CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-051

PHARMACOLOGY REVIEW(S)

Supervisory Pharmacologist Review

NDA: 22-051 – Fluticasone furoate nasal spray
FROM: Timothy J. McGovern, Ph. D., Supervisory Pharmacologist
DATE: March 15, 2007

I concur with the recommendation by Dr. Huiqing Hao, the pharmacology/toxicology reviewer, that the pharmacology and toxicology of fluticasone furoate (GW685698X) have been adequately studied and evaluated and that the drug product is approvable from a nonclinical standpoint (see NDA review dated March 5, 2007).

Pharmacology: Fluticasone furoate is a synthetic glucocorticoid with similar structural and pharmacological activity to that of fluticasone propionate. The drug showed binding affinity to glucocorticoid receptors (kd = 0.3 nmol/L) that was similar to fluticasone propionate. In *in vitro* assays, fluticasone furoate showed greater steroid receptor nuclear translocation than fluticasone propionate and similar activity in transrepression of TNF-alpha induced NFKB response and transactivation of mouse mammary tumor virus-luciferase reporter.

Fluticasone furoate demonstrated similar activity to fluticasone propionate and mometasone in inhibiting TNF-alpha induced IL-8 release in human bronchial epithelial cells. In vivo studies in mice and rats demonstrated that fluticasone furoate inhibits antigen-induced nasal symptoms with comparable potency and longer duration than fluticasone propionate in a rat allergic rhinitis model and inhibits the inflammatory response in an oxazolone-induced ear skin delayed type hypersensitivity model and an ovalbumin-induced lung eosinophilia model.

The primary metabolite (M10) demonstrated minimal glucocorticoid effects.

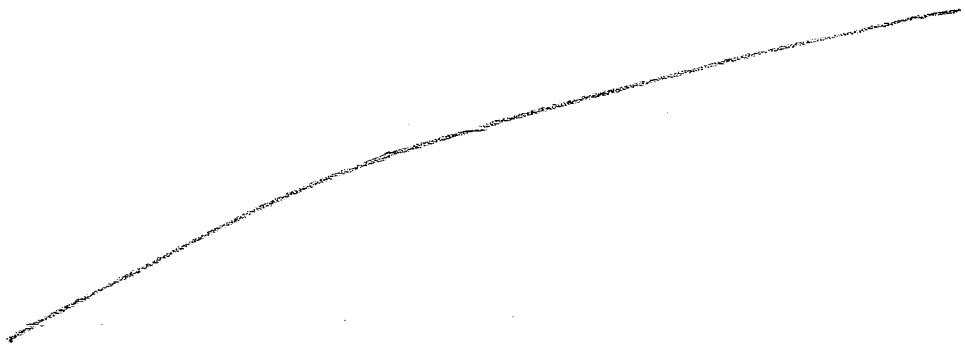
ADME: Primary tissues of distribution include the liver, kidney, spleen and GI tract following oral dosing in rats. Plasma protein binding is similar across species. Fluticasone furoate was extensively metabolized in rats and dogs and the major pathway included loss of the fluoromethyl carbothioate group to form the carboxylic acid GW694301X (M10). Most other metabolites are formed from M10. Fluticasone furoate is primarily eliminated through bile as metabolites. The elimination half-life is relatively short: 3 hours in rats and 3-13 hours in dogs.

General toxicology: The nonclinical toxicology program for NDA 22-051 included a complete program using the inhalation route of administration and a bridging program to support the intranasal route of administration. Inhalation studies were conducted in rats and dogs. Typical glucocorticoid-related effects were primarily observed although some unique findings were also noted initially for which no NOAEL was identified. These findings included increased eosinophilic inclusions in the bronchiolar epithelium in rats, and testicular degeneration, focal nephropathy, chronic stomach inflammation, and biliary tract epithelial vacuolation in dogs. Of note, the kidney and biliary tract findings

were also noted in a 26-week intranasal dog study. The sponsor submitted information to address the Division's concerns regarding these findings. Based on the information submitted and the lack of a similar finding in a 2-week intranasal study in rats, the Division concluded that the bronchiolar finding in rats was not a significant adverse response. In dogs, the testicular finding was concluded to not be drug-related based on submission of historical control data, a NOAEL for the focal nephropathy was identified in a 26-week intranasal study based on evaluation of historical control data, the Division concluded that, while the biliary tract finding may be drug related, the biliary tract lesion is not a clinical safety concern based on the evaluations and conclusions of a pathology working group convened to evaluate the findings across studies, and the systemic exposure level associated with the NOAEL for the stomach lesion provided an adequate safety margin (~132-fold) for the maximum proposed human intranasal dose. Airway inflammatory lesions that were identified in the inhalation studies are not considered relevant for the proposed intranasal use of the drug product.

In order to bridge the intranasal use of the proposed drug product to the complete inhalation toxicology program, the sponsor conducted a 2-week intranasal study in rats and 4-week and 6-month intranasal studies in dogs. The 6-month study identified typical glucocorticoid-related findings as well as the kidney and biliary tract findings noted above. Additionally, local findings, including nasal associated lymphoid tissue atrophy and nasal cavity goblet cell hypertrophy, and heart purkinje fiber vacuolation were observed. The heart findings were within historical background incidence and the local findings were considered clinically monitorable.

An additional issue that arose during the development program was the use of different



Overall, the toxicology program supports the proposed clinical use of the intranasal drug product. The findings in dogs and rats are predominantly typical steroid-related effects that are clinically monitorable. The NOAELs for the non-typical effects noted above provide adequate safety margins based on systemic exposure comparisons for the proposed clinical intranasal dosing up to 110 g/day.

Reproductive toxicity: Fluticasone furoate did not impair fertility in rats. Interestingly, the drug was not teratogenic or embyrocidal in rats or rabbits, a finding commonly observed with other steroids. Based on the known potential of steroids as a class to affect

reproductivity, the drug should be categorized as a pregnancy category C and the standard labeling text should be included in this product label.

Genotoxicity: GW685698X was tested in an Ames test, a mouse lymphoma assay and in 3 in vivo rat micronucleus assays. All of the genotoxicity studies demonstrated negative results. The original 2 micronucleus assays did not test doses up to the maximum recommended limit doses. The sponsor repeated the study a third time up to doses of 40 mg/kg, IV, and the maximum dose induced clinical signs that approximated an MTD.

Carcinogenicity: In two 2-year inhalation carcinogencity studies in mice and rats, fluticasone furoate did not induce any tumors.

Labeling: The sections "Mechanism of Action", "Carcinogenesis, mutagenesis and impairment of fertility", "Pregnancy" and "Overdosage" sections were revised in a preliminary label review by Dr. Huiqing Hao. The changes were made to maintain consistency with other recently approved nasal corticosteroid formulations, delete information in the "Pregnancy" and "Mechanism of Action" sections that are not deemed relevant and modify animal to human exposure ratios based on the Division's standard calculations when body surface area comparisons ($\mu g/m^2$) are used.

In conclusion, all nonclinical issues raised during the drug development program regarding fluticasone furoate-related toxicities observed in the inhalation studies and intranasal bridging studies have been resolved and there are no outstanding nonclinical issues. It is noted that an ONDQA-solicited consultation regarding the safety of various drug substance impurities and leachables in the drug product is currently ongoing. Therefore, this application is considered approvable from a nonclinical perspective pending inclusion of the recommended changes to the product label and successful resolution of the safety issues related to the ONDQA consultation.

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/s/

Timothy McGovern
3/15/2007 02:54:37 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-051**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**06/28/06 (original), 10/05/06 (PWG report), 10/18/2006 (additional nonclinical studies including rat micronucleus assay), 12/20/2006 (updated mouse carcinogenicity study report)**
PRODUCT:	**Fluticasone Furoate Nasal Spray**
INTENDED CLINICAL POPULATION:	**Adults and children 2 years of age and older with symptoms of seasonal and perennial allergic rhinitis**
SPONSOR:	**GlaxoSmithKline**
DOCUMENTS REVIEWED:	**E-submission, Module 4.**
REVIEW DIVISION:	**Division of Pulmonary and Allergy Products**
PHARM/TOX REVIEWER:	**Huiqing Hao, Ph.D.**
PHARM/TOX SUPERVISOR:	**Timothy McGovern, Ph.D.**
DIVISION DIRECTOR:	**Badrul Chowdhury, MD., Ph.D.**
PROJECT MANAGER:	**Ladan Jafari**

Date of review submission to Division File System (DFS): March 2, 2007

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
This NDA (fluticasone furoate nasal spray) is recommended for approval from a nonclinical perspective.

B. Recommendation for nonclinical studies
There are no additional nonclinical studies recommended for this NDA submission.

C. Recommendations on labeling
Modified language pertaining to teratogenic effects (under Section 8 Use in Specific Populations - Pregnancy), Overdosage (Section10), Mechanism of Action (Section 12.1 under Clinical Pharmacology) and Nonclinical Toxicology (Section 13) is recommended as detailed in the suggested labeling section of this review (see section Overall Conclusions and Recommendations).

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings
GW685698X (fluticasone furoate, FF) is a synthetic glucocorticoid compound. The sponsor completed a full battery of general toxicology studies via the inhalation route and conducted a bridging toxicology program with intranasal administration. Animals exposed to GW685698X by inhalation administration (up to 6 months in rats and up to 9 months in dogs), and by intranasal administration (2 weeks in rats, one month and 6 months in dogs) showed typical glucocorticoid effects: decrease of body weight gain or body weight loss, changes in clinical pathology (decrease of WBC/lymphocyte counts, increase of RBC count, increase of blood cholesterol and/or triglycerol levels), and histopathology findings of adrenal atrophy (dogs only), lymphoid depletion (thymus, spleen, lymph nodes, and lymphoid tissues of lung and nasal passage), fatty bone marrow, hair loss and dermal thinning, increased liver glycogen deposition, and hepatocyte rarefaction and/or hypertrophy (dogs only). Pituitary acidophilic cells, increased adipose cell infiltrations in heart, pancreas, parotid salivary gland and skeletal muscles, and epiphyseal plate retention were also observed in dogs after 39 weeks of treatment. Incidence and severity of the glucocorticoid effects are dose and treatment duration dependent.

Beside the typical glucocorticoid findings, non-typical glucocorticoid effects were also observed in animals and these effects include findings of increased eosinophilic inclusions in bronchiolar epithelium of rats, and dog findings of focal nephropathy (tubular basophilia and increased mitotic figures), changes in blood chemistry parameters (increase of alkaline phosphatase, gamma glutamyl transferase and/or glutamyl dehydrogenase), biliary tract (bile duct and gall bladder) epithelial vacuolation, airway inflammatory lesions, nasal cavity goblet cell hypertrophy (dog intranasal studies only)

and chronic stomach inflammation. Most of these findings were of a minimum to slight degree.

None of the above drug related findings are of significant clinical concern for the proposed route of administration because of the extensive clinical experience (typical steroid effects), the minimal severity of findings and their lack of clinical significance (eosinophilic inclusions in rat lungs, biliary tract epithelial vacuolation), irrelevancy to clinical route of administration (airway inflammation in dog inhalation study as compared to human intranasal use), the ability to monitor findings clinically (nasal cavity goblet cell hypertrophy) or sufficient safety margins (nephropathy, 29-fold; chronic stomach inflammation, 132-fold for the proposed clinical maximum dose of 110 mcg/day) based on AUC values.

In a standard battery of genotoxicity tests, GW685698X was negative in an Ames test, a mouse lymphoma assay and in rat micronucleus assays under the conditions tested. Two-year inhalation carcinogenicity studies in rats and mice revealed that GW685698X is not tumorigenic.

Reproductive toxicity studies demonstrated that GW685698X is devoid of effects on fertility and early embryo-fetal development in rats, embryo-fetal development in rabbits, and prenatal and postnatal development in rats.

In Guinea pigs, GW685698X does not induce respiratory hypersensitivity reactions.

B. Pharmacologic activity

In vitro, GW685698X showed a high binding affinity to glucocorticoid receptors (kd=0.3 nM), potent glucocorticoid receptor agonist effects such as transrepression of TNFalpha-induced NFKB response (pIC50=10.5), and transactivation of MMTV-luciferase reporter (pIC50=9.9). In cultured cells, GW685698X protected bronchial epithelial cells from protease- and mechanically-induced cellular damage as measured by transcutaneous electrical resistance and cell membrane permeability, and inhibition of LPS-induced TNF release from PBMCs.
In rats and mice, topical application of GW685698X inhibited oxazolone-induced ear skin delayed type hypersensitivity (DTH) responses as measured by ear swelling (0.001 mcg reduced 50% in mice; 5 mcg reduced 75% in rats). In ovalbumin induced rat lung eosinophilia model, intratracheal administration of GW685698X (30 mcg/rat) inhibited 75% of lung eosinophilia. Lastly, intranasal administration of 10 mcg GW685698X to rats resulted in 60-70% reduction of egg albumin induced allergic symptoms (nasal rubbing and sneezing).

C. Nonclinical safety issues relevant to clinical use
None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-051
Review number: 1
Sequence number/date/type of submission: 000/6/28/2006/original, 10/05/06/BP, 10/18/2006/SU, 12/20/2006/BP

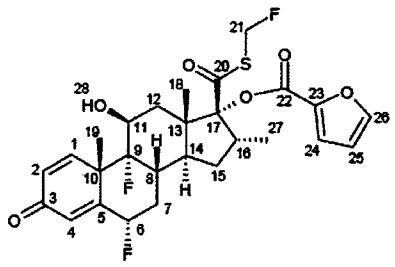
Information to sponsor: Yes (X) No ( )

Sponsor and/or agent: GlaxoSmithKline
Manufacturer for drug substance: Glaxo Operations UK Limited (trading as Glaxo Wellcome Operations), Harmire Road, Barnard Castle Co. Durham DL12 8DT, United Kingdom

Reviewer name: Huiqing Hao, Ph.D.
Division name: Pulmonary and Allergy Products
Review completion date: 3/2/2007

Drug:

Trade name: Proposed tradenames are Veramyst and ———.
Generic name: Fluticasone Furoate Nasal Spray
Code name: GW685698X
Chemical name: CAS name- Androsta-1,4-diene-17-carbothioic acid, 6,9-difluoro-17-[(2-furanylcarbonyl)oxy]-11-hydroxy-16-methyl-3-oxo-, S-(fluoromethyl) ester, (6α,11β,16α,17α)- (9CI)
CAS registry number: 397864-44-7
Molecular formula/molecular weight: 538.58
Structure:



Relevant INDs/NDAs/DMFs: IND 48,647 (intranasal use) and IND 70,297 (inhalation use)

Drug class: Corticosteroid

Intended clinical population: Adults and children 2 years of age and older with symptoms of seasonal and perennial allergic rhinitis. The usual starting dosages are proposed to be 110 mcg (2 sprays per nostril) once daily for adults and adolescents ≥12 years and 55 mcg (1 spray per nostril) once daily in children 2-11 years.

Clinical formulation: Aqueous suspension, with the composition presented below:

Component	27.5 Micrograms/spray					
	Theoretical Quantity per Spray through Nozzle (mg)	(% w/w)	Amount per Container (mg)		Function	Reference to Standard
			120 Sprays	Sprays		
Active Ingredient						
Fluticasone Furoate (micronised)	0.0275				Active	GlaxoSmithKline
Other Components						
Dextrose Anhydrous						USP
Microcrystalline Cellulose and Carboxymethylcellulose Sodium [1]						NF
Polysorbate 80						NF
Benzalkonium Chloride Solution [2]						NF
Edetate Disodium						USP
Purified Water						USP

Notes:
1.
2.
3. Benzalkonium chloride equivalent to mg/spray and %w of suspension.

Route of administration: Nasal spray

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

	Study No
Pharmacodynamics	
The in vitro preclinical pharmacology of GW685698X, a potent and selective glucocorticoid receptor agonist	SR2006/00001/01
Characterisation of binding to the glucocorticoid receptor, inhibition of the NFkB pathway, association with lung cells and tissue and in vitro duration of action for GW685698X	SH2005/00036/00
Protection Against Protease- and Mechanically-induced Cellular Damage and Inhibition of LPS-induced TNF Release by GW685698X and Other Glucocorticoids	WM2006/00016/00
The Effect of GW685698X on the Translocation of the Glucocorticoid Receptor from the Cytoplasm to the Nucleus of Live Cells	SH2006/00012/00
Comparative effect of fluticasone furoate and fluticasone propionate on nasal symptoms in allergic rhinitis models in rats	RD2006/01507/00
Pharmacokinetics	
Investigation of the Binding of [14C]-GW685698 in Human Plasma and Selected Plasma Proteins In Vitro	WD2005/01123/00 (05DMW083)
Quantification and identification of the circulating metabolites of GW685698X in the	WD2005/00557/00

male CD-1 mouse following a single intravenous administration of [14C]-GW685698 at a target dose of 1mg/kg	(04DMW106)
Investigation into the long term stability of GW685698 in human plasma	WD2006/01727/00 (YAY/031)
Characterization of the major metabolites of GW685698X following a single oral administration and a single intravenous administration of [14C]-GW685698 to healthy adult male subjects	WD2005/01496/00 (05DMW004)
Pharmacokinetic drug interaction	
An In Vitro Investigation into the Inhibition by GW685698X and a metabolite GW694301X of Xenobiotic Transport via Human OATP1B1 Heterologously Expressed in CHO Cells	WD2005/00763/00 (05DMW042)
An In Vitro Investigation of the Inhibition by GW685698X and GW694301X of Xenobiotic Transport via Human P- Glycoprotein, Heterologously Expressed in MDCKII Cells	FD2005/00368/00 (05DMF052)
An in vitro investigation of both the transport via heterologously expressed human P-glycoprotein and the passive membrane permeability of GW685698X in MDCKIIMDR1 Cells	WD2006/00293/00 (04DMW085)
The inhibition of human cytochrome P450 enzymes by GW694301 in vitro	WD2005/00543/00 (05DMW006)
Toxicology	
GW685698X: Additional pathology investigations to a toxicity study by inhalation administration to Wistar rats for 13 weeks	WD2006/01990/00 (V26805)
GW685698X: Additional pathology investigations to a toxicity study by inhalation administration to Wistar rats for 26 weeks	WD2006/01991/01 (V26806)
GW685698X: Additional investigations into target organ toxicity using tissues generated from study R24142	WD2006/01906/00 (V26765)
GW685698X: Additional pathology investigations to a carcinogenicity study by inhalation administration	WD2006/01992/00 (V26862), 10/18/06 submission
Genotoxicity	
GW685698X: Additional rat bone marrow micronucleus assay in rats	WD2006/02023/00 (R26808)
Carcinogenicity study	
GW685698X Carcinogenicity study by inhalation administration to CD-1 mouse for 104 weeks	WD2005/00894/01 (M24141)
GW685698X Carcinogenicity study by inhalation administration to Han Wistar rats for 104 weeks	WD2005/00895/02 (R24142)
Local tolerance	
GW685698X: Primary eye irritation study in rabbits	RD2005/00377/01 (L41628)
Special toxicology	
GW685698X: A 5- Day Inhalation Tolerability Study of a Powder Aerosol Formulation in the Guinea Pig Followed by an Immunologic Sensitization Potential Study in Guinea Pigs via Inhalation	CD2005/00215/00 (G05086)

Studies not reviewed within this submission:

The following studies were previously reviewed under INDs 48,647 or 70,297 as listed in the table below:

Study Title	Study No	Review location
Pharmacodynamics		
The effects of GW685698X in human NFkB, human AP1, human GRE, human MMTV, rat TAT, human bronchial IL8, human estrogen receptor, human progesterone receptor, human mineralocorticoid receptor and human andrenogen receptor functional assays	SH2002/00038/00	48647, Rev. 1
The effects of GW685698X in oxazolone induced mouse ear skin delayed type hypersensitivity model	SH2003/00031/00	48647, Rev. 1
The effects of GW685698 in the rat ovalbumin induced lung eosinophilia model, oxazolone induced rat ear skin delayed type hypersensitivity model and the rat model of thymus involution	SH2002/00044/00	48647, Rev. 1
Safety pharmacology		
GW685698X Safety Pharmacology: Overt central and peripheral pharmacodynamic effects following acute subcutaneous administration in conscious Wistar Han Rats	WD2001/00889/00 (R23287)	48647, Rev. 1
GW685698X Safety Pharmacology: Overt central and peripheral pharmacodynamic effects following acute subcutaneous administration in the conscious dog	WD2002/00077/00 (D23351)	48647, Rev. 1
GW685698X: Evaluation of tissues from dogs from overt central peripheral pharmacodynamic effects following acute subcutaneous administration in the conscious dog	FD2002/00012/00 (I01693)	48647, Rev. 1
GW685698X: Evaluation of effect on respiration in the unrestrained conscious rat following single subcutaneous administration	FD2001/00004/00 (G01654)	48647, Rev. 1
Single subcutaneous dose cardiovascular study in rats	FD2002/00033/00 (G01646)	48647, Rev. 1
Investigation of single intravenous dose in the dog	FD2002/00019/00 (I01702)	48647, Rev. 1
GW685698X: Single intravenous dose cardiovascular study in dogs	FD2002/00011/01 (G01668)	48647, Rev. 1
GW685698X Safety Pharmacology: Effect of GW685698X on action potential parameters in dog isolated cardiac purkinje fibres	WD2001/01020/00 (V23207)	48647, Rev. 1
Absorption		
The pharmacokinetics of 3H-GW685698 in the Wistar Han Rat following a single oral dose of 3H-GW685698 (0.1mg/kg)	WD2001/00769/00 (B30535)	48647, Rev. 1
The pharmacokinetics of 3H-GW685698 in the Wistar Han Rat following a single intravenous dose of 3H-GW685698	WD2001/00701/01 (B30534)	48647, Rev. 1

