NO_4.C_{11}H_8O_3$, 603.8
Fluticasone propionate, $C_{25}H_{31}F_3O_5S$, 500.6

Structures:

Salmeterol xinafoate



Fluticasone propionate



Relevant IND and NDAs:

IND 57,151, Salmeterol and Fluticasone propionate HFA Inhalation Aerosol
NDA 21-077, Salmeterol and Fluticasone Propionate Diskus Aerosol DPI
NDA 20-236 and NDA 20-692, Salmeterol Inhaler and Diskus Aerosol DPI
NDA 20-121, Fluticasone Propionate Intranasal Inhaler Aerosol
NDA 20-548 and NDA 20-549, Fluticasone Propionate Inhaler and Rotadisk DPI Aerosol

Drug Class: Salmeterol xinafoate, β_2 Receptor agonist.
Fluticasone propionate, Glucocorticoid.

Indication: ——— maintenance treatment of asthma.

Formulation: Suspension of various concentrations fluticasone propionate and a fixed concentration of salmeterol xinafoate in a hydrofluoroalkane propellant (HFA-134a, 1,1,1,2-tetrafluoroethane).

Dose/Actuation:

Product	Fluticasone propionate mcg Ex Valve	Ex Actuator	Salmeterol xinafoate mcg of Salmeterol Base Ex Valve	Ex Actuator
Advair HFA 44/21	50	44	25	21
Advair HFA 110/21	125	110	25	21
Advair HFA 220/21	250	220	25	21

HFA-134a: 75 mg/actuation ex valve

Route of Administration: Oral inhalation

Recommended Dose: The proposed maximum daily dose propionate delivered from the actuator is 4 actuations equivalent to 84 μg of salmeterol base and up to 880 μg of fluticasone propionate .

Proposed Use: Maintenance Treatment of asthma in adullts and children ≥ 12 years old.

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

Introduction and Drug History

Salmeterol xinafoate and fluticasone propionate have been used as marketed product alone (Salmeterol: NDA 20-236 as an as an inhalation aerosol, NDA 20-692 as a diskus DPI; Fluticasone propionate: NDA 20-121 as a nasal spray, NDA 20-548 as an inhalation aerosol, NDA 20-549 as a rotadisk DPI, NDA 20-833 as a diskus DPI and in combination (NDA 21-077 as a diskus DPI). In this NDA, the combination is an inhalation aerosol using a hydrofluoroalkane (HFA-134a, 1,1,1,2-tetrafluoroethane) as the propellant. This was undertaken since this HFA is less ozone depleting to the atmosphere than the chlorofluorohydrocarbon (CFC) propellants.

Studies Submitted were previously reviewed in IND 57,151 by L. Pei (Submission Date, 10/23/98; Review Date, 1/11/99

Toxicology

Multidose

Pilot 35-day inhalation toxicity in rats, WPT/93/176 vol. 5.2.

13-Week inhalation study in Wistar rats, WPT/96/075, vol. 5.3-5.4.

Pilot 14-day inhalation toxicity in dogs, WPT/93/189 vol. 5.5.

OVERALL SUMMARY AND EVALUATION

Salmeterol and fluticasone propionate have been used as an inhaler alone and together for the treatment of asthma. The pharmacology and toxicology of salmeterol and fluticasone propionate alone and in combination as a dry powder have been summarized in the NDAs 20-236 and 20-121, 21-077 reviews. The review for IND 57,151 and NDA 21-077 are attached to this report.

This NDA is an inhalation aerosol containing salmeterol and fluticasone propionate using HFA-134a (HFA) as the propellant. This formulation was initially developed since the propellant (HFA) was less ozone depleting to the atmosphere than the CFC propellant.

Salmeterol

Salmeterol xinafoate (salmeterol hydroxynaphthoate) is a racemic $beta_2$ receptor agonist producing in bronchodilitation predominantly due to the R+ enantiomer. It is a modification of the albuterol molecule resulting in a longer onset and duration of activity. In acute studies in dogs and cats, salmeterol produces tachycardia. In rats and humans, hydroxylated salmeterol is a common metabolite. Its metabolism in humans is via the P450 enzymes, CYP3A4 and CYP2E1, indicating potential adverse effects when administered with drugs that inhibit the P450 enzymes. Salmeterol is highly bound to mouse, rat, rabbit, dog and human plasma proteins, and excretion in animals is mainly fecal by way of the bile.

In multidose oral toxicity studies, salmeterol was more toxic in juvenile than in adult rats. In long-term inhalation studies in rats, salmeterol was irritating to the respiratory tract. Other changes observed were characteristic of $beta_2$ agonism, i.e., hypoglycemia, hypokalemia, increased body weight gained and leiomyomas. Plasma levels indicate enzyme induction. In dogs, salmeterol was administered orally and by inhalation in the multidose and long-term studies. The effects seen were attributed to $beta_2$ agonism, i.e., tachycardia, vasodilation, increased muscle mass, hypoglycemia, hypokalemia and myocardial papillary necrosis with and without calcification. In the 52-week study, death occurred in one animal following seizures. In carcinogenicity studies in mice, salmeterol by the oral route was not carcinogenic in males, but produced leiomyomas and lymphosarcomas in the F. Other changes were myocardial fibrosis, uterine smooth muscle hyperplasia, cystic glandular hyperplasia and follicular cysts. In the rat, salmeterol administered by the oral and inhalation routes produced changes in the male reproductive system, liver and thyroid gland; females also manifested mesovarian leiomyomas and ovarian cysts similar to that seen in mice. Inhaled salmeterol was irritating to the larynx. In rats, salmeterol did not affect fertility, was not teratogenic, but was fetotoxic and decreased the fertility of the offsprings. In pregnant rats and rabbits, salmeterol and/or its metabolites crossed the placenta, and they were also present in the

milk of lactating rats. Salmeterol was teratogenic in rabbits and not genotoxic or mutagenic.

Fluticasone propionate

Fluticasone propionate is a potent flourinated glucocorticoid. Unlike other corticosteroids, fluticasone propionate increases sodium and potassium urinary excretion. Safety pharmacology studies indicate no potential adverse effects. Orally in mice, salmeterol was not bioavailable. In rats, the bioavailabliity by the intranasal route was comparable to the inhaled route in humans. Rats, dogs and humans were similar, showing a high degree of protein binding and a low degree of binding to the red blood cells. In mice, rats, dogs and humans, fluticasone propionate undergoes hydrolysis to the non toxic –COOH derivative. Excretion of unchanged fluticasone propionate and its metabolites were fecal.

