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. In the single dose study, ventricular myocardial degeneration was observed in all salmeterol treated groups, but the addition of fluticasone elicited atrial myocarditis. 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	Single dose	5 weeks	13 weeks
Dose (μg/kg/day) Salmeterol/fluticasone	0/0, 5200/0, 3300/1900	0/0, 74/140, 75/370, 140/710, 380/760	0/0, 37/0, 0/75, 7/71, 41/75
Control (salm. Only)	Ventricular myocardial degeneration (VMC)	Minimal increase in heart rate	None
Treated (salm./flut.)	VMC + atrial myocarditis	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/ [redacted] 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.

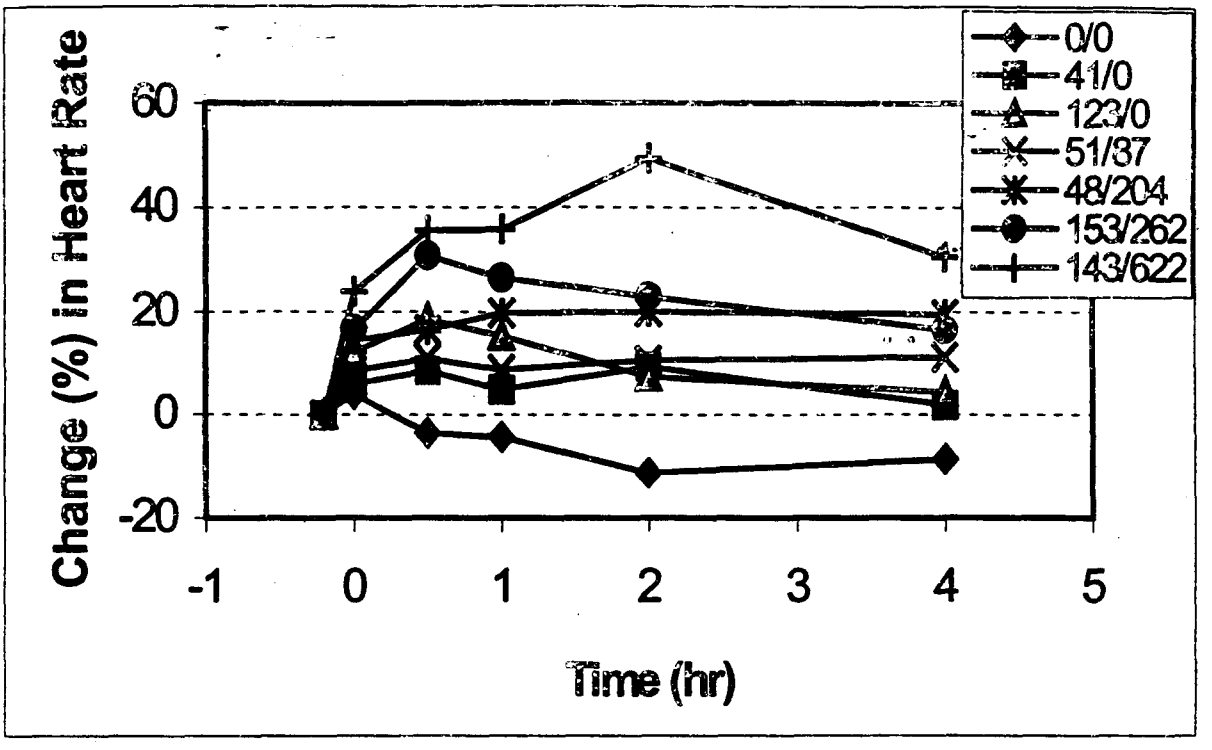

Figure 2. Time-course of changes in heart rate (in percent) after salmeterol and fluticasone exposure. Each time point is the average of the adjusted means of the group from all 5 measuring occasions. Pre-dosing measurements (the first time point) is considered as the base line (100%). Numbers in legends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

Individual toxicity profiles of salmeterol, fluticasone and ——— are well known. The major target organs of toxicity are the heart for salmeterol, the immune system and growth for fluticasone, and]

[

Toxicity profile of the salmeterol/fluticasone/ ——— combination, however, is less known. Toxicity of salmeterol and fluticasone in combination, although in a different (dry powder) formulation, has been tested previously in laboratory animals. Rats exposed to this combination by inhalation for less than 5 weeks showed atrial myocarditis that was absent in the salmeterol control [Study Nos. WPT/93/175 (5-wk), WPT/93/397 (1x), WPT/93/395 (1x), Pharm/tox Review by Mark Vogel on Oct 22, 1996 in IND ——— This finding suggests that fluticasone could potentially enhance the cardiac toxicity of salmeterol; however, no such finding was found in studies with the longer treatment duration (13-week) in rats or dogs. Note that studies with longer treatment duration usually employ lower exposure than the short-term testing.

Toxicity of fluticasone and ——— combination has also been assessed in laboratory animals previously. A 13-week inhalation study in dogs (WPT/92/425) showed lesions in the respiratory system that were absent with other formulations (Pharm/tox review by Dr. Shannon Williams on September 12, 1997 in IND ——— These lesions included epithelial hypertrophy and hyperplasia in the carina and hypertrophy in the bronchioles. A re-evaluation of these data and historic background information indicated that the above pathological findings could be secondary to immunosuppression of steroids (Pharm/tox review by Dr. Mark Vogel on August 5, 1997 in IND ——— Clinically, three-month phase 3 clinical trials are underway under IND ———

The toxicity profile of the salmeterol, fluticasone and ——— 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. The sponsor also submitted an acute inhalation toxicity study in dry powder formulation.

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. In rats, the acute inhalation exposure of both fluticasone (1.9 mg/kg) and salmeterol (3.2 mg/kg) causes atrial myocarditis and ECG abnormalities that are absent in the salmeterol control (5.2 mg/kg). The functional ECG abnormalities correlate with the morphologic abnormalities. In fact, increased cardiac toxicity occurs at a lower salmeterol dose level. 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 ——— 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 ——— 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/ ——— : 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 ——— 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.

/S/ 1/11/99

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

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

/S/ 1/11/99

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Histopathology Inventory For IND —— (Salmeterol/Fluticasone ——

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					

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Jan.

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REVIEW AND EVALUATION OF PHARMACOLOGY/ TOXICOLOGY DATA
Chemistry Consult

NDA No. 21-077

Date of Consult Request: 8/20/99

Reviewer: Lawrence F. Sancilio, Ph.D.

Division: PULMONARY DRUG PRODUCTS, HFD-570

Reviewer Completion Date: 12/17/99

Dates of Submissions by the Sponsor: 3/24/99, 12/6/99

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 ——— in patients 12 years of age and older.

Clinical Formulation Components: Each blisterpack contains 50 μg of salmeterol and 100, 250 or 500 μg of fluticasone propionate in lactose.

Route of Administration: Oral inhalation

Recommended Dose: The proposed maximum daily dose is 100 μg of salmeterol xinafoate and up to 1000 μg of fluticasone propionate.

Request by Dr. D. Koble to evaluate the safety of ——— . and 'the most abundant other impurity' in the drug substance and ——— in the drug product. In the drug substance, their proposed respective specifications were — and — in the drug substance and —— in the drug product.

Summary and Evaluation

According to the ICH Guideline, each impurity in the drug substance should be –

— if the dose of the drug is ≤ 2 gm. For the drug product, the degradant should be – — if the dose of the drug is ≥ 10 gm. Otherwise, the impurity should be qualified.

Dr. Koble verified that ——— was present in the preclinical batches from the analysis presented in the 12/6/99 submission. In the long-term preclinical studies, ——— was present at —— (12/6/99 submission) in the salmeterol batches used in the dog. The results in the following table show that the daily dose of ——— at the proposed —— specification is approximately 1/10 that of the safe dose. Thus, the proposed specification of ——— in the salmeterol drug substance is qualified.

Species/Study NOAEL	Safe Dose of Salmeterol µg/kg[a]	Safe Dose of ——— µg/kg[b]	Anticipated Daily Human Dose of ——— µg/kg[c]
Dog 52-Week 190 µg/kg mg/kg (40 µg/kg by inhalation and 150 µg/kg p.o.).	31.7	———	———

[a] Determined from the NOAEL
[b] Determined from the safe dose of salmeterol containing —— of the impurity, ———
[c] Determined from the daily human dose of salmeterol (2 µg/kg) containing the proposed —— of the impurity. ———

The Most Abundant Other Impurity, —

The following table show that the daily dose of the proposed specification of —— of this impurity in the drug substance exceeds the acceptable safe dose. This was determined from its —— presence in the preclinical batch. Consequently, the proposed specification was not qualified.

Species/Study NOAEL ———	Safe Dose of Salmeterol µg/kg[a]	Safe Dose of The Most Abundant Other Impurity µg/kg[b]	Anticipated Daily Human Dose of The Most Abundant Other Impurity µg/kg[c]
Mouse 80-Week 200 µg/kg, p.o.	20	———	———

[a] Determined from the NOAEL
[b] Determined from the safe dose of salmeterol containing —— of 'The Most Abundant Other Impurity'.
[c] Determined from the daily human dose of salmeterol (2 µg/kg) containing the proposed —— of The Most Abundant Other Impurity.

The safety of this degradant was previously reviewed and qualified at a proposed specification of — (NDA 20-692, Review of L. Sancilio, May 14,1999) in the drug product at the same dosage. Consequently, the proposed specification of — for salmeterol in the drug product in this NDA is qualified.

Recommendation

The proposed specification of — for ——— in the drug substance and for — of ——— in the drug product is qualified.

The proposed specification of — for the 'the most abundant other impurity' in the drug substance was not qualified and should be lowered to —

/S/ 12/17/99

Lawrence F. Sancilio, Ph.D.
Pharmacologist/Toxicologist

/S/ Dec. 17, 1999

CC:
Div. Files NDA 21-077
HFD-570/Division File
HFD-570/CSO
HFD-570/LFSancilio
HFD-570/SJohnson
HFD-570/DKoble

Attachment
Approved by J. Sun

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OCT 18 1996

DIVISION OF PULMONARY DRUG PRODUCTS

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Review

IND No. ——— **Serial No.** 000 **Submission date:** 24 MAY 96

Reviewer: W. Mark Vogel, Ph.D. **Review Completed:** 18 OCT 96

Information to be Conveyed to Sponsor: Yes ( ), No (✓)

Sponsor: Glaxo Wellcome, Research Triangle Park, NC

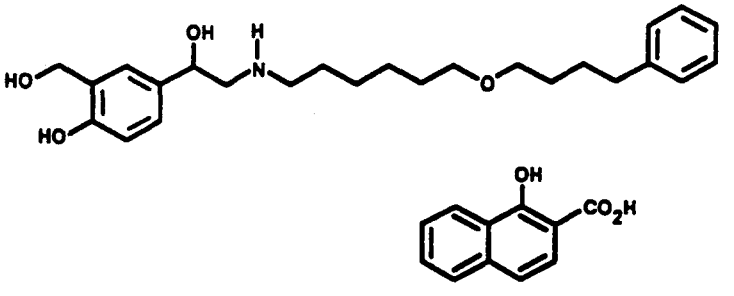
Drug Name: Salmeterol xinafoate /fluticasone propionate Diskus™ dry powder inhaler

Chemical Names:

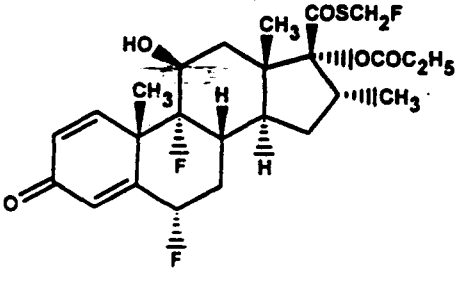
Salmeterol xinafoate — 4-hydroxy-α^1-[((6-(4-phenylbutoxy)hexyl)-amino)methyl]-1,3-benzenedimethanol, 1-hydroxy-2-naphthoate

Fluticasone propionate — S-fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate

Structures:



Salmeterol xinafoate MW = 603.8



Fluticasone propionate MW = 500.6

Related INDs/NDAs/DMFs:

IND	[redacted]	[redacted]
IND	[redacted]	[redacted]
NDA	20-121	Flonase nasal spray, 0.05%
NDA	20-236	Serevent inhalation aerosol
NDA	20-548	Flovent inhalation aerosol
NDA	20-549	Flovent Rotadisk for inhalation

Class: *Salmeterol:* long-acting β_2-adrenergic agonist, bronchodilator
Fluticasone: glucocorticoid steroid

Indication: Asthma

Formulation: Multiple dose dry powder inhaler: 50 μg salmeterol, 100 or 250 μg fluticasone, made up to 12.5 mg with lactose [redacted]

Route: Oral inhalation

Proposed Clinical Protocol:

Objective: Safety and efficacy of combination *vs* placebo and individual components.
Dose: Salmeterol/Fluticasone = 50μg/10μg and 50μg/250μg
Frequency: Twice daily for 12 weeks
Population: 640 asthma patients > 12 year old, already using inhaled corticosteroids and β-adrenergic agonists.