(0.1mg/kg)		
The pharmacokinetics of 3H-GW685698 in the Wistar Han rat following a single subcutaneous dose of 3H-GW685698 (0.1mg/kg)	WD2001/00875/00 (B30536)	48647, Rev. 1
Pharmacokinetics of GW685698X in Wistar Han rat following subcutaneous administration of GW685698X in various formulations	WD2001/00846/00 (B30485)	48647, Rev. 1
The pharmacokinetics of GW685698X in the female New Zealand White Rabbit following single oral and single intravenous administration of GW685698X at 100 µg/kg	WD2001/01091/00 (B30756)	48647, Rev. 1
Single dose toxicokinetic study in the female rabbit by inhalation administration	WD2002/01053/00 (L23429)	48647, Rev. 1
The rates and routes of elimination of total radioactive drug-related material and the pharmacokinetics of 3H-GW685698 and total radioactive drug-related material in the beagle dog following a single intravenous, a single oral and a single subcutaneous administration at 0.1mg/kg bodyweight	WD2001/00850/00 (B30557)	48647, Rev. 1
The pharmacokinetics of GW685698X in the male and female beagle dog after a single subcutaneous dose at 1 mg/mg	WD2001/00692/01 (B30486)	48647, Rev. 1
Investigation of the plasma protein binding of 3H-GW685698 in human, beagle dog, New Zealand White Rabbit, Wistar Han Rat and CD-1 Mouse in vitro using an ultra-filtration method	WD2003/01268/00 (03DMW044)	48647, Rev. 1
The in vitro binding and distribution of GW685698X to plasma proteins and whole blood from rat, dog, rabbit, mouse and human	WD2001/00979/00 (B30588)	48647, Rev. 1
The tissue distribution of radioactivity in the albino (Wistar Han) and pigmented (Random Hooded) male rat following a single intravenous administration of 3H-GW685698 at 133 µg GW685698X/kg nominal dose	WD2001/00584/00 (B30493)	48647, Rev. 1
The tissue distribution of radioactivity in the albino (Wistar Han) and pigmented (Random Hooded) male rat following a single intravenous administration of 3H-GW685698 at 133 µg GW685698X/kg nominal dose	WD2001/00574/01 (B30511)	48647, Rev. 1
A qualitative assessment of the distribution of drug-related material using whole-body autoradiography following a single intravenous administration of [14C]GW685698X to male pigmented rats at a target dose level of 1 mg/kg	FD2003/00138/00 (1990/062)	48647, Rev. 1
Quantitative whole- body autoradiography following a single intravenous administration (30 minute infusion) of [14C]GW685698X to male pigmented rats at a target dose level of 1 mg/kg	FD2003/00264/00 (1990/161)	48647, Rev. 1
Metabolism		
Investigations in to the in vitro metabolism of GW685698X in liver microsome preparations	WD2002/00231/00 (B30625)	48647, Rev. 1
In vitro metabolism of GW685698X by cytochrome P450 enzymes in human liver microsomal preparations and individually expressed cytochrome P450 enzyme preparations	WD2002/00297/00 (B30739)	48647, Rev. 1

Metabolism of GW685698X by sphaeroplasts containing expressed human cytochrome P450 Enzymes	WD2002/00978/01 (B30879)	48647, Rev. 1
Investigations into the metabolism of GW685698X in vitro hepatocytes from man, rat and dog	WD2002/00003/00 (B30509)	48647, Rev. 1
In vitro investigations into the metabolism of [14C]GW685698 and [3H]GW685698 in human, mouse, rat, female rabbit and dog	WD2004/00004/00 (B30977)	48647, Rev. 8
The metabolism of 3H GW685698X in isolated perfused rat liver model (IPRL)	WD2003/00002/00 (B30878)	48647, Rev. 1
The metabolism of 14C-GW685698X in male isolated perfused rat liver model (IPRL)	WD2003/00828/00 (B30935)	48647, Rev. 1
Profiling of the major metabolites of GW685698X following incubation with high- density, complex, metabolically-active, anaerobic microbiotas of human, rat, and dog origin	WD2004/01155/00 (04DMW028)	48647, Rev. 8
The potential of GW685698X to inhibit cytochrome P450 activity in human hepatic microsomes in vitro	WD2001/00374/00 (B30575)	48647, Rev. 1
The inhibition of human cytochrome P450 enzymes by GW685698X in vitro	FD2003/00126/00 (B30972)	48647, Rev. 8
Investigation to determine the stability of GW685698X in whole blood at 37°C in mouse, rat, rabbit, dog and man.	WD2002/00516/00 (B30787)	48647, Rev. 1
Preliminary quantification and characterisation of the major metabolites of GW685698X following intravenous administration of [14C]GW685698 to the male rat	WD2004/01053/01 (B30976)	48647, Rev. 8
Profiling of the metabolites of GW685698X in the Wistar Han rat following a single intravenous administration of 3H-GW685698 at a dose of 1 mg/kg	WD2004/00040/00 (03DMW042)	48647, Rev. 8
The biliary excretion and metabolism of 3H GW685698 in male Wistar Han Rats (9 mg/kg iv, 6 mg/kg po)	WD2001/01130/01 (B30607)	48647, Rev. 1
Identification of the metabolites of GW685698X in the bile from biliary cannulated male Wistar Han rat following either a single intravenous or oral administration of [14C]-GW685698 at a target dose of 1 mg/kg	WD2004/01197/00 (03DMW114)	48647, Rev. 8
Preliminary quantification and characterisation of the major metabolites of GW685698X following intravenous administration of [14C]-GW685698 to the male dog	WD2004/01054/00 (03DMW069)	48647, Rev. 8
Profiling of the metabolites of GW685698X in the beagle dog following a single intravenous administration of 3H-GW685698 equivalent to 1 mg GW685698X/kg	WD2004/00036/00 (03DMW045)	48647, Rev. 8
The biliary excretion and metabolism of 3H-GW685698 in the male beagle dog (4.5mg/kg iv)	WD2001/01133/01 (B30678)	48647, Rev. 1
Excretion, quantification and identification of the metabolites of GW685698X in the bile and faeces from biliary cannulated beagle dog following either a single intravenous or oral administration of [14C]- GW685698 at a target dose of 0.25 and 0.5 mg/kg, respectively	WD2004/00983/00 (03DMW115)	48647, Rev. 8
The effect of GW685698X on hepatic levels of cytochrome P450 and related parameters in the male and female Wistar Han rat after administration by inhalation at 0 and nominally 64 µg/kg/day for 28 days	FD2002/00013/00 (—069)	48647, Rev. 1
Investigation into the Metabolic Products of GW685698X Present in Human Urine Following a Single Inhaled (4mg) or Intravenous (0.25mg) Administration of GW685698X	WD2002/00954/00 (B30901)	48647, Rev. 1
Excretion		
The rates and routes of elimination of 3H-GW685698 in the	WD2001/00854/00	48647,

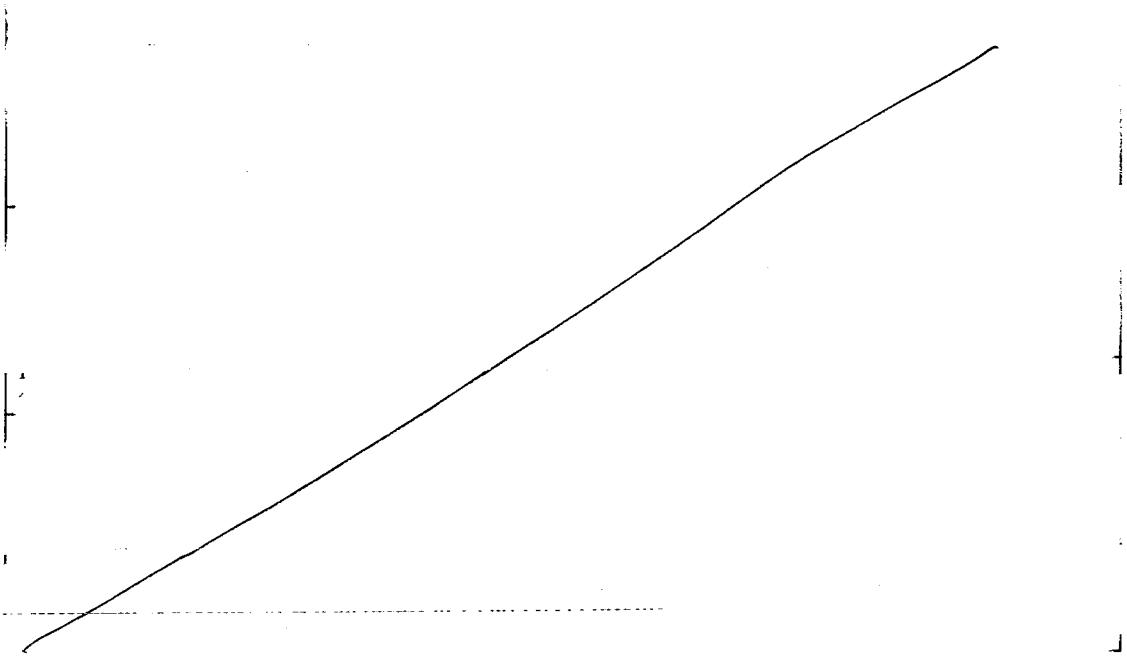
Wistar Han rat following a single oral administration of 3H-GW685698 equivalent to 0.1 mg GW685698X/kg	(B30537)	Rev. 1
The rates and routes of elimination of 3H-GW685698 in the Wistar Han Rat following a single intravenous administration of 3H-GW685698 equivalent to 0.1 mg GW685698X/kg	WD2001/00780/00 (B30530)	48647, Rev. 1
Investigation into the excretion of radioactivity following a single bolus intravenous administration (1.0 mg/kg) of 14C-GW685698X to the Wistar Han rat	WD2003/00127/00 (B30945)	48647, Rev. 8
Elimination of drug-related material following a single intravenous administration of [14C]GW685698X to male Sprague Dawley Rats at a target dose level of 1 mg/kg	FD2003/00374/01 ([redacted] 284)	48647, Rev. 8
The rates and routes of elimination of 3H-GW685698 in the Wistar Han Rat following a single subcutaneous administration of 3H-GW685698 equivalent to 0.1 mg GW685698X/kg	WD2001/00856/00 (B30553)	48647, Rev. 1
Excretion of radioactivity following a single intravenous bolus administration (0.1 mg/kg) of 14C-GW685698X to the male beagle dog	WD2003/00126/00 (B30946)	48647, Rev. 1
Elimination of drug-related material following a single intravenous administration of [14C]GW685698X to male beagle dogs at a target dose level of 0.1 mg/kg	FD2003/00241/00 ([redacted] 370)	48647, Rev. 8
Toxicology		
Single Dose Toxicity		
GW685698X (Corticosteroid receptor agonist): Acute oral toxicity study in the mouse	WD2001/00583/00 (M23212)	48647, Rev. 1
GW685698X (Corticosteroid receptor agonist): Acute intravenous toxicity study in the mouse	WD2001/00686/00 (M23209)	48647, Rev. 1
GW685698X (Corticosteriod receptor agonist) 30% w/w: acute inhalation toxicity study in CD-1 mice	WD2001/01017/00 (M23196)	48647, Rev. 1
GW685698X (Corticosteroid receptor agonist): Acute oral toxicity study in the Wistar Han rat	WD2001/00582/00 (R23211)	48647, Rev. 1
GW685698X (Corticosteroid receptor agonist): Acute intravenous toxicity study in the rat	WD2001/00936/00 (R23210)	48647, Rev. 1
GW685698X (Corticosteriod receptor agonist) 30% w/w: Acute inhalation toxicity study in Wistar Han rats	WD2001/01018/00 (R23195)	48647, Rev. 1
Repeat Dose Toxicity		
GW685698X: Study to determine the maximum repeatable inhalation dose in the CD-1 mouse	WD2002/01063/00 (M23601)	48647, Rev. 4
GW685698X: Toxicity study by inhalation administration to CD-1 mice for 13 weeks	WD2003/00100/00 (M23602)	preIND 48,647
GW685698X: A 14-day intranasal toxicity study in the Wistar Han rat	WD2004/00128/01 (R24885)	48647, Rev. 5
GW685698X (Corticosteroid receptor agonist): Maximum repeatable dose study in Wistar Han rats by inhalation administration for 7 days	WD2001/00459/00 (R23108)	48647, Rev. 1
GW685698X (Corticosteroid receptor agonist): 4-Week inhalation toxicity study in the Wistar Han rat	WD2001/01019/00 (R23246)	48647, Rev. 1
GW685698X (Corticosteroid receptor agonist): A further 30 day inhalation toxicity study in the Wistar Han rat	WD2002/00525/02 (R23525)	48647, Rev. 1
GW685698X; Toxicity study by inhalation administration to Wistar Han rats for 13 weeks	WD2003/00099/00 (R23603)	preIND 48,647
GW685698: Toxicity study by inhalation administration to Wistar Han Rats for 26 weeks	WD2003/01044/01 (R23653)	48647, Rev. 4
GW685698X/ GW857238X: A 28-day inhalation toxicity study of a powder aerosol formulation in the rat	WD2004/00655/00 (R24928)	48647, Rev. 5

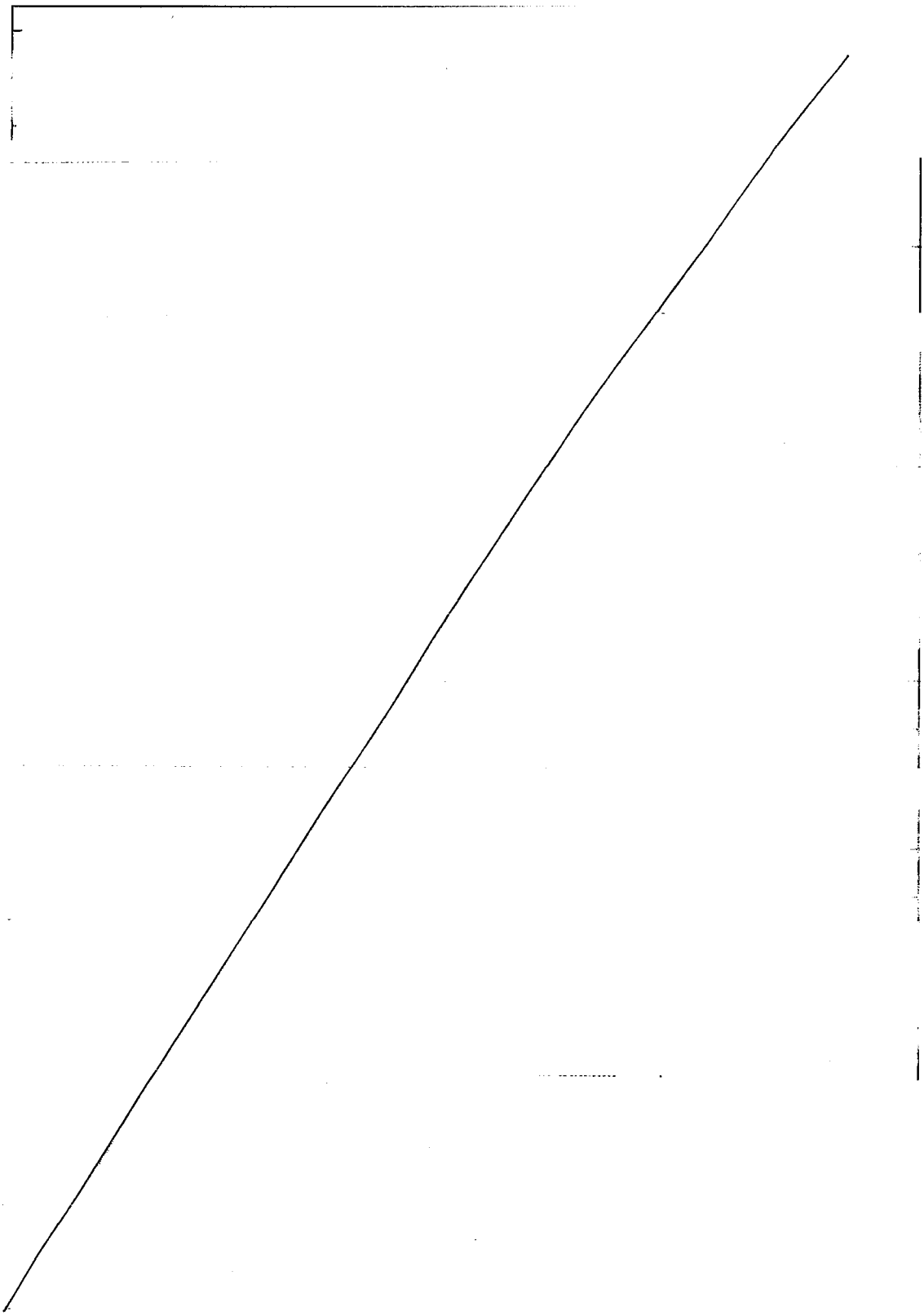
GW685698 and GSK159797C: Toxicity study by inhalation administration to rats for 4 weeks	WD2005/00299/00 (R25789)	70,297 Rev. 1
GW685698X: 28 Day Nasal administration toxicity study in the dog	WD2002/01366/01 (D23901)	48647, Rev.1
GW685698X: 26 week intranasal administration toxicity study in the dog with a 13 week interim kill	WD2004/01625/00 (D25132)	48647, Rev. 4
GW685698X: 7-Day maximum repeatable dose inhalation study in dogs	WD2001/00685/00 (D23109)	48647, Rev. 4
GW685698X: 7-Day inhalation tolerability study of a powder formulation in the beagle dog	WD2002/00980/00 (D23515)	48647, Rev. 4
GW685698X (Corticosteriod receptor agonist): 4-week inhalation toxicity study in the dog	WD2001/01015/00 (D23245)	48647, Rev. 4
GW685698X: A 28- day inhalation toxicity study of a powder formulation in the beagle dog (with 28-day recovery period)	WD2002/00981/00 (D23514)	48647, Rev. 4
GW685698X; Toxicity study by once daily inhalation administration to beagle dogs for 13 weeks	WD2003/00645/00 (D23588)	48647, Rev. 4
GW685698X: A 39- week inhalation toxicity study of a powder formulation in the beagle dog	WD2004/00523/01 (D24159)	48647, Rev. 4
Review of the Occurrence of the Morphologic Changes in Kidneys from Control Dogs, as Well as from Treated Groups of Project Number 78232	WD2005/01501/00 (D24159)	48647, Rev. 7
Pathology working group (PWG) report on epithelial vacuolation in bile ducts and gall bladder in the beagle dog after administration of GW685698X	Not designated	70,297 Rev. 4
2 Week study to examine the influence of GW857238X on the inhalation toxicology and toxicokinetics of GW685698 in beagle dogs	WD2004/00820/00 (D25157)	I70297 Rev. 1
GW685698X and GSK159797C: A 28 day inhalation toxicity study of a powder aerosol formulation in the beagle dog	WD2005/00669/00 (D25790)	70297, Rev. 1
Genotoxicity		
GW685698X: Fluticasone furoate; Ames agar plate assay with Salmonella typhimurium and Escherichia coli	WD2001/01058/00 (V23233)	48647, Rev. 1
GW685698X: Fluticasone furoate; mammalian cell mutation test at the thymidine locus in mouse lymphoma L5178Y cells	WD2001/01059/01 (V23249)	48647, Rev. 1
GW658698X: Corticosteroid receptor agonist; An investigation of its potential to induce micronuclei in the bone marrow of Han Wistar rats after intravenous administration	WD2002/00528/00 (R23581)	48647, Rev. 1
GW685698X: repeat intravenous rat bone marrow micronucleus assay	WD2004/00558/01 (R25156)	48647, Rev. 5
Reproductive and developmental toxicity		
Fertility and early embryonic development toxicity		
GW695698X : A male fertility inhalation toxicity study in the Wistar Han rat	WD2003/01271/00 (R24208)	48647, Rev. 5
GW685698X Combined study of effects on fertility and embryo-fetal development in female Wistar Han rats by inhalation administration	WD2002/01055/00 (R23393)	48647, Rev. 4
Embryofetal development		

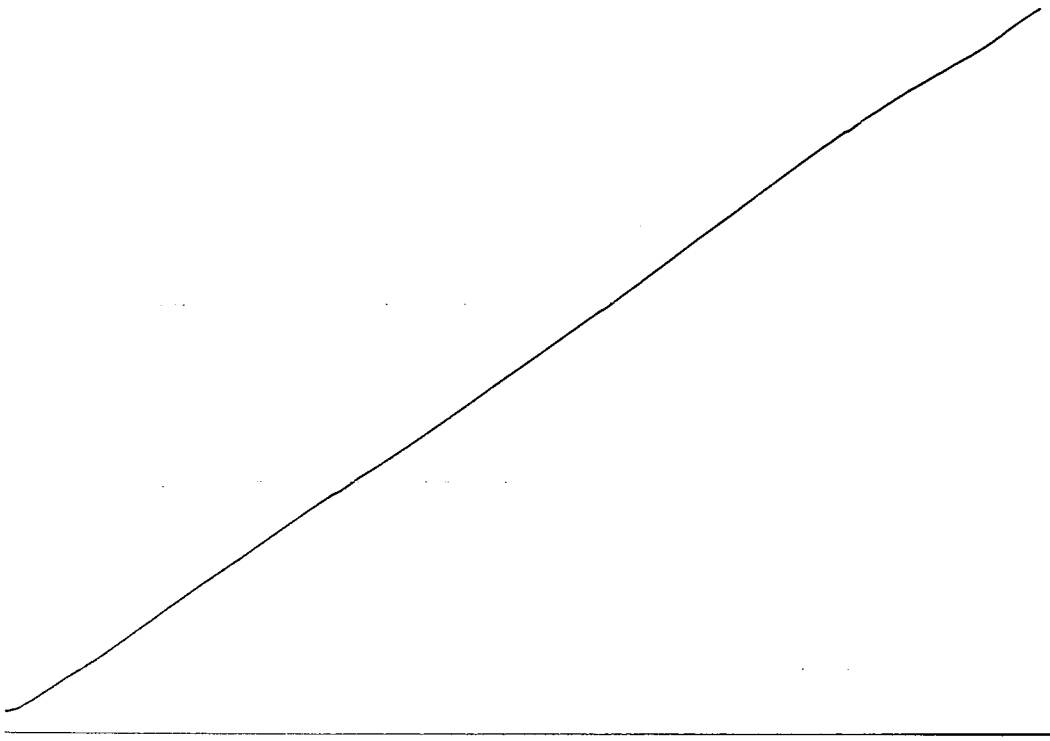
GW685698X: Preliminary embryo-fetal toxicity study in the rabbit by inhalation administration	WD2001/01016/00 (L23306)	48647, Rev. 4
GW685698X: Study of effects on embryo-fetal toxicity in the New Zealand White rabbit by inhalation administration	WD2002/00882/00 (L23338)	48647, Rev. 4
Prenatal and postnatal development, including maternal function		
A nose-only inhalation pre- and postnatal study of GW685698X in the rat	WD2003/01783/00 (R24209)	48647, Rev. 5
Local Tolerance		
GW685698X: Single dose oral irritancy study in male Sprague Dawley rats	WD2004/00866/00 (R25440)	48647, Rev. 5
GW685698X (Corticosteroid receptor agonist): 3 day intranasal irritancy study in the dog	WD2002/00304/00	48647, Rev. 5
Other		
GW685698X- Evaluation of increased eosinophilic inclusions in the bronchiolar epithelium observed post treatment of corticosteroid in rats	WD2004/00419/00 (V24494)	48647, Rev. 5
– **GW685698X: A Maximum Tolerated Dose Intranasal Study in** Juvenile Beagle Dogs	WD2003/00120/00 (D23996)	48647, Rev. 4

The following studies were not reviewed as they are considered irrelevant to the proposed indication, redundant to the studies already reviewed, or having no impact on the approvability of the NDA: the general toxicology studies included oral studies for fluticasone propionate alone, exploratory studies for combination of FF and beta-2 agonists, toxicity studies of beta-2 agonists, toxicity comparison of FF and other steroids in human bronchiolar epithelium in vitro, and FF dose ranging study in juvenile dogs.