In acute toxicity studies in mice, rats and dogs, the manifestations were similar to those seen with glucocorticoids, i.e., weight loss, decreased thymus weight and/or decreased cortisol levels. By the oral route, fluticasone propionate was not toxic in rats and mice. Juvenile rats were markedly more sensitive by the subcutaneous than the oral route. This was attributed to the high first pass metabolism. By the s.c. route, mice showed cardiac inflammation. In multidose and long-term non-lethal inhalation studies in rats, the typical glucocorticoid effects were seen. In addition to the above effects, there were hair loss, decreased food consumption and lymphocyte levels, hyperglycemia, increased liver and kidney weights, decreased adrenal and spleen weights and histological changes in the liver, thymus and spleen. Similar findings were seen in multidose and long-term non-lethal inhalation studies in dogs. However, in the long-term study, the dogs showed lung inflammation due to local irritation and infection due to the compromised immune system. In a one-year inhalation study, juvenile dogs showed greater sensitivity than the adult animals. In the carcinogenicity studies in rats and mice, fluticasone propionate was not tumorgenic. In reproductive studies, fertility was not affected, but fetotoxicity and teratogenicity was seen in mice, rats and rabbits. In rats and rabbits, radioactivity was found in the fetuses and in the milk of lactating rats. Fluticasone propionate was not genotoxic or mutagenic.

Salmeterol and Fluticasone Propionate

Bridging inhalation toxicity studies were conducted in M and F rats (35-days and 13-weeks) and dogs (14-days) with the salmeterol/fluticasone propionate HFA formulation. In a pilot 35-day study the doses of salmeterol was 2 and 5 times the fluticasone propionate doses as shown in the following table. A 2-week recovery period was included in this study.

Group No.	Dose, mcg/kg	
	Salmeterol	Fluticasone propionate
Control	0	0
1	74	140
2	75	370
3	140	710
4	380	760

Since salmeterol and fluticasone propionate were not tested alone in this study, no conclusion could be made regarding interaction between the two drugs. However, the changes seen were characteristic of beta$_2$-agonist and the glucocorticoids. Histologically, all treated groups showed the signs of local irritation, i.e., hyperplasia in the epithelium of the nasal area and larynx, squamous metaplasia in the larynx, necrosis of the ventral cartilage in the larynx and macrophage aggregation in the lungs. No cardiac histopathology was observed. Glomerulonephrosis occurred in the M in Group 4 suggesting a drug interaction. This was not seen in the 13-week study where lower dosed of both drugs were used. M. Plasma levels determined within 30 min following for both salmeterol and fluticasone propionate were dose related but not dose proportional. At the end of 2 weeks, there was partial recovery of the inflammatory manifestations in the respiratory tract and the histological signs of hypercorticoidism. The kidneys were not examined to determine whether the renal effects were reversible.

In the 13-week study in Wistar rats, the doses of salmeterol were 2 and 10 times the fluticasone propionate dose, and there was a four-week recovery period with the C and HD combination group. The results are shown in the following table. In F, squamous epithelial hyperplasia was seen in the larynx of the salmeterol alone and HD combination group. However, drug interaction was observed in the M as there was an enhancement in the incidence of squamous epithelial hyperplasia at the HD M combination group. In addition, in the M groups receiving the combination, there was an increase in the incidence of macrophage aggregation around the terminal bronchioles; this was not seen in the M receiving either drug alone. In the HD, M and F combination recovery group (the only treated groups examined), the macrophage aggregating effect was still evident (M, C,0/10, HD, 2/10; F, C, 0/10; HD, 5/10) indicating a sustained effect. All other treatment-related changes seen in the HD combination group were reversible.

	Incidence or % Change									
	Male					Female				
Salmeterol, µg/kg	C	37.4	0	7.2	40.5	C	37.4	0	7.2	40.5
Fluticasone propionate, µg/kg		0	75.1	71.4	75.1		0	75.1	71.4	75.1
Histopathology										
Lungs										
Macrophage Aggregation Around the Terminal Bronchioles	0/14	0/15	0/15	3/14	4/15	3/14	0/15	1/15	3/14	2/15
Heart										
Myocardial degeneration [a]	0/14	0/0	0/0	0/14	3/15	0/15	0/0	0/0	0/15	0/15
Myocarditis [a]	0/14	0/0	0/0	0/14	1/15	0/15	0/0	0/0	0/15	1/15
Myocardial fibrosis [a]	0/14	0/0	0/0	0/14	1/15	0/15	0/0	0/0	0/15	1/15
Larynx										
Squamous Epithelial Hyperplasia	0/14	3/15	0/15	3/14	**7/15**	0/14	**7/15**	0/15	0/14	**5/15**

[a] minimal
Bold indicates statistical significance ($P < 0.05$)

The M, HD combination group showed an increased incidence of myocardial degeneration. In this study, the hearts from the animals receiving salmeterol, which alone is known to induce cardiotoxicity and fluticasone propionate, were not examined to make an assessment of drug interaction. When confronted by the presence of myocardial degeneration in the HD M combination groups, the sponsor indicated that this cardiac toxicity was not uncommon in this rat strain and for this reason did not examine the hearts of the animals receiving the compounds alone. To support this claim, they also submitted historical control data for nine 13-week studies in this strain (Han Wistar rats) spanning from 1992-1998. In six of these studies, the incidence of myocarditis in M ranged from 1/10 to 5/15, and in two of these studies, myocardial fibrosis occurred in one animal (1/10 and 1/12). No myocardial degeneration was seen in these historical controls. Further, a submitted article (Ruben et al., Non-Proliferative Lesions of the Heart and Vasculature in Rats CV-1, In Guides for Toxicologic Pathology. STP/ARP/AFIP, pp 1-5, 2000) indicated that older Sprague-Dawley and F-344 M manifest spontaneous cardiomyopathy consisting of myocarditis, myocardial fibrosis and myocardial degeneration; these may also occur in young rats as early as 3-4 months. However, no incidences were given for the young and old rats and the strain of rats was different from the strain used in the study submitted. The historical myocardial toxicity data and the cited article submitted by the sponsor did not support their claim that there was no interaction between salmeterol and fluticasone propionate. There was a difference in the degree of cardiac toxicity seen in the historical controls and in the HFA combination-treated animals. Myocarditis seen in the historical control data indicates an early stage of toxicity while myocardial degeneration observed in the combination treated animals is evidence of a later stage of toxicity. The 1-hour plasma levels of salmeterol and fluticasone propionate alone and in the combination groups indicated no pharmacokinetics interaction.