Previous Review(s), Date(s) and Reviewer(s): None

Preclinical Studies Submitted and Reviewed in this IND:

Study	Report #	Vol	Page
Safety Pharmacology:			
Guinea pig, intravenous cardiovascular safety study	WPT/95/140	3	008
Toxicology:			
Rat, acute inhalation toxicity (study-1)	WPT/93/395*	3	078
Rat, acute inhalation toxicity (study-2, for myocarditis)	WPT/93/397*	3	218
Rat, preliminary, 15-day inhalation toxicity study	WPT/92/182	5	031
Rat 14-day inhalation tox. (Sprague-Dawley *vs* Wistar)	WPT/92/318	5	182
Rat, pilot, 35-day inhalation toxicity study	WPT/93/175	6	196
Rat, 13-week inhalation toxicity study	WPT/95/011	8	001
Dog, preliminary, 14-day inhalation toxicity study	WPT/92/178*	10	001

Study	Report #	Vol	Page
Dog, pilot, 14-day inhalation toxicity study	WPT/93/089*	10	168
Dog, 13-week inhalation toxicity study	WPT/95/233	11	001
Reproductive Toxicology:			
Mouse, organogenesis dose-ranging study	WPT/92/179*	12	022
Mouse, oral/subcutaneous organogenesis study	WPT/92/196	12	066
Rat, preliminary toxicity in pregnant females	WPT/92/125*	13	001
Rat, oral/subcutaneous organogenesis study	WPT/92/371	13	103
Toxicokinetics:			
Mouse, oral + subcutaneous organogenesis study	GDM/93/045	16	335
Mouse, placental transfer study	GDM/92/040	16	348
Rat, acute inhalation toxicity study	GDM/94/019	16	361
Rat, preliminary, 15-day inhalation toxicity study	GDM/92/052	17	001
Rat 14-day inhalation tox. (Sprague-Dawley *vs* Wistar)	GDM/94/020	17	015
Rat, pilot, 35-day inhalation toxicity study	GDM/94/011	17	088
Rat, fetal and post-natal development	GDM/93/050	17	143
Dog, pilot single dose inhalation study	GDM/93/058*	17	192
Dog, preliminary, 14-day inhalation toxicity study	GDM/92/051	17	291
Dog, pilot, 14-day inhalation toxicity study	GDM/94/158	17	319

* Previously reviewed submission to IND ——— or NDA 20-549, Flovent Rotadisk.

Studies Not Reviewed in this IND: None

Studies Previously Reviewed: Studies marked by asterisk above were previously reviewed; results are briefly summarized herein, with reference to the original review.

Note: Portions of this review were excerpted directly from the sponsor's submission.

Throughout review, bold highlighted values in data tables indicate statistically significant difference compared to vehicle control.

Guinea Pig Intravenous Cardiovascular and Respiratory Safety Study
Glaxo Wellcome report WPT/95/140, vol 3, pg 8

Methods: Two groups of anesthetized (ketamine/xylazine/pentobarbital) male guinea pigs were treated with cumulative doses of salmeterol, 0.01-100μg/kg, i.v., at 10 minute intervals. One of the groups was pretreated with fluticasone, 10 mg/kg, s.c., at 24 hr and 3 hr before salmeterol dosing, the other received fluticasone vehicle. The following measurements were made: arterial blood pressure, heart rate, EKG, and tracheal inflation pressure. Fluticasone plasma concentration was determined at the end of the experiment.

Results: Fluticasone plasma concentration was 9.0 ng/mL. Baseline heart rate was slightly increased in fluticasone pre-treated animals *vs* vehicle controls (12%↑, $P<0.05$%). In both groups, salmeterol caused dose-related decreases in systolic and diastolic arterial blood pressure, and increases of heart rate. Guinea pigs pre-treated with fluticasone were slightly more sensitive to the heart rate effects of salmeterol at lower doses. This small effect was not biologically important despite statistical significance. Salmeterol did not affect tracheal pressure nor cause arrhythmias in either group.

Comment: No biologically relevant cardiovascular interaction between salmeterol and fluticasone was observed. It should be noted that the baseline arterial systolic pressure (range = 26 to 56 mmHg) and diastolic pressure (range = 15 to 42 mmHg) were low in these animals. This is probably not an optimal model for investigating a drug like salmeterol, for which decreased blood pressure is the primary cardiovascular effect.

PHARMACOKINETICS AND TOXICOKINETICS

Rat Inhalation Studies: Toxicokinetic results from 1 single-dose and 4 multiple-dose studies are summarized in table 1, page 5. Salmeterol and fluticasone were co-administered by nose-only inhalation of dry powder with lactose. Drug exposure was for 20 minutes in the 3-month study and for one hour in all other studies. A single post-dosing blood sample was obtained "as soon as possible" (6-20 minutes) after inhalation. Salmeterol was measured by —— fluticasone by —— Particle sizes were —— —— The sponsor's dose calculations assume deposition of all particles —— and estimate respiratory minute volume based on body weight. This should be considered a "total deposition"; pulmonary deposition is probably ~7-10% and upper respiratory deposition ~3-4% There were no gender related differences, values in table 1 are for combined males and females. Plasma concentrations increased in proportion to dose for both drugs. Plasma concentrations for each drug were independent of the dose of the other drug, indicating a lack of interaction between the drugs. With repeated administration there was little drug accumulation, ~2-fold at most; after 2 weeks plasma concentrations declined slightly with continued administration.

Table 1. Systemic exposure in rats after inhalation of salmeterol and fluticasone.

Study & Report No.	Exposure (μg/L air)		Dose (μg/kg)		Day	Cp (ng/mL)*	
	Salmeterol	Fluticasone	Salmeterol	Fluticasone		Salmeterol	Fluticasone
Acute inhalation toxicity WPT/93/397 GDM/94/019	1	2	28	73	1	5.9	>2.8
	2	4	64	166		7.7	>4.7
	5	10	36	92		6.3	11.4
	10	20	556	1083		9.3	19.5
	20	40	2815	5441		16.3	24
Multi-dose Pilot WPT/92/318 GDM/94/020	20	0	810	0	14	7.6	—
	20	0.02	750	1		10.6	<0.025
	20	0.2	770	8		10	0.11
	20	2	910	77		14.5	1.05
	0	2	0	60		—	1.72
Multi-dose Pilot WPT/92/182 GDM/92/052	2	0.2	69	7	1	3.2	<0.25
					15	2.3	0.31
	20	2	902	71	1	10.1	1.13
					15	14	1.40
Preliminary 5-week inhalation toxicity WPT/93/175 GDM/94/011	2	4	95	180	1	2.8	6.7
					14	3.4	7.4
					35	2.8	6.2
	2	10	87	400	1	2.4	<10
					14	4.5	17.4
					35	3.0	14.7
	4	20	170	750	1	3.4	17.8
					14	11.7	37.6
					35	7.6	32.7
	10	20	410	770	1	7.4	11
					14	>18.4	23
					35	15.2	21.6
3-Month Inhalation Toxicity WPT/95/011	3	0	36	0	1	1.28	<0.13
					35	2.66	0.92
					90	1.69	0.82
	0	6	0	71	1	<0.5	1.14
					35	<0.5	3.89
					90	<0.5	1.86
	0.6	6	7.8	72	1	<0.5	1.43
					35	0.6	2.36
					90	0.5-	2.37
	3	6	37	70	1	1.37	1.36
					35	2.35	2.56
					90	2.55	2.8

* Cp = plasma concentration measured as soon as possible after inhalation exposure.
Dose assumes "total deposition" of all particles

Dog Inhalation Studies. Toxicokinetic results from 1 single dose and 3 multiple dose studies are summarized in table 2, page 7. Plasma concentration-time curves are shown in figure 1, below. Salmeterol and fluticasone were co-administered by oral inhalation (oral-pharyngeal tube) of dry powder with lactose. Inhalation exposure was six minutes. Blood samples were obtained at multiple intervals from 2 minutes through 6 hours, and at 24 hours, after dosing. This allowed determination of C_{max}, but AUCs could not be measured reliably because many values were below the limit of quantification after 6 hours. Fluticasone was sporadically detected in plasma of animals not administered the drug. It is not clear whether this is due to cross reaction of ——— with endogenous steroids, contamination during sample processing, or a flaw in the analytical method. Particle sizes ranged from ——— Doses were calculated as described above for rats. No good deposition data are available for oral inhalation in dogs; pulmonary deposition might differ significantly with different devices and techniques of administration. There were no gender related differences, values in table 2 are for combined males and females. The shape of the concentration-time curve for inhaled salmeterol resembled intravenous administration. C_{max} occurred at the earliest measured time point, followed by rapid redistribution and slower elimination. Absorption of fluticasone was slower than for salmeterol; T_{max} occurred 5-40 minutes after the end of inhalation. Using data points from 2-6 hours, individual plasma $t_{1/2}$ values ranged from ——— hr for salmeterol and ——— hr for fluticasone. For fluticasone, plasma half-life appeared to increase with increasing dose. This would be consistent with slow continued absorption from the lung. Plasma concentrations increased in proportion to dose for salmeterol; for fluticasone the increase in plasma concentration was less than proportional to dose. Plasma concentrations for each drug were independent of the dose of the other drug, indicating a lack of interaction between the drugs. With repeated administration there was no accumulation.

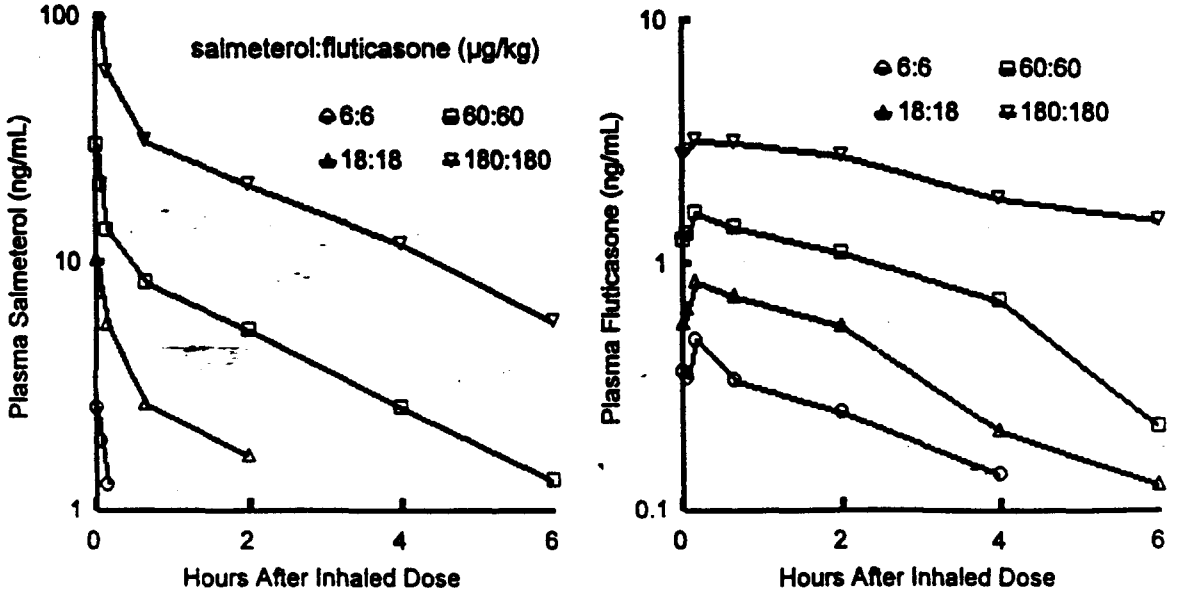


Figure 1. Plasma concentrations after salmeterol + fluticasone inhalation in dogs. Data are from pilot absorption study (report GDM/93/058).

Table 2. Systemic exposure in dogs after inhalation of salmeterol and fluticasone.

Study & Report No.	Exposure (μg/L air)		Dose (μg/kg)		Day	C_{max} (ng/mL)	
	Salmeterol	Fluticasone	Salmeterol	Fluticasone		Salmeterol	Fluticasone
Pilot Absorption Study GDM/93/058	5	5	6.4	6.6	1	2.6	0.49
	15	15	18.7	18.7		10.2	0.84
	50	50	61.5	60.6		29.9	1.65
	150	150	184	179		116	3.69
Preliminary 14-Day Tox. WPT/92/178 GDM/92/051	15	15	17.5	16.4	1	9.6	1.62
					14	7.8	2.55
	150	150	178	171	1	78	7.6
					14	60	7.8
Pilot 14-Day Toxicity WPT/93/089 GDM/94/158	5	0	4.5	0	1	3.1	0.32
					14	2.5	0.09
	15	0	16.6	0	1	17.4	0.29
					14	10.7	0.13
	5	10	5.4	9.1	1	3.8	1.18
					14	3.2	0.97
	5	25	5.8	21.9	1	3.4	2.21
					14	2.9	1.66
	15	30	15.0	23.9	1	11.0	1.94
					14	8.2	2.07
	15	75	15.9	61.0	1	12.2	4.32
					14	6.4	3.88
3-Month Inhalation Toxicity WPT/95/233	15	0	14.6	0	1	5.3	
					35	2.7	<LQ
					90	7.7	
	0	30	0	30.2	1		0.57
					35	<LQ	0.99
					90		0.84
	3	30	3.2	30.9	1	1.6	1.57
					35	1.9	1.11
					90	1.4	1.44
	15	30	15.9	30.3	1	16.0	1.27
					35	6.1	1.07
					90	5.7	1.25

LQ = limit of quantitation. Dose assumes "total deposition" of all particles ——

Oral (Salmeterol) & Subcutaneous (Fluticasone) Administration in Pregnant Rodents

In mouse and rat organogenesis studies, salmeterol was administered by oral gavage and fluticasone by subcutaneous injection. Single post-dose plasma samples were obtained for quantitation of drug levels. Results are summarized in table 3, page 8-9.

Comparisons between species are precluded by differences in sampling times (0.25 – 0.5 hr in mouse, 1 hr in rat). For salmeterol, measurable plasma concentrations were only achieved at the high dose of 10 mg/kg in both rat and mouse. There was appreciable accumulation of drug with repeated administration at this dose. For fluticasone, concentrations increased with increasing dose but the increases were less than proportional to dose. There was only slight accumulation with multiple doses in mid- and high-dose groups.