Pharmacology	







2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

GW685698X (fluticasone furoate, FF) is a synthetic glucocorticoid compound with a high binding affinity to glucocorticoid receptors (kd=0.3 nM). In vitro studies demonstrated that FF induced glucocorticoid nuclear receptor translocation, has a potent glucocorticoid receptor agonist effects (transrepression of TNFalpha-induced NFKB response, and transactivation of MMTV-luciferase reporter), protected cells (human bronchial epithelium cell line) from protease- and mechanically-induced cellular damage, and inhibited LPS-induced TNF release from PBMCs. A study on steroid receptor selectivity showed that FF has high binding affinity and agonist effects to progesterone receptors. However, for other steroid receptors including androgen receptors, minerocorticoid receptors, and estrogen receptors, FF showed either low binding affinity or negative of receptor agonist effects. The above properties of FF are generally similar to fluticasone propionate.

In vivo anti-inflammatory effects of GW685698X were demonstrated in oxazolone-induced ear skin delayed type hypersensitivity (DTH) responses, topical application of GW685698X inhibited skin inflammation based on measured edema extent in mice (50% @ 0.001mcg and 100% @ 0.03 mcg) and rats (75% @ 5 mcg). Single intratracheal administration of GW685698X at 30 mcg to rats inhibited 75% of lung eosinophilia (50%

by fluticasone propionate 30 mcg) induced by ovalbumin. In a rat allergic rhinitis model, intranasal administration of GW685698X inhibited antigen induced nasal symptoms (50-70% inhibition of nasal rubbing and sneezing at combined intranasal doses of 1 and 10 mcg).

GW685698X produced no effects regarding CNS and respiratory functions. However, a slight increase of heart rate and decrease of blood pressure were seen in rats given 4 mg/kg subcutaneously, and wasting conditions (polyuria and muscle wasting) were seen in dogs given single subcutaneous doses of 4 and 10 mg/kg at 2-4 weeks postdosing.

2.6.2.2 Primary pharmacodynamics

Note: pIC50 (or pEC50) described in the following text represents negative log value of IC50 (or EC50) of molar concentrations. Therefore, there are no units associated with the endpoint.

Mechanism of action:

The precise mechanism through which GW685698X affects rhinitis symptoms is not known. However, as a glucocorticoid, the anti-inflammatory activities of GW685698X may contribute to its effects on rhinitis symptoms.

1. Binding with glucocorticoid receptors: The receptor binding method was not provided other than a reference to a publication which further referenced other publications (Hogger P et al, Steroids, 1994, 59, Oct., 597-602). The binding affinity of GW685698X with human glucocorticoid receptor is similar to fluticasone propionate (FP) and higher (30 fold) than Dexamethesone (see the data below, from Report SH2005/00036/00)

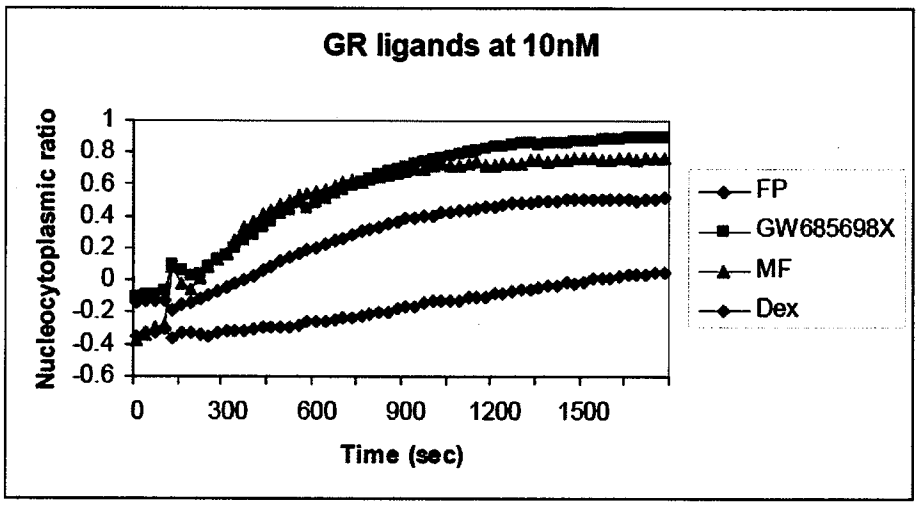
Glucocorticoid	k_1 x 10^5 (l/[mol/min])	k-1 x 10-4 [l/min]	kd [nmol/l]	$t_{½}$ [h]	RRA
Dexamethasone	10.53 ± 0.35	94.67 ± 5.43	8.80 ± 0.41	1.23 ± 0.04	100 ± 5
FP	21.17 ± 0.56	10.73 ± 0.65	0.51 ± 0.03	10.82 ± 0.64	1775 ± 130
GW685698X	37.46 ± 0.73	11.22 ± 0.62	0.30 ± 0.02	10.34 ± 0.59	2989 ± 135

Data is the mean and mean deviation of the mean of at least three independent experiments.
RRA stands for relative receptor affinity and is a measure of binding potency standardised to a value of 100 for dexamethasone.

2. Steroid receptor nuclear translocation (SH2006/00012/00): In live EYFP-GR COS-1 cells (enhanced yellow fluorescent protein-glucocorticoid receptor transfected COS-1 cells), different steroids (fluticasone propionate, fluticasone furoate (GW685698X), mometasone furoate (MF), and Dexamethasone) were compared on the rate of glucocorticoid receptor (GR) nuclear translocation and the maximal responses. Real time GR nuclear translocatin was preformed taking

images every 30 seconds for half an hour using a PMT (photomultiplier tubes) of 1000 and YFP (yellow flurosecence protein) filter of 10%; Analysis of results was conducted using the quantification function as part of the confocal software; The fluorescence intensity value of the cytoplasmic area (C) was subtracted from the one of the nuclear area (N) and then divided by the value of total fluorescence to calculate the nucleocytoplasmic ratio [(N-C)/(N+C)]. At a concentration of 10 nM, MF and GW685698X showed a greater nuclear translocation than FP and FP showed a greater nuclear translocation than Dexamethasone (see figure below). Compounds at 100 nM induced a GR nuclear translocation with similar translocation rate and maximal response (results not shown).

Nuclear translocation of EYFP-GR in live COS-1 cells at 10 nM steroids



3. Glucocorticoid receptor agonist effects in vitro: Effects of transrepression of TNFalpha induced NFKB response in A549 SPAP cells, and transactivation of MMTV-luciferase reporter (MMTV, mouse mammary tumor virus promoter) in A549 cells were studied (Report SR2006/00001/01). The potency of GW685698X (FF) is similar to FP, but higher than Dexamethasone (see the table below).

Compound	NFKB-agonist, pIC50 ±SD	GR-MMTV agonist, pEC50±SD
FF	10.5±0.3	9.9±0.2
FP	10.4±0.3	9.7±0.2
Ciclesonide	9.7±0.2	9.2±0.2
MF	10.6±0.2	10.1±0.2
Dexamethesone	8.9±0.1	8.2±0.1
Global data for Dexamethasone	9.0 ± 0.1	8.2 ± 0.1

FF, fluticasone furoate; FP, fluticasone propionate; ciclesonide, ciclesonide active principle; MF, mometasone furoate.

Inhibition of TNFalpha induced NFKB response in human A549 cells were also observed in another study for GW685698 and comparators (SH2005/00036/00) and the potency for each of compound was similar to that presented above.

An earlier study (SH2002/00038/00, review 1) has compared GW685698X with FP, MF and metabolite of GW685698X on a series of glucocorticoid receptor agonist effects (transrespression of TNFalpha induced NFKB, and EGF induced AP1 activity, transactivations of GRE-luciferase readout, MMTV-luciferase readout and rat hepatocyte GRE driven tyrosine aminotransferase activity) which are not presented in this review because the sponsor reported that the data underestimates the activity of the test drug due to a solubility problem (drug precipitation was observed in aqueous solution).

The primary metabolite of GW685698X, M10 (GW694301) demonstrated minimal glucocorticoid effects. M10 showed a weak inhibition on TNFalpha induced NFKB response (pEC50 values of 6.57 and 5.9 in Study SH2005/00036/00 and SH2002/00038/00, respectively). Compared to the parent compound, GW694301 was found at least 6000-fold less potent in inhibition of pro-inflammatory activity (transrepression of NFKB and AP-1 responses, and transactivation of GRE-Luciferase readout, MMTV-Luciferase readout, rat TAT induction) (IND 48,647 review-1 for Study SH2002/00038/00).

4. Steroid receptor selectivity (SR2006/00001/01)

a). Androgen and estrogen receptors: GW685698X has an affinity to androgen receptor which is similar to that of FP and MF. Androgen agonist response determined by androgen receptor (AR) transactivation assay using MMTV luciferase reporter showed that GW685698X and FP have no agonist effects while MF and ciclesonide showed partial agonist responses as compared to reference compound DHT. As to estrogen receptors (ER), none of the steroids tested showed affinity for either ERalpha or ERbeta.

GW685698X binding and activity on androgen and estrogen receptors in comparison with its comparators

Compound	AR binding –**FP, pIC50** ±SD	AR-agonist, pIC50 ±SD	ERalpha-SPA, pIC50 ±SD	ERbeta-SPA, pIC50 ±SD
FF	6.4±0.3	<5	<5.0	<5.0
FP	5.9±0.3	<6	<6.0	<6.0
Ciclesonide	Not tested	6.2±0.4	<5.0	<5.0
Mometasone	6.9±0.1	6.7±0.6	<5.0	<5.0
DHT (ref.)	8.3±0.1	7.9±0.3		
DHT global data	8.3 ± 0.1	8.0 ± 0.3		
Estradiol (ref.)			8.6±0.2	8.5±0.1
Estradiol global data			8.6 ± 0.2	8.5 ± 0.1

b). Mineralocorticoid receptors (MR) and progesterone receptors (PR):
GW685698X has a minimum activity (pIC50=7.6) in the MR transactivation assay using the MMTV luciferase reporter agonist mode (see table below). The potency is similar to fluticasone propionate. Mometasone and ciclesonide showed higher potency in the MR agonist assay. In the MR antagonist assay, GW685698X does not have quantifiable activity, similar to MF or ciclesonide. FP partially inhibited the agonist response.

GW685698X has a high binding affinity for the human PR, and this activity is similar to FP, ciclesonide and MF. The comparison of binding affinity to glucocorticoid receptors and progesterone receptors could not be presented because of the limit of assay (test compound lying on the tight binding limit resulted in an underestimate of the true affinity). Consistent with the binding affinity, GW685698 and FP showed a potent PR agonist effect (PRb transactivation assay using MMTV luciferase reporter). Ciclesonide is less potent while MF is more potent in this assay. Similar results were observed in the second PR agonist assay (T47D cells using the endogenous alkaline phosphatase reporter).

GW685698X binding and activity on mineralocorticoid receptors and progesterone receptors in comparison with its comparators

Compound	MR agonist, pIC50 ±SD	MR antagonist, pIC50 ±SD	PR binding#, pIC50 ±SD	PRb agonist, pEC50 ±SD	PR agonist (T47D)*, pEC50 ±SD
FF	7.6±0.4	<5	7.9±0.3	9.0±0.2	9.0±0.2
FP	7.6±0.4	7.3	7.6±0.5	9.0±0.4	8.6±0.2
Ciclesonide	8.7±0.4	<5	7.3	8.4±0.4	NA
MF	9.3±0.4	<5	8.3±0.1	10.7±0.3	9.7
Aldosterone	10.0±0.2				
Aldosterone Global data	10.0 ± 0.4				
GSK259492		6.6±0.1			
GSK259492 global data		6.7 ± 0.2			
CCI5122			8.3±0.2	9.4±0.2	8.9±0.3
CCI5122 global data			8.2 ± 0.2	9.4±0.2	8.9±0.3

Reference compounds: aldosterone for MR agonist assay; GSK259492, for the MR antagonist assay; CCI5122 (progesterone) for the PR binding and PR agonist assays. *, Single experiment data

#, The tight binding limit for this assay is approximately 10 nM. This compound lies on the tight binding limit indicating that the value is an underestimate of the true affinity at PR

Drug activity related to proposed indication:

1. Protection against protease- and mechanically-induced cellular damage and inhibition of LPS-induced TNF Release (WM2006/00016/00):

a). Effects on protease induced damage: In cultured 16HBE14o epithelial cells, cell damage was measured by decreases of transcutaneous electrical resistance (TER). Exposure of the epithelial cells to GW685698 (0.1 to 10 nM) increased TER in healthy cells and prevented the elastase (5 mcg/mL, from human sputum) induced TER reduction (see the tow tables below). GW685698X, fluticasone propionate and mometasone furoate had similar potency with EC_{50} values of approximately 25 pM, whereas dexamethasone was less potent with an EC_{50} of 2 nM (table below).

TER values (mean±SD) of cell monolayers 24 hours after application of steroids calculated as a percentage of the individual start values and expressed as % of vehicle control value (n=4)

Steroid, nM	FP	GW685698X	MF	Dexamethasone
Vehicle	100±7	100±7	100±7	100±8
0.001	104±9	105±7	105±3	
0.01	113±7	104±3	107±2	
0.1	140±4	154±12	132±14	102±5
1	160±11	151±14	139±8	113±5
10	171±11	146±15	147±3	144±10
100				152±19
1000				142±20

TER values (mean±SD) of cell monolayers at 24 hours following application of 5 mcg/mL elastase to the basolateral bathing medium (steroids were applied 24 hours prior to applications of elastase). Of note, there is no information provided regarding the units of the values.

Steroid, nM	FP	GW685698X	MF	Dexamethasone
Vehicle	0	0	0	0
0.01	0	0	0	
0.1	4±5	73±62		
1	153±13	140±31	103±39	
10	120±40	139±35	135±24	111±23
100				140±18
1000				126±16

Addition of elastase to the basolateral surface of the cells resulted in the cell monolayer becoming detached from the underlying membrane and rolling up as a complete sheet. All of the steroids tested, protected against this cell detachment. The relative order of potency was the same as that seen in TER values.

The same relative order of potency was also seen with inhibition of IL8 spontaneous secretion from the cells and reduction of permeability of normal 16HBEa2 cells (see the data below).

Table 4 IL-8 secretion into the basolateral bathing medium from cell monolayers 24 hours after application of steroids

	FP		GW685698X		MF		Dexamethasone	
[steroid](nM)	mean	sd	mean	sd	mean	sd	mean	sd
Vehicle	452	25	516	47	437	29	508	15
0.001	410	66	461	38	418	30		
0.01	401	20	388	21	418	25		
0.1	223	22	189	10	204	12	452	33
1	164	10	169	9	168	16	397	43
10	148	8	147	4	143	19	205	7
100							172	7
1000							172	11

Table 5 Effect of steroids on 16HBEa2 epithelial permeability: Experiment 1

[Steroid], nM	FP				GW685698X			
	Flux, pmol/h	sd	TER kΩ	sd	Flux, pmol/h	sd	TER kΩ	sd
0.003	151.53	0.24	0.405	0.014	114.51	19.43	0.496	0.057
0.01	135.36	19.80	0.435	0.044	84.47	11.95	0.583	0.076
0.10	99.60	11.82	0.511	0.058	46.20	1.52	0.794	0.050
1	67.66	10.76	0.633	0.059	66.40	11.08	0.619	0.058
10	47.21	21.67	0.773	0.151	53.73	11.02	0.708	0.068
0	148.22	7.55	0.442	0.021				
[Steroid], nM	Budesonide				Dex			
	Flux, pmol/h	sd	TER kΩ	sd	Flux, pmol/h	sd	TER kΩ	sd
0.03	151.32	5.91	0.465	0.015				
0.10	135.45	9.94	0.406	0.057				
1	99.93	10.26	0.537	0.009	132.12	17.45	0.468	0.024
10	69.34	4.59	0.655	0.014	71.44	5.32	0.629	0.019
100	89.38	7.2	0.558	0.014	60.81	5.01	0.666	0.032
1000					62.22	17.92	0.586	0.020
10000					74.65	6.13	0.637	0.039

Table 6 Effect of steroids on 16HBEa2 epithelial permeability: Experiment 2

[Steroid], nM	FP				GW685698X			
	Flux, pmol/h	sd	TER kW	sd	Flux, pmol/h	sd	TER kW	sd
0.003	58.74	8.13	0.766	0.039	52.06	0.95	0.865	0.025
0.01	49.76	3.74	0.843	0.023	45.30	3.18	0.909	0.045
0.10	34.50	5.18	1.070	0.081	28.96	3.41	1.184	0.106
1	28.98	2.08	1.163	0.036	29.66	1.85	1.204	0.097
10	42.44	2.07	0.937	0.033	25.86	4.28	1.307	0.159
0	67.89	3.79	0.720	0.017				
[Steroid], nM	Budesonide				Dex			
	Flux, pmol/h	sd	TER kW	sd	Flux, pmol/h	sd	TER kW	sd
0.03	63.94	4.56	0.761	0.031				
0.10	58.34	4.92	0.787	0.032				
1	37.37	2.75	1.032	0.051	47.56	5.69	0.930	0.041
3					44.86	13.00	0.945	0.154
10	25.48	3.41	1.306	0.143	36.83	4.90	1.039	0.108
100	32.53	4.64	1.098	0.087	30.44	1.66	1.123	0.055
1000					29.63	3.97	1.170	0.083

b). Effects on mechanical damage (by a wounding device): Morphological changes in 16HBE14o epithelial cells (irregular central island of cells) were induced by mechanic damage. With increasing FF concentrations (0.001-10 nM) the central island of epithelium is better preserved and the wound area decreases until its width is equivalent the diameter of the wound probe. The rank order of potency was the following: GW685698X>FP>mometasone furoate >budesonide >Dexamethsaone >BMP (beclomethasone monopropionate).

c). LPS-induced TNFalpha release from PBMCs was inhibited by steroids. PBMC was treated with LPS (1ng/mL) and appropriate FF concentrations (the series of concentration started with 1 mcM, no further information was provided) and incubated for 20 hours at 37°C. TNF in the supernatant was determined by electrochemiluninescence assay using the —— technology. The potency (pIC50) observed was GW685698X 9.93, FP 9.63, budesonide 8.41, ciclesonide-active principle 8.5, and prednisolone 7.23.

2. In vivo study: In mouse and rat oxazolone-induced ear skin delayed type hypersensitivity (DTH) responses, topical application of GW685698X inhibited the inflammatory response based on the ear skin swelling reduction (50% @ 0.001 mcg and 100% @ 0.03 mcg in mice and 75% @ 5 mcg in rats). Single intratracheal dose of GW685698X at 30 mcg inhibited lung eosinophilia by 75% (50% by fluticasone propionate 30 mcg) in ovalbumin-induced rat lung eosinophilia model (review 1 for IND 48,647 original submission).

3. In vivo study: In a rat egg albumin induced allergic rhinitis model (RD2006/01507/00), intranasal administration of GW685698X inhibited antigen induced nasal symptoms (nasal rubbing decreased approximately 47% and 62% at 1 mcg and 10 mcg, respectively; sneezing decreased approximately 56 and 70% at 1 mcg and 10 mcg, respectively) with comparable potency and longer duration compared with fluticasone propionate. Significant inhibition of the nasal rubbing and

sneezing were sustained up to 12 hours postdoing for GW685698X, whereas the inhibition on sneezing had disappeared by 12 hours for fluitcasone propionate.

2.6.2.3 Secondary pharmacodynamics
No studies were performed.

2.6.2.4 Safety pharmacology
All safety pharmacology studies were submitted and reviewed under IND 48,647 (review 1 for the original submission). The following is a brief summary of the safety pharmacology studies.

Neurological effects:
Neurological effects of GW685698X were tested in rats and dogs using modified Irwin test. Within 48 hours of observation period, there were no changes in behavior, skeletal tone, reflexes or overt gastrointestinal (abnormal defecation), neurological and autonomic activities (salivation, lacrimation) in rats or dogs given s.c. doses of 4 and 10 mg/kg (plasma levels at 1.66 to 4.13 ng/ml in rats and 1.39 to 2.88 ng/ml in dogs).

Cardiovascular effects:
Subcutaneous administration of single dose GW685698X (4 mg/kg) to rats resulted in mild increase of mean arterial blood pressure (12.1 mmHg, 24 hours post-dosing) for 14 days, and slightly reduced heart rate (-15 bpm, 7 days post-dosing) for 11 days. Intravenous doses of GW685698X at 0.03 and 0.1 mg/kg in dogs caused transient (15 min) cardiovascular effects in both the drug treated and control dogs (decreased arterial blood pressure 46 mmHg and increased heart rate 40 bpm) and these effects are probable not drug related. Investigation in isolated dog Purkinje fibers revealed that GW685698X up to 2200 pg/ml had no effects on QT interval.

Pulmonary effects:
Conscious rats received 4 or 10 mg/kg of GW685698X subcutaneously were not observed with changes in respiratory parameters (respiratory rate, peak inspiration and expiration flow, inspiration and expiration times, minute volume and tidal volume).

Renal effects: Dogs given GW685698X (4 or 10 mg/kg) subcutaneously demonstrated declining conditions in week 2 and thereafter. Polyuria and muscle wasting became apparent in 2-4 weeks and 4-5 weeks postdosing, respectively. Rapid progression in the severity of this condition resulted in the animals being killed in extremis 5 weeks post-dosing. Plasma drug concentration examined post mortem were 0.65 and 1.4 ng/ml for the doses of 4 and 10 mg/kg.