A 13-week inhalation study with the dry powder (NDA 21-077) was conducted with Wistar rats with salmeterol and fluticasone propionate alone and in combination. In the M rats, the incidence of myocardial fibrosis was similar in the salmeterol (2/15) and salmeterol-fluticasone propionate treated (1/15) animals indicating no drug interaction.

A pilot 14-day inhalation study was conducted in dogs (2 M and 2F/group) with salmeterol and fluticasone propionate alone and in combination using HFA as the propellant. The changes seen in the combination groups were attributed to the glucocorticoid effects, i.e., decreased body weight gained, hypokalemia, decreased thymus weight and atrophy, decreased serum cortisol levels, adrenal atrophy and hepatocyte rarefaction. Plasma levels of the individual drugs were not different from those in the combination groups indicating no pharmacokinetics interaction. However, there was a drug interaction regarding the pulse rate. As seen in the following table, the glucocorticoid enhanced the increased the pulse rate due to salmeterol, characteristic of $beta_2$-agonists.

	Average Pulse Rate						
Salmeterol, mcg /kg	0	41	123	51	48	153	143
Fluticasone propionate, mcg /kg	0	0	0	87	207	262	622
Average Pre-Dose Pulse Rate, beats/min, M and F	116	109	111	109	102	107	103
↑ in Pulse Rate[a]	-9	+7	+13	+11	+18	+24	+33

[a] Difference between the average pre-dose pulse rate and the average group means pulse rate on days 1, 2, 3, 7 and 14 determined immediately after and 0.5, 1, 2 and 4 h following administration of inhaled drugs.

In the 5- and 13-week inhalation toxicity studies in rats, the salmeterol alone and in combination with fluticasone propionate were irritating to the respiratory tract, which was still evident at the end of the 4-week recovery period. No signs of irritation to the respiratory tract were observed in the 2-week study in dogs. Drug interaction between salmeterol and fluticasone propionate occurred in rats and dogs. There was an increased incidence of myocardial degeneration and an increased incidence of macrophage aggregation around the terminal bronchioles in M rats in the 13-week toxicity study and an increased tachycardia in the 2-week study in dogs. Since these drug interactions were not seen when salmeterol and fluticasone propionate were administered as a dry powder in the combination studies, the propellant, HFA, may have contributed to this drug interaction. To confirm this potential interaction, a 3-month inhalation toxicity study in dogs was recommended (IND 57,171: 1/11/99 review of 10/23/98 submission by L. Pei). The sponsor in their 3/01/01 letter indicated that this 13-week inhalation study in dogs using salmeterol in combination with fluticasone propionate was not necessary. Reference was made to the lack of cardiac toxicity in the 13-week inhalation combination toxicity study in dogs using the dry powder formulation and to the cardiac toxicity observed in the 13-week inhalation combination study in rats was due to the spontaneous

background lesion (Ruben et al., Non-Proliferative Lesions of the Heart and Vasculature in Rats CV-1, In Guides for Toxicologic Pathology. STP/ARP/AFIP, pp 1-5, 2000) and not due to a drug interaction. This response did not support the concept that there was no drug interaction between salmeterol and fluticasone propionate when administered by the propellant, HFA. Although the sponsor did not conduct the13-week inhalation study in dogs as recommended by the Agency, there was a drug interaction between salmeterol and fluticasone propionate based on the findings in the 13-week inhalation study in rats and the pilot 2-week inhalation study in dogs. Thus, an additional study in a second species would have no impact on these findings. Since these cardiac findings may potentially be an adverse effect

Label Review

The recommended changes are in **BOLD;** deletions are ~~strikeout.~~

4 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

RECOMMENDATIONS

This NDA is approvable with the above labeling revisions.

Lawrence F. Sancilio, Ph.D.

Attachments (2)
Cc LGilbert-McClain

Drug: **Salmeterol NDA 21254**
Advair

	Age	mg/dose	# daily Doses	mg/day	kg	mg/kg	Factor	mg/m²
Adult	>12	0.021	4	0.084	50	0.0017	37	0.06

	Route	mg/kg/d	Conv. Factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
Mouse	p.o.							
Mouse	p.o.							
Mouse	p.o.	10	3	30	482.63	63.16	480	
rat	p.o./inh	0.21	6	1.26	20.27	2.65	20	
rat	p.o. /inh	0.68	6	4.08	65.64	8.59	65	
Reproduction and Fertility:								
rat	s.c.	0.01	6	0.06	0.97	N/A	1/1	N/A
rat	p.o.	2	6	12	193.05	N/A	190	N/A
rat	p.o.	1	6	6	96.53	N/A	95	N/A
rat	p.o.	10	6	60	965.25	N/A	970	N/A
Teratogenicity:								
Mouse	p.o.	10	3	30	482.63	N/A	480	N/A
rat	p.o.	1.4	6	8.4	135.14	N/A	140	N/A
rabbit	p.o.							
rabbit	p.o.							
rabbit	p.o.	10	12	120	1930.50	N/A	1900	N/A
Overdosage:								
Mouse	p.o.	150	3	450	7239.38	947.37	7200	
rat	p.o.	1000	6	6000	96525.10	12631.58	97000	
rat	inh	3.6	6	21.6	347.49	45.47	350	
rat	inh	2.9	6	17.4	279.92	36.63	280	
Other:	(Describe studies here)							
dog	inh	0.7	20	14	225.23	29.47	230	
dog			20	0	---	---	---	---
dog			20	0	---	---	---	---
extra			---	---	---	---	---	---
extra			---	---	---	---	---	---

Drug: **Fluticasone propionate ADVAIR**

	Age	mg/dose	# daily Doses	mg/day	kg	mg/kg	Factor	mg/m²
Pediatric				0				
Adult	>12	0.44	2	0.88	50	0.0176	37	0.65