Placental transfer was measured in 12- or 18-day pregnant rats, using single doses of radiolabeled ^{14}C–salmeterol and ^{3}H–fluticasone. Data are presented in table 3, page 9, concentrations are expressed as gram equivalents for total radioactivity. For fluticasone, maternal plasma radioactivity concentrations at 100 μg/kg, s.c., were about twice the concentration predicted from measurements of fluticasone by —— the mouse organogenesis study. This suggests that about half the fluticasone-related radioactivity was parent drug. Fluticasone-related radioactivity appeared quickly in the fetus at total concentrations eventually exceeding those in maternal plasma. For salmeterol, at 10 mg/kg, maternal plasma radioactivity concentrations were more than 100-fold higher than the concentration of parent salmeterol measured by —— the mouse organogenesis study. This suggests that most of the salmeterol-related radioactivity consisted of salmeterol metabolites. From 0.33 to 2 hr after dosing only low salmeterol-related radioactivity concentrations were observed in the fetus, 0.7% to 10% of maternal plasma concentrations. Low transfer of radioactivity across the placenta might be expected if most of the radioactivity is relatively polar salmeterol metabolites.

Table 3. Plasma drug concentrations in rodent reproduction studies.

Study & Report No.	Salmeterol p.o. dose (mg/kg)	Fluticasone s.c. dose (μg/kg)	Pregnancy Day	Salmeterol		Fluticasone	
				Hr. post-dose	Conc. (ng/mL)	Hr. post-dose	Conc. (ng/mL)
Mouse Organogenesis Study WPT/92/179 GDM/93/045	0	40	6 15	---	---	0.5 0.5	3.07 3.29
	1.4	0	6 15	0.25 0.25	<LQ <LQ	---	---
	0.2	10	6 15	0.25 0.25	<LQ <LQ	0.5 0.5	1.46 1.24
	1.4	40	6 15	0.25 0.25	<LQ <LQ	0.5 0.5	3.00 5.02
	10	150	6 15	0.25 0.25	6.8 15.1	0.5 0.5	4.95 8.59

Table 3. Plasma drug concentrations in rodent reproduction studies.

Study & Report No.	Salmeterol p.o. dose (mg/kg)	Fluticasone s.c. dose (μg/kg)	Pregnancy Day	Salmeterol		Fluticasone	
				Hr. post-dose	Conc. (ng/mL)	Hr. post-dose	Conc. (ng/mL)
Rat Organogenesis Study WPT/92/371	1	0	7 16	1.0 1.0	<LQ <LQ	---	---
	0	30	7 16	---	---	1	0.29 0.36
	0.1	10	7 16	1.0 1.0	<LQ <LQ	1	0.22 0.14
	1	30	7 16	1.0 1.0	<LQ 1.3	1	0.30 0.35
	10	100	7 16	1.0 1.0	6.6 26.7	1	0.28 0.52
Mouse Placental Transfer Study* GDM/92/040	10	100			Dam	Fetus	Dam	Fetus
			12	0.33	970	100	0.5	8.4	10.5
			18	0.33 2.0 24	1420 1910 90	10 30 120	0.5 2.0 24	7.1 3.8 1.0	4.9 10.3 2.8

*Concentration is total radioactivity, ng/mL plasma for dams, ng/g total for fetus.
LQ = limit of quantitation.

TOXICOLOGY

SINGLE DOSE STUDIES

Rat Acute Inhalation Toxicity (Study-1)
Glaxo-Wellcome Report WPT/93/395, vol 3, pg 78

This study was reviewed previously (NDA 20-549); results are summarized below:

Drug was administered by nose-only inhalation of dry powder with lactose vehicle. Estimated doses are shown in table 4, page 10. For the high dose group and their concurrent controls 5/sex/group were examined on day 3 and 5/sex/group were examined on day 15. For the low- and mid-dose groups and their concurrent controls 5/sex/group were examined on day 3. There were no deaths. Body weight was decreased in all

treated groups. Histopathology findings were typical of β_2-adrenergic agonists and glucocorticoids. Changes due to fluticasone were lymphoid depletion in thymus and spleen and glycogen vacuolation in the liver. Changes attributed to salmeterol were atrial myocarditis in all treated groups and focal ventricular myocardial degeneration; in 3/5 high-dose males. Local irritant effects were attributed to salmeterol. These included squamous metaplasia, keratinization, and inflammatory cell infiltrate in the larynx, and localized squamous metaplasia and epithelial degeneration in the nasal cavity. In animals killed on day 15 most of the findings were absent or reduced in severity, suggesting that the changes were reversible. For the high dose, no atrial myocarditis was observed on day 15 but atrial fibrosis was seen in one male. The atrial myocarditis seen in this study was not observed with either drug alone in previous studies using different strains of rats. The atrial myocardial lesions were similar, however to known effects of β_2-adrenergic agonists, including salmeterol, on ventricular myocardium. Another acute inhalation study, described below was done to clarify this observation.

Table 4. Myocarditis in first rat inhalation study.

Salmeterol mg/kg	0	3.6	0	0.25	0.49
Fluticasone mg/kg	0	1.9	0	0.46	0.91
Atrial myocarditis	0/10	10/10	0/10	5/10	5/10

Rat Acute Inhalation Toxicity (Study-2, for Atrial Myocarditis)
Glaxo Wellcome Report WPT/93/397, vol 3, pg 218

This study was reviewed previously (NDA 20-549); results are summarized below:

The study was done to characterize the atrial myocarditis observed in the previous rat acute inhalation study. Drug was administered by nose-only inhalation of dry powder with lactose vehicle. Six groups received combined salmeterol:fluticasone at the estimated doses shown in table 5; due to variations in aerosol concentration dose for group 4 was less than expected. These limited observations were made: body weight, electrolytes, serum enzymes (LDH, AST, CPK), creatine, and heart histology (day 3). Body weight gain was decreased in the higher dose groups, with females being more sensitive. Plasma potassium and inorganic phosphate were decreased in several of the treated groups, without a clear dose-response relationship. There were no increases in the serum activity of myocardial enzymes. This study shows that atrial myocarditis was dose related, but it is not known whether it is due to salmeterol alone, and a NOAEL for this effect was not established.

Table 5. Myocarditis in second rat inhalation study.

Group	1	2	3	4	5	6
Salmeterol µg/kg	0	2815	556	36	64	28
Fluticasone µg/kg	0	5441	1083	92	166	73
Atrial myocarditis	0/10	6/10	5/10	1/10	4/10	4/10

MULTIPLE DOSE STUDIES

Rat Preliminary, 15-day Inhalation Toxicity Study
Glaxo Wellcome Report WPT/92/182, vol 5, pg 031

Study Dates: Animal dosing from 09 MAR 92 to 23 APR 92
Testing Lab: ———
Test Articles: Salmeterol xinafoate (GR33343G)
Fluticasone propionate (CCI18781)
Lactose vehicle BP/NF, batch SN 9164
0.8:0.08 % salmeterol:fluticasone in lactose, batch U92/300A
8.0:0.8 % salmeterol:fluticasone in lactose, batch U92/301A
GLP: The study was accompanied by a signed GLP statement.

Methods: Three groups of Wistar rats were assigned to the following treatments:

	vehicle	low dose	high dose
Number/sex main study	5	5	5
Number/sex satellite	1	2	2
Estimated salmeterol dose* (μg/kg)	0	69	902
Estimated fluticasone dose* (μg/kg)	0	7.2	71
Salmeterol particle size (μm)	—	—	—
Fluticasone particle size (μm)	—	—	—

* Assumes a total deposition for all particles

Drug was given by nose-only inhalation during 1-hour daily exposures. Samples for plasma drug levels were obtained (at 7-36 minutes after exposure) from satellite animals on day-1 and main study animals on day 15. The following observations were made:

Mortality . . . . . twice daily
Clinical observation recorded daily as noticed, careful examination weekly
Body weight . . . . . twice prestudy, study days 1, 4, 8, 11, and 15
Food intake . . . . . weekly
Clinical chemistry . . day 13
Hematology . . . . . day 13
Urinalysis . . . . . overnight collection day 13
Drug levels . . . . . single post-dosing sample days 1 and 15
Necropsy . . . . . terminal
Histopathology . . . . gross lesions + presumed target organs in all groups

Results: (summarized in table 6, pg 13; bold shaded values are statistically significant)

Mortality: None

Clinical Observations: No toxicologically significant treatment-related effects.

Body Weight: Body weight gain was decreased at the high dose. There is no explanation for the slight weight loss in control and low-dose females.

Food Intake: No toxicologically significant treatment-related effects.

Hematology: There was a small increase in hematocrit at the high dose and in low-dose males. Eosinophils were low in all treated groups; lymphocytes were not decreased.

Clinical Chemistry: Plasma glucose was decreased, particularly in females. Slight increases occurred in plasma globulins, ALT, Na^+, Cl^-, and cholesterol. Those increases may have been secondary to mild hemoconcentration rather than specific effects.

Urinalysis: Urine volume was increased about 2-fold with a slight decrease in specific gravity. This diuresis may have contributed to the mild hemoconcentration.

Organ Weights: Thymus weight was decreased at the high dose and in low-dose females.

Gross Pathology: Small thymus was noted in high-dose males and females. Fluid distention of the uterus was seen at the low and high dose.

Histopathology: Changes suggestive of local irritation were observed in the nasal turbinates at the high dose and in the larynx at low and high doses. Intramural coronary arteritis was observed at the high dose; arterial mural disorganization was noted in intramural coronary arteries and in arteries lying in close association with the larger bronchioles. Ventricular myocarditis was somewhat more frequent in treated than in control rats. Atrial myocarditis seen in single dose studies was not observed here. Atrophy of thymus was dose related; adrenal atrophy was not observed. Renal tubular basophilia and dilation of the renal pelvis were more common in treated rats but the incidence was not clearly dose-related. Distention of the uterus seen on gross examination was confirmed microscopically (this occurs during normal estrus).

Toxicokinetics: Plasma levels from single samples obtained soon after inhalation exposure (7-36 minutes) are summarized in table 7, page 14. Plasma concentration increased with increasing dose. There was no effect of gender and no significant accumulation from day 1 to day 15.

~~Table 6. Rat preliminary 15-day inhalation study: Treatment-related findings.~~

	males			females		
Salmeterol (μg/kg)	0	69	902	0	69	902
Fluticasone (μg/kg)	0	7.2	71	0	7.2	71
Body weight gain (g)	11	17	4	-6	-4	-16
Clinical Pathology:						
Hematocrit (%)	51	54	54	50	52	55
Eosinophils ($10^3/mm^3$)	0.13	0.05	0.02	0.11	0.03	0.04
Plasma glucose (mg/dL)	100	94	86	93	78	74
Plasma globulins (mg/dL)	2.9	3.4	3.4	3.2	3.6	3.4
Plasma ALT (mU/mL)	25	26	31	21	24	34
Plasma Na^+ (mEq/L)	139	143	140	140	141	142
Plasma Cl^- (mEq/L)	97	100	98	100	101	101
Plasma cholesterol (mg/dL)	56	77	84	73	70	86
Urine volume (mL)	3.1	8.0	7.3	2.2	4.4	3.6
Urine specific gravity	1039	1027	1027	1050	1031	1038
Gross Pathology:						
Abs. thymus weight (mg)	259	250	129	261	220	104
Small thymus	0	0	3/5	0	0	3/5
Fluid distention of uterus				0	2/5	3/5
Histopathology:						
Nasal turbinates						
parakeratosis, squamous epithelium	0	0	4/5	0	0	4/5
epithelial erosion	0	0	2/5	0	0	2/5
epithelial hyperplasia c̄ goblet cell loss	0	0	1/5	0	0	4/5
Larynx						
ventral epithelial necrosis	0	3/5	2/5	0	1/5	4/5
ventral epithelial hyperplasia	0	2/5	5/5	0	4/5	3/5
necrosis of ventral cartilage	0	0	4/5	0	1/5	4/5
Lung: arterial mural disorganization	0	0	2/5	0	0	0
Coronary arteritis	0	0	1/5	0	0	2/5
Coronary arterial mural disorganization	0	1/5	2/5	0	0	0
Ventricular myocarditis	0	2/5	1/5	1/5	0	1/5
Thymus atrophy	1/5	1/5	3/5	0	1/5	4/5
Renal tubular basophilia	0	0	2/5	0	1/5	0
Dilation of renal pelvis	0	0	2/5	0	1/5	0
Uterine luminal dilation				0	2/5	3/5

Table 7. Rat preliminary 15-day inhalation study toxicokinetics.

		Males		Females	
Salmeterol dose (μg/kg)		69	902	69	902
Fluticasone dose (μg/kg)		7.2	71	7.2	71
Salmeterol plasma concentration (ng/mL)	day 1	4.8	10.5	1.5	9.6
	day 15	2.4	14.0	2.2	14.0
Fluticasone plasma concentration (ng/mL)	day 1	<0.25	1.11	<0.25	1.14
	day 15	0.31	1.22	0.34	1.58

Rat 14-day Inhalation Toxicity (Sprague-Dawley *vs* Wistar)
Glaxo Wellcome Report WPT/92/318, 14 NOV 94, vol 5, pg 182

Study Dates: Animal dosing from 08 JUL 92 to 24 JUL 92
Testing Lab: ———
Test Articles: Lactose vehicle BP/NF, batch WJ 6437

Salmeterol xinafoate	Fluticasone propionate	Batch number
0% w/w	0.8% w/w	U92/347A
8% w/w	0% w/w	U92/346A
8% w/w	0.008% w/w	U92/350A
8% w/w	0.08% w/w	U92/349A
8% w/w	0.8% w/w	U92/348A

GLP: The study was accompanied by a signed GLP statement.