Gastrointestinal effects:
There were no gastrointestinal effects (abnormal defecation) or autonomic effects (salivation, lacrimation) in rats or dogs given s.c. doses of 4 and 10 mg/kg (plasma levels at 1.66 to 4.13 ng/ml in rats and 1.39 to 2.88 ng/ml in dogs).

Abuse liability: No information provided

Other: N/A

2.6.2.5 Pharmacodynamic drug interactions
No studies were performed.

2.6.3 PHARMACOLOGY TABULATED SUMMARY
N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary
Oral absorption in animals were essentially complete, but due to an extensive first-pass effects, systemic exposures to the parent compound were minimal (bioavailability of parent drug is approximately 1-4% in rats and rabbits).

Intravenous administered 3H-GW685698X was distributed rapidly and widely with a volume of distribution of 8L/kg in rats and 10 L/kg in dogs. In rats received an i.v. dose, the radioactive drug related material (RDM) levels in most tissues were higher than corresponding blood levels within 24 hours postdosing, and the highest levels at 0.5 hour postdosing were seen in the small intestine and small intestine wall demonstrating biliary clearance. At 168 hours post-dosing, levels of radioactivity were only detectable in the liver and kidney cortex. Following a single oral dose of 3H-GW685698X to rats, RDM was not widely distributed, with liver, kidney, spleen and GI tract being the only tissues with higher levels than the blood in 1-24 hours post-dosing. The circulating drug-related materials were predominantly associated with the plasma fraction and plasma protein binding was 99% in humans, rabbits, mice and dogs, and 98% in rats.

The major metabolic route of GW685698X was loss of the fluoromethyl carbothioate group to form the carboxylic acid GW694301X (M10). All other metabolites are formed from M10. The second metabolic event often observed is oxidative defluoronation in the C6 position which occurred in M10 to form hydroxylated metabolite at the C-6 position (rats, in vitro; dogs, in vitro and in vivo; human hepatocytes, in vitro) or to form a ketone at C6 position (seen in rat hepatocyte culture and in vivo, as well as mouse hepatocyte culture; but not seen in human and dog hepatocyte cultures). Oxidative defluoronation of parent compound at C6 position was also seen in hepatocytes of rats and dogs but not in human hepatocytes and accounted for 12-18% of the total metabolism in these species. Thus, there may be differences in the in vitro metabolic routes between species but these are minor. A unique human metabolite, a thioester glutathione conjugate (M4) of the acid metabolite M10 (3-9% of the total metabolism) was identified in vitro in human hepatocyte incubations at a GW685698X concentration of 50 mcM. Otherwise, no unique human metabolites were identified. Phase II metabolites, glucuronide conjugate of M10 (rats, mice, dogs, rabbits and humans in vivo and/or hepatocytes), glutathione conjugate of M10 (rats), and glycylcysteine conjugate of M10 (dogs in vivo, and rabbit, mouse and human hepatocytes) were observed in animal bile and/or hepatocyte incubations. Phase II metabolites of GW685698X were not present in the feces following intravenous

administration, indicating these were not stable on passage through the gastrointestinal tract.

The primary elimination of GW685698X is via liver metabolism and bile secretion as indicated by the findings that drug-related materials in 0-24 hour bile accounted for 78% and 68% of i.v. dose in rats (1 mg/kg) and dogs (0.1 mg/kg), respectively. There is no unchanged parent compound detected in bile from rats and dogs. Elimination T1/2 is 3 hours in rats and 3-13 hours in dogs. The urinary elimination is a minor route, accounting for less than 4% of dose in rats, dogs and humans.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Oral absorption in animals was essentially complete but due to an extensive first-pass metabolism, systemic exposure to the parent compound was minimal. An AUC of GW685698X at 0.1 mg/kg by oral administration was 0.4% of that by intravenous administration in rabbits. Following a 0.1 mg/kg oral dose of ^{3}H-GW685698X to rats, an AUC of radioactive drug related material (DRM) were approximately one thousand-fold that for GW685698X and was similar to that seen following IV administration. In dog plasma, DRM was present up to 1 week after oral dosing (0.1 mg/kg) whereas GW685698X was not detectable (IND 48,647, review 1).

2.6.4.4 Distribution

Most of the drug distribution studies were reviewed under IND 48,647 (review 1). Following an IV administration of ^{3}H-GW685698X (0.1 mg/kg) radioactive drug related materials (RDM) was found distributed rapidly and widely with the volume distributions of 8L/kg in rats and 10 L/kg in dogs. In rats, the RDM levels in most tissues were higher than corresponding blood levels at 0.5 to 24 hour after dosing. The highest levels of RDM were seen 0.5 hours after dosing in the small intestine and small intestine wall demonstrating biliary clearance. At 168 hours post-dosing, levels of radioactivity were only detectable in the liver and kidney cortex. Following a single oral dose of ^{3}H-GW685698X to rats, RDM was not widely distributed, with liver, kidney, spleen and GI tract being the only tissues with higher levels than the blood at 1-24 hours post-dosing.

Plasma protein binding in vitro was 99% in humans, rabbits, mice and dogs, and 98% in rats. The whole blood/plasma concentration ratio ranged from 0.58 to 0.79 in human, rat, dog, mouse and rabbit indicating that circulating radioactivity was predominantly associated with the plasma fraction (review 1).

The in vitro binding of GW685698X to human plasma albumin, alpha1-acid glycoprotein, and γ-globulin was 96%, 90%, and 33% respectively, across a drug concentration range of 20 to 250 ng/mL [WD2005/01123/00].

2.6.4.5 Metabolism

Most of the metabolism studies were reviewed under IND 48,647 (original and review 8). GW685698X was extensively metabolized in rats and dogs, as 0-24 hourbile contained 70-80% of i.v. dose as drug related materials without unchanged drug. The route of metabolism for GW685698X was studied in animals and in hepatocyte cultures. The major metabolic route of GW685698X was loss offluoromethyl carbothioate group to form the carboxylic acid GW694301X (M10). All other metabolites are formed from M10. The liver homogenate from mice treated with FF 1 mg/kg intravenously produced only metabolite M10 (30% and 24% of the radioactivity at 1 and 3 hours after dosing). The parent compound, FF accounted for 8.5% of the dose at either sampling time (WD2005/00557/00). Analysis of human feces indicated that the hydrolysis of the thioester moiety to give M10 being the predominant route of metabolism (WD2005/01496/00, no quantitative data provided). This major metabolic route was observed in hepatocyte incubations in all species tested (rats, mice, dogs, rabbits and humans) and in vivo studies in rats and dogs. The second metabolic event often observed is oxidative defluoronation in the C6 position. Oxidative defluoronation of M10 to form the hydroxylated metabolite at the C-6 position was observed in the rat (in vitro), dog (in vitro and in vivo) and human (in vitro). Oxidative defluoronation of M10 to form a ketone at C6 position was seen in rats (in vitro and in vivo) and mice (in vitro), but not other species (dogs and humans, in vitro). Oxidative defluoronation of parent compound at C6 position was also seen in hepatocytes of rats and dogs but not in human hepatocytes and accounted for 12-18% of the total metabolism in these species. Thus, there may be differences in the in vitro metabolic routes between species but these are minor. A unique human metabolite, a thioester glutathione conjugate (M4) of the acid metabolite M10 (3-9% of the total metabolism), was identified at low levels in vitro in human hepatocyte incubations at a GW685698X concentration of 50 mcM. Otherwise, no unique human metabolites were identified. The low levels observed in vitro for metabolite M4 and the lack of confirming data from in vivo studies suggest that there is no significant safety concern regarding this metabolite. The figure below presents metabolite scheme for the major in vivo metabolites of GW685698X.

Phase II metabolites, glucuronide conjugate of M10 (rats, mice, dogs, rabbits and humans in vivo and/or hepatocytes), glutathione conjugate of M10 (rats), and glycylcysteine conjugate of M10 (dogs in vivo, and rabbit, mouse and human hepatocytes), were observed in animal bile and/or hepatocyte incubations. Phase II metabolites of GW685698X were not present in the feces following intravenous administration, indicating these were not stable on passage through the gastrointestinal tract.

Rapid and extensive metabolic turnover of ^{3}H-GW685698X was seen in the presence of NADPH, indicating that metabolism was performed by a cytochrome P450 enzyme(s). Cytochrome P450 3A4 was identified in the metabolism of GW685698X in vitro using human derived enzymes.

The principal radio-labeled component in the plasma of mice (WD2005/00557/00, submitted in the current NDA), rats, dogs and humans (WD2005/01496/00 submitted in the current NDA) following intravenous administration was the parent drug (mice, 21% and14% of plasma radioactivity at 1 and 3 hours postdosing; rats, 68% and 15% of dose at 5 min and 2 hours postdosing; dogs, 35% and 25% of plasma radioactivity at 40 minutes and 2 hours postdosing, no quantitative data provided for humans). A large amount of radioactivity was non-extractable from plasma and this explains that AUC of radioactive DRM) was significantly higher than that of the parent compound (7-fold in rats and 23-fold in dogs) following an intravenous administration of radio-labeled GW685698X.

2.6.4.6 Excretion

Excretion studies were reviewed under IND 48,647 (reviews 1 and 8).
GW685698 is primarily eliminated through bile as metabolites. Drug-related materials in 0-24 hour bile accounted for 78% and 68% of i.v. dose in rats and dogs, respectively. There was no unchanged parent compound detected in bile. In non-biliary-cannulated animals (rats and dogs) given an i.v dose, most of drug-related material was eliminated via feces and in which minimum or no parent compound was detected. Similar to these animal data, fecal elimination of radioactivity accounted for 101% and 90% of dose following an oral (2 mg) and intravenous dose (0.25 mg) in humans. The urinary elimination is a minor route, accounting for less than 4% of dose in rats, dogs and humans. Elimination T1/2 is 3 hours in rats and 3-13 hours in dogs.

2.6.4.7 Pharmacokinetic drug interactions

Pharmacokinetic drug interactions were studied in vitro and most of these studies are reviewed below except those reviewed previously as specified.

For transport systems, GW685698 is a substrate for human P-glycoprotein (P-gp) and has moderate passive membrane permeability. Neither GW685698X (up to 30 mcM) nor its metabolite GW694301 (up to 100 mcM) inhibits human P-gp. GW685698X and its metabolite GW694301 (M10) inhibit human organic anion transporting polypeptide 1B1, a human liver-specific uptake transporter protein (IC50 values were 0.2 mcM and 3 mcM for GW685698X and GW694301, respectively).

For metabolic enzymes, GW685698 inhibited (IC_{50} <10 mcM) the activity of human CYP3A4, 2B6, 2C9, 2C19, 2C8 and 2D6 but not 1A2, 2A6 and 2E1. The most potent inhibition was on CYP2C8 and 3A4 ($IC_{50} \leq 1.5$ mcM) with less effects on others (IC_{50}<10 mcM). However, no time or NADPH dependency was observed [FD2003-00126-00, IND 48,647 review 1]. Inhibition of all of the above enzymes by M10 was also studied

and M10 had no direct inhibition or metabolism-based inhibition of any of the P450 enzymes (IC50 >80 mcM). In vivo, rats received GW685698X for 4 weeks by inhalation at dose of 64 mcg/kg (AUC 4.4 ng.h/ml); GW685698X had no significant effects on hepatic protein, cytochrome P450 concentrations, or on the activities of CYP1A, 2B, 3A, 2E and 4A enzymes. The reason for the discrepancy between in vitro (inhibition of CYP 2C8 and 3A4) and in vivo data (void of inhibition to any P450 enzymes) is unclear.

Overall, this drug may interfere with metabolism of other molecules in humans that require organic anion transporting protein, or cytochrome P450 enzymes (CYP3A4, 2B6, 2C9, 2C19, 2C8 and 2D6), but unlikely through P-gp.

The following are details of the above studies:

1. GW685698 is a substrate of human P-gp and has a moderate passive membrane permeability (average $P_{7.4}$ of 80 ± 45 nm/s (mean ± SD)) [2006/00293/00]
   The potential for human P-glycoprotein (P-gp) to transport GW685698 was investigated using stable transfected MDCKII-MDR1 cells cultured as monolayers on 24-well PET membrane inserts. Directional transport was determined by measurement of apical to basolateral ([A→B]) and basolateral to apical ([B→A]) rates of transport using 0.5 µM GW685698 in the absence and presence of 2 µM GF120918, a potent P-gp inhibitor. The passive membrane permeability of GW685698 was determined in the presence of GF120918 (a potent P-gp inhibitor).

   The apical efflux ratio of GW685698 at 0.5 µM was determined as 3.0 and 0.5 in the absence and presence of 2 µM GF120918, respectively. These results indicate that, under the assay conditions used, GW685698 is a substrate of human P-gp. In the presence of GF120918, GW685698 demonstrated moderate passive membrane permeability in the MDCKII-MDR1 cell line with an average permeability coefficient at pH7.4 ($P_{7.4}$) of 80 ± 45nm/s (mean ± SD) based on the passive membrane transport. The mass balance for GW685698 was low (40-60%). The poor mass balance suggests there may be non-specific binding of this compound to the plates used in this system. This is not believed to affect the conclusions of the study as the poor mass balance is seen in both directions.

Compound	Rate A→B (nmoles/h/cm²)	Rate B→A (nmoles/h/cm²)	Apical Efflux Ratio	P-gp Substrate	A→B Mass Balance (%)	B→A Mass Balance (%)	$P_{7.4}$ (nm/s)	Passive Permeability Class
0.5 µM GW685698	0.0040± 0.00039	0.012 ± 0.0035	3.0	Y	56 ± 1.2	61 ± 0.82	-	-
0.5 µM GW685698 + 2 µM GF120918A	0.0060 ± 0.00090	0.0032 ± 0.00065	0.52	-	43 ± 4.9	58 ± 3.3	80 ± 45	Moderate
3 µM [³H]-amprenavir	0.016± 0.0015	0.54 ± 0.0012	34	Y	85 ± 1.1	89 ± 0.80	-	-
3 µM [³H]-amprenavir + 2 µM GF120918A	0.26 ± 0.0037	0.30 ± 0.0043	1.1	-	91 ± 4.6	92 ± 1.1	306 ± 24	High

Data are the mean ± standard deviation from three monolayers, except for $P_{7.4}$ (where n=6).
All donor compartments contained Lucifer yellow CH to determine monolayer integrity (pass criterion $P_{7.4} \leq 50$ nm/s) and wells designated for P-glycoprotein (P-gp) inhibition contained 2 µM GF120918 in both donor and receiver compartments.
[³H]-amprenavir was used as positive control (criterion for assay acceptability: apical efflux ratio for amprenavir ≥15 collapsing to approximately 1 in the presence of inhibitor).
A compound is classified as a P-gp substrate if the apical efflux ratio in the absence of inhibitor GF120918 is ≥ 2 and this efflux collapses to ~1 in the presence of inhibitor, GF120918.
Passive membrane permeability was classified as low (≤ 50 nm/s), moderate (50 - 250 nm/s) or high (≥ 250 nm/s)

Calculated $P_{7.4}$ is ranked as low (<50 nm/s), moderate (50 - 250 nm/s) or high (> 250 nm/s)

2. FF and M10 do not inhibit transport of digoxin via human P-glycoprotein in vitro, at concentrations up to 30 and 100 μM, respectively [2005/00368/00]

   The effect of GW685698X and GW694301X (FF, 0.1- 30 mcM; M10, 0.1-100 mcM) on the Pgp-mediated transport of digoxin (30 nM) was assessed by determining the basolateral to apical ([B→A]) transport of [3H]-digoxin by MDCKII-MDR1 cells transfected with the human MDR1 gene, which produces the Pgp protein. GW685698X does not inhibit transport of digoxin via human P-glycoprotein in vitro, at concentrations up to 30 μM. GW694301X does not inhibit transport of digoxin via human P-glycoprotein in vitro, at concentrations up to 100 μM.

3. FF and its metabolite M10 inhibit human organic anion transporting polypeptide 1B1 with IC_{50} values of 0.2 μM and 3 μM, respectively [2005/00763/00]

   The human organic anion transporting polypeptide 1B1 (OATP1B1) is a liver-specific uptake transporter protein found on the sinusoidal membrane of hepatocytes. Culture of the stable transfected cell line CHOOATP1B1, probe substrate [^{3}H]-Estradiol 17β-D-glucuronide ([^{3}H]-EG), and GW685698 (concentration range 0.01 - 10 μM) or GW694301 (concentration range 0.1 - 50 μM) showed that GW685698 and GW694301 inhibited human OATP1B1 under these assay conditions with calculated IC_{50} values of 0.2 μM and 3 μM, respectively.

4. Studies on inhibition of human cytochrome P450 enzymes by FF and M10:
   Direct inhibition was examined by incubating test compound, appropriate probe substrate, pooled human liver microsomes and an NADPH regenerating system over a known time-course at 37°C. The production of metabolite in each incubation was quantified by LC/MS/MS and IC_{50} values for the direct inhibition of each enzyme activity were determined.
   Metabolism-dependent inhibition is a recognized effect that requires enzymatic activation of the inhibitor in the presence of cofactor (NADPH) and results in an increased potency of inhibition. In this study, the test compound (FF or M10) and microsomes were pre-incubated with NADPH for 20 minutes prior to initiation of reaction by addition of substrate. The corresponding control incubations were designed to provide a pre-incubation without NADPH while incorporating the 20 minutes pre-incubation of test compound with microsomes to ensure consistent equilibration of test compound in both control and NADPH pre-incubations. The control pre-incubations were therefore performed for 20 minutes with GW694301, microsomes and substrate and the reaction initiated by the addition of NADPH.

The following table presents the substrates and inhibitors for each enzyme.

P450	Probe substrate	Positive inhibitor (direct)	Positive inhibitor (metabolism based)
1A2	Phenacetin	Fluvoxamine	Furafylline
2A6	Coumarin	Tranylcypromine	
2B6	Bupropion	Orphenadrine	Ticlopidine
2C8	Paclitaxel	Quercetin	Phenelzine
2C9	Diclofenac	Sulphaphenazole	Tienilic acid
2C19	mephenytoin	Ticlopidine	Ticlopidine
2D6	Bufuralol	Quinidine	Paroxetine
3A4	Atorvastatin	Ketoconazole	Troleandomycin
3A4	Midazolam	Ketoconazole	Troleandomycin
3A4	Nifedipine	Ketoconazole	Troleandomycin

GW685698X inhibited (IC_{50} <10 mcM) the activity of CYP3A4, 2B6, 2C9, 2C19, 2C8 and 2D6 but not 1A2, 2A6 and 2E1. The most potent inhibition was on CYP2C8 and 3A4 ($IC_{50} \leq 1.5$ mcM) and less potent on others (IC_{50} <10 mcM). However, no time and NADPH dependency were observed. The following table presents the IC_{50} values for each P450 enzyme (Review 1 for FD2003/00126/00)

Inhibition of Cytochrome P450 Enzymes by GW685698X			
P450	IC50 (uM)	Time- and NADPH-Dependent Inhibition: IC50 (uM)	
		Control pre-Incubation[1]	NADPH Pre-Incubation[2]
1A2	> 100	> 100	> 100
2A6	> 100	> 100	> 100
2B6	4.0	4.6	4.0
2C8	0.58	1.6	3.3
2C9	2.4	2.2	4.1
2C19	5.5	5.4	7.1
2D6	3.2	3.2	7.5
2E1	> 100	> 100	> 100
3A4 (atorvastatin)	1.3	1.1	1.5
3A4 (midazolam)	0.74	0.70	1.0
3A4 (nifedipine)	1.5	1.7	1.6

1. Microsomes, buffer and GW685698X pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.
2. Microsomes, buffer and GW685698X pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.

The metabolite GW694301 does not have any direct inhibition or metabolism-based inhibition to cytochrome P450 enzymes (IC_{50} >80 mcM for CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) [WD2005/00543/00].

Inhibition of cytochrome P450 enzymes by GW694301			
P450	Direct Inhibition IC_{50} (μM)	Metabolism-dependent inhibition: IC_{50} (μM)	
		Control pre-incubation[1]	NADPH pre-incubation[2]
1A2	>100	>100	>100
2A6	>100	>100	>100
2B6	>100	>100	>100
2C8	80	>100	>100
2C9	>100	>100	>100
2C19	>100	>100	>100
2D6	>100	>100	>100
3A4 (atorvastatin)	>100	>100	>100
3A4 (midazolam)	98	>100	>100
3A4 (nifedipine)	>100	>100	>100

1. Microsomes, buffer and GW694301 pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.
2. Microsomes, buffer and GW694301 pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.

2.6.4.8 Other Pharmacokinetic Studies

GW685698X at 10-1000 pg/mL is stable in human plasma for at least 18 months at -20°C [WD2006/01727/00].

2.6.4.9 Discussion and Conclusions

In general pharmacokinetic behavior across different species is similar.

Oral bioavailability is very low (<1% in rabbits, rats, and dogs) due to first pass effect. Intravenously administered GW685698X is rapidly and widely distributed with a Vd value of 8L/kg in rats and 10 L/kg in dogs. The highest levels are in small intestine and small intestine wall in rats. Plasma protein binding is greater than 98% in all species studied (rats, mice, rabbits, dogs and humans). GW685698X was extensively and rapidly metabolized by the liver; 0-24 bile contained no detectable parent drug in rats and dogs. The main route of GW685698X metabolism is loss of -fluoromethyl carbothioate group to form the carboxylic acid GW694301X (M10) in rats, dogs, rabbits, mice and humans. All other metabolites are formed from M10 in most of species. An additional metabolic event occurs in most species including humans is oxidative defluoration in C6 position of M10. However, oxidative defluoration in C6 position of the parent compound was seen in rat and dogs but not humans, which account for 12-18% if total metabolism observed in vitro in these species. Other than a thioester glutathione conjugate (M4) of the acid metabolite M10 (3-9% of the total metabolism) identified in vitro in human hepatocyte

incubations at a GW685698X concentration of 50 mcM, no unique human metabolites were identified.

Approximately 58% and 51% of i.v. doses (1 mg/kg in rats, 0.1 mg/kg in dogs) were eliminated to bile in rats and dogs, respectively. Elimination T1/2 is 3 hours in rats and 3-13 hours in dogs. Urine elimination is a minor route, accounting less than 4% of dose in rats and dogs.