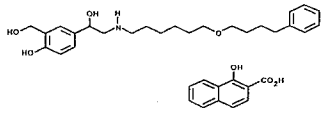
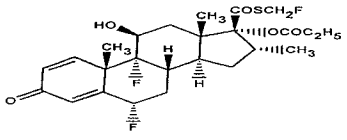
	Route	Mg/kg/d	Conv. Factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
mouse	p.o.	1	3	3	4.61	N/A	5	N/A
rat	p.o.	0.057	6	0.342	0.53	N/A	½	N/A
hamster			4	0	---	---	---	---
rat			6	0	---	---	---	---
rat			6	0	---	---	---	---
Reproduction and Fertility:								
mouse	s.c.	0.1	3	0.3	0.46	N/A	½	N/A
rat	s.c.	0.05	6	0.3	0.46	N/A	½	N/A
rat	p.o.		6	0	---		---	
rat	s.c.	0.01	6	0.06	0.09	N/A	1/11	N/A
Teratogenicity:								
mouse	s.c.	0.15	3	0.45	0.69	N/A	1/1	N/A
rat	s.c.	0.03	6	0.18	0.28	N/A	¼	N/A
rabbit	s.c.	0.004	12	0.048	0.07	N/A	1/14	N/A
rabbit	p.o.	0.3	12	3.6	5.53	N/A	6	N/A
mouse	s.c.	0.04	3	0.12	0.18	N/A	1/5	N/A
Overdosage:								
mouse	s.c.	1000	3	3000	4606.88	N/A	4600	N/A
mouse			3	0	---	---	---	---
rat	p.o.	1000	6	6000	9213.76	N/A	9200	N/A
rat	inh	1.9	6	11.4	17.51	N/A	20	N/A
Other:	(Describe studies here)							
rat	p.o.	10	6	60	92.14	N/A	90	N/A
rat	p.o. s.c.	0.1	6	0.6	0.92	N/A	1/1	N/A
mouse	s.c.	0.045	3	0.135	0.21	N/A	1/5	N/A
extra			---	---	---	---	---	---
extra			---	---	---	---	---	---

REVIEW AND EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
Original Review

Redacted Review Posted on FDA website under NDA 21-077

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.	57,151
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** **GR106642X, HFA 134a**
Generic Name:	Salmeterol/fluticasone/GR 106642X 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
DNA 20-292 Serevent Diskus
IND 50703 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:

	Strength			Function
Formulation	21/44 µg	21/110 µg	21 µg	
Salmeterol xinafoate (micronised, mg)				Active ingredient
Fluticasone propionate (micronised, mg)				Active ingredient
GR106642X (g)				Excipient

Document submitted and reviewed in this IND:

Study	Report #	Vol.	Page
[redacted]	[redacted]	[redacted]	[redacted]
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 propellant HFA 134a (GR106642X).

Document not reviewed in this IND:

[redacted]

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 50,703).

GR106642X is Glaxo Wellcome's code name for HFA-134a. HFA-134a is a less ozone depleting propellant developed to replace the CFC propellant. Toxicity profile of the HFA-134a has been well characterized (Drug Master File

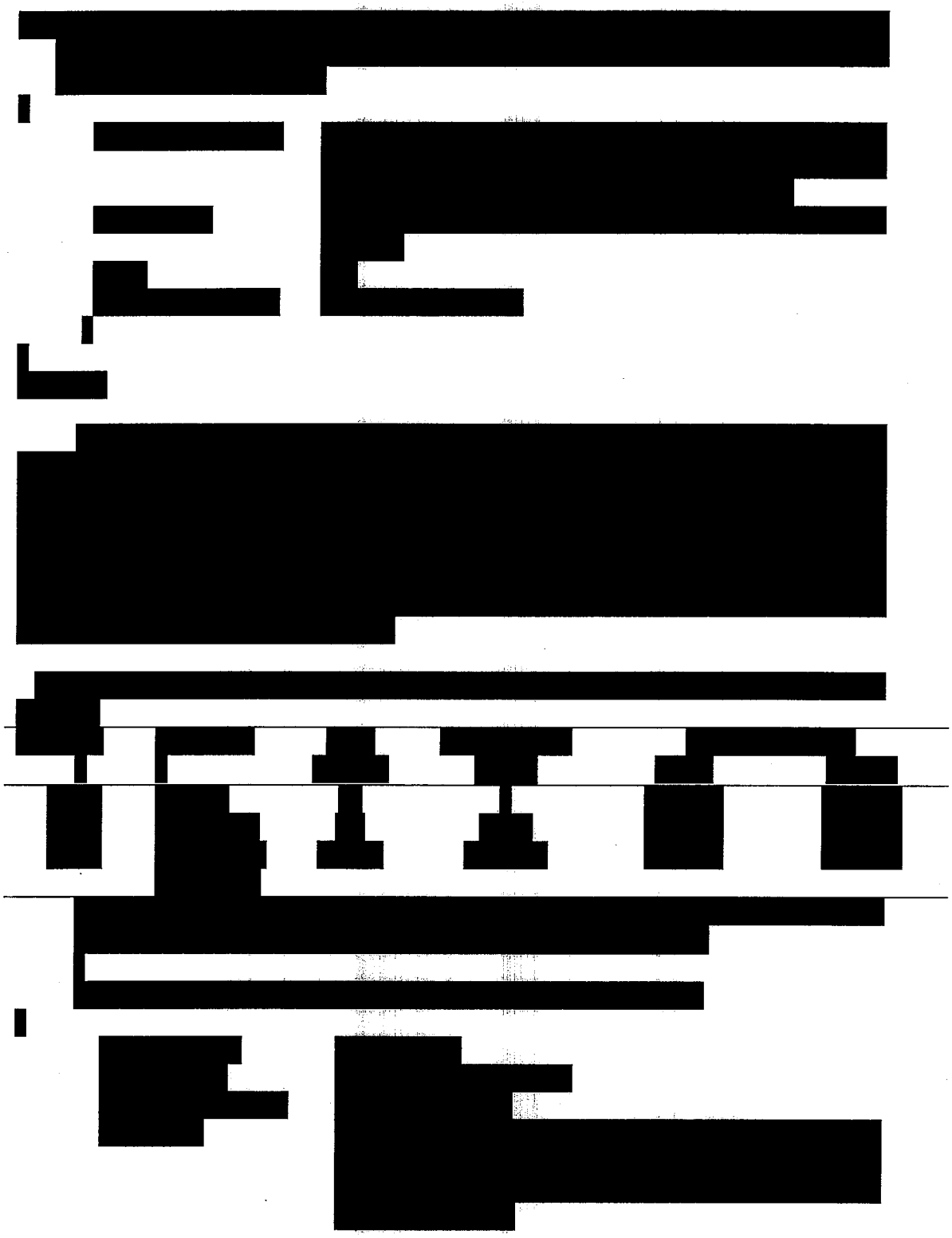
In the current submission, Glaxo Wellcome submitted 3 protocols (Protocols SAS30001, SAS30003, and SAS30004) to study the safety and efficacy of salmeterol/fluticasone combination in asthmatic patients. The proposed dose levels of salmeterol/fluticaosne were (in μg): 42/88, 42/220, 42/440 —— Treatment 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