Methods: Six groups of rats were assigned to the following treatments:

Group #	1	2	3	4	5	6
Nº Wistar rats/sex	5	5	5	5	5	5
Nº Sprague Dawley rats/sex	5	5	5	5	5	5
Estimated salmeterol dose* (mg/kg)	0	0	0.81	.75	.77	.91
Estimated fluticasone dose* (μg/kg)	0	60	0	.95	8	77
Salmeterol particle size (μm)	———					
Fluticasone particle size (μm)	———					

* Assumes a total deposition for all particles ———; NM = not measurable.

Half of the rats in each group were Wistar strain and half were Sprague Dawley. Drug was given by nose-only inhalation during 1-hour daily exposures. Samples for plasma drug levels were obtained (at 6-22 minutes after exposure) in 3/sex/group on day 14. In this pilot study only a few clinical chemistry parameters were measured and a limited list of potential target organs was subject to gross and microscopic evaluation.

~~The following observations were made:~~

Mortality	twice daily
Clinical signs	recorded daily as noticed, careful examination weekly
Body weight	twice prestudy, study days 1, 4, 8, 11, and 14
Food intake	weekly
Electrocardiogram	within 20 minutes of dosing days 1 and 13
Clinical chemistry	day 13 (AST, CPK, and corticosterone only)
Drug levels	single post-dosing sample day 14
Necropsy	terminal
Histopathology	gross lesions + presumed target organs in all groups

Results: (Summarized in table 8, pg 16; bold shaded values are statistically significant, blank cells indicate zero incidence for enumerative data.

Mortality: None
Clinical Signs: No toxicologically significant treatment-related effects.

Body Weight: In males of both strains body weight gain was decreased in the two high-dose fluticasone groups (2 and 6) with and without salmeterol. In females net body weight loss occurred in group 2, high-dose fluticasone alone. Females in groups 3,4,and 5, (salmeterol alone or with low or mid-dose fluticasone) had increased body weight gain relative to controls. In group six (salmeterol plus high-dose fluticasone) the two effects canceled each other so that body weight gain was just slightly less than in controls.

Food Intake: Food intake for high-dose fluticasone was slightly less than in controls (9 to 18%↓); statistical significance could not be determined because there was only one cage per group.

Heart Rate: Contrary to expectation, heart rate, on day-1, was slightly decreased in groups receiving salmeterol (8%↓ overall). This might be a compensatory response to increased heart rate during the 1-hour inhalation period. Fluticasone dose did not influence this response. On day-13 the effect was still present but much smaller.

Clinical Chemistry: In Sprague Dawley rats only, corticosterone was decreased in groups that received salmeterol. There is no obvious explanation for this effect.

Organ Weights: Thymus weight was decreased in high-dose fluticasone groups; in Wistar females thymus weight was also decreased at the lower doses of fluticasone. Relative heart weight was increased in groups receiving salmeterol; an additive effect of fluticasone was probably due to decreased body weight rather than further cardiac hypertrophy. Adrenal weight was occasionally decreased in high-dose fluticasone groups.

Gross Pathology: Small thymus was observed in several high-dose fluticasone groups.

Table 8. Rat pilot 14-day inhalation study: Treatment-related findings.

		Wistar						Sprague Dawley					
Salmeterol (mg/kg)		0	0	.8	.8	.8	.9	0	0	.8	.8	.8	.9
Fluticasone (μg/kg)		0	60	0	1	8	77	0	60	0	1	8	77
Body weight gain (grams)	♂	35	10	41	42	28	20	54	13	40	54	55	28
	♀	12	-12	28	25	14	8	10	-8	28	24	27	5
Heart rate, day 1 (beats/min)	♂	554	546	517	520	522	502	545	503	517	462	504	517
	♀	549	535	503	498	507	516	561	547	495	511	501	504
Corticosterone (ng/mL)	♂	48	47	47	49	44	49	39	35	30	30	30	28
	♀	67	70	71	68	71	53	76	64	56	55	56	52
Thymus weight (absolute grams)	♂	.36	.22	.32	.32	.24	.22	.36	.13	.28	.30	.33	.16
	♀	.36	.09	.30	.27	.24	.12	.26	.08	.25	.27	.28	.11
Heart weight (relative grams)	♂	.88	.92	1.01	1.04	1.04	1.07	1.03	1.07	1.08	1.01	1.07	1.16
	♀	.67	.71	.72	.72	.73	.74	.69	.76	.79	.72	.73	.82
Adrenal weight (absolute mg)	♂	54	49	54	55	48	46	53	43	48	49	48	47
	♀	76	58	68	71	70	60	51	47	58	57	56	54
Small thymus	♂								2				1
	♀		3				1		3				1
Nasal parakeratosis of squamous epithelium	♂			1	3	2	3			2		2	3
	♀			2	1	2	3			1	2		1
Nasal hyperplasia of respiratory epithelium	♂			4	3	4	4			4	4	5	2
	♀			5	3	4	4			2	2	2	5
Nasal squamous metaplasia	♂			1									1
	♀												
Larynx squamous epithelial hyperplasia	♂			3	4	3	3			3	3	3	1
	♀			3	2	1	4			2	2	3	2
Larynx squamous metaplasia	♂			5	3	2	2			1	4	2	1
	♀			5	3	2	3			3	2	4	2
Larynx parakeratosis	♂			2	2	2	3			1		3	1
	♀			2	2	3	3			1	1	2	2
Thymus atrophy	♂		2				2		5				3
	♀								5				5
Coronary artery mural disorganization	♂				1		1			2			
	♀	1		2		1	3						
Intramural coronary arteritis.	♂												
	♀						1						
Lung arterial mural disorganization	♂					1						1	
	♀	1		1	2	2	3			1			1
Myocarditis	♂		1		2		1	1	1	1	1	1	2
	♀					1							

Histopathology: Numerous signs of local irritation were seen in nasal passages and larynx of salmeterol-treated groups. Frequent findings are indicated in table 8. Additional findings in nasal passages were: erosion of respiratory epithelium, ulceration of squamous epithelium, low basophilic epithelium, and rosette formation in olfactory epithelium. Additional findings in larynx were: hyperplasia, ulceration, and necrosis of epithelium, and necrosis or regeneration of cartilage. Atrophy of thymus was seen in high-dose fluticasone groups; adrenal atrophy was not seen. Arterial mural disorganization of coronary arteries in right ventricle or apex was observed in several salmeterol-treated groups and in one lactose control rat. This was characterized by enlarged nuclei in the media, with mural basophilia and increased cellularity of the adventitia. Similar findings occurred in arteries lying near larger bronchioles. These findings were more common in Wistar females. Coronary arteritis was seen in one group-6 Wistar female.

Toxicokinetics: Plasma concentrations were measured only on day-14 (Table 9). There was no effect of gender or strain on salmeterol concentration. Salmeterol concentrations were somewhat higher in the presence of increasing fluticasone but, even if real, the differences are unlikely to be biologically significant. Fluticasone concentrations increased with increasing dose, with no effect of gender or strain. Fluticasone concentrations were slightly decreased in the presence of salmeterol but, again, the magnitude is of doubtful biological significance.

Table 9. Rat pilot 14-day inhalation study: Toxicokinetics day-14.

		Wistar					Sprague Dawley				
Salmeterol (mg/kg)		0	.8	.8	.8	.9	0	.8	.8	.8	.9
Fluticasone (μg/kg)		60	0	.95	8	77	60	0	.95	8	77
Salmeterol concentration (ng/mL)	♂	---	<8.0	10.6	8.2	18.2	---	<8.0	9.7	11.4	13.9
	♀	---	10.7	9.2	11.2	12.6	---	9.2	13.1	9.5	13.5
Fluticasone concentration (pg/mL)	♂	1334	---	<50	<50	920	1664	---	<25	130	1118
	♀	2217	---	<25	124	1089	1672	---	<25	119	1089

Rat Pilot, 35-day Inhalation Toxicity Study
Glaxo Wellcome Report WPT/93/175, vol 6, pg 196

Study Dates: Animal dosing from 09 FEB 93 to 30 MAR 93
Testing Lab:
Test Articles: Lactose vehicle BP/NF, batch WJ 8088

Salmeterol xinafoate	Fluticasone propionate	Batch number
2% w/w	4% w/w	U92/364A
2% w/w	10% w/w	U92/366A

GLP: The study was accompanied by a signed GLP statement.

Methods: Five groups of Wistar rats were assigned to the following treatments:

Group #	1	2	3	4	5
Nº rats/sex autopsied after 2-wk Rx	5	5	5	5	5
Nº rats/sex autopsied after 5-wk Rx	10	10	10	10	10
Nº rats/sex 5-wk Rx + 2-wk recovery	5	---	---	5	5
Nº rats/sex satellite for plasma levels	6	6	6	6	6
Estimated salmeterol dose* (mg/kg)	0	0.1	0.1	0.2	0.4
Estimated fluticasone dose* (mg/kg)	0	0.2	0.4	0.8	0.8
Salmeterol particle size					
Fluticasone particle size					

* Assumes a total deposition for all particles

Drug administration was by nose-only inhalation for 1 hour daily exposure. Subgroups were autopsied after 2-weeks and 5-weeks of treatment, and after 5-weeks treatment plus 2-weeks drug-free recovery. Two satellite rats/sex were used on day-1, week-2, and week-5 for drug levels; single plasma samples were obtained 6 - 27 minutes after exposure. The following observations were made:

Mortality	twice daily
Clinical observation	recorded daily as noticed, careful examination weekly
Body weight	once prestudy, weekly thereafter
Food intake	weekly
Electrocardiogram . .	recovery groups only, post-dosing on day-1, wk-2, wk-5, recovery
Clinical chemistry . .	week-2, week-5, and 2-weeks recovery
Hematology	week-2, week-5, and 2-weeks recovery
Urinalysis	overnight collection week-2, week-5, and 2-weeks recovery
Drug levels	single post-dosing sample day-1, week-2, and week-5
Necropsy	2-weeks, 5-weeks, and recovery
Histopathology	gross lesions + presumed target organs in all groups

Results:

Mortality: One female vehicle control rat died in week 4; cause of death is unknown.

Clinical Observations: Hair loss, a known corticosteroid effect, was observed during weeks 4 and 5 in 5 females and 1 male of group-5 and in one group-2 female. Most group-4 and group-5 rats of both sexes had hair loss during the recovery period. Poor grooming was noted in most animals in groups 3, 4, and 5 during weeks 3 and 4.

Body Weight: Most treated groups lost body weight during treatment.
Food Intake: Food intake was decreased in high dose animals.
Electrocardiography: In week 5, heart rate increased in females of groups 3 and 4.
Hematology: Hematocrit, hemoglobin, and red cell number were increased in weeks 2

and 5. Mean corpuscular volume and mean corpuscular hemoglobin concentration were decreased. In weeks 2 and 5 lymphocyte count decreased and neutrophil count increased in most treated groups; eosinophil count decreased in several treated groups.

Clinical Chemistry: In weeks 2 and 5, total protein, globins and albumin increased in various treated groups; particularly in males; total protein is shown for illustration in table 10. In weeks 2 and 5 glucose was increased in various groups; the effect was not as great in week 5. ALT and AST values were increased in various treated groups in weeks 2 and 5. K^+, and PO_4^- were increased in weeks 2 and 5; in week 5 Cl^- was decreased. Cholesterol was increased in weeks 2 and 5. Corticosterone decreased in treated groups. Most of these effects exhibited only weak dose dependency.

Urinalysis: Urine K^+ and PO_4^- decreased in treated groups.

Table 10. Rat pilot 5-week inhalation study: Treatment-related *in vivo* findings at week 5.

	Male					Female				
Salmeterol (mg/kg)	0	0.1	0.1	0.2	0.4	0	0.1	0.1	0.2	0.4
Fluticasone (mg/kg)	0	0.2	0.4	0.8	0.8	0	0.2	0.4	0.8	0.8
Body weight gain (g)	90	**0**	**-23**	**-38**	**-23**	37	**-7**	**-24**	**-33**	**-26**
Food intake (% control)	---	**90**	**91**	**92**	**89**	---	99	96	96	**93**
Heart rate (beats/min)	546	568	583	560	570	555	585	**586**	**592**	567
Hematocrit (%)	54	**60**	**61**	**61**	**61**	54	**61**	**61**	**58**	**60**
Lymphocytes ($10^3/mm^3$)	6.4	**3.7**	**1.7**	**1.8**	**1.7**	4.9	**1.9**	**1.4**	**1.1**	**1.3**
Neutrophils ($10^3/mm^3$)	2.2	**3.2**	2.9	**4.9**	**3.5**	1.4	**2.1**	**2.2**	**3.4**	**3.0**
Fibrinogen (mg/dL)	207	**254**	**267**	**263**	**264**	179	**230**	**228**	**214**	**231**
Total protein (g/dL)	6.4	**7.1**	**7.1**	**7.4**	**7.1**	6.4	6.4	6.5	6.5	6.6
Glucose (mg/dL)	88	87	91	**130**	109	87	94	**120**	**140**	109
ALT (U/L)	26	**37**	33	**45**	**39**	23	32	35	**48**	**36**
AST (U/L)	52	66	64	65	67	50	57	55	**64**	**68**
Plasma K^+ (mEq/L)	3.2	**3.9**	**3.9**	**4.2**	**4.0**	2.9	**3.9**	**3.8**	**4.0**	**3.9**
Plasma PO_4 (mEq/L)	4.1	**4.6**	**4.5**	**4.8**	**5.1**	3.4	**4.2**	**4.3**	**4.4**	**4.4**
Plasma Cl^- (mEq/L)	97	**95**	**95**	**93**	**95**	99	99	98	**96**	**97**
Cholesterol (mg/dL)	71	85	**101**	**150**	**106**	71	81	84	**103**	89
Corticosterone (μg/dL)	85	**45**	**34**	**36**	**33**	129	58	**40**	**33**	**45**
Urine K^+ (mEq/vol)	0.97	**0.63**	**0.60**	**0.57**	**0.49**	0.49	0.44	0.44	0.37	0.37
Urine PO_4 (mEq/vol)	0.64	**0.23**	**0.13**	**0.18**	**0.11**	0.45	**0.19**	**0.22**	**0.14**	**0.16**

Bold shaded values are statistically significant.