GW685698X and M10 are inhibitors for human organic anion transporting polypeptide 1B1 (IC50: 0.2 μM and 3 μM, respectively). GW685698 is a direct inhibitor (IC_{50}<10 mcM) for human CYP3A4, 2C8, 2B6, 2C9, 2C19, and 2D6 but not 1A2, 2A6 and 2E1. There is no similar inhibition effects found for GW694301. A 4-week rat inhalation study at 64 mcg/kg showed no significant effects on hepatic protein or cytochrome P450 concentrations or on the activities of CYP1A, 2B, 3A, 2E and 4A enzymes. Therefore, GW685698X may interfere with metabolisms of other molecules in humans that require organic anion transporting protein, or cytochrome P450 enzymes (CYP3A4, 2B6, 2C9, 2C19, 2C8 and 2D6), but interference is unlikely through the P-gp pathway.

2.6.4.9 Tables and figures to include comparative TK summary

Toxicokinetic parameters from intranasal and inhalation studies of up to 39 weeks duration were evaluated in dogs or rats showed no gender related difference and no plasma drug accumulation over time. Systemic exposure of the drug increases with dose, in a proportional or subproportional manner. There is no species related pattern on the **TK parameters as AUC's were comparable at similar doses. Intranasal administration of** GW685698X produced lower systemic exposures as compared to inhalation administration (50-180 fold) based on 4-week studies.

TK parameters (male and female combined data) in preclinical studies

		Rats			Dogs		
4-wk IH	Dose, mcg/kg	7	18	72	9	22	74
	AUC0-t*, ng.h/mL	0.82	1.80	4.42	1.76	2.06	4.38
	Cmax, ng/mL	0.172	0.462	1.50	0.68	1.17	2.52
13-wk IH	Dose, mcg/kg				11	33	65
	AUC0-t, ng.h/mL				0.81	1.88	2.23
	Cmax, ng/mL				0.32	1.02	1.02
26-wk IH	Dose, mcg/kg	3	8	20			
	AUC0-t, ng.h/mL	0.481	0.494	1.238			
	Cmax, ng/mL	0.224	0.206	0.338			
39-wk IH	Dose, mcg/kg				13	30	60
	AUC0-t, ng.h/mL				1.16	2.34	4.62
	Cmax, ng/mL				0.66	1.37	2.59
4-wk IN	Dose, mcg/kg					40	120
	AUC0-t, ng.h/mL					0.020	0.141
	Cmax, ng/mL					67#	51
26-wk IN	Dose, mcg/day					1200	2400
	AUC0-t, ng.h/mL					0.258	0.753
	Cmax, ng/mL					0.094	0.193

Above are male and female combined data. Inhalation doses presented are delivered dose (not pulmonary deposited doses); AUC and Cmax data presented are the mean values after steady level is reached: Day 28 for 4 week study, mean values of weeks 4 and 13 for 13-week study, mean values of weeks 6 and 26 for 26-week study, mean values of weeks 4, 26 and 39 for 39-week study.
* AUC0-24h for dog 4-week inhalation study; # data from females only.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A.

2.6.6 TOXICOLOGY

Note: doses referred to in all inhalation studies are delivered doses rather than pulmonary deposited doses unless specified otherwise.

2.6.6.1 Overall toxicology summary

General toxicology:
Single dose toxicity studies were conducted in two species (rats and mice) using three routes of administration (intravenous, oral and inhalation). The common findings observed on Day 3 sacrifice were body weight loss or lower body weight gain and lymphoid atrophy (thymus and spleen). There was no mortality observed. Therefore, the maximum non-lethal dose if greater than the high dose in each study, namely, i.v. doses of 18 mg/kg in CD1 mice, 12 mg/kg in male rats and 18 mg/kg in female rats; oral dose of 2 g/kg in CD-1 mice and Wistar Han rats; and inhalation doses (pulmonary deposited dose) of 0.14 mg/kg in CD-1 mice and 0.087 mg/kg in Wistar Han rats (review of IND 48,647 original submission).

Most of the repeat-dose studies were conducted by inhalation administration and additional studies up to 6-months duration using intranasal administration were conducted to bridge the data characterizing inhalation exposure to the proposed route of intranasal administration. These studies were reviewed under IND 48,647 (reviews 1, 4 and 5). The major toxicology studies completed include the following:

Species	Administration route	Treatment duration	Dose (mcg/kg)	IND 48,647 Review #
Rat	Inhalation	4 weeks	6.9, 17.6, 71.7	Rev. 1
		30 days	6.5, 19.5, 72	Rev. 1
		13 weeks	5, 9, 24	preIND 48,647
		26 weeks	3.2, 8.3, 20.3	Rev. 4, 6
	Intranasal	2 weeks	80, 160*	Rev. 5
Dog	Inhalation	4 weeks (1)	10.57, 30.59, 104.6	Rev. 4
		4 weeks (2)	9.23, 22.1, 74.3	Rev. 4
		13 weeks	11.3, 33.0, 64.7	Rev. 4, 6, 7
		39 weeks	13.3, 30.1, 59.6	Rev. 4, 6, 7
	Intranasal	4 weeks	40, 120	Rev. 1
		26 weeks	1200, 2400*	Rev. 4
Mouse	Inhalation	13 weeks	7.3, 18.6, 76.9	preIND 48,647

Note: inhalation doses presented above were delivered doses rather than pulmonary deposited doses; *mcg/day

Rats:

The toxicity findings in the rat inhalation and intranasal studies were mostly typical for glucocorticoids including lower body weight gain or body weight loss, changes in hematology and blood chemistry parameters (increased RBC, decreased WBC/lymphocyte, increased cholesterol and triglyceride), and histopathology findings of lymphoid depletion (thymus, spleen, lymph nodes, and lymphoid tissues of lung and nasal passage), fatty bone marrow, and hair loss and dermal thinning (26-week). Laryngeal epithelial hyperplasia/metaplasia was seen in the 2-week intranasal, and 13- and 26-week inhalation studies. This finding was considered procedure related and not clinically relevant (review 1 of IND 48,647 original submission, review 6 of IND 48,647 submission of 11/2/05).

A non-typical glucocorticoid finding, increased eosinophilic inclusions in bronchiolar epithelial cells, was observed in all drug-treated groups in the 13- and 26-week rat inhalation studies (no similar findings were seen in the 4-week and 30-day studies). Upon **the Division's request, GSK** conducted additional studies and reported a summary (detailed study reports are included in the present NDA submission) for their findings and a review of the summary data can be found in review 6 for IND 48,647 submission of 11/2/05, and a preliminary review for resubmission of IND70,297 on 6/12/06. GSK identified that the inclusions are in Clara cells and the inclusion components are predominantly the natural secretory products of Clara cells including SP-D (surfactant protein D) and CCSP (Clara cell secretory protein). This finding is GW685698X related and no NOEL was identified in any rat studies conducted. However, the proportions of

affected Clara cells are small in all studies (2-mos, 3-mos, 6-mos, and 2-year) with the maximum of 0.78% (control, 0.025%) of the total approximately 4000-5000 Clara cells in four standard sections. Additionally, the proportion of affected cells does not progress with treatment duration (13-wk and 26-wk inhalation studies with similar doses were compared). GSK concluded that this finding is treatment related but not toxicological significant. The Division concurred that this conclusion. Thus, the finding is not considered to be adverse in nature.

A rat 2-week intranasal study used doses of 80 and 160 mcg/day. The study identified no unusual toxicities compared to inhalation studies. The increase of eosinophilic inclusion in bronchiolar epithelium observed in subchronic and chronic inhalation studies were not seen in this 2-week intranasal study. As this study identified no dose limiting toxicity or toxicity that was specific to intranasal administration, **the sponsor's selection of the dog** for the chronic (6 months) intranasal study was considered acceptable.

Dogs:
The major toxicity findings in dog inhalation and intranasal studies were also typical glucocorticoid effects: decreased body weight gain or body weight loss, increased blood triglyceride and /or cholesterol, adrenal atrophy, lymphoid depletion (thymus, spleen, lymph nodes, and other lymphoid tissues), increased liver glycogen deposition, hepatocyte rarefaction and/or hypertrophy, fatty bone marrow, and skin epidermal atrophy. In the dog 39-week inhalation study, additional glucocorticoid effects including pituitary acidophilic cells, increased adipose cell infiltrations in heart, pancreas, parotid salivary gland and skeletal muscles, and epiphyseal plate retention were also observed.

The sponsor was asked to address some of the non-typical glucocorticoid effects observed in dogs with no NOAEL defined. Those toxicities included purkinje fiber vacuolation (26-week intranasal), testicular degeneration/atrophy (39-week inhalation), focal nephropathy (tubular basophilia in 26-week intranasal, and 39-week inhalation), a slight change in blood chemistry parameters (2-3 fold increase in alkaline phosphatase, gamma glutamyl transferase and/or glutamyl dehydrogenase), and biliary tract (bile duct and gall bladder) epithelial vacuolation (13- and 39-week inhalation, 26-week intranasal). **Based on GSK's responses these issues were resolved.**

1. Purkinje fiber vacuolation finding was justified by the historical control data. The Division concluded this finding not treatment related (review 6 for IND 48,647 of 11/2/05 submission).
2. The finding of testicular degeneration/atrophy was considered not treatment related based on the historical background finding provided by the sponsor and the absence of dose-relationship in the 9-month study with GW685698X (review 6 for IND 48,647 of 11/2/05 submission).
3. For the finding of focal nephropathy, GSK submitted historical control data and tissue slide re-evaluation for the 9-month inhalation study. In light of historical control data, a NOAEL of 1200 mcg/day (AUC of 258 pg.h/mL) was concluded for the 6-month intranasal study. A discrepancy was noted in the comparison of NOAELs for this finding in the 6-month intranasal study and 9-month inhalation study, as that a higher NOAEL (13 mcg/kg, AUC of 1160 pg.h/mL) was defined

in a study with a longer treatment duration (9-month inhalation). This discrepancy remains to be clarified. Nevertheless, the NOAEL of 6-month intranasal study provides an adequate safety margin (approximately 29-fold for the recommended 110 mcg/day based on AUC comparison, 258 versus 8.8 pg.h/mL). Therefore, focal nephropathy is not a clinical concern (review 6 for IND 48,647 of 11/2/05 submission).

4. Safety reassessment for biliary tract epithelial vacuolation was through a re-evaluation by an independent pathology working group (PWG). The PWG's evaluation confirmed this finding being treatment related based on dose-response relationship and historical control data. However, the severities of the findings are of a low degree, and are only slightly higher than historical background (less than grade 2 in a 5 point scale (1, minimum; 2, slight; 3, moderate; 4, marked; 5, severe), in most cases, except gall bladder finding at the high dose of the 39-week study where the mean severity was in the degree of 2.3. The incidence and severity appeared not to progress significantly as compared to the 13-week and 39-week inhalation studies. There were no associated lesions (inflammation or degeneration) in these studies. The PWG's confirmation alleviated the Division's concern and it concluded that this finding was treatment related but not a safety concern (review 4 for IND 70,297 submission of 9/26/2006).

Airway inflammatory lesions (epithelial hyperplasia, pseudogland formation, and inflammation) in dogs were observed in 13- and 39-week inhalation studies. There was no similar finding observed in the 4- or 26-week dog intranasal studies. These findings are considered local effects of the test article and are not clinically relevant for the intended intranasal administration applied in this NDA. Additionally, these findings are not considered to be a clinically significant concern based on the similar findings in dogs exposed to fluticasone propionate by inhalation and extensive clinical experience with fluticasone propionate (preliminary safety review, IND 70,297, resubmission of 6/12/06).

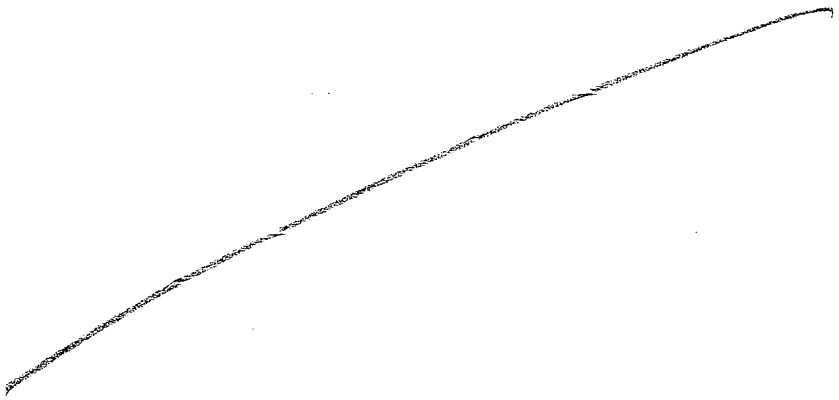
A non-typical glucocorticoid effect, chronic stomach inflammation was observed in the dog 39-week study. The NOAEL is the low dose, 13 mcg/kg (AUC = 1160 pg.h/mL) which provides a 132-fold safety margin for the human dose of 110 mcg/day based on AUC (1160 versus 8.8 pg.h/mL).

A 6-month IN study was conducted in dogs to bridge the IH and IN toxicity programs based on the results of short-term IN studies in rats and dogs. The doses in the 26-week study were 1200 and 2400 mcg/dog. The toxicities observed were similar to that seen in inhalation studies except the findings of nasal associated lymphoid tissue atrophy and nasal cavity goblet cell hypertrophy. These local toxicities showed dose-related incidence or severity. These findings were considered local effects that are clinically monitorable.

Mice:

Mice were used in a 13-week inhalation study only and this study served a purpose of dose ranging for a 2-year carcinogenicity study. Toxicity findings in this 13-week study were similar to those found in the rat and/or dog studies.

NOAELs for Chronic use:
Based on typical glucocorticoid effects, there are no NOAELs defined in the rat and dog studies. However, extensive clinical experience with this class of drugs and the ability to monitor for the effects precludes a necessity for establishment of NOAELs for these effects. Non-typical glucocorticoid adverse effects, nephropathy and chronic stomach inflammation, were seen in dogs. The NOAELs for these effects were defined as 1200 mcg/day (AUC of 258 pg.h/mL) for nephropathy based on the 6-month intranasal study (higher NOAEL in the 9-month inhalation study), and 13 mcg/kg (AUC of 1160 pg.h/mL) for chronic stomach inflammation based on the 39-week inhalation study. These NOAELs represent 29- and 132-fold safety margins, respectively, for the proposed human intranasal dose up to 110 mcg/day based on AUC comparisons.



Genetic toxicology:
GW685698X was tested in an Ames test, a mouse lymphoma assay and in 3 rat micronucleus assays. All studies but the third micronucleus assay were reviewed under IND 48,647. The original 2 micronucleus assays did not test doses up to the maximum recommended limit doses. The sponsor repeated the study a third time up to doses of 40 mg/kg, IV, and the maximum dose induced clinical signs that approximated an MTD. It is evaluated in this review. All of the genotoxicity studies demonstrated negative results.

Carcinogenicity: Two-year carcinogenicity studies were conducted in rats and mice by inhalation administration. Exposure of GW685698X in rats at doses up to 8.61 mcg/kg and in mice up to18.8 mcg/kg, the maximum tolerated doses in both species, resulted in no significant increases in tumor incidences. These doses represent 0.6-0.7 fold of the maximum recommended human dose. The CDER Executive CAC concurred that the studies were acceptable and that no drug-related tumors were produced. See the attached CAC meeting minutes for Jan 16, 2007 meeting on page 90.

Reproductive toxicology:
Male and female fertility studies were reviewed in Review 5 and Review 4, respectively, for IND 48,647. Fertility and early embryonic development investigations were conducted in two rat studies where GW685698X was given to male rats only (6.6, 12.9 and 29.4 mcg/kg) in one study and was given to females rats only (11, 23 and 91 mcg/kg) in the other study. The doses employed in these studies were adequate based on maternal or paternal body weight loss or lower body weight gain (females, body weight loss 4% prior to mating and 34% lower body weight gain during gestation; males, lower body weight gain of 69% prior to mating). There were no drug related findings in regard to mating performance, precoital interval or fertility, nor evidence of major skeletal or visceral abnormalities other than incomplete ossification in the fetuses born to drug-treated high dose females that was likely associated with maternal toxicity. For fertility and early embryonic development, the NOAELs are 29.4 mcg/kg for male rats and 91 mcg/kg for female rats. On a mg/m^2 basis, these NOAELs represent 2- and 7-fold safety margins for the recommended human dose of 110 mcg/day. In regard to the proposed product label, the sponsor calculated the high dose in the female fertility study as 91 mcg/kg based on application of the inhalable particle in the chamber with a corresponding aerodynamic diameter. This calculated dose is acceptable.

The effect of GW685698X on embryo-fetal development was studied in rabbits and the study was reviewed in review 4 for IND 48,647. A preliminary study (inhalation delivered doses of 14, 79 and 149 mcg/kg for 14 days) demonstrated lower body weight gains of pregnant rabbits (LD gained 44% and MD gained 16% of gain in control animals) and suspected abortion at the two upper doses with high dose animals sacrificed early. The doses of 1.8, 3.2 and 8.1 mcg/kg were used in the definitive study where a dose-related decrease of maternal body weight gain (up to 61% during the treatment period, i.e., Days 8 to 20 of pregnancy) was observed. There were no major or minor fetal abnormalities other than increased incidence of incomplete ossification in some bones at all doses. Therefore, this drug at 8.1 mcg/kg was devoid of teratogenic effects in rabbits. The rabbit NOAEL of 8.1 mcg/kg provides a 1 fold safety margin for human dose of 110 mcg/day on an mg/m^2 basis.

A pre- and post-natal development study in rats was conducted and reviewed in review 5 for IND 48,647. GW685698X does not affect rat prenatal and postnatal development. Female rats were given GW685698X at delivered doses of 5.5, 15.7 and 27.2 mcg/kg by inhalation from Day 6 post-coitum to Day 20 (or 21) post partum resulted in decreases of F0 maternal body weight gain (up to 25%) and food consumption (up to 9%) during gestation. Examinations on F1 physical development, behavior and reproduction and examinations on F2 survival indices, body weights and autopsy revealed no drug-related finding. Therefore, the NOAEL for pre- and post-natal toxicity is 27.2 mcg/kg in rats. This NOAEL provides a 2-fold safety margin for the human dose of 110 mcg/day on a mg/m^2 basis.

Although no definitive drug-related developmental effects were noted with GW685698X, a Pregnancy Category of C is appropriate based on the known class effects of glucocorticoids.

Special toxicology:
GW685698X did not induce respiratory hypersensitivity reactions in guinea pigs that were treated with inhalation dose of 70.6 mcg/kg for five days followed by a single inhalation challenge (67 mcg/kg) 17 days later.

Based on an intranasal dose finding study in juvenile dogs (1/sex, 8 weeks old), the sponsor concluded that one actuation per session should be used because the subsequent actuations were mostly breathed out by the animals (IND 48647, Rev. 1).

Formulation containing ——— 5%) produced strong local irritation by intranasal administration in dogs and are precluded from further studies with this formulation.

2.6.6.2 Single-dose toxicity
There are no new studies submitted in the NDA.

2.6.6.3 Repeat-dose toxicity

Most repeat dose studies were reviewed under IND 48,647 (reviews 1, 4 and 5). The current NDA provides supplemental toxicology studies that are reviewed below.

Review of supplemental toxicity studies

Study titles:

1. Additional pathology investigations to a toxicity study by inhalation administration to Wistar Han rats for 13 weeks (report WD2006/01990/00)
2. Additional pathology investigations to a toxicity study by inhalation administration to Wistar Han rats for 26 weeks (report WD2006/01991/00)
3. Additional pathology investigations to a carcinogenicity study by inhalation administration (Study V26862, Report No. WD2006/01992/00)
4. Additional investigations into target organ toxicity using tissues generated from study R24142 (V26765) (report WD2006/01906/00)

Key study findings: The GW685698X-induced increase of eosinophilic inclusions in bronchiolar epithelium affected less than 0.8% of Clara cells. The major component of the inclusion is surfactant protein D, with a low level of Clara cell secretary protein. There is no substantive difference between inclusions seen in control and treated animals.

Study summary:

GW685698X was associated with increased eosinophilic inclusions present in the cytoplasma of non-ciliated bronchiolar epithelial cells (Clara cells). These four studies were designed to provide quantitative data on the affected Clara cells and characterize the proteins of the inclusions. The first three studies were designed to quantitate eosinophilic inclusions and the affected proportion of cells. The fourth study was to evaluate the protein content of the eosinophilic inclusions.

In the first three studies, the numbers of eosinophilic inclusions were counted by the study pathologist (———————), in the lung sections from all terminal killed animals from the 13- and 26-week inhalation studies. The numbers of Clara cells were counted in the lung sections from four standard sections of 3 male and 3 female control rats of each study using immunohistochemistry method, by GSK. The average Clara cell count of the control animals was used as a reference value to calculate the proportion of Clara cells showing inclusions. Obviously, the actual Clara cell numbers in treated animals were not reflected in the results and no information was provided regarding actual Clara cell number in these treated animals. Nevertheless, the number of cells affected in each treated group, and the proportion of affected over the Clara cells in control animals, reflect the frequency of this finding. The proportion of affected Clara cells derived from the above method, indicated that less than 1% of Clara cells were affected by GW685698X in the 13- and 26-week inhalation studies and the 2-year carcinogenicity study (see the details below).