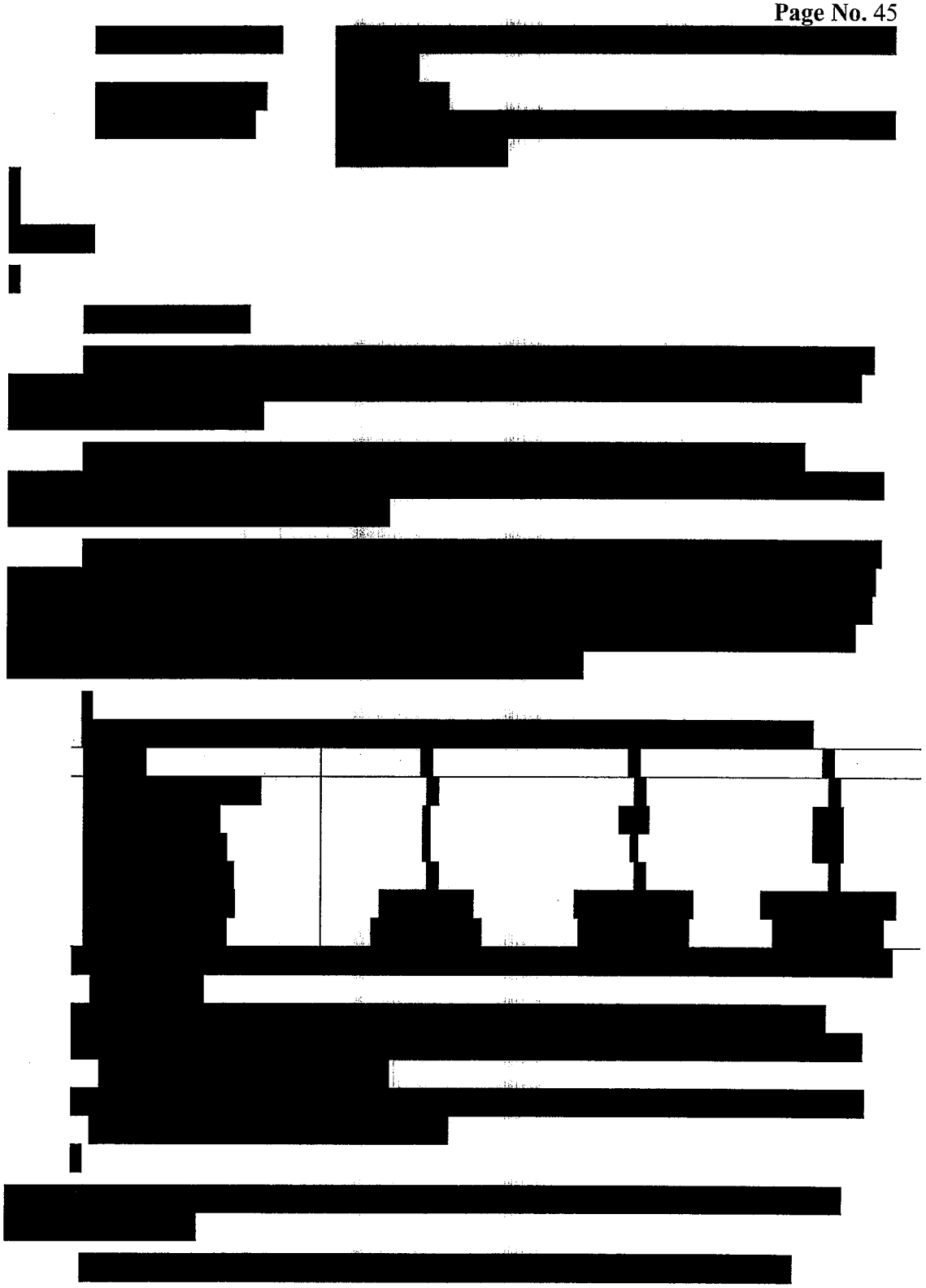
I. Safety Pharmacology

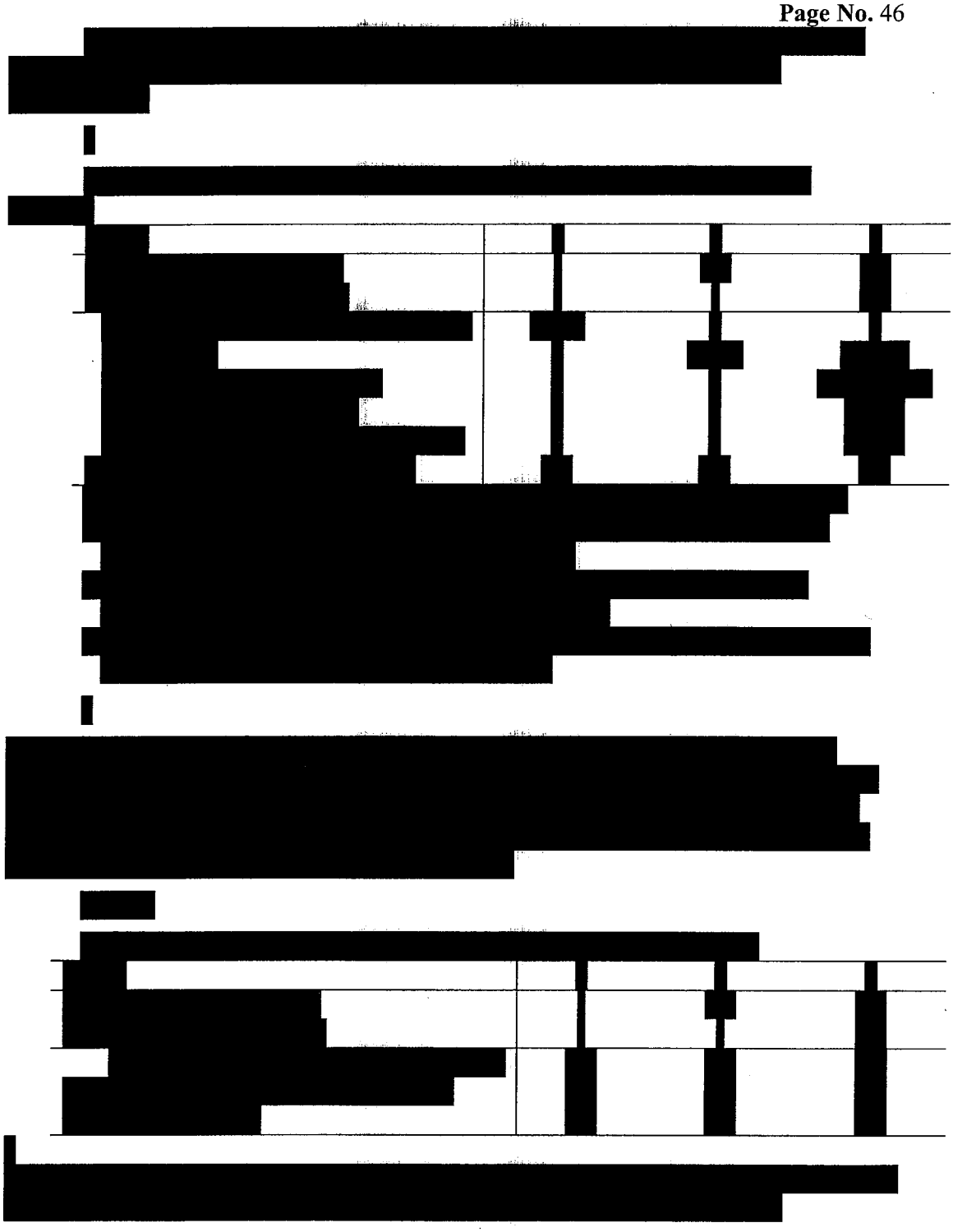
[redacted]