Organ Weights: Organ weight changes were mostly predictable and present at both weeks 2 and 5 (table 11, below). Decreases were observed in relative and absolute thymus, spleen and adrenal weights. Increases were observed in relative weights of various organs, including: heart, liver, lung, and kidney. None of these weighs were increased in relation to brain weight and may be artifacts of decreased body weight.

Gross Pathology: Abnormalities (table 11, below) included: alopecia, small thymus, small adrenals, small spleen, pale subpleural foci, decreased adipose tissue (males only), depressions or white raised areas in the forestomach, and fluid distension of the uterus.

Table 11. Rat pilot 5-week inhalation study: Post-mortem findings.

	Male					Female				
Salmeterol (mg/kg)	0	0.1	0.1	0.2	0.4	0	0.1	0.1	0.2	0.4
Fluticasone (mg/kg)	0	0.2	0.4	0.8	0.8	0	0.2	0.4	0.8	0.8
Adrenal weight (mg)	32	**18**	**11**	**9**	**11**	40	**17**	**11**	**9**	**10**
Thymus weight (mg)	338	**39**	**28**	**22**	**28**	293	**39**	**20**	**19**	**21**
Spleen weight (g)	0.90	**0.57**	**0.50**	**0.46**	**0.50**	0.64	**0.45**	**0.37**	**0.34**	**0.34**
Macroscopic exams total	10	10	10	10	10	10	10	10	10	10
Skin: alopecia		1	5	1	2		3	3	6	6
Thymus: small		10	10	10	10		10	10	10	10
Lungs: pale foci		5	5	6	6		5	6	5	8
Adipose tissue: decreased		2	4	1	5					
Forestomach: depressions		3	3	3			2	1	4	1
Adrenals: small	1	8	10	10	10		10	10	10	10
Uterus: fluid distension						2	6	3	4	3

Bold shaded values are statistically significant. Blanks indicate zero incidence.

Histopathology: Known target organs and gross lesions were examined. Findings are summarized in table 12, pg. 21. Airway irritation was common in treated groups, particularly groups 4 and 5. In the nasal passages there was: squamous metaplasia, hyperplasia of respiratory epithelium, and erosion or atrophy of olfactory epithelium. In larynx there was: hyperplasia and metaplasia of squamous epithelium, necrosis of cartilage, and epithelial hyperplasia. There was epithelial hyperplasia and presence of non-ciliated cells at the tracheal bifurcation. These findings increased in incidence from 2 to 5 weeks. Aggregated alveolar macrophages were more common in treated rats. Myocardial fibrosis was seen in one group-4 male at 2 weeks and in another at 5 weeks. Coronary arteritis was noted in one group-5 male at 2 weeks. It is not certain whether a slight increase in the incidence of myocarditis was treatment related. Coronary arterial wall thickening and disorganization occurred in 4 treated animals in various groups.

Most treated animals exhibited atrophy of thymus (severe in all but the low dose group), adrenals (most severe in high dose females) and tracheobronchial lymph node. Periportal hepatocyte vacuolation or rarefaction were seen in liver. Gross stomach lesions were associated with epithelial erosion, ulceration or hyperplasia.

Table 12. Rat pilot 5-week inhalation study: Histopathology at 5-weeks.

	Male					Female				
Salmeterol dose (mg/kg)	0	0.1	0.1	0.2	0.4	0	0.1	0.1	0.2	0.4
Fluticasone dose (mg/kg)	0	0.2	0.4	0.8	0.8	0	0.2	0.4	0.8	0.8
Total number of animals	10	10	10	10	10	10	10	10	10	10
Nasal abnormalities*	1	2	2	4	10	2	1	1	3	7
Larynx abnormalities*	2	5	8	7	10	1	8	7	7	8
Abnormalities tracheal carina*			1		5	1				2
Alveolar macrophage aggregates	3	7	9	10	10	2	9	9	10	9
Myocarditis	2	4	5	3	6	2		3	2	
Coronary arterial disorganization					2		1			1
Thymus atrophy		10	9	9	9	1	9	10	9	10
Adrenal atrophy:		10	10	10	10		10	10	10	10
Tracheobronchial L.N. atrophy		9	8	9	8		9	9	9	9
Periportal hepatocyte vacuolation			2	4	3		3	7	9	9
Periportal hepatocyte rarefaction		5	3	3	6		1			1

* See text for specific lesions. Blank spaces indicate zero incidence.

Toxicokinetics: Results are summarized in table 12 below. Plasma levels of both drugs increased with increasing dose. At the two higher doses plasma levels of both drugs were modestly higher in weeks 2 and 5 than on day 1. Given the range of variability this was not biologically significant. Plasma levels were similar in males and females.

Table 11. Rat pilot 5-week inhalation study: Toxicokinetics

		Male				Female			
Salmeterol dose (μg/kg)		0.1	0.1	0.2	0.4	0.1	0.1	0.2	0.4
Fluticasone dose (μg/kg)		0.2	0.4	0.8	0.8	0.2	0.4	0.8	0.8
Salmeterol plasma concentration (ng/mL)	day 1	3	2	4	7	2	3	3	8
	week 2	3	5	12	>21	4	8	12	15
	week 5	3	3	10	18	4	3	6	12
Fluticasone plasma concentration (ng/mL)	day 1	6	>10	25	8	7	>9	11	14
	week 2	8	18	37	16	7	17	39	30
	week 5	6	<13	38	18	6	14	27	25

Recovery: Most treatment effects at 5 weeks returned toward but not completely back to normal after 2 weeks of withdrawal from treatment. There was a possible "rebound" effect, such that plasma glucose was lower in treated groups vs control after 2 weeks of withdrawal. No histopathology was observed in nasal passages, abnormalities of the larynx were seen only in high-dose groups 4 and 5. No abnormalities were seen in heart or liver. Lymphoid and adrenal atrophy were present but decreased in severity.

Rat 13-week Inhalation Toxicity Study
Glaxo Wellcome Report WPT/95/011, 30 JAN 96, vol 8, pg 001

Study Dates: Animal dosing from 22 FEB 95 to 21 JUN 95
Testing Lab: ——
Test Articles: Lactose vehicle BP/NF, batch WL 9903

Salmeterol xinafoate	Fluticasone propionate	Batch number
5% w/w	---	U95/403A
---	10% w/w	U95/404A
1% w/w	10% w/w	U95/402A
5% w/w	10% w/w	U95/401A

GLP: The study was accompanied by a signed GLP statement.

Methods: Five groups of Wistar rats were assigned to the following treatments:

Group #	1	2	3	4	5
Nº rats/sex main study groups	15	15	15	15	15
Nº rats/sex recovery groups	10	---	---	---	10
Nº rats/sex satellite for plasma levels	16	16	16	16	16
Estimated salmeterol dose* (μg/kg)	0	36	0	8	37
Estimated fluticasone dose* (μg/kg)	0	0	71	72	71
Salmeterol particle size					
Fluticasone particle size					

* Assumes total deposition of all particles

Drug was given by nose-only inhalation during 20 minute daily exposures. Samples for plasma drug levels were obtained 1 hour after exposure in 4/sex/group.

Clinical observation . . twice daily, with careful external exam weekly
Body weight . . . . . prestudy, weekly
Food intake . . . . . weekly
Ophthalmology . . . . prestudy and week 12
Clinical chemistry . . weeks 4 and 12
Hematology . . . . . weeks 4 and 12
Urinalysis . . . . . overnight collections, weeks 4 and 12
Drug levels . . . . . 1 hour post-dosing, day 1 and weeks 4 and 5.

Necropsy . . . . . terminal
Histopathology . . . . comprehensive list of tissues plus gross lesions in all groups

Results:

Mortality: None was considered treatment related. One group 4 female died of apparent asphyxiation in the inhalation restraint tube. Two males and two females from groups 1, 3 and 5 were killed on humane grounds after eye injury during orbital sinus bleeding.

Clinical Observations: Increased hair loss was seen in all fluticasone treated groups.

Body Weight: Body weight gain was decreased in all fluticasone treated groups. (Table 13, pg. 24)

Food Intake: Food intake decreased mildly in all fluticasone treated groups. (Table 13, pg. 24)

Ophthalmoscopy: No toxicologically significant treatment-related effects.

Hematology: There was a modest but consistent increase of hematocrit, red cell count, and hemoglobin concentration in fluticasone treated groups. Lymphocyte count decreased in fluticasone treated groups; this effect was less in the presence of salmeterol, which exerted an opposite effect. Neutrophil count was increased in some fluticasone treated groups, particularly in males. Platelet count decreased with either fluticasone or salmeterol alone; the effect was additive but mild, with a decrease of ~18% for the high dose combination. Fibrinogen increased in fluticasone treated groups; it is not clear whether this was a specific effect or secondary to mild hemoconcentration. (Table 13, pg. 24)

Clinical Chemistry: In fluticasone treated males albumin, globulins, and total plasma proteins all increased. Blood glucose decreased with salmeterol but increased with fluticasone; results with the combination were variable, tending to cancel out at the high dose combination. There were small but consistent increases of ALT and AST in fluticasone treated females. In fluticasone treated groups there were modest increases of plasma K^+ and PO_4 with decreased Cl^-; these may reflect decreased mineralocorticoid activity secondary to decreased ACTH. In fluticasone treated males triglycerides and cholesterol increased and corticosterone decreased. (Table 13, pg. 24)

Urinalysis: In fluticasone treated groups urinary pH increased and K+ decreased. There were several additional small, statistically significant differences from control but these followed no clear patterns and were not biologically significant. (Table 13, pg. 24)

Table 13. Rat 13-week inhalation study: Treatment-related *in vivo* findings at week 13.

	Male					Female				
Salmeterol (μg/kg)	0	36	0	8	37	0	36	0	8	37
Fluticasone (μg/kg)	0	0	71	72	71	0	0	71	72	71
Body weight gain (g)	183	192	**74**	**75**	**86**	67	73	**-4**	**-6**	**5**
Food intake (%Δ)	—	+4	**-7**	**-7**	**-5**	—	+4	**-6**	**-6**	**-4**
Hematocrit (%)	54	53	**58**	**58**	**59**	50	51	**52**	**54**	**55**
Lymphocytes ($10^3/mm^3$)	5.4	6.4	**3.5**	**3.9**	**4.0**	3.3	**4.4**	**1.6**	**2.1**	2.8
Neutrophils ($10^3/mm^3$)	1.9	2.0	**2.6**	**2.7**	**2.7**	1.5	**1.0**	1.85	1.75	**2.3**
Platelets ($10^3/mm^3$)	750	**649**	**656**	**663**	**624**	679	**567**	678	**585**	**547**
Fibrinogen (mg/dL)	263	290	**309**	**324**	**340**	179	191	**222**	**220**	**237**
Total protein (g/dL)	6.7	6.8	**7.4**	**7.4**	**7.6**	7.2	7.1	7.2	7.2	7.1
Glucose (mg/dL)	123	**97**	133	123	122	106	94	**150**	**134**	103
ALT (U/L)	37	35	39	40	42	31	32	**50**	**53**	**48**
AST (U/L)	60	**49**	57	56	62	46	48	**61**	**62**	**67**
Plasma K^+ (mEq/L)	3.4	**3.8**	**3.7**	3.6	**4.0**	3.0	**3.6**	3.2	**3.5**	**3.8**
Plasma PO_4 (mEq/L)	3.9	3.7	4.4	4.5	4.5	3.1	**3.4**	**8.4**	**3.9**	**4.2**
Plasma Cl^- (mEq/L)	99	99	98	**98**	**97**	100	**101**	**97**	**97**	**99**
Triglyceride (mg/dL)	71	77	**99**	**99**	**102**	59	57	76	59	66
Cholesterol (mg/dL)	64	80	78	**87**	**94**	70	78	83	77	76
Corticosterone (μg/dL)	26	24	**16**	22	**21**	38	46	46	33	37
Urine pH	6.4	6.6	**7.0**	**6.8**	**7.1**	6.0	5.8	**6.3**	**6.5**	**6.3**
Urine K^+ (mEq/vol)	1.1	1.3	**0.5**	**0.8**	1.1	0.8	0.7	**0.5**	**0.5**	**0.5**

Bold shaded values statistically significant vs lactose control.

Organ Weights: Absolute weights of thymus, spleen and adrenals were decreased in fluticasone treated groups. Salmeterol by itself increased absolute heart weight in males. Changes from control in relative weights of lung, liver, and kidney appear to be incidental or artifacts of reduced body weight. (Table 14, pg. 25)

Gross Pathology: Findings related to fluticasone treatment included: hair loss, decreased incidence of enlarged cervical lymph nodes, small thymus, decreased body fat, pale subpleural foci in lung, and depressed areas in the forestomach. No gross findings were attributed to salmeterol. (Table 14, pg. 25)

Histopathology: Airway irritation attributed to salmeterol was less severe than in the pilot studies (probably due to the much lower doses used in the present study); common findings were erosion of olfactory epithelium and epithelial hyperplasia of the ventral larynx.

~~Atrophy of nasal associated lymphoid tissue occurred in fluticasone treated groups.~~ Macrophage aggregates around terminal bronchioles were more common in fluticasone treated groups. Seven animals had myocardial fibrosis; it is unexpected that 3 of these were treated with fluticasone alone. Coronary arteritis or mural disorganization noted in the pilot studies were not seen in this study (possibly due to the lower doses). Common findings in fluticasone treated groups included: adrenal atrophy; lymphoid atrophy in thymus, cervical and mesenteric lymph nodes, and spleen; vacuolation of hepatocytes (centrilobular in males, periportal in females); and epithelial hyperplasia of the non-glandular region of the stomach.