Rat 13-week inhalation study

Estimated Achieved Dose[a] (µg/kg/day)	Male				Female			
	0	4.3	8.5	24.3	0	4.3	8.5	24.3
Achieved Aerosol Concentration (µg/L)	0	0.111	0.214	0.600	0	0.111	0.214	0.600
Numbers of animals evaluated for eosinophilic inclusions in the lungs	12	12	12	12	12	12	12	12
Mean number of eosinophilic inclusions	0.7	13.4	11.3	37.8	1.1	7.3	11.3	18.7
Number of animals examined to determine number of Clara cells in lung	3	0	0	0	3	0	0	0
Mean Total Clara cell count in 4 lobes	4875	ND	ND	ND	4073	ND	ND	ND
Mean % of Clara cells showing inclusions	0.014	0.27[b]	0.23[b]	0.78[b]	0.027	0.18[b]	0.28[b]	0.46[b]

a. Doses expressed in terms of pure active moiety (estimated achieved dose – males and females combined)
b. Derived from reference values (Total Clara cell count) obtained from control group
ND Not determined

Rat 26-week inhalation study

Estimated Achieved Dose[a] (µg/kg/day)	Male				Female			
	0	3.2	8.3	20.3	0	3.2	8.3	20.3
Achieved Aerosol Concentration (µg/L)	0	0.087	0.222	0.530	0	0.087	0.222	0.530
Numbers of animals evaluated for eosinophilic inclusions in the lungs	12	12	12	12	11	12	12	12
Mean number of eosinophilic inclusions	1.5	5.1	6.8	15.8	0.9	6.4	9.6	12.0
Number of animals examined to determine number of Clara cells in lung	3	0	0	0	3	0	0	0
Mean Total Clara cell count in 4 lobes	4148	ND	ND	ND	3811	ND	ND	ND
Mean % of Clara cells showing inclusions	0.036	0.12[b]	0.16[b]	0.38[b]	0.024	0.17[b]	0.25[b]	0.31[b]

a. Doses expressed in terms of pure active moiety (estimated achieved dose – males and females combined)
b. Derived from reference values (Total Clara cell count) obtained from control group
ND Not determined

2-year rat carcinogenicity study

	Males					Females				
Dose (µg/kg/day)	0	0	1.00	3.19	8.61	0	0	1.00	3.19	8.61
Range	0 to 17	0 to 8	1 to 27	1 to 60	4 to 90	0 to 5	0 to 9	0 to 31	1 to 55	2 to 75
Mean	2.3	2.2	6.9	14.9	24.3	1.8	1.9	6.6	13.3	18.3
Mean % of Clara cells showing inclusions	0.042	0.041[a]	0.13[a]	0.27[a]	0.45[a]	0.052	0.055[a]	0.19[a]	0.38[a]	0.52[a]
Number of animals examined	37	40	48	39	38	43	39	43	32	30

a. Derived from reference values obtained from Group 1 animals

In the fourth study, the protein content of the eosinophilic inclusions in the rat lung tissues from a 2-year carcinogenicity study was evaluated using immunohistochemistry methods. The results of antibody staining indicated that the major component of the inclusion is surfactant protein D. Additionally, Clara cell secretary protein is present at a

low level in a proportion of inclusions. There is no substantive difference between inclusions seen in control and treated animals.

The following table presents the antibody staining results.

Test antibody	Cytoplasm	Inclusion (treated)	Inclusion (control)
SP-D	+	+	+
CCSP (UPSTATE)	+	+	+
CCSP (USB)	+	+	+
Superoxide dismutase	+	-	-
TOM20	+	-	-
IgA	-	-	-
IL-6	+	-	-
MIM	+	-	-
Annexin-1	-	-	-
Mast cell tryptase	+	-	-
SP-A	+	-	-
SP-B	+	-	-
SP-C	+	-	-
Lysozyme	-	-	-
Galectin-1	+	-	-
COX-1	+	-	-
COX-2	-	-	-

2.6.6.4 Genetic toxicology

Study title: GW685698X: Additional rat bone marrow micronucleus assay in rats

Key findings: This in vivo rat micronucleus test is considered valid and the test drug is negative for clastogenic effects under the test conditions.

Study no.: R26808, Report WD2006/02023/00

Volume #, and page #: E-submission, Module 4
Conducting laboratory and location: GlaxoSmithKline, Park Road, Ware Hertfordshire, SG12 0DP, UK

Date of study initiation: 3/20/2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: GW685698X, lot 051062997, 99.6% pure

Methods

Strains/species/cell line: Rat: Han Wistar (—WI (Han))

Doses used in definitive study: 0, 10, 20, 40 mg/kg/dose, two doses administered intravenously, 24 hours apart, 6 male rats per group

Basis of dose selection: The report states that the high dose is the maximum feasible dose based on solubility (20 mg/mL in 100% PEG 400)), and the maximum achievable dose volume (without inducing clinical signs of toxicity attributable only to the vehicle itself) of 2 mL/kg for this vehicle). The report referenced a stability study (FD2004-00155-00) which tested the stability of concentrations of 0.025 to 20 mg/ml in polyethylene glycol; all concentrations were concluded to be stable for up to 4 days at ~ 4 degrees Celsius or ambient temperatures. However, higher concentrations were not tested so it is unknown if higher concentrations could have been used in the current study.

Negative controls: 100% PEG 400

Positive controls: Cyclophosphamide monohydrate, 20 mg/kg, once on Day 2 (Day 1 is the first day when animal were given test compound) orally.

Incubation and sampling times: 24 hours after the final dose

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): This study is considered valid based on the following:

1. Doses appear to approximate the maximum tolerated dose based on clinical signs; **the sponsor's contention that the MFD was** employed is unsubstantiated as the supporting solubility data assessed concentrations only up to 20 mg/ml. It is, therefore, unknown if higher concentrations could have been administered.
2. The number of micronucleated polychromatic erythrocytes (MPCE), from a population of at least 2000 PCE, was recorded for each animal, 6 male rats per group was employed.
3. Results of negative and positive controls were expected.
4. Criteria for positive results: If any treatment group shows a response (mean frequency of MPCE) which is >4 times the concurrent vehicle control value, the result is considered positive, or if any treatment group shows a response (mean frequency of MPCE) which is <4 times the concurrent vehicle control value but exceeds the historical control range (98% confidence limits), an additional 2000 PCE (where possible) will be analyzed from each animal (vehicle and all treated groups). If the additional data shows that one or more treatment groups show a response <4 times the concurrent vehicle control but this exceeds the historical control range (98% confidence limits), the data will be subjected to statistical analysis to determine statistical significance as compared to vehicle group, as well as dose-relationship (trend test).

Study outcome:
All doses induced clinical signs including restless/agitated, eyes closed, breathing abnormalities (irregular/labored/rapid), jerky movements, low posture, slow movements,

piloerection, reluctance to move, subdued behavior, tremors (1 male at 10 mg/kg/day) and unsteady gait. Additionally, group mean bodyweight loss of 11-13% in all treated groups were observed, compared to control of increase of 4.7% body weight in 2 days. These clinical findings and body weight changes showed no dose relationship and the sponsor concluded that the data indicated that the animals could tolerate the doses, although the doses appear to be close to the maximum tolerated dose.

GW685698X showed no bone marrow toxicity as the slight reduction of % PCE (control, 52%; treated 40-42%) in all treated groups were within historical control range (37-57% PCE) and was not considered biological relevant.

The group mean MPCE/2000 PCE for 10 mg/kg/day exceeded the historical control range (mean MPCE/2000 PCE is 2.28. and ranged 1.16-3.41), however, given the increase was marginal (3.46 MPCE/2000 compared with the upper confidence limit of the historical range of 3.41 MPCE/2000), the relative increase was only 1.7-fold, no animal exhibited an individual MPCE/2000 PCE value greater than those seen in the individual animal data range and that there was no increase at the higher doses of 20 and 40 mg/kg/day, the increase was not considered to be of any biological relevance. Group mean values for 20 and 40 mg/kg/day were within the historical control range. Since all treated group mean values were less than 4 times the concurrent vehicle control, and within or close to the historical control range, no statistical analysis was performed and the sponsor concluded the result being negative. This reviewer agrees with the sponsor that this drug is negative of clastogenic effects under the test conditions. The following table presents the results of this micronucleus assay.

Treatment	Dose (mg/kg)	Animal n	% PCE	MPCE
Vehicle	0	6 male rats	54	2.0
GW685698X	10	6 male rats	42	3.5
	20	6 male rats	40	3.0
	40	6 male rats	42	2.5
Cyclophosphamide	20	3 male rats	39	57.6

MPCE: group mean micronucleated PCE (MPCE) per 2000 PCE analysed.

Laboratory Historical Vehicle Control Data (calculated on Jan. 9, 006 based on 320 animals (256 males and 64 females))

	MPCE/2000PCE[1]	% PCE[1]
Mean	2.28	46
Range[2]	1.16-3.41	37-57

1. Group mean value, male and female combined
2. %MPCE range based on 98% confidence limits of group mean values; %PCE range based on observed group mean value

Observed individual animal data (% of animals with 0, 1, 2 etc MN)*

0	1	2	3	4	5	6	7	8	9
5.5	22.2	32.1	20.7	14.6	4.4	0.3	0.0	0.0	0.3

*male and female combined

2.6.6.5 Carcinogenicity

Study title: Carcinogenicity study by inhalation administration to Han Wistar rats for 104 weeks

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: This 2-year rat carcinogenesis study is deemed valid as the doses tested were adequate and the observations made were appropriate. An MTD was reached as evidenced by the findings of dose related decrease of body weight which reached about 10% at the HD groups (compared to two control groups, males, 9.7-11.4%; females, 4.6-8.3%) , as well as corticosteroid effects. Additionally, lower survival rates in treated females were observed (control, 60-67%, MD females, 26%, HD females, 45%).

Evaluation of tumor findings: Rats given GW685698X at 1.00, 3.19, and 8.61 mcg/kg by inhalation showed no significant increases in tumor incidences.

Study no.: GSK document No. WD2005/00895/02
Volume #, and page #: e-submission
Conducting laboratory and location: [redacted]

Date of study initiation: Dec. 16, 20023
GLP compliance: Yes, a signed GLP statement was included in the study report
QA report: yes (X) no ( )
Drug, lot #, and % purity: GW685698X, batch No.E02B348, E02L2040, E02B347, purity of 97.9-99.2%.
CAC concurrence: The CAC concurred that this study is acceptable. The CAC concurred that GW685698X did not increase tumor incidence in this study. The CAC meeting minutes of 1/16/07 meeting is attached on page 90.

Methods
Doses: estimated achieved inhalation doses of 0 (lactose, 2 groups), 1.00, 3.19, and 8.61 mcg/kg

Basis of dose selection (MTD, MFD, AUC etc.): MTD was identified in a 13-week inhalation study (5, 9, 24 mcg/kg) where a dose-related reduction of body weight gain (males, 1, 20 and 33% at the LD, MD and HD, respectively; females, 15, 24 and 54% at the LD, MD and HD, respectively) and typical glucocorticoid effects were observed. On the basis of the effect on body weight gain, 24 mcg/kg was considered by the sponsor too

high for use in a carcinogenicity study and therefore, 9 mcg/kg was selected as the target high dose.

Species/strain: Han Wistar rats

Number/sex/group (main study): 60/sex/group

Route, formulation, volume: Snout only inhalation, GW685698X powder was blended with lactose powder BP/USNF at a nominal concentration of 0.4% (w/w). Animals were exposed to a designated aerosol concentration for 60 minutes per day. The two control groups were treated with lactose aerosol at a concentration that is similar to that for the high dose groups.

Frequency of dosing: 60 min inhalation daily for 104 weeks

Satellite groups used for toxicokinetics or special groups: 3/sex/time point for control and HD groups

Age: 8-9 weeks (initial age)

Animal housing: The rats were housed in groups of 4 (main study animals) or 3 (toxicokinetic study animals) of the same sex and treatment group in stainless steel cages with grid floors.

Restriction paradigm for dietary restriction studies: N/A

Drug stability/homogeneity:
GW685698X was supplied pre-formulated by GSK (blend batch E02B139) or blended at [redacted] GW685698X powder was blended with lactose powder BP/USNF at a nominal concentration of 0.4% (w/w) (as base) and stored at room temperature. The details of the blends were as follows:

Blend batch number	Input drug batch number	Prepared by	Expiry date	Dates of use		Content uniformity
				From	To	
E02B139		GSK	30 Apr 03	10 Mar 03	30 Apr 03	#
[redacted]	E02B348	[redacted]	31 Jan 04	30 Apr 03	27 Jan 04	98.6
	E02L2040	[redacted]	31 Dec 04	26 Jan 04	29 Nov 04	99.2
	E02B347	[redacted]	31 Jul 05	30 Nov 04	10 Mar 05	97.9

* Blended at [redacted] under a separate study ([redacted] to the principles of GMP
\# Data not present, assumed pure

Dual controls employed: Two lactose control groups were employed.

Interim sacrifices: No.

Deviations from original study protocol: No major deviations from original protocol occurred.

Observation times
Mortality: Twice daily at each working day. A necropsy was performed on those main study animals that died or were killed during the treatment period. Toxicokinetic animals that died or were killed during the treatment period were discarded without necropsy.

Clinical signs: Daily, throughout the pretreatment period and during treatment. Detailed clinical examinations were performed prior to initiation of dosing, and weekly until necropsy. Palpation - The animals were examined weekly in conjunction with the physical examination. Masses were recorded every 4 weeks from Week 10. New masses were recorded at detection in respect of the mass number and location, date, size and description.

Body weights: The animals were weighed at least once during the pretreatment period, on the day that treatment commenced, each week for the first 16 weeks, thereafter once every 4 weeks and on the day of necropsy.

Food consumption: Cage food consumption was recorded weekly during the week before treatment commenced, each week for the first 16 weeks and thereafter once every four weeks.

Ophthalmoscopy: All rats were examined prior to initiation of dosing, 10/sex/group were examined during Week 78, prior to inhalation dosing of the day.

Hematology: 10 animals/sex/goup (main study) were examined during Weeks 31 and 103.

Clinical Chemistry: At the same time as that for hematology examinations.

Histopathology: All animals including those subjected to terminal sacrifice and unscheduled deaths in the main study were examined histopathologically.
Peer review: yes (X), no ( )
Selected (no details provided) microscopic tissue sections and pathology data interpretation subjected a peer review. The histopathologic diagnoses and the interpretation reported reflect a concurrence of peer review pathologist and the study pathologist.

The tissues subjected microscopic examinations included the following:

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Ovaries	X
Adrenals	X	Pancreas	X
Animal identification		Parathyroids	X
Aorta (thoracic)	X	Pituitary	X
Bone marrow		Preputial/clitoral gland	X
Brain	X	Prostate	X
Caecum	X	Rectum	X
Colon	X	Salivary gland	
Duodenum	X	mandibular	X[1]
Epididymides	X	sublingual	X[1]
Eyes/Optic nerves	X	parotid	X[1]
Femur (Stifle joint)	X	Sciatic nerve	X[1]
Harderian glands	X	Seminal vesicles	X
Head		Skeletal muscle (thigh)	X[1]
Heart	X	Skin	X
Ileum	X	Spinal cord (whole cord)	X[2]
Jejunum	X	lumbar	X
Kidneys	X		
Lachrymal glands		Spleen	X
Larynx and oropharynx	X	Sternum with bone marrow	X
Liver (all main lobes)	X	Stomach	X
Lung (all lobes and bronchi)	X	Testes	X
Lymph node -		Thymus	X
Cervical	X	Thyroids	X
Mesenteric	X	Tongue	X
Tracheo-bronchial	X	Trachea	X
Regional to masses	X	Tracheal Bifurcation (with main stem bronchi)	X
Mammary gland (inguinal)	X	Urinary bladder	X
Nasal cavities and Nasopharynx with skull	X	Uterus with cervix	X
Oesophagus	X	Vagina	X

1. Only one examined
2. Retained in situ at necropsy. TS and LS sections prepared and examined from the lumbar region

Following the initial microscopic examination of the lung tissue sections, the sponsor conducted an investigation specifically designed to evaluate the protein content of the eosinophilic inclusions present in the cytoplasm of non-ciliated bronchiolar epithelial cells (Clara cells) in the rat lung. However, this investigational effort failed to accomplish such a goal.

Toxicokinetics: Blood samples were obtained from toxicokinetic study animals during Weeks 4, 26, 45 and 58 at the following time points: predose, 5 min, 30 min, 1 hour, 2 hour and 4 hours after dosing for the HD animals and at 5 min, 30 min, and 1 hour post dosing for control animals.

Results

Estimated delivered doses:

Group	Aerosol conc. (ug/L)	Inhaled dose (ug/kg)	MMAD (um)	Pulmonary dose (ug/kg)
1	0	0		0
2	0	0		0
3	0.030	1.00		0.1
4	0.095	3.19		0.32
5	0.252	8.61		0.86

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)
D dose (µg/kg)
RMV(mL/min) 4.19xBW(g) $^{0.66}$[McMahon et al, 1977]
T Duration of exposure/day (minutes)
C Aerosol GW685698X concentration (µg/L)
BW Group average body weight for study (g)
Pulmonary dose was estimated to be 10% of inhaled dose.

Mortality: The mortality and cumulative survival throughout the study are presented in the tables below (from the statistics review by Dr. Steve Thomson on 11/15/2006):

Male rats

Period (Weeks)	Control 1	Control 2	Low 1.00 µg/kg/day	Medium 3.19 µg/kg/day	High 8.61 µg/kg/day
1-50	0/60 [1] 100%[2]	2/60 [1] 97%[2]	2/60 97%	1/60 98%	1/60 98%
51-78	7/60 88%	2/58 93%	1/58 95%	7/59 87%	5/59 90%
79-91	7/53 77%	7/56 82%	2/57 92%	3/52 82%	7/54 78%
92-104	12/46 57%	12/49 62%	11/55 73%	13/49 60%	12/47 58%
Terminal	34	37	44	36	35

[1] number deaths / number at risk in each cell.
[2] In each cell, Kaplan-Meier estimate of cumulative survival at end of interval.

Female rats

Period (Weeks)	Control 1	Control 2	Low 1.00 µg/kg/day	Medium 3.19 µg/kg/day	High 8.61 µg/kg/day
1-50	0/60 [1] 100%[2]	0/60 [1] 100%[2]	1/60 98%	1/60 98%	0/60 100%
51-78	4/60 93%	6/60 90%	7/59 87%	6/59 88%	5/60 92%
79-91	6/56 83%	7/54 78%	1/52 85%	10/53 72%	8/55 78%
92-104	10/50 67%	11/47 60%	12/51 65%	14/43 26%	20/47 45%
Terminal	40	36	39	29	27

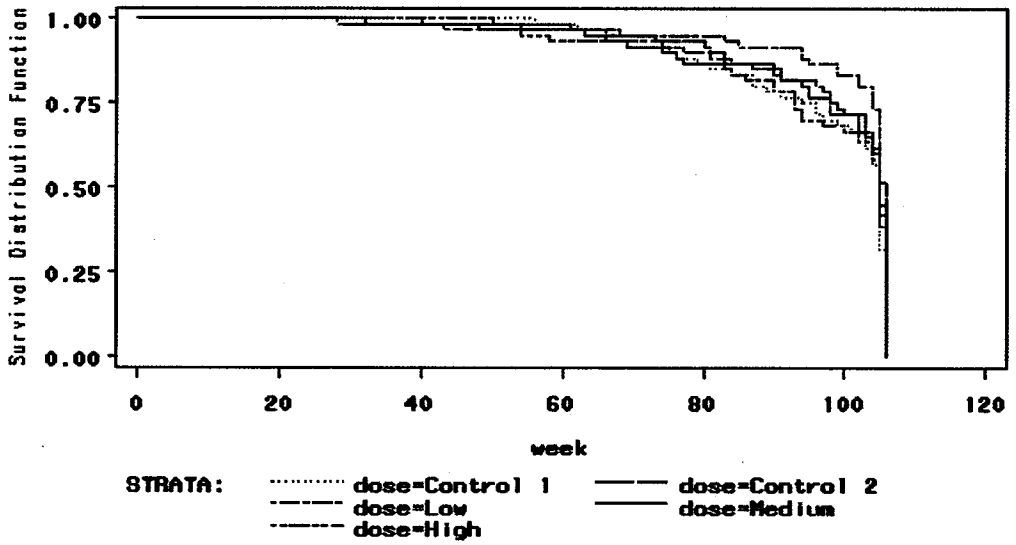
[1] number deaths / number at risk in each cell.
[2] In each cell, Kaplan-Meier estimate of cumulative survival at end of interval.

Note that the terminal sacrifice occurred in mainly in weeks 105-106, although generally three animals per treatment group were sacrificed at week 104. To be consistent with the treatment period for the carcinogenicity analysis, statistics reviewer pooled these three sacrifices with the natural deaths in weeks 92-103 the mortality table. Thus the mortality tables indicate mortality across weeks 92-104, not 92-103.

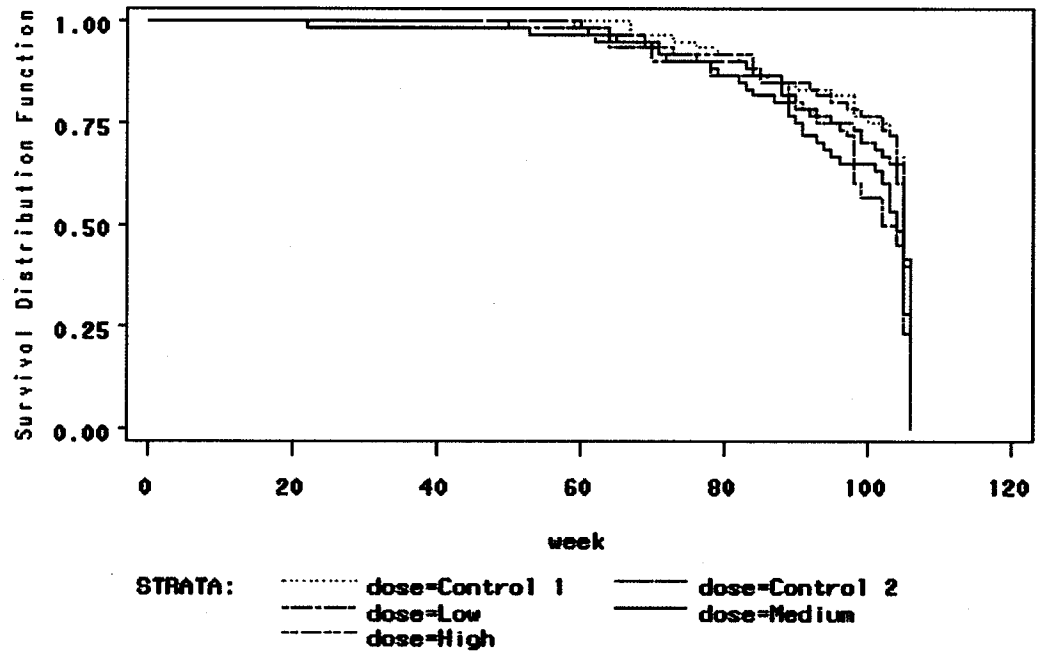
The mortality data presented in the study report is slightly different in that the animals sacrificed in Week 104 were included in the terminal sacrifice category in the study report.