II. Toxicology



APPEARS THIS WAY ON ORIGINAL





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 MDI

Method:

Wistar Rats ([redacted] 10/sex/dose) were exposed by nose-only inhalation to salmeterol/fluticasone/GR33343G 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 = [redacted] The MMAD of particles ranged between [redacted] μm, with geometric standard variation of [redacted] The percentages of inhalable particles [redacted] μm) were estimated between [redacted] total deposition).

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)/ Fluticasone (mg/kg)	0 /0	74 /140	75 /370	140 /71 0	380 /760	0 /0	74 /140	75 /370	140 /71 0	380 /76 0
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)/ Fluticasone (mg/kg)	0 /0	74 /140	75 /370	140 /71 0	380 /760	0 /0	74 /140	75 /370	140 /71 0	380 /76 0
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

	Male					Female				
Salmeterol (μg/kg)/ Fluticasone (μg/kg)	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

	Delivered dose: salmeterol / Fluticasona (μg/kg)				
Drug	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 (WI BR, 10 weeks of age) were exposed by nose-only inhalation to salmeterol/fluticasone/GR33343G 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 MMAD of particles in the 13-week study ranged between — µm, with geometric standard variation of — . The percentages of inhalable particles (— m) were estimated between — (total deposition).

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) Fluticasona (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
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:	[redacted]
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 in FHA 134a 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 [redacted] for salmeterol and fluticasone respectively (Vol. 8, page 115). The recovery study showed that [redacted] of administered salmeterol and [redacted] 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.

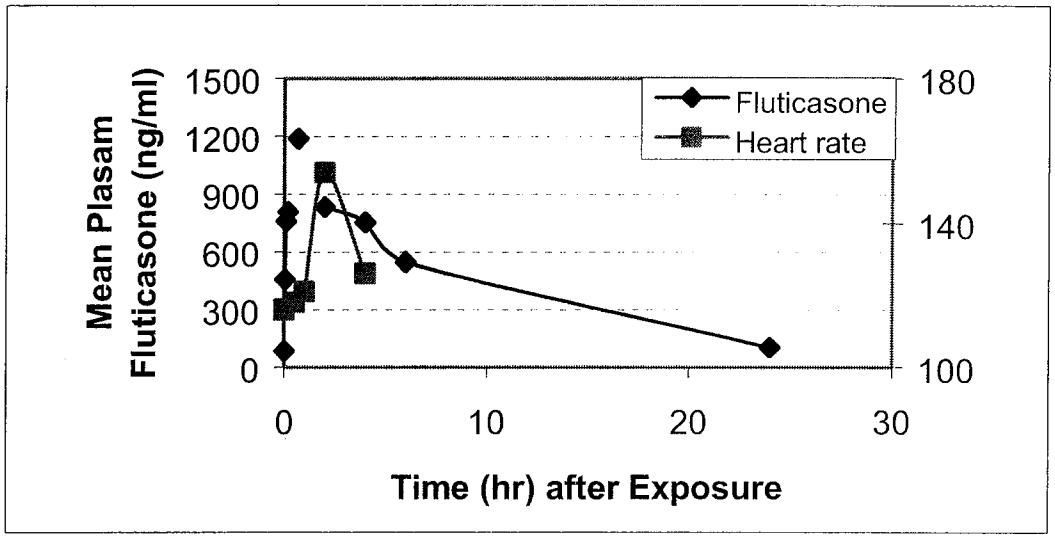


Figure 1. Time course of plasma salmeterol concentration and Heart rate in dogs.

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/HFA 134a 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 HFA 134a, however, is less known.

[redacted]

[redacted]

APPEARS THIS WAY
ON ORIGINAL

Rat:

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/HFA 134a 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/HFA 134a 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 period of 4 weeks. At the end of the recovery period, there was an 8 - 15% decrease in absolute body weight in the high dose groups. The only noticeable salmeterol effect was the myocardial degeneration in three (of fifteen) high dose males that received both salmeterol and fluticasone and were sacrificed at the end of exposure. This finding was absent in control groups that received similar amount of either fluticasone or salmeterol only. Again, no NOEL levels were demonstrated.

Table 30 (below) summarizes the major findings of cardiac toxicity in rats. [redacted] Myocardial degeneration (high dose males) was also associated with the addition of fluticasone in the 13-week study, confirming the single dose study. Minimal cardiac effects were seen in all treated groups in the 5-week study, suggesting that its dose selection for salmeterol be too low.

Table 30. Summary of Cardiotoxicity of Salmeterol and Fluticasone in Rats

Duration	[redacted]	5 weeks	13 weeks
Dose (µg/kg/day) Salmeterol/fluticasone		0/0, 74/140, 75/370, 140/710, 380/760	0/0, 37/0, 0/75, 7/71, 41/75
Control (salm. Only)	[redacted]	Minimal increase in heart rate	None
Treated (salm./flut.)	[redacted]	Minimal increase in heart rate	Myocardial degeneration

Dog:

In a 2-week pilot inhalation study, Beagle dogs (2/sex/group) were given through an orolaryngeal tube 2 – 6 actuations of the clinical formulation of salmeterol/fluticasone/HFA 134a MDI. The estimated dose levels were (salmeterol/fluticasone): 0/0, 41/0, 48/87, 51/207, 123/0, 153/262, and 143/262 µg/dog (total deposition). Noticeable and dose-related changes included body weight losses (0.1 to 0.7 kg), and decreases in serum cortisol levels (43 – 52%)

and in thymus weight (52-72%), and increases in serum potassium levels (10 - 12%) starting from the mid dose (48/87 μg/dog) and/or higher dose groups. Atrophy of adrenals and thymus were also evident in these groups. Atrophy and adrenal glands and thymus correlated well with the changes in serum cortisol levels and thymus weight. Hepatocyte rarefaction in the periportal zone in the liver was observed in the mid low, mid high, and high fluticasone dose groups. The only noticeable salmeterol effect was the increases in heart rate, but this increase in heart rate was also fluticasone dose-dependent given approximately the same levels of salmeterol (Figure 2). Once more, no NOEL levels were demonstrated.