Table 14. Rat 13-week inhalation study: Treatment-related post-mortem findings.

	Male					Female				
Salmeterol (μg/kg)	0	36	0	8	37	0	36	0	8	37
Fluticasone (μg/kg)	0	0	71	72	71	0	0	71	72	71
Thymus weight (mg)	268	270	**106**	**112**	**96**	212	**253**	**54**	**59**	**73**
Heart weight (g)	1.4	**1.6**	1.3	1.4	1.4	1.0	1.0	0.9	0.9	0.9
Spleen weight (g)	0.9	1.0	0.8	0.7	0.7	0.7	0.8	0.4	**0.5**	**0.6**
Adrenal weight (g)	64	60	**51**	**54**	**54**	78	80	**51**	**53**	**62**
Number of animals examined	15	15	14	15	14	14	15	14	14	15
Alopecia			11	15	13	1	7	14	14	15
Enlarged cervical lymph node	14	14	2	7	4	14	10	4	1	4
Small thymus	1		8	12	11			14	14	15
Pale foci in lung	3	3	4	7	4	1	3	4	8	4
Minimal adipose tissue		2	10	10	12	0	1	11	13	15
Depression in forestomach			1	5	1			6	1	2
↓ Nasal lymphoid cellularity			11	7	6			9	6	3
Olfactory epithelium erosion					1	2	6	1	1	2
Epithelial hyperplasia in larynx	1	10		2	4		7	1		9
Macrophage aggregates around terminal bronchioles		2	6	10	8		1	5	6	5
Myocardial fibrosis		2			1		1	3		
Thymus involution	1	2	14	15	12	3	1	14	14	15
Cervical lymph node atrophy			13	14	11	1		11	11	12
Lymphoid atrophy of spleen			12	11	10			14	13	14
Adrenal cortical atrophy			10	5	4			9	6	6
Centrilobular hepatocyte vacuolation			13	10	9					
Periportal hepatocyte vacuolation								6	6	2

Bold shaded values indicate statistical significance vs control; blanks indicate zero incidence.

Toxicokinetics: Plasma levels are shown in table 15, below. Fluticasone levels were similar in the three fluticasone treated groups; there was no effect of gender or concurrent salmeterol exposure. Salmeterol increased with increasing dose; there was no effect of gender or concurrent fluticasone exposure. Plasma levels for both drugs was lowest on day 1 but the accumulation by week 5 and 13 was only modest.

Table 15. Rat 13-week inhalation study: Plasma levels immediately after exposure.

		Male				Female			
Salmeterol (μg/kg)		36	0	8	37	36	0	8	37
Fluticasone (μg/kg)		0	71	72	71	0	71	72	71
Salmeterol plasma level (ng/mL)	day 1	1.2	---	0.4	1.2	1.3	---	0.4	1.5
	week 5	2.1	---	0.5	2.4	3.4	---	0.6	2.3
	week 13	2.0	---	0.5	2.2	1.4	---	0.6	2.8
Fluticasone plasma level (ng/mL)	day 1	---	1.1	1.4	1.5	---	1.1	1.4	1.2
	week 5	---	3.6	2.6	2.9	---	4.2	2.1	2.3
	week 13	---	2.1	2.6	2.5	---	1.5	2.1	3.1

Recovery: Control and high-dose subgroups were withdrawn from treatment for 4 weeks of recovery. Most of the treatment related effects returned toward but not completely back to normal. Weight gain increased during recovery but was still less than control (13%↓ in males, 8%↓ in females). Food intake exceeded control values during recovery. Lymphocyte count recovered in females but not in males. In males, glucose was decreased (12%↓) and corticosterone was increased (~2-fold ↑). Triglycerides were increased in females (34%↑). Adrenal, thymus, and spleen weights were normal after recovery. Body fat was decreased in 3/20 treated rats. No treatment related histopathology was evident during recovery except for persistence of macrophage aggregates near terminal bronchioles in 7/20 treated rats.

Dog Preliminary, 14-day Inhalation Toxicity Study
Glaxo Wellcome Report WPT/92/178, vol 10, pg 001

This study was reviewed previously (NDA 20-549); results are summarized below:

Three groups (2/sex/group) received: lactose vehicle, or salmeterol:fluticasone at 17:16 and 178:164 μg/kg/day. Salivation was observed at the high dose. Effects related to salmeterol were increased heart rate (~20%↑) and myocardial necrosis. Effects related to fluticasone were decreased lymphocyte count, decreased hematocrit, decreased adrenal weight, and increased liver weight, with microscopic evidence of adrenal atrophy, lymphoid atrophy in thymus and lymph nodes, and swelling and rarefaction of hepatocytes. Blood urea nitrogen was increased 29% at the high dose but there was no histologic indication of kidney damage. Adverse effects were dose related and occurred at both the low and high dose.

Dog Pilot, 14-day Inhalation Toxicity Study
Glaxo Wellcome Report WPT/93/089, vol 10, pg 168

This study was reviewed previously (NDA 20-549); results are summarized below:

Seven groups (2/sex/group) were allotted to the treatments shown below:

Group	1	2	3	4	5	6	7
Salmeterol dose (μg/kg)	0	4.5	16.6	5.4	5.8	15.0	15.9
Salmeterol C_{max} (ng/mL)*	---	2.5	10.7	3.2	2.9	8.2	6.4
Fluticasone dose (μg/kg)	0	0	0	9.1	21.9	23.9	61
Fluticasone C_{max} (ng/mL)*	---	0.09	0.13	0.97	1.66	2.07	3.88

*Day 14

There were no significant clinical observations. Body weight gain was decreased in all groups except group 3 (high dose salmeterol alone), which showed increased weight gain in the males. Heart rate was increased on day 1 in all treated groups (50-100% ↑). By day 14 tolerance had developed and heart rate increased 0-4% in treated groups. Decreased hematocrit and decreased lymphocyte count were observed in groups 5-7; increased neutrophil count was seen in group 7. Inorganic phosphate was decreased in groups 4-7 (16-27%↓). In group 7, K^+ was increased (16%↑) and Ca^{++} was slightly decreased (10%↓). Absolute liver weight increased in groups 5-7 (14-52%↑), thymus weight decreased in groups 4-7 (53-67%↓), adrenal weight decreased in groups 6 and 7 (20-24%↓), ovary weight decreased in groups 5-7 (18-39%↓). Only potential target organs were examined histologically. Findings included: atrophy of thymus and adrenals in groups 4-7, pigmented alveolar macrophages in groups 2 and 3, swelling and rarefaction of hepatocytes in groups 5-7.

Dog 13-week Inhalation Toxicity Study
Glaxo Wellcome Report WPT/95/233, 09 JAN 96, vol 11, pg 001

Study Dates: Experimental work phase 07 MAR 95 to 04 JUL 95
Testing Lab: ———
Test Articles: Lactose vehicle BP/NF, batch WL 9903

Salmeterol xinafoate	Fluticasone propionate	Batch number
5% w/w	---	U95/403A
---	10% w/w	U95/404A
1% w/w	10% w/w	U95/402A
5% w/w	10% w/w	U95/401A

GLP: The study was accompanied by a signed GLP statement.

Methods: Five groups of beagle dogs were assigned to the following treatments:

Group #	1	2	3	4	5
Nº dogs/sex main study groups	4	4	4	4	4
Nº rats/sex recovery groups	2	---	---	---	2
Estimated salmeterol dose* (μg/kg)	0	15	0	3	16
Estimated fluticasone dose* (μg/kg)	0	0	30	31	30
Salmeterol particle size					
Fluticasone particle size					

*Assumes total deposition of all particles

Drug was administered by oral inhalation via an oropharyngeal tube for 6 minutes daily exposure. Drug treatment was withdrawn for 4 weeks in control and high dose recovery groups. The following observations were made:

Clinical observation	daily as noted, careful examination weekly
Mortality	twice daily
Body weight	prestudy, weekly
Food intake	daily by weight
Ophthalmology	prestudy and week 12
Electrocardiogram . .	prestudy and week 12; EKG was recorded only before dosing
Clinical chemistry . .	prestudy and weeks 4 and 12
Hematology	prestudy and weeks 4 and 12
Urinalysis	overnight collections prestudy and weeks 4 and 12
Drug levels	0.03, 0.08, 0.17, 0.67, 2, 4, and 6 hr post-dose on day 1 and week 13; 0.03 and 2 hr post-dose in week 5.
Necropsy	terminal
Histopathology	comprehensive list of tissues plus gross lesions in all main study groups; only presumed target tissues in recovery groups.

Results:

Mortality: One group 4 female was found prostrate and killed for humane reasons on day 50. Findings were considered to be consistent with lobar bronchopneumonia secondary to the immunosuppressant activity of fluticasone.

Clinical Observations: Three dogs from group 4 exhibited signs which might have been treatment related. One male was subdued with rapid respiration and mild fever; another male was dehydrated and lethargic; one female had rapid respiration, audible breathing sounds, and slight salivation. These dogs were treated with antibiotics and recovered without incident after one or two days suspension of dosing. These observations were considered to be consistent with infections secondary to the immunosuppressant activity of fluticasone.

Body Weight: Weight loss was seen in the first week of exposure to fluticasone. Weight gain over the course of the study was decreased in fluticasone treated groups.

Food Intake: No toxicologically significant treatment-related effects.

Ophthalmoscopy: No toxicologically significant treatment-related effects.

Electrocardiography: No abnormalities were reported but recordings were only made before dosing.

Note: For clinical pathology values, group averages were reported for combined males and females; effects were similar for both sexes.

Hematology: Low lymphocyte counts and high neutrophil counts were observed in fluticasone treated groups in weeks 4 and 12. Increased fibrinogen levels were observed in fluticasone treated groups.

Clinical Chemistry: In fluticasone treated groups there was decreased plasma glucose, increased alkaline phosphatase, decreased creatinine, decreased CPK, increased cholesterol and markedly decreased cortisol. With the combination there were numerous small changes in electrolytes that were not biologically significant.

Urinalysis: Urine pH increased in group 5, the high dose combination.

Organ Weights: In fluticasone treated groups thymus and adrenal weights were markedly decreased and liver weight was increased. There was a decrease in lung weight, statistically significant only for the high dose combination. Spleen weight was highly variable with a significant decrease only for the low dose combination.

Gross Pathology: There were numerous nonspecific lesions in lung, such as red, brown, or pale discoloration, adhesions, and raised or depressed areas. When summed together, these lesions were more common in drug treated dogs. In animals that received fluticasone, 10/24 had small adrenals and 5/24 had a large proportion of adipose tissue in thymus. Joints containing clear gelatinous fluid were observed in 2/8 (25%) controls and in 7/15 (47%) treated with the combination; the significance of this is unknown.

Histopathology: In groups treated with fluticasone, lymphoid atrophy was evident in thymus, spleen, lymph nodes and tonsils. Adrenal cortical atrophy was seen in all dogs treated with fluticasone. Centrilobular hepatocyte swelling or rarefaction was observed in fluticasone treated groups. Pneumonitis occurred in control animals with a somewhat higher incidence in treated animals. There were no effects specifically attributable to salmeterol.

Table 16. Treatment-related effects in dog 13-week inhalation toxicity study.

Group #	1	2	3	4	5
Salmeterol dose (μg/kg)	0	15	0	3	16
Fluticasone dose (μg/kg)	0	0	30	31	30
Body weight gain (kg)	3.15	3.20	**1.85**	**1.85**	**1.95**
Lymphocyte count ($10^3/mm^3$)	4.31	4.01	**2.87**	**2.94**	**2.54**
Eosinophil count ($10^3/mm^3$)	0.18	0.25	**0.01**	**0.01**	0.08
Neutrophil count ($10^3/mm^3$)	6.17	6.85	7.64	**8.34**	6.65
Fibrinogen (mg/dL)	204	223	**270**	**306**	**263**
Glucose (mg/dL)	115	113	**108**	**104**	**106**
Creatinine (mg/dL)	0.8	0.9	**0.6**	**0.6**	**0.6**
Alkaline Phosphatase (U/L)	173	180	**276**	**306**	**258**
CPK (U/L)	112	114	97	**62**	**75**
Cholesterol (mg/dL)	107	125	**155**	**167**	**166**
Cortisol (μg/dL)	1.7	2.4	**<0.3**	**<0.2**	**<0.2**
Urine pH	6.8	7.0	6.9	7.3	**7.6**
Adrenal weight (g)	1.19	1.13	**0.59**	**0.68**	**0.71**
Thymus weight (g)	16.6	12.4	**7.6**	**5.6**	**8.3**
Spleen weight (g)	79.9	68.6	69.3	**48.2**	77.7
Lung weight (g)	102.0	92.6	81.1	80.8	**80.8**
Liver weight (g)	370	351	**404**	**497**	**415**
Number of animals	8	8	8	7	8
All lung lesions*	4	8	10	13	7
Small adrenals	0	0	5	4	1
↑ Adipose content of thymus	0	0	2	2	1
Clear gelatinous fluid in joints	2	0	0	2	5

Bold shaded values statistically significant *vs* control. * Total lesions can exceed total number of animals since some dogs had more than one type of lung lesion.