In spite of the slight difference in the mortality numbers, the FDA statistics reviewer agrees with the sponsor that there was treatment related mortality in females but not males. As stated in the review, the estimated survival curves for male rats were generally rather interwined, with no particular treatment group having the uniformly higher survival. Starting early in the study, male rats in the low dose treatment group (1.00 µg/kg/day) tended to have the highest survival, while for female rats, at the end of the study the control groups tended to have the highest survival. The difference in survival in the mid- and high-dose groups was noted primarily from week 92 on. The log rank tests of homogeneity in survival was statistically significant ($p=0.0317$) in female rats at the two highest doses, as was the overall test of trend in survival in ($p=0.0169$). No information regarding the cause of death was available. In male rats differences in survival were not statistically significant.
The following two figures display the Kaplan-Meier estimated survival curves including the time of censoring, including sacrifice or accidental death, as an event (Statistics Review by Dr. Steve Thomson, 11/15/2006).

Male rats



Female rats



Clinical signs: A higher incidence of hairloss (chiefly on head) was noted in treated animals. The number of animals affected was as follows:

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose	0	0	1	3.19	8.61	0	0	1	3.19	8.61
n	60	60	60	60	60	60	60	60	60	60
Hair loss	13	19	26	41	60	29	29	34	55	59

There was no treatment-related effect on the incidence and distribution of palpable swellings detected in life.

Body weights:
By the end of the study, the body weights of the HD animals were lower than combined two controls (males, 10.6%; females6.6%). The decreased body weights in the mid and low dose groups were less significant than that of the HD groups.

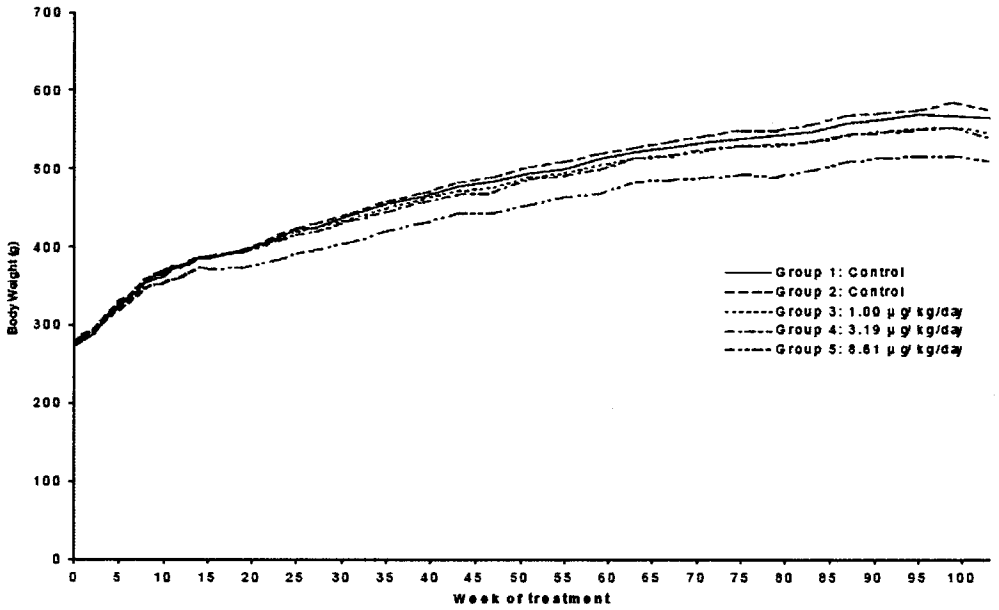
Body weights in the 2-year study rats (mean ±SD, grams)

		Dose (ug/kg)	0	0	1	3.19	8.16
Male	Week 0	N	60	60	60	60	60
			275 ±19.3	276 ±19.7	278 ±19.8	279 ±20.2	279 ±18.8
	Week 103	N	39	42	48	40	38
			566 ±59.4	577 ±63.5	548* ±64.2	541* ±60.1	511** ±65.3
	WK103 % of control 1, % of control 2		100		96.8 95.0	95.5 93.8	90.3 88.6
Female	Week 0	N	59	60	60	60	60
			179 ±11.8	182 ±13.5	181 ±13.1	182 ±12.5	181 ±12.0
	Week 103	N	44	39	43	32	30
			337 ±38	350 ±44.5	354 ±46.3	324* ±35.5	321* ±33.7
	WK103 % of control 1, % of control 2		100		105.0 101.1	96.1 92.6	95.3 91.7

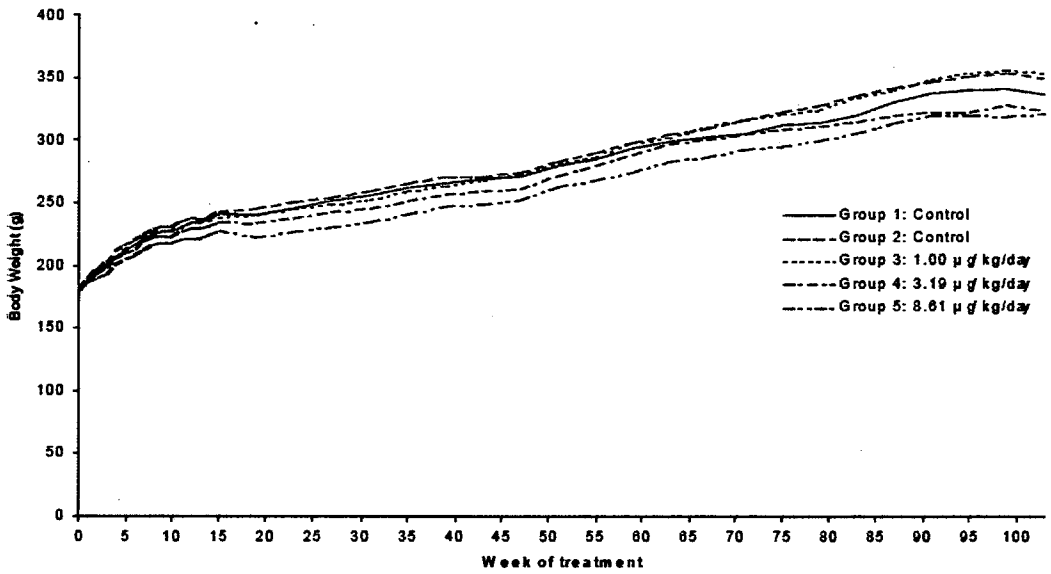
Significantly different from combined control, * P<0.05, **, P<0.01

In general the reduced rate of weight gain in treated males was seen up to Week 67 of exposure after which rate of weight gain was similar to or exceeded that of controls. In females statistically significant differences from control weight gains were seen at the MD and the HD only up to Week 19. The following two figures show the changes of body weights throughout the 2 years of treatment period.

Body Weight – group mean values (g) for males



Body Weight – group mean values (g) for females



Food consumption: There were no treatment related effects.

Ophthalmoscopy: No remarkable findings

Hematology: In Week 31 total white cells, lymphocytes, eosinophils, basophils and monocytes were statistically significantly lower than combined control values by 28, 34, 47, 50 and 30% respectively, in males at 8.61 mcg/kg/day. In Week 104 total white cells, lymphocytes, basophils and large unclassified cells were statistically significantly lower than combined control values by 26, 29, 80 and 38% respectively, in males at 8.61 mcg/kg/day.

Clinical Chemistry: Potassium levels in males dosed at 3.19 and 8.61mcg/kg/day were 11% higher than combined controls. Bilirubin levels in females dosed at 3.19 and 8.61 mcg/kg/day were 50% higher than combined controls.

Gross pathology:
An increased incidence of forestomach depression, hair loss), and decreased incidence of pale areas on the lungs were observed in treated groups. Note that the values for hair loss presented in the following table are the incidence in terminal killed animals and the values reported in the clinical signs section included all animals. There were no histopathological findings associated with these gross lesions. These lesions might be treatment related. However, the toxicological significances are not clear. The following table presents the incidences of gross lesions in the terminal sacrifice.

Incidence of gross lesions in rats killed after 104 weeks of treatment

	males					females				
Dose (ug/kg)	0	0	1	3.19	8.16	0	0	1	3.19	8.16
n	37	40	48	39	38	43	39	43	32	30
Forestomach depression	1	0	1	6	5	0	1	4	3	7
Hairloss	10	13	22	31	38	21	18	24	30	30
Pale areas on lungs	30	30	34	16	19	30	28	36	11	10

Histopathology:

Non-neoplastic:
Non-neoplastic findings were typical glucocorticoid effects and primarily affected the respiratory tract. These changes were decreased cellularity of lymphoid tissues (nose-associated lymphoid tissue and bronchiole-associated lymphiod tissue), decreased incidences of inflammatory cells in lamina propria or subepithelium in nasal turbinate, nasal pharynx, larynx, and tracheal bifurcation. Secondary to immune suppression, fungal rhinitis in nasal turbinate (one female at each of the MD and HD), and dilated invaginations from ventral pouch were also recorded (see details in the relevant part below).

Additional findings in the respiratory tracts included the increased pigmented macrophages in the lamina propria or subepithelium of larynx and trachea, and the lung, increased perivascular/peribronchiolar inflammatory cells and decreased incidences of foamy alveolar macrophages in the lung, as well as the changes of eosinophilic inclusions that was decreased in the olfactory epithelium and increased in bronchiolar epithelium.

The nature and the toxicological significance of pigmented macrophages, perivascular/peribronchiolar inflammatory cells are not clear. Eosinophilic inclusion changes and decreased alveolar foamy macrophages are not considered toxicologically significant (the decreased eosinophilic inclusions possibly reflect a decrease of age-related increase of the inclusions, and increased eosinophilic inclusions affect only up to 0.78% Clara cells in rats given 6 month inhalation of this drug, though the specific proportion of affected cells in the current study is not provided. (See IND 48,647, review 6 of the submission dated 11/2/05).

The only systemic finding, increased incidence of mastocytosis in the mesenteric lymph nodes was seen in a dose-related fashion. This finding was not observed in previous toxicity studies. The sponsor considered this finding an expected steroid effect based on a literature report (Gopinath C, Prentice, DE and Lewis, DJ. Chapter 8: The Lymphoid System. Atlas of Experimental Toxicological Pathology. MTP Press Limited, 1987:130). This reviewer considered this finding being not significant on the evaluation of carcinogenesis potential although its toxicological significance is unknown.

The incidences and severities of the non-neoplastic lesions are presented below.

1. Nasal turbinates: Decreased cellularity of the nose-associated lymphoid tissue (NALT), decreased incidences of inflammatory cells in the lamina propria of the respiratory epithelium and decreased incidences and/or degrees of eosinophilic inclusions in the olfactory epithelium

		Males					Females				
Dose (µg/kg/day)		**0**	**0**	**1.00**	**3.19**	**8.61**	**0**	**0**	**1.00**	**3.19**	**8.61**
Cellularity of NALT	Total	56	60	59	60	60	58	60	59	57	58
	Minimal	9	11	16	34	45	7	14	24	36	44
	Slight	47	49	43	26	15	51	46	35	21	14
Respiratory epithelium – inflammatory cells in lamina propria	Total	19	17	23	8	9	22	14	15	12	7
	Minimal	17	14	20	6	6	21	13	14	12	4
	Slight	2	2	3	2	3	1	0	0	0	3
	Moderate	0	1	0	0	0	0	1	1	0	0
Olfactory epithelium – eosinophilic inclusions	Total	22	28	10a	9a	9a	13	11	10	3a	4a
	Minimal	7	10	5	6	6	8	8	5	3	3
	Slight	8	9	2	3	3	1	2	5	0	1
	Moderate	7	6	3	0	0	3	1	0	0	0
	Marked	0	3	0	0	0	1	0	0	0	0
Number of nasal turbinates examined		**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**59**

a – $p < 0.05$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.

2. Nasopharynx: Dose-related decreases of incidences of subepithelial inflammatory cells

		Males					Females				
Dose (µg/kg/day)		0	0	1.00	3.19	8.61	0	0	1.00	3.19	8.61
Subepithelial inflammatory cells	Total	36	31	27	9b	12b	35	24	19	10b	6b
	Minimal	18	14	13	6	11	21	12	13	9	5
	Slight	15	17	14	3	1	12	11	6	1	1
	Moderate	3	0	0	0	0	2	1	0	0	0
Number of nasopharynges examined		60	60	60	60	60	60	60	60	60	59

b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.

3. Larynx: Decreased incidences of inflammatory cells in the lamina propria, increased pigmented macrophages in the lamina propria, and increased incidences of dilated invagination(s) from the ventral pouch were reported. The dilated invaginations usually contained inflammatory cells and foreign materials. Inflammatory reactions in the ventral pouch were seen in control rats, associated with the presence of foreign particles, and with the passage of time, the foreign material might be expected to become localized in invaginations from the pouch. The increased incidences of these lesions in all three treated groups are probably relate to immunosuppression, and delayed healing. The incidence and severity of the findings in the larynx are presented below.

		Males					Females				
Dose (µg/kg/day)		0	0	1.00	3.19	8.61	0	0	1.00	3.19	8.61
Inflammatory cells in lamina propria	Total	55	56	54	39b	35b	57	52	49	41a	30b
	Minimal	30	18	37	26	21	32	32	36	34	19
	Slight	24	32	14	11	12	24	16	10	7	5
	Moderate	1	5	3	2	2	1	4	3	0	6
	Marked	0	1	0	0	0	0	0	0	0	0
Pigmented macrophages in lamina propria	Total	0	0	0	2	5	0	0	7a	20b	22b
	Minimal	0	0	0	2	5	0	0	7	20	21
	Slight	0	0	0	0	0	0	0	0	0	1
Dilated invagination(s) from ventral pouch	Total	31	34	39	46a	47a	15	17	31a	33b	26
	Minimal	6	4	3	4	2	2	3	2	3	1
	Slight	15	13	16	15	14	11	6	10	14	12
	Moderate	8	14	15	17	25	2	7	15	7	10
	Marked	2	3	5	10	6	0	1	4	9	3
Number of larynges examined		60	60	60	60	60	60	59	60	60	60

a – $p < 0.05$, b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately. Where the significance differs, the lesser degree of significance is indicated (i.e. a rather than b)

4. Trachea: Decreased incidences of inflammation

		Males					Females				
Dose (µg/kg/day)		0	0	1.00	3.19	8.61	0	0	1.00	3.19	8.61
Inflammation	Total	9	15	5	2	6	13	10	1b	0b	2a
	Minimal	7	13	4	2	2	13	10	1	0	1
	Slight	2	2	1	0	3	0	0	0	0	1
	Moderate	0	0	0	0	1	0	0	0	0	0
Number of tracheas examined		60	60	60	60	60	60	59	60	60	60

a – $p < 0.05$, b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately. Where the significance differs, the lesser degree of significance is indicated (i.e. a rather than b)

5. Tracheal bifurcation: Decreased incidences of subepithelial inflammatory cells at the point of bifurcation and in the adjacent trachea and bronchi, increased

incidences of subepithelial pigmented macrophages in the trachea and bronchi, and an increased incidence of dilated glands in the trachea.

		Males					Females				
Dose (µg/kg/day)		**0**	**0**	**1.00**	**3.19**	**8.61**	**0**	**0**	**1.00**	**3.19**	**8.61**
Subepithelial inflammatory cells	Total	15	12	1b	0b	0b	17	15	4b	2b	0b
	Minimal	11	7	0	0	0	12	7	3	2	0
	Slight	4	5	1	0	0	5	8	1	0	0
Trachea – subepithelial inflammatory cells	Total	18	21	4b	4b	0b	14	12	0b	0b	2b
	Minimal	16	19	4	3	0	14	11	0	0	2
	Slight	2	2	0	0	0	0	1	0	0	0
	Moderate	0	0	0	1	0	0	0	0	0	0
Bronchi – subepithelial inflammatory cells	Total	36	38	9b	2b	1b	33	31	6b	2b	0b
	Minimal	36	36	9	2	1	33	27	6	2	0
	Slight	0	2	0	0	0	0	4	0	0	0
Trachea – subepithelial pigmented macrophages	Total	0	1	0	1	9a	0	2	3	7	20b
	Minimal	0	1	0	1	9	0	2	3	7	19
	Slight	0	0	0	0	0	0	0	0	0	1
Bronchi – subepithelial pigmented macrophages	Total	0	1	0	1	4	3	6	2	4	16a
	Minimal	0	1	0	1	4	3	6	2	4	14
	Slight	0	0	0	0	0	0	0	0	0	2
Trachea – dilated glands	Total	6	12	11	17	27b	11	9	17	18	15
	Minimal	0	2	0	0	1	0	1	0	0	0
	Slight	2	8	6	8	13	6	4	9	8	5
	Moderate	4	2	4	7	10	5	2	6	9	6
	Marked	0	0	1	2	3	0	2	2	1	4
Number of tracheal bifurcations examined		**60**	**60**	**60**	**60**	**60**	**60**	**59**	**60**	**59**	**60**

a – $p < 0.05$, b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately. Where the significance differs, the lesser degree of significance is indicated (i.e. a rather than b)

6. Lungs: Decreased cellularity of the bronchiole-associated lymphoid tissue (BALT), increased incidence of eosinophilic inclusions in the bronchiolar epithelium (minimal focal or multifocal).

		Males					Females				
Dose (µg/kg/day)		**0**	**0**	**1.00**	**3.19**	**8.61**	**0**	**0**	**1.00**	**3.19**	**8.61**
BALT absent		0	1	6	23b	36b	0	0	4	19b	38b
Cellularity of BALT	Total	56	59	53	37b	24b	60	59	56	39b	21b
	Minimal	11	7	39	28	20	14	10	38	31	20
	Slight	41	52	14	8	4	45	43	18	8	1
	Moderate	4	0	0	1	0	1	6	0	0	0
Bronchiolar epithelium – eosinophilic inclusions	Total	8	7	20a	32b	27b	10	10	33b	38b	50b
	Minimal	8	7	20	32	27	10	10	33	38	50
Number of lungs examined		**60**	**60**	**60**	**60**	**60**	**60**	**59**	**60**	**60**	**60**

a – $p < 0.05$, b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.

Additionally, a decreased incidence of foamy alveolar macrophages, and an increased incidence of inflammatory cells and pigmented macrophages in perivascular/peribronchiolar areas were recorded as the following.

		Males					Females				
Dose (µg/kg/day)		**0**	**0**	**1.00**	**3.19**	**8.61**	**0**	**0**	**1.00**	**3.19**	**8.61**
Foamy alveolar macrophages	Total	41	35	40	29	25	30	35	29	27	21
	Minimal	28	15	26	20	20	18	19	13	20	19
	Slight	13	20	12	8	5	10	16	16	7	2
	Moderate	0	0	2	1	0	2	0	0	0	0
Cholesterol cleft granuloma(ta)	Total	10	10	4	5	8	4	5	4	5	2
	Minimal	7	6	3	2	3	3	4	4	3	1
	Slight	2	3	1	3	5	1	1	0	2	1
	Moderate	1	1	0	0	0	0	0	0	0	0
Pigmented alveolar macrophages	Total	11	16	20	13	13	10	11	20	15	23a
	Minimal	11	14	19	12	12	10	11	20	15	20
	Slight	0	1	1	1	1	0	0	0	0	3
	Marked	0	1	0	0	0	0	0	0	0	0
Perivascular/peribronchiolar inflammatory cells	Total	36	39	47	46	50a	41	42	44	47	46
	Minimal	34	36	45	42	47	37	38	41	46	44
	Slight	2	3	2	4	3	4	4	3	0	2
	Moderate	0	0	0	0	0	0	0	0	1	0
Perivascular/peribronchiolar pigmented macrophages	Total	3	2	4	2	6	21	17	23	26	39b
	Minimal	3	2	4	2	6	21	17	23	25	32
	Slight	0	0	0	0	0	0	0	0	1	7
Number of lungs examined		**60**	**60**	**60**	**60**	**60**	**60**	**59**	**60**	**60**	**60**

a – $p < 0.05$, b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.

7. Mesenteric lymph node: A dose-related increase of mastocytosis

		Males					Females				
Dose (µg/kg/day)		**0**	**0**	**1.00**	**3.19**	**8.61**	**0**	**0**	**1.00**	**3.19**	**8.61**
Mastocytosis	Total	13	17	27	28	39b	19	16	19	31a	37b
	Minimal	10	10	21	17	20	11	11	14	17	20
	Slight	2	7	5	8	13	8	3	3	13	14
	Moderate	0	0	1	3	6	0	2	2	1	3
	Marked	1	0	0	0	0	0	0	0	0	0
Number of mesenteric lymph nodes examined		**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**

a – $p < 0.05$, b – $p < 0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately. Where the significance differs, the lesser degree of significance is indicated (i.e. a rather than b)

Neoplastic:
Tumors with increased incidences in treated groups were seen in liver, pancreas and adrenal gland (see below for details). However, these increased tumor incidences did not reach statistically significant levels based on the statistics analysis performed by FDA statistics review.

Liver tumors: The number of hepatocellular carcinoma and of adenoma and carcinoma combined was higher in male rats receiving 8.61 μg/kg/day than in controls (see the table below).

	Males				
Dose (μg/kg/day)	**0**	**0**	**1.00**	**3.19**	**8.61**
Hepatocellular adenoma	1	4	2	4	4
Hepatocellular carcinoma	0	0	0	0	2
Hepatocellular adenoma and/or carcinoma	1	4	2	4	6
Number of livers examined	**60**	**60**	**60**	**60**	**60**

Pancreas tumors:
The incidence of islet cell adenoma was higher in the mid dose (3.19 mcg/kg) males and low dose (1.00 mcg/kg) males compared to controls. One male rat in each of the low dose and high dose (8.61 mcg/kg) had islet cell carcinoma, and the controls showed zero incidences. The table below presents the incidences of pancreatic tumors.

	Males				
Dose (μg/kg/day)	**0**	**0**	**1.00**	**3.19**	**8.61**
Islet cell adenoma	2	1	3	6	2
Islet cell carcinoma	0	0	1	0	1
Islet cell adenoma and/or carcinoma	2	1	4	6	3
Number of pancreases examined	**60**	**60**	**60**	**60**	**60**

Adrenal tumors: There was a higher incidence of cortical adenoma in female rats compared to the controls (see the table below).

	Females				
Dose (μg/kg/day)	**0**	**0**	**1.00**	**3.19**	**8.61**
Cortical adenoma	2	0	0	0	4
Number of adrenals examined	**60**	**60**	**60**	**60**	**60**

FDA statistical reviewer's analysis revealed that prior to adjusting for multiplicity, only three test of tumor incidence were found to be statistically significant (see the table below). For the Haseman-Lin-Raham rules for adjusting for the large number of comparisons, those tumor-organ combinations with one or fewer tumors in the control group would be classified as rare tumors, the remainder as common. Then, using these rules, no pairwise comparison or test of trend would be considered as statistically significant (trend test, P>0.025 for rare tumors and P>0.005 for common tumors; pair wise test, P>0.05 for rare tumors and P>0.01 for common tumors). Therefore, it is concluded that these results are consistent with the notion of no particular carcinogenic signals. This conclusion is in agreement **with the sponsor's conclusion that treatment** with GW685698X did not increase the incidence of any neoplastic finding.