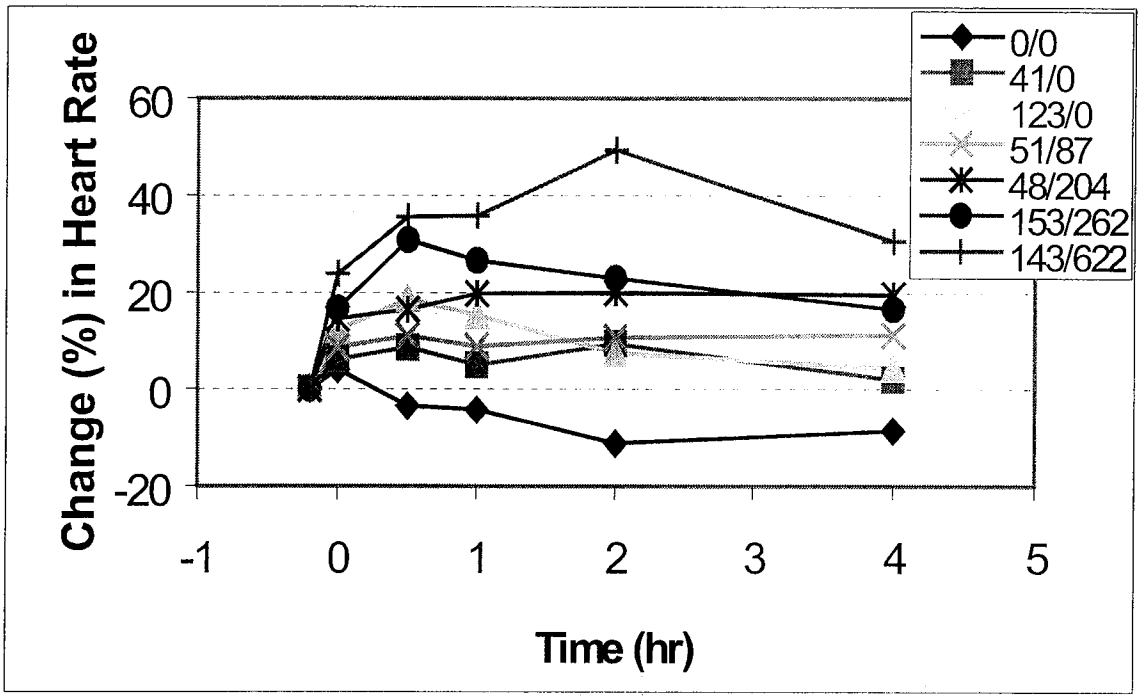


Figure 2. Time-course of changes in heart rate (in percent) after salmeterol and fluticasone exposure. Each time point is the average of the adjusted means of the group from all 5 measuring occasions. Pre-dosing measurements (the first time point) is considered as the base line (100%). Numbers in legends represent salmeterol (before the slash) and fluticasone (after the slash) dose levels during the experiment.

Available toxicity studies suggest that fluticasone may enhance cardiac effect of salmeterol when they are used in combination by inhalation. This is indicated by the findings that 1) addition of fluticasone to salmeterol-treated animals causes cardiac lesions that are absent in the salmeterol control animals in rats; and 2)

fluticasone exaggerates the chronotropic effect of salmeterol in a dose-dependent manner in dogs.

Evaluation

Salmeterol is a beta-adrenergic bronchodilator and fluticasone a corticosteroid. Both drugs have been approved, although separately, for the asthma indication. Examples of their products are Serevent Inhalation Aerosol (NDA 20-236) and Flovent Inhalation Aerosol (NDA 20-548). No combination product of these two drugs has been approved. Glaxo Wellcome proposes to conduct a phase 3 clinical trial to study safety and efficacy of salmeterol/fluticasone/HFA 134a metered dose inhaler.

Individual toxicity profiles of salmeterol, fluticasone and HFA 134a are well known. The major target organs of toxicity are the heart for salmeterol, the immune system and growth for fluticasone, and the central nervous system for HFA 134a. Like many other aliphatic compounds, HFA 134a may potentially sensitize the heart.

[redacted]

The toxicity profile of the salmeterol, fluticasone and HFA 134a in combination is assessed in this submission. Glaxo Wellcome conducted a few bridging toxicity studies in compliance with the Division's Points-To-Consider document (*Reg. Toxicol. Pharmacol.,* 1997;25:189-193). Their studies included inhalation toxicity studies with the treatment duration of up to 13 weeks in rats, and 2 weeks in dogs using the to-be-marketed formulation. [redacted]

Data in this submission further confirms the previous observation that fluticasone may enhance the cardiac effect of salmeterol. This drug interaction occurs cross species as evident in rats, dogs and humans. [redacted] The 13-week inhalation study shows myocardial degeneration that is not present in either salmeterol or fluticasone control groups. Although no cardiac abnormalities are observed in all treated groups in the 5-week inhalation toxicity study, the appropriateness of salmeterol dose selection is questionable. Overall, addition toxicity studies confirm the previous observation that fluticasone enhances cardiac toxicity of salmeterol in rats.

In dogs, the 2-week pilot study (2/sex/group) shows that: 1) salmeterol causes dose-dependent increases in heart rate; and 2) the fluticasone causes dose-dependent and further increases in heart rate when salmeterol doses are similar (Figure 2). However, no treatment-related histologic finding is apparent in any treated groups. Unfortunately, this is such an under powered (rather small sample size and selected histology panel) that toxicity profiles of the fluticasone/salmeterol combination may not be fully evaluated. Nonetheless, data indicate that fluticasone enhances chronotropic effect of salmeterol in dogs.

Clinical experience with the salmeterol and fluticasone combination is available, although mostly in the dry powder. A total of 769 asthma patients (including 125 children of 4 – 11 years old) have been given salmeterol and fluticasone in a dry powder inhaler (IND 50,703). Dose levels are 50 μg of salmeterol and 100 – 500 μg of fluticasone, bid. According to the sponsor, adverse events of these clinical trials indicate typical effects of salmeterol and fluticasone. Despite good experience with the dry powder formulation, limited clinical experience for HFA 134a formulation exists. In fact, the only available data is a single dose tolerability study in healthy male subjects (Protocol No. C92-029). Each testing subject (12 total) receives a total of 336 μg of salmeterol and 1760 μg fluticasone within 5 hours. An increase in heart rate (3.7 beats/minute/100 μg salmeterol) is seen after salmeterol administration, but the co-administration of fluticasone results in a further increase in heart rate (5.6 beats/minute/100 μg salmeterol). This finding is consistent with the findings in dogs.