~~Table 17. Histopathology in dog 13-week inhalation toxicity study.~~

Group #	1	2	3	4	5
Salmeterol dose (μg/kg)	0	15	0	3	16
Fluticasone dose (μg/kg)	0	0	30	31	30
Total number of animals (♂ + ♀)	8	8	8	8	8
Thymus involution	0	0	7	8	7
Decreased/absent germinal centers:					
spleen	0	0	7	7	8
cervical lymph nodes	0	0	8	7	6
mesenteric lymph nodes	0	0	7	8	8
tracheobronchial lymph nodes	0	0	2	1	3
tonsil	0	0	8	7	7
Adrenal cortical atrophy	0	0	8	8	8
Centrilobular hepatocyte swelling/rarefaction	0	0	8	6	4
Pneumonitis	3	5	5	7	5

Toxicokinetics: Plasma levels were determined in satellite groups (2/sex/group). There were no obvious differences between males and females and combined results are summarized in table 18, below. Plasma levels did not change significantly over time for either drug. Plasma levels of salmeterol and fluticasone were not affected by co-administration of the other drug.

Table 18. Toxicokinetics in dog (♂ + ♀) 13-week inhalation toxicity study.

Group #		1	2	3	4	5
Salmeterol dose (μg/kg)		0	15	0	3	16
Fluticasone dose (μg/kg)		0	0	30	31	30
Salmeterol C_{max} (ng/mL)	day 1	–	5.3	–	1.6	16.0
	day 35	–	2.7	–	1.9	6.1
	day 90	–	7.7	–	1.4	5.7
Fluticasone C_{max} (ng/mL)	day 1	–	–	0.57	1.57	1.27
	day 35	–	–	0.99	1.11	1.07
	day 90	–	–	0.84	1.44	1.25

Acute and multiple-dose toxicity of combined salmeterol and fluticasone was attributable to the known effects of either drug given alone with no evidence for synergism, potentiation, or unique effects of the combination. The studies were not designed such that NOAEL levels for the combination could be determined. Instead, the approach was to give the drugs in combination at doses with known toxic effects to see if toxicity was altered in the presence of the other drug.

Acute Inhalation Toxicity in Rats: Effects attributed to fluticasone were decreased body weight, lymphoid depletion in thymus and spleen, and glycogen vacuolation in the liver. Effects attributed to salmeterol were local airway irritation, focal ventricular myocardial degeneration, and atrial myocarditis. The only effect not observed previously for either drug alone is atrial myocarditis. The atrial lesion is attributed to salmeterol because a similar lesion is seen with other vasodilator drugs, such as minoxidil and theobromine (see review in Toxicologic Pathology 17:94-108, 1989). Atrial myocarditis was seen in 10/10 rats examined 3 days after inhalation of 3.6 mg/kg salmeterol but was not seen in any of 10 rats examined 15 days after inhalation of 3.6 mg/kg salmeterol. Atrial myocarditis was never observed in 2-, 5-, or 13-week multiple-dose studies even though high doses in the 2- and 5-week studies were greater than some associated with atrial myocarditis in the acute studies). Because the lesion is reversible and not progressive, the biological significance is questionable. For focal ventricular myocardial fibrosis, the NOAEL was a salmeterol dose of 0.5 mg/kg in one study (3/5 incidence at next highest dose of 3.6 mg/kg) and at a dose of 64 µg/kg in a subsequent study (1/5 incidence at the next highest dose of 556 µg/kg).

Multiple-dose Toxicity in Rats: Effects attributed to fluticasone were: decreased body weight; lymphoid atrophy in circulating lymphocytes, thymus, spleen, and lymph nodes; increased incidence of pale foci in lungs possibly reflecting immunosuppressant activity; adrenal atrophy with decreased serum corticosterone; increased serum transaminases and histology consistent with hepatic glycogen deposition; metabolic effects such as increased glucose, triglycerides and cholesterol, and decreased body fat; mild hemoconcentration; serum and urine electrolyte changes consistent with suppression of mineralocorticoid activity; gross and microscopic stomach lesions; and hair loss. These are all recognized effects of fluticasone and glucocorticoids as a class. Effects attributed to salmeterol were: increased body weight (which tended to antagonize the effect of fluticasone); local irritation of the nasal passages, larynx (including squamous metaplasia), and tracheal bifurcation; decreased glucose and slightly increased lymphocyte count opposing the effects of fluticasone. Squamous metaplasia of laryngeal epithelium was seen in a previous 2-year rat carcinogenicity study of oral+inhalation salmeterol (NDA 20-263, original Pharm/Tox review, L. Sancilio, 13 JUL 92). Because there were no laryngeal tumors associated with life-time salmeterol inhalation this squamous metaplasia cannot be considered a preneoplastic change. Squamous metaplasia may be species specific since it was not observed in the 1-year dog inhalation toxicity study of salmeterol.

~~Cardiovascular effects attributed to salmeterol included: cardiac hypertrophy, occasional~~ myocardial fibrosis, coronary arteritis, and mural thickening and "disorganization" in coronary arteries and arteries close to large bronchioles; effects on heart rate were inconsistent. All of these except the effects on arteries are known effects of salmeterol. "Periarteritis" was seen previously with salmeterol alone at the high dose in the 2-year rat carcinogenicity study (2mg/kg p.o. + 0.58 mg/kg by inhalation). Coronary arteritis has been observed for other β-agonists and vasodilators (reviewed by Detweiler in *Toxicologic Pathology* 17:94-108, 1989 and by Greaves in *Histopathology of Preclinical Toxicity Studies*, Elsevier, Amsterdam, 1990, pp 250-260). There is some strain selectivity for the effect in the present studies, with Wistar rats being more sensitive. The coronary arteritis is a relatively rare, high-dose effect. In a pilot 2-week study it occurred in 3/10 combined males and females at 900 μg/kg and in none at 69 μg/kg; in a second 2-week study it occurred only in 1/40 females at 800 μg/kg; in a 5-week study with interim sacrifice at 2 weeks it occurred at 2 weeks in 1/10 males at 200 μg/kg but in none at 400 μg/kg, it was not observed at 200 or 400 μg/kg at 5 weeks. In the 13-week study neither coronary arteritis nor arterial mural disorganization was observed at the high dose of 36 μg/kg. There was a small but consistent increase in the observation of "myocarditis" in rats treated with the combination; the details of this lesion were not discussed in any of the reports. Greaves notes that "Small foci of necrosis, focal inflammation and fibrosis are occasionally observed in young untreated rats and become more common with increasing age. It is generally believed that these foci are due to focal ischaemia as a result of myocardial vascular disease" (*Histopathology of Preclinical Toxicity Studies*, Elsevier, Amsterdam, 1990, pp 233-241). Ischemic events related to clinical administration of combined salmeterol and fluticasone can be monitored by the electrocardiogram.

Multiple-dose Toxicity in Dogs: Effects attributed to fluticasone included: immunosuppression manifested as increased incidence of infections, lymphopenia, decreased eosinophil count, and histologic lymphoid atrophy of thymus, spleen, lymph nodes and tonsils; adrenal atrophy with decreased plasma cortisol; increased liver weight with swelling or rarefaction of hepatocytes; increased alkaline phosphatase possibly indicating altered bone metabolism; decreased creatinine and CPK probably reflecting decreased skeletal muscle mass; decreased glucose and increased cholesterol and fibrinogen. These effects have all been observed previously with fluticasone alone. A small decrease in lung weight in one of these studies was also seen previously with fluticasone alone. Effects attributed to salmeterol in the preliminary studies included: increased heart rate and myocardial necrosis; and increased body weight. The local irritation seen in rat airways due to salmeterol was not seen in dogs. In the 13-week study there was no significant toxicity specifically attributable to salmeterol. There was a small increase in the incidence of lung lesions and diagnosis of pneumonitis with either drug alone or in combination. In the 13-week study, the incidence of clear gelatinous fluid in joints was increased in the high dose combination but not with either drug alone or with the low dose combination; the toxicological significance of this finding is unknown. The atrial myocarditis and coronary arteritis in rats were not seen in dogs.

REPRODUCTIVE TOXICITY

Mouse, Organogenesis Dose-ranging Study
Glaxo Wellcome Report WPT/92/179, vol.12, pg. 022

This study was reviewed previously (IND —— results are summarized below:

Six groups of pregnant mice were allotted to the treatments shown below:

Group	1	2	3	4	5
Salmeterol p.o. dose (mg/kg)	0	0.2	0.2	10	10
Fluticasone s.c. dose (μg/kg)	0	15	150	15	150
Maternal body wt. gain (% Δ vs control)	---	8%↓	22%↓	3%↓	31%↓

Maternal body weight gain was decreased in groups 3 and 5 (high-dose fluticasone). There were no significant differences among the groups in number of implantations, early or late resorptions, post-implantation loss, live and dead fetuses. Fetal weight was slightly decreased in group 5 only (16%↓). Cleft palate was seen in 1 fetus in each of groups 3 and 5.

Mouse, Oral Gavage/Subcutaneous Organogenesis Study
Glaxo Wellcome Report WPT/92/196, 26 APR 95, vol.12, pg. 066

Study Dates: Drug dosing 06 MAY 92 to 18 MAY 92
Testing Lab: ——
Test Article: Salmeterol xinofoate as a suspension in ——
—— drug substance batch number L039A86
Fluticasone propionate as a suspension in ——
—— drug substance batch number EPPG 12/40
GLP: The study was accompanied by a signed GLP statement.

Methods:

Six groups of 26 mated female CD1 mice were assigned to the following treatments:

Group #	1	2	3	4	5	6
Salmeterol oral gavage dose (mg/kg/day)	0	1.4	0	0.2	1.4	10
Fluticasone s.c. dose (μg/kg/day)	0	0	40	10	40	150

Mice were dosed daily from day 6 to 15 of pregnancy. Mating was confirmed by the presence of sperm plugs (designated day 0). Dose was adjusted daily for changes in body weight.

~~The following observations were made:~~

Clinical observation . . .	daily
Body weight	days 0, 3, 6-15 daily, and 18
Food intake	days 0-6, 6-9, 9-12, 12-15, 15-18
Drug levels	in satellite groups (5/time point) at 0.25, 0.5 and 24 hr after dosing on days 6 and 15.
Maternal necropsy . . .	day 18: pregnancy status, early resorptions, late resorptions, dead fetuses, live fetuses.
Fetal examination . . .	all: weight, external exam, sex 2/3: visceral exam followed by preparation and skeletal exam 1/3: Bouin's fixation, sectioning & exam of head & major organs

Results: (summarized in table 19, page 36)

Mortality: None

Maternal Body Weight: Body weight gain was retarded in fluticasone treated groups; salmeterol caused a slight increase in body weight and counteracted the effect of fluticasone in the low and mid dose combination.

Maternal Food Intake: Food intake was slightly greater with salmeterol alone and slightly less with fluticasone alone; differences were not statistically significant.

Maternal Gross Pathology: No toxicologically significant treatment-related effects.

Uterine Exam: At the high dose combination (group 6) the post-implantation loss was increased (3.7 fold), number of live fetuses was decreased (19%↓).

Fetal Weights: Fetal weight was mildly decreased at the high dose; statistically significant for males (4.4%↓).

Fetal Abnormalities: The incidence of cleft palate (the most common major abnormality) was somewhat greater in the high dose combination (group 6) but neither cleft palate nor total major abnormalities differed significantly among groups. Delayed ossification of the occipital bones was more frequent in fluticasone treated groups; this was highly significant for the high dose combination. This was considered a minor variant and was probably related to decreased maternal weight gain and decreased fetal weight.

Toxicokinetics: Salmeterol plasma concentration was below the limit of quantitation except in the high dose combination (group 6). Fluticasone increased with increasing dose; the increase was less than proportional to dose. For both drugs there was about a 2-fold increase in plasma level from day 6 to day 15.

Table 19. Mouse organogenesis study

Group number	1	2	3	4	5	6
Salmeterol dose (mg/kg)	0	1.4	0	0.2	1.4	10
Fluticasone dose (μg/kg)	0	0	40	10	40	150
Maternal body wt gain (g)						
days 6-9	2.6	2.9	**1.7**	**2.1**	**1.9**	**1.3**
days 12-15	7.9	**8.6**	**7.1**	8.0	7.5	**6.8**
Nº of live fetuses	12.9	13.1	12.8	12.3	12.2	**10.4**
Post implantation loss (%)	4.9	6.4	3.3	6.2	9.2	**18.1**
Male fetal weight (g)	1.37	1.39	1.38	1.40	1.35	**1.31**
Female fetal weight (g)	1.33	1.32	1.33	1.33	1.30	1.28
Cleft palate (% of fetuses)	1.0	0.0	0.9	0.3	0.0	2.1
Any major visceral or skeletal abnormalities (%)	1.0	0.6	0.9	1.0	0.4	2.1
Delayed occipital ossification (%)	4.3	3.6	7.8	5.6	5.8	**30.7**
Salmeterol C_{max} (ng/mL) day 1	<LQ	<LQ	---	<LQ	<LQ	6.8
day 15	<LQ	<LQ	---	<LQ	<LQ	15.1
Fluticasone C_{max} (ng/mL) day 1	<LQ	---	3.07	1.46	3.00	4.95
day 15	<LQ	---	3.29	1.24	5.02	8.59

Bold shaded values are statistically significant *vs* control. LQ=limit of quantitation.

Comment: The increase of cleft palate at the high dose combination may be real despite the lack of statistical significance; this is a known class effect of glucocorticoids. The NOAEL for the combination is the mid dose. The fluticasone concentration at the NOAEL is 20 to 30 times the clinical C_{max} of 146 pg/mL after 250:500 μg salmeterol:fluticasone Diskus DPI. Salmeterol plasma level was blow the limit of quantitation —— at the NOAEL. Extrapolating from the high dose, assuming dose proportionality, salmeterol concentration at the NOAEL is estimated at 0.95 to 2.1 ng/mL; this is about 6 to 14 times the clinical C_{max} of 150 pg/mL after 42 μg salmeterol — 2x daily. Plasma levels of salmeterol after inhalation of the combination via the Diskus DPI have not yet been reported. Previously, plasma levels after oral salmeterol alone were below the limit of detection at the NOAEL dose for developmental effects in rabbits and at the low and mid doses of the mouse oral carcinogenesis study (NDA 20-263, original Pharm/Tox review, L. Sancilio, 13 JUL 92).

~~Rat, Oral Fertility in Pregnant Females~~
Glaxo Wellcome Report WPT/92/125, vol. 13, pg. 1

This study was reviewed previously (IND ——); results are summarized below:

Six groups of pregnant mice were allotted to the treatments shown below:

Group	1	2	3	4	5
Salmeterol p.o. dose (mg/kg)	0	0.1	0.1	10	10
Fluticasone s.c.. dose (μg/kg)	0	10	100	10	100
Maternal body wt. gain (% Δ vs control)	---	3%↓	99%↓	36%↑	74%↓
Placental weight (% Δ vs control)	---	11%↓	24%↓	15%↓	20%↓

Maternal body weight gain was increased in group 4 (high salmeterol/low fluticasone). In the two high-dose fluticasone groups (3 and 5) maternal body weight gain was markedly reduced. Food intake was also decreased in groups 3 and 5 (~14%↓). Placental weight was decreased, particularly in the two high-dose fluticasone groups. In group 5 there was increased incidence of left sided umbilical artery and changes in ossification of occipital bones. Skeletal development was delayed in groups 3 and 5.

Rat, Oral/Subcutaneous Organogenesis Study
Glaxo Wellcome Report WPT/92/37, 07 JUN 95, vol.13, pg. 103

Study Dates: Drug dosing commenced 26 MAY 92
Testing Lab: Glaxo Group Research Ltd., Ware, Hertfordshire, England, UK
Test Article: Salmeterol xinofoate as a suspension in ——; batch number R6376/005
Fluticasone propionate as a suspension in —— batch number R6384/008
GLP: The study was accompanied by a signed GLP statement.

Methods:

Six groups of 24 mated female AHA rats were assigned to the following treatments:

Group #	1	2	3	4	5	6
Salmeterol oral gavage dose (mg/kg/day)	0	1	0	0.1	1	10
Fluticasone s.c. dose (μg/kg/day)	0	0	30	10	30	100

Rats were dosed daily from day 7 to 16 of pregnancy. Mating was confirmed by the presence of sperm in vaginal smears (designated day 1). Dose was adjusted daily for changes in body weight.

~~The following observations were made:~~

Clinical observation	daily
Body weight	days 1, 4, 7-16 daily, 18, and 20 (confirmed mating = day 1)
Food intake	days 1, 4, 7, 10, 13, 16, 18, and 20
Drug levels	in satellite rats (3/group) at 1 hr after dosing on days 7 and 16.
Maternal necropsy	day 21: pregnancy status, early resorptions, late resorptions, dead fetuses, live fetuses, placental weight.
Fetal examination	all: weight, external exam, sex 1/2: visceral exam followed by preparation and skeletal exam 1/2: Bouin's fixation, head sectioning and exam

Results: (summarized in table 20, page 39)

Mortality: None

Maternal Body Weight: Weight gain increased in the group that received salmeterol alone (group 2) and decreased in the fluticasone treated groups; the greatest effect was with the high dose combination (group 6).

Maternal Food Intake: Food intake decreased with combination treatment at the mid dose (9% ↓) and high dose (14% ↓).

Maternal Gross Pathology: An increased incidence of subcutaneous hemorrhage appeared to be related to the subcutaneous fluticasone injection site.

Uterine Exam: At the high dose combination (group 6) pre- and post-implantation losses increased and the number of live fetuses decreased. These changes were not statistically significant but the value for post-implantation loss exceeded the historical control range.

Fetal Weights: Fetal weight decreased with fluticasone alone (group 3) and with the high dose combination (group 6). Placental weight decreased with the high dose combination.

Fetal Abnormalities: The incidence of umbilical hernia (a major abnormality) increased with the high dose combination; this is a known class effect for glucocorticoids. The incidence of left-sided umbilical artery (a minor variation) increased slightly with fluticasone alone and significantly with the high dose combination. In the normal rat embryo, the umbilical arteries are paired during early embryonic development but during the second half of gestation the left artery regresses and the right artery alone persists (Teratology 7:237-242, 1973). Delayed skeletal development was evident with the high dose combination and, to a lesser extent, with fluticasone alone. Occipital changes included bipartite, incompletely ossified and butterfly shaped occipitals. Incomplete ossification was noted for sternebral elements, 5th metatarsal, thoracic vertebral centra, and cervical arches. These changes are most likely related to decreased maternal food intake and retarded weight gain.

~~*Toxicokinetics:* Salmeterol was quantifiable only in the high dose combination and on~~ day 16 with the mid-dose combination. Fluticasone plasma levels increased in a less than dose-proportional manner. With the high dose combination there was drug accumulation from day 7 to 16 ~~for~~ both agents.

Table 20. Rat organogenesis study.

Group #		1	2	3	4	5	6
Salmeterol dose (mg/kg/day)		0	1	0	0.1	1	10
Fluticasone dose (μg/kg/day)		0	0	30	10	30	100
Maternal body wt. gain days 7-16 (g/day)		**4.8**	**6.7**	**2.6**	**4.1**	**3.1**	**0.8**
Maternal food intake days 7-16 (g/day)		27.8	29.8	26.7	28.9	**25.4**	**23.8**
Maternal subcutaneous hemorrhage		1/24	0/24	3/24	0/24	5/24	3/24
Pre-implantation loss (%)	{2.9-14.1}	3.4	6.4	7.9	4.5	7.0	8.4
Post-implantation loss (%)	{1.1-7.7}	6.2	3.9	5.4	5.9	3.5	8.8
Mean no. live fetuses/litter	{12.2-14.8}	14.5	15.3	13.9	14.5	13.9	12.9
Mean fetal weight (g)	{3.5-3.8}	3.6	3.7	**3.4**	3.6	3.6	**3.3**
Placental weight (g)		0.51	0.52	0.49	0.50	0.49	**0.43**
Umbilical hernia (%)		0	0	0.3	0	0	**2.9**
Left sided umbilical artery (%)	{1.5-5.6}	3.1	3.1	5.1	0.6	3.3	**16.1**
Occipital changes (%)	{0.7-5.3}	0.6	0	1.8	2.5	1.1	**28.8**
Incomplete ossification (%)							
nasals	{0-8.9}	1.8	1.8	**9.9**	3.1	2.9	**7.5**
sternebrae	{42-69}	57	40	64	47	53	**81**
5th metatarsal	{0-1.2}	1.2	0	3.5	1.2	0	**5.0**
thoracic vertebral centra	{1.4-9.3}	4.3	0.6	2.9	2.5	1.1	**11.9**
any cervical arch	{0-4.1}	0	0	1.2	0	0.6	**18.8**
Salmeterol plasma conc. (ng/mL)	day 7	<1.0	<1.0	<1.0	<1.0	<1.0	6.6
	day 16	<1.0	<1.0	<1.0	<1.0	1.3	26.7
Fluticasone plasma conc. (pg/mL)	day 7	<50	<50	291	218	296	279
	day 16	<50	<50	361	142	350	525

Bold shaded values P < 0.05 *vs* control. { } Brackets indicate historical range.

Comment: The toxicological significance of left-sided umbilical artery is unknown. Unlike rats, both left and right umbilical arteries are maintained during normal human

~~gestation (Acta Obstet Gynecol Scand 195-214, 9). Except for the latter observa~~ tion, all of the effects of the combination are known effects of salmeterol or fluticasone given alone. The NOAEL for developmental effect for the combination was the mid dose (1 mg/kg salmeterol, 30 μg/kg fluticasone). The fluticasone plasma concentration at the NOAEL dose is about twice the clinical C_{max} of 146 pg/mL after 250:500 μg salmeterol:fluticasone Diskus DPI. Salmeterol plasma level on day 7 was blow the limit of quantitation —— at the NOAEL. Extrapolating from the high dose and the data from day 16, salmeterol concentration at the NOAEL is estimated at 0.33 ng/mL. Thus, salmeterol plasma concentrations at the NOAEL were about 2 to 9 times the clinical C_{max} of 150 pg/mL after 42 μg salmeterol MDI 2x daily. Plasma levels of salmeterol after inhalation of the combination via the Diskus DPI have not yet been reported.

OVERALL SUMMARY AND EVALUATION

The sponsor proposes a combination of two approved, marketed, inhalation drug products for treatment of asthma. The following preclinical bridging data were submitted in support of the combination.

Pharmacokinetics: Toxicokinetic data from single- and multiple-dose toxicity studies were provided. Accurate AUC's could not be determined because plasma concentrations were often below the limit of quantitation. C_{max} data showed no pharmacokinetic interactions in studies up to 3-months in dog and rat at plasma concentrations ≥ 5 times the maximum anticipated clinical plasma concentrations of 0.15 ng/mL for each. There was no multiple dose accumulation in dog and minimal (~2-fold) accumulation in rat.

Safety Pharmacology: There was no effect of fluticasone (10 mg/kg, s.c., 24 and 3 hr pretreatment) on cardiovascular responses (↑HR, ↓BP) to i.v. salmeterol (0.01-100 μg/kg) in guinea pigs.

Note on Inhalation Doses: Doses reported throughout the review for inhalation in rats and dogs are those calculated by the investigators assuming 100% deposition of all particles —— This should be considered a "total deposition". Pulmonary deposition in rats is probably ~7-10% and upper respiratory deposition ~3-4%. No good deposition data are available for oral inhalation in dogs; pulmonary deposition might differ significantly with different devices and techniques of administration.

Acute Toxicity: At doses up to 3.6:1.9 mg/kg salmeterol:fluticasone inhalation in rats there were no deaths. Toxicity was characteristic of glucocorticoids (lymphoid atrophy, ↑ hepatic glycogen) and β_2-agonists (focal myocardial degeneration). Sporadic atrial myocarditis was observed with the combination, a toxicity not seen previously with either drug alone. A NOAEL for this was not determined (< 28:73 μg/kg salmeterol: fluticasone); but the effect was transient; it was never observed at 2-week to 3-months.

~~**Multiple-Dose Toxicity: In rat, 2-, 5-, 13-wk studies (salmeterol:fluticasone 2.6:2.8**~~ ng/mL C_{max} at week 13) revealed known effects of salmeterol (local irritation, myocardial hypertrophy, ↓K^+, ↑glucose) and fluticasone (lymphoid and adrenal suppression). In dog, 2- & 13-wk studies (salmeterol:fluticasone 5.7:1.2 ng/mL C_{max} at week 13) revealed characteristic effects of fluticasone and no effect of salmeterol. NOAELs for the combination were not determined but toxicities were expected and were not more serious for the drug combination than for each alone. **Coronary arteritis** seen in 2-week rat studies was not seen previously but it was a sporadic, high-dose effect; not observed in studies of 5-weeks (high dose = 400 μg/kg) or 13-weeks (high dose = 36 μg/kg).

Developmental Toxicity: Data were submitted from oral:subcutaneous salmeterol:fluticasone organogenesis studies in rat and mouse. In rats, there were decreased maternal and fetal weight, umbilical hernia, and delayed ossification, all seen before with fluticasone and other steroids. There was also increased fetal weight seen before with salmeterol. Salmeterol alone caused β-adrenergic class effects in a previous rabbit study. The findings were: precocious eye opening, cleft palate, sternebral fusion, limb and paw flexures, and delayed ossification of the frontal cranial bones (NDA 20-263, original Pharm/Tox review, L. Sancilio, 13 JUL 92). The effects of salmeterol alone in mice was not studied previously. Left sided umbilical artery and altered occipital ossification, not seen before with either drug alone, were seen with the combination; these were considered minor developmental variations. The NOAEL (1 mg/kg salmeterol, 30 μg/kg fluticasone) was associated with salmeterol plasma levels 2-9 times the expected clinical C_{max} and fluticasone levels about twice the clinical C_{max}. In mice, the effects of the combination were all seen previously with fluticasone alone (↑ post-implantation loss, ↓ maternal and fetal weights, variations in ossification, and 1 incidence of cleft palate). The NOAEL (1.4 mg/kg salmeterol, 40 μg/kg fluticasone) was associated with estimated salmeterol plasma levels 6-14 times the expected clinical C_{max} and fluticasone levels 20-30 times the clinical C_{max}. A placental transfer study in mouse showed accumulation of salmeterol and fluticasone related radioactivity in fetus comparable to maternal plasma concentrations.

RECOMMENDATIONS

- **Preclinical studies support the proposed clinical studies.**

Points discussed with Medical Officer: The following points were noted:

- Most of the preclinical studies were not designed to establish NOAELs.
- Atrial myocarditis and coronary arteritis are novel effects of the combination; but atrial myocarditis was transient and coronary arteritis was a sporadic effect occurring only at high dose levels.

Cardiac changes attributed to salmeterol are probably due to exaggerated pharmacological effect and subsequent ischemia. Examination of the EKG for excessive heart rate and characteristic ischemic changes should provide adequate monitoring.

Impurities, Extractables, and Excipients: The chemist noted that there is a salmeterol degradation product that accumulates to a significant extent —— in the salmeterol multiple-dose dry powder Diskus™ formulation. The sponsor is planning to submit dog and rat studies addressing the toxicity of the degradant to the forthcoming NDA for the salmeterol multiple-dose dry powder formulation. When submitted, those studies should be cross referenced to this IND. After the review of those studies consideration should be given as to whether bridging studies will be needed to examine the effects of the salmeterol degradant when co-administered with fluticasone.

File Name: N:\IND —— PHARM\96-05-24.REV

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18 Oct 96

Mark Vogel, Ph.D., Pharmacologist

Oct 18, 1996

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Original IND ——
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