Available data indicate that potential drug interaction between salmeterol and fluticasone exists. The sponsor, however, interprets this drug interaction (enhanced cardiac toxicity) in rats as a rodent species-specific finding and as no clinical relevance in the safety evaluation of these drugs in humans (Vol. 1.1, p 90). Such an interpretation is not a true reflection of all the available data in this submission (e.g. data in dogs and humans) and therefore, may be flawed. Studies show that addition of fluticasone to salmeterol treated-animals are associated with atrial myocarditis, cardiac arrhythmia and myocardial degeneration in rats. Studies also show that addition of fluticasone is associated with the potentiation of the chronotropic effect of salmeterol in both dogs and humans, although histologic abnormalities are lacking in dogs (this study is not a robust due to the small sample size of n=2/sex/dose). The increase in heart rate is known to be associated with cardiac toxicity of beta agonists. That the enhanced cardiac effects of salmeterol by fluticasone are observed not only in rats but also in dogs and humans shows that potential drug interaction between salmeterol and fluticasone exists across species. Thus the interpretation of cardiotoxic finding in rats as a rodent specific phenomenon is flowed. The findings of drug interaction in rats are relevant to the safety evaluation of salmeterol and fluticasone combination and should be of safety concern for the proposed clinical trials.

Conclusion:

Safety evaluation of the proposed salmeterol/fluticasone/HFA 134a formulation has been completed. Available preclinically and clinical data show that this new formulation exhibits a toxicity profile in typical mixture of fluticasone and salmeterol toxicity: cardiac toxicity, immune system and growth suppression. However, they also suggest the existence of potential pharmacological and/or toxicological drug interaction between salmeterol and fluticasone: the former may enhance cardiac toxicity of the latter when used in combination by inhalation. Cardiac toxic findings (atrial myocarditis and arrhythmia) that are absent in the salmeterol control are observed in the salmeterol/fluticasone treatment in rats; stronger chronotropic effect (e.g. increases in hear rate) is seen in salmeterol/fluticasone groups than salmeterol control in dogs and humans. There is little evidence that HFA 134a may further enhance this cardiac effect.

The sponsor proposes the maximal recommended clinical doses of each individual ingredient in their clinical trials. Given the possible enhancement of the cardiac toxicity of salmeterol by fluticasone, cardiac toxicity of the new formulation should be of safety concern, especially in the patients with the compromised cardiovascular function. Therefore, the cardiac toxicity of the new formulation needs to be carefully evaluated. This can be achieved through closer monitoring cardiac function in the clinical trials and conducting additional preclinical studies. Preclinical studies may include a three-month inhalation

toxicity study in dogs, and pharmacological studies (in vitro and/or in vivo). The toxicology study will compensate for the inadequacy of the 2-week pilot study in dogs.

Recommendation:

1. The proposed trials may proceed from the preclinical viewpoint.

2. Cardiovascular function of the patients should be closely monitored.

3. Additional pharmacology and/or toxicity studies should be conducted to further evaluate this potential drug interaction between salmeterol and fluticasone. Pharmacology studies may be in vitro and/or in vivo studies. Toxicology studies may include at least a 3-month inhalation toxicity study in dogs.

Points discussed with the Medical Reviewer:

1. Potential drug interaction exists between salmeterol and fluticasone. This interaction is indicated by the possible enhancement of cardiac toxicity of salmeterol by fluticasone.

2. Close monitoring of cardiovascular function is suggested in the clinical trials.

Draft letter for the sponsor:

We believe that available data suggest potential drug interaction between salmeterol and fluticasone. We strongly suggest you conduct additional pharmacology and toxicology studies to further evaluate this observation. Pharmacology studies may be in vitro and/or in vivo mechanistic or efficacy studies. Toxicology studies may include at least a 3-month inhalation toxicity study in dogs.

Luqi Pei, D.V.M., Ph.D.
Pharmacologist/Toxicologist

Ori: IND HFD-570/Division File
HFD-570/Dr. Pei/ Zoetis/Burns

Histopathology Inventory For IND 57,151 (Salmeterol/Fluticasone/HFA 134a)

Study No.	WPT/93/176	WPT/96/075	WPT/93/189
Description	5 weeks, IH	3-month, IH	14 day, IH
Species	Rat (WI)	Rat (WI)	Dog (pilot)
Adrenals	x	X	X
Aorta		X	
Bladder			
Bone marrow smear			
Bone (femur)		X	
Brain	x	X	
Caecum		X	
Cervix			
Colon (large intestine)		X	
Diaphragm			
Duodenum		X	
Epididymis		X	
Esophagus		X	
Eye		X	X
Fallobian tube			
Gall bladder			
Gross lesions			x
Harderian gland		X	
Heart	X	X	X
Hypophysis			
Ileum (small intestine)		X	
Injection site			
Jejunum		X	
Kidneys		X	X
Laryngeal gland			
Larynx	X	X	X
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, cervical		X	X
, mandicular			
, mesenteric		X	X
, submaxillary			
, tracheobronchial		X	
Mammary gland			
Nasal cavity		X	
Optic nerves		X	
Ovaries		X	
Pancreas		X	
Parathyroid			
Peripheral nerve			
Pharynx	X	X	X
Pituitary	X	X	X
Prostate		X	
Rectum		X	
Salivary gland		X	
Sciatic nerve		X	
Seminal vesicles		X	
Skeletal muscle		X	
Skin		X	
Spinal cord		X	
Spleen		X	
Sternum		X	
Stomach		X	
Testes		X	
Thymus	X	X	X
Thyroid		X	
Tongue		X	

Trachea	X	X	X
Uterus		X	
Vagina		X	
Zymbal gland			
Others			
Paranasal sinus			
Oral cavity			
Middle ear			
Teeth			
Nasal pharynx		X	
Abdominal tissue			
Incisor			
molar			
Sublingual gland			
Haw gland			
Bronchia		X	
Vertebra			
Coagulating gland			

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

/s/

Lawrence Sancilio
10/19/01 08:58:52 AM
PHARMACOLOGIST

Joseph Sun
10/19/01 09:53:38 AM
PHARMACOLOGIST
I concur.