Organ / Tumor	Con-trol1	Con-trol2	Low	Med-ium	High	p-values: Trend	Hi vs Cntrl
Male Rats							
LIVER							
HEPATOCELLULAR CARCINOMA	0	0	0	0	2	0.0346	0.1072
Hep. Adenoma/Carcinoma	1	4	2	4	6	0.0381	0.0644
Female Rats							
ADRENALS							
CORTICAL ADENOMA	2	0	0	0	4	0.0199	0.1504

Tumors with decreased incidences including hemangioma and lymphangioma in the mesenteric lymph node of both sexes at 8.61 mcg/kg and thymoma (lymphoid) were reported (see the tables below). These decreases of tumor incidence are not biologically significant.

Incidence of hemangioma and lymphangioma in mesenteric lymph nodes

	Males					Females				
Dose (µg/kg/day)	0	0	1.00	3.19	8.61	0	0	1.00	3.19	8.61
Haemangioma	9	12	8	9	2	4	6	2	2	0
Lymphangioma	2	3	0	1	0	1	0	0	0	0
Focal endothelial hyperplasia	1	0	1	0	0	1	1	1	0	0
Number of mesenteric lymph nodes examined	60	60	60	60	60	60	60	60	60	60

Incidence of thymoma in rat 2-year inhalation study with GW685698X

	Males					Females				
Dose (µg/kg/day)	0	0	1.00	3.19	8.61	0	0	1.00	3.19	8.61
Thymoma (lymphoid)	3	1	1	2	0	4	7	5	0	2
Number of thymuses examined	57	59	58	57	57	60	59	58	60	60

Toxicokinetics:

The analysis of samples collected in Weeks 4 and 26 gave spurious results due to contamination and, therefore, are not reported. The data of control and the HD group in Weeks 45 and 58 were obtained. There was no quantifiable concentration of GW685698X in control rats. Systemic exposure of GW685698X (AUC_{0-t} and Cmax) in the HD rats was similar in Weeks 45 and 58; exposure increased by approximately 40% at the later time point. No significant gender difference was noted. The mean Cmax was observed within one hour after the end of the exposure period. The table below summarizes the TK data obtained.

Sex	Target Dose (µg/kg/day)	Sampling Occasion	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)
Male	9	Week 45	0.275	0.094	1.17
		Week 58	0.387	0.144	1.14
Female	9	Week 45	0.257	0.118	1.17
		Week 58	0.361	0.130	1.54

Study title: Carcinogenicity study by inhalation administration to CD-1 mice for 104 weeks

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: This mouse 2-year inhalation carcinogenicity study is deemed adequate. An MTD was reached based on the decreased body weight at HD (males, 5.7-9.6%; females, 6.5-7.8%) compared to lactose controls, as well as corticosteroid effects.

Evaluation of tumor findings: Mice given GW685698X by inhalation (2.22, 6.09, and 18.8 mcg/kg) showed no significant increase in tumor incidence.

Study no.: GSK document No. WD2005/00894/01
Volume #, and page #: e-submission
Conducting laboratory and location: [redacted]

Date of study initiation: March 10, 2003
GLP compliance: Yes, a signed GLP statement was included in the study report
QA report: yes (X) no ( )
Drug, lot #, and % purity: GW685698X, batch No.E02B139, [redacted] and purity of 97.9-99.2%.
CAC concurrence: The CAC concurred that this study is acceptable. The CAC concurred that GW685698X did not increase tumor incidence in this study.

Methods
Doses: estimated inhalation doses of 0 (lactose, 2 groups), 2.22, 6.09, and 18.8 mcg/kg

Basis of dose selection (MTD, MFD, AUC etc.): MTD was identified in a 13-week inhalation study (7.3, 18.6 and 76.9 mcg/kg) where a dose related reduction of body weight gain/ or body weight loss were observed (males: lower body weight gain of 18, 43 and 94%, for the LD, MD and HD, respectively; females, lower body weight gain of 27% at the MD; females at the HD lost body weight, 3.1%). On the basis of the effect on body

weight gain, 76.9 mcg/kg was considered too high for use in a carcinogenicity study and therefore, 18 mcg/kg was selected as the target high dose.

Species/strain: CD-1 mice

Number/sex/group (main study): 60/sex/group

Route, formulation, volume: Snout only inhalation, GW685698X powder was blended with lactose powder BP/USNF at a nominal concentration of 0.4% (w/w). Each drug treatment group was exposed to a designated aerosol concentration (see results section for details) for 60 minutes per day. Animals in the two control groups were treated with lactose aerosol at a concentration that is similar to that for the high dose groups.

Frequency of dosing: 60 min inhalation daily for 104 weeks

Satellite groups used for toxicokinetics or special groups: 18/sex/dose for treated and 3/sex for control groups

Age: 6-7 weeks (initial age)

Animal housing: The mice were housed in groups of 2 (main study animals) or 3 (toxicokinetic study animals) of the same sex and treatment group in [redacted] Stainless steel cages.

Restriction paradigm for dietary restriction studies: N/A

Drug stability/homogeneity:
GW685698X, was supplied pre-formulated by GSK or blended [redacted] in a GMP environment as part of a separate study. GW685698X powder was blended with lactose powder BP/USNF at a nominal concentration of 0.4% (w/w) (as base). GW685698X/lactose formulations were stored at room temperature. The details of the blends were as follows:

Blend batch number	Input drug batch number	Prepared by	Expiry date	Dates of use		Content uniformity
				From	To	
E02B139		GSK	30 Apr 03	10 Mar 03	30 Apr 03	#
[redacted]	E02B348	[redacted]	31 Jan 04	30 Apr 03	27 Jan 04	98.6
	E02L2040		31 Dec 04	26 Jan 04	29 Nov 04	99.2
	E02B347		31 Jul 05	30 Nov 04	10 Mar 05	97.9

* Blended at [redacted] under a separate study (. .o the principles of GMP
\# Data not present, assumed pure

Dual controls employed: Yes, two lactose control groups were included.

Interim sacrifices: No.

Deviations from original study protocol: No major deviations from original protocol occurred.

Observation times
Mortality: Twice daily at each working day. A necropsy was performed on those main study animals that died or were killed during the treatment period. Toxicokinetic animals that died or were killed during the treatment period were discarded without necropsy.

Clinical signs: Daily throughout the pretreatment period and during treatment. Palpation - The animals were examined weekly in conjunction with the physical examination. Masses were recorded every 4 weeks from Week 0. New masses were recorded at detection in respect of the mass number and location, date, size and description.

Body weights: The animals were weighed at least once during the pretreatment period, on the day that treatment commenced, each week for the first 16 weeks, thereafter once every 4 weeks and on the day of necropsy.

Food consumption: Cage food consumption was recorded weekly during the week before treatment commenced, each week for the first 16 weeks and thereafter 1 week in every four throughout the treatment period.

Ophthalmology: The eyes of all mice were examined prior to initiation of dosing. The eyes of 10 (main study) mice/sex/group were examined during Week 78, prior to inhalation dosing on the day.

Hematology: Blood samples were collected during Week 104 or prior to necropsy at scheduled termination, in 10 animals/sex/group and a different 10 animals/sex/group for clinical chemistry.

Clinical chemistry: See Hematology part.

Histopathology: Peer review: yes (X), no ( )
A peer review of selected microscopic tissue sections (tissue type reviewed was not provided), and pathology data interpretation was completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.

The tissues subjected microscopic examinations included the following:

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Parathyroids	X
Adrenals	X	Pituitary	X
Animal identification		Preputial/clitoral gland	X
Aorta (thoracic)	X	Prostate	X
Brain	X	Salivary gland	
Caecum	X	mandibular	X[1]
Colon	X	sublingual	X[1]
Duodenum	X	parotid	X[1]
Epididymides	X	Sciatic nerve	X[1]
Eyes/Optic nerves	X	Seminal vesicles	X
Femur (Stifle joint)	X	Skeletal muscle (hindlimb)	X[1]
Harderian glands	X	Skin	X
Heart	X	Spinal cord (whole cord)	X[2]
Ileum	X	cervical	
Jejunum	X	thoracic	
Kidneys	X	lumbar	
Lachrymal glands		Spleen	X
Larynx	X	Sternum (with bone marrow)	X
Liver (all main lobes) and gallbladder	X	Stomach	X
Lung	X	Testes	X
Lymph node -		Thymus	X
Cervical	X	Thyroids	X
Mesenteric	X	Tongue	X
tracheo-bronchial	X	Trachea	X
Mammary gland (inguinal)	X	Tracheal Bifurcation (with main stem bronchi)	X
Nasal cavities and nasopharynx with skull	X	Urinary bladder	X
Oesophagus	X	Uterus with cervix	X
Ovaries	X	Vagina	X
Pancreas	X		

1. Only one examined
2. Retained in situ at necropsy. TS and LS sections prepared and examined from all regions

Toxicokinetics: Blood samples were obtained from toxicokinetic study animals during Week 39 at the following time points: predose, immediately after dosing, 1, 2, 3 and 4 hours after dosing. Blood samples from vehicle control animals were collected at one timepoint only, immediately after dosing.

ResultsEstimated delivered doses:

Group	Aerosol conc. (ug/L)	Inhaled dose (ug/kg)	MMAD (um)	Pulmonary dose (ug/kg)
1	0	0		0
2	0	0		0
3	0.03	2.22		0.222
4	0.082	6.09		0.609
5	0.25	18.8		1.88

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)
D dose (µg/kg)
RMV(mL/min) 4.19xBW(g) $^{0.66}$[McMahon et al, 1977]
T Duration of exposure/day (minutes)
C Aerosol GW685698X concentration (µg/L)
BW Group average body weight for study (g)
Pulmonary dose was 10% of inhaled dose.

Mortality: No drug-related differences in survival were noted. The mortality and cumulative survival throughout the study are presented tables below (excerpted from the statistics review by Dr. Steve Thomson on 11/15/2006):

Male mice

Period (Weeks)	Control 1	Control 2	Low 2.22 µg/kg/day	Medium 6.09 µg/kg/day	High 18.8 µg/kg/day
1-50	3/60 [1] 95%[2]	5/60 92%	6/60 90%	7/60 88%	4/60 93%
51-78	6/57 85%	8/55 78%	9/54 75%	9/53 73%	13/56 72%
79-91	13/51 65%	8/47 65%	12/45 55%	7/44 62%	7/43 60%
92-103	8/38 50%	14/39 42%	15/33 30%	10/37 45%	13/36 38%
Terminal	30	25	18	27	23

[1] number deaths / number at risk in each cell.
[2] In each cell, Kaplan-Meier estimate of cumulative survival at end of interval.

Female mice

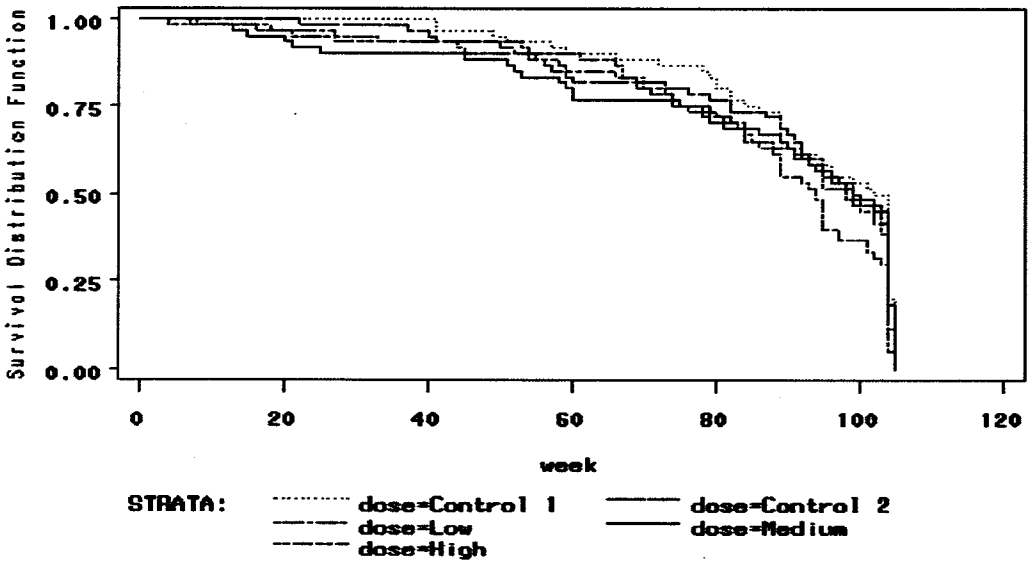
Period (Weeks)	Control 1	Control 2	Low 2.22 µg/kg/day	Medium 6.09 µg/kg/day	High 18.8 µg/kg/day
1-50	9/60 [1] 85%[2]	6/60 [1] 90%[2]	5/60 92%	5/60 92%	9/60 85%
51-78	7/51 73%	13/54 68%	8/54 78%	10/55 75%	8/51 72%
79-91	11/40 55%	9/41 53%	11/45 60%	9/45 60%	9/43 57%
92-103	12/29 35%	13/32 32%	17/33 32%	15/36 35%	13/34 35%
Terminal	21	19	19	21	21

[1] number deaths / number at risk in each cell.
[2] In each cell, Kaplan-Meier estimate of cumulative survival at end of interval.

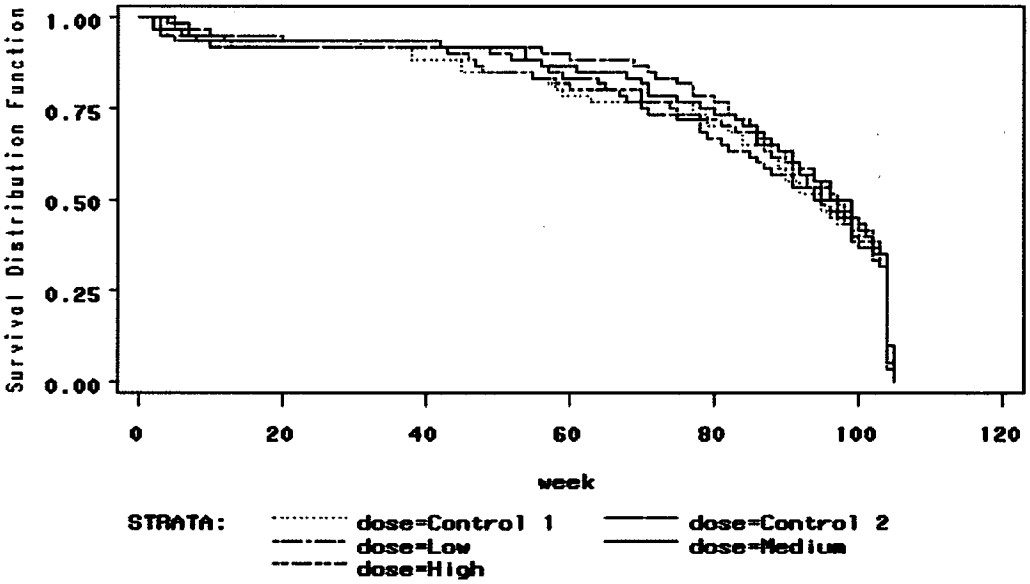
The mortality data reported in the study report were the same as the above.

Kaplan Meier estimated survival curves for the male and female mice are provided as the two figures below (excerpted from the statistics review by Dr. Steve Thomson on 11/15/2006):

Male mouse survival curve



Female mouse survival curve



The survival curves presented in the study report were slightly different in that the sponsor did not include the terminal sacrifice into the curve.

In spite of the slight difference in the data presentation, both FDA statistics reviewer and the sponsor concluded that there is no drug related mortality in both genders. The FDA statistics reviewer stated that for both mouse genders, the estimated survival curves were generally rather intertwined, with no particular treatment group having the uniformly higher survival. In male mice the control tended to have slightly higher survival than the actual dosing groups. For female mice this slight tendency was reversed with the control tending to have slightly lower survival than the three dosing groups. Tests of the hypothesis of homogenous survival curve were not rejected (males, Logrank p=0.2441, p=0.3434, females: Logrank p=0.6721, p=0.9111).

Clinical signs: A higher incidence of hairloss (head, forelimbs, dorsal and ventral surface) was noted in treated animals. The number of animals affected was as follows (60 animals/sex/dose were examined):

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (µg/kg/day)	0	0	2.22	6.09	18.8	0	0	2.22	6.09	18.8
Forelimbs	24	25	34	29	52	37	22	36	39	49
Head	46	44	57	58	58	47	47	54	56	57
Dorsal surface	21	17	23	24	25	5	2	5	11	28
Ventral surface	10	12	13	13	33	5	7	13	15	38

There was no evidence of a treatment-related effect on the incidence and distribution of palpable swellings detected in life.

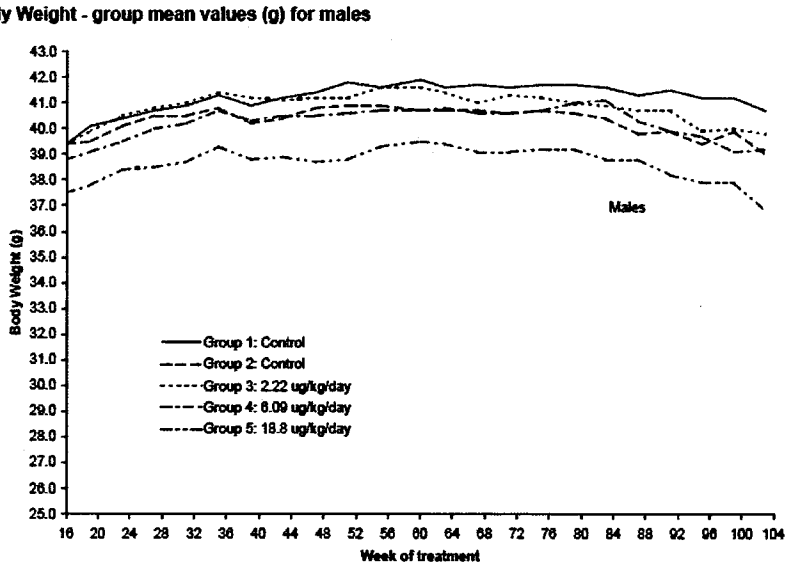
Body weights: By the end of the study, the body weights of the HD animals were lower than combined controls (males, 7.7%; females, 7.2%). The body weights in MD and LD groups were not significantly different from the controls.

Body weights in the 2-year study mice (mean ±SD, grams)

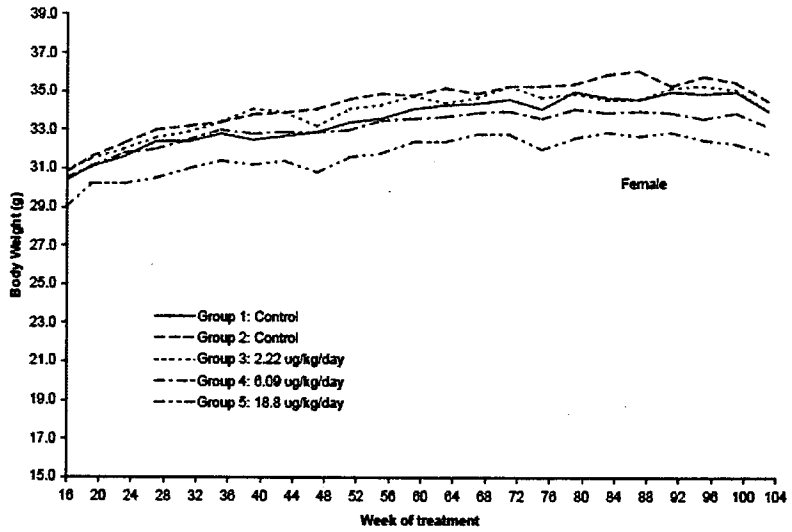
		Dose (ug/kg)	0	0	2.22	6.09	18.8
Male	Week 0	N	60	60	60	60	60
			33.8 ±2.09	33.6 ±1.86	33.9 ±2.10	33.3 ±2.06	34.4* ±1.92
	Week 103	N	30	26	18	27	23
			40.7 ±2.87	39.0 ±2.19	39.8 ±3.63	39.2 ±2.45	36.8** ±2.83
	wk 103		100				
	% of C 1				97.7	96.3	90.4
	% of C 2				102	100.5	94.3
Female	Week 0	N	60	60	60	60	60
			26.3 ±1.84	25.9 ±1.97	26.6 ±1.99	26.7 ±2.03	26.5 ±1.57
	Week 103	N	22	20	19	22	21
			34.0 ±2.68	34.5 ±3.66	34.0 ±2.30	33.2 ±3.76	31.8** ±2.37
	wk 103		100				
	% of C1				--	97.6	93.5
	% of C2				98.5	96.2	92.2

Significantly different from combined control, * P<0.05, **, P<0.01

In general the reduced rate of gain was present for the first 27 weeks of the study and less significant or no difference from the control thereafter. The following two figures show the changes of body weights from Week 16 till the end of the study.



Body Weight - group mean values (g) for females



Food consumption: There were no treatment related effects.

Ophthalmology: There were no treatment related effects.

Hematology: Lymphocyte levels were statistically significantly reduced in Week 104 for all treated males (by 32 to 33%) compared with the combined Control. The change is considered to be treatment related and was similar in all groups and not dose-related. No such effect was present in females. Platelet levels were statistically significantly increased for females receiving 18.8 µg/kg/day (by 43%) compared with combined control.
Clinical Chemistry: Compared to combined controls, the HD females had 26% lower urea and 9% higher total protein.

Gross pathology:
The macroscopic examination of mice that died, sacrificed in extremis and killed at termination revealed lesions in skin and stomach.

The skin lesions included nasal region hair loss in all treated groups (males, 57-59/60 versus 93/120 of control; females, 52-56/60 versus 84/120 of control), nasal scabs in the HD and MD groups (males, 19-22/60 versus 16/120 of control; females, 8-13/60 versus 2/120 of control) and skin depressions (no detail description provided) in treated male groups (HD, 9/60; MD, 4/60; LD, 4/60; control, 1/120).

The stomach finding was forestomach depressions (in the esophageal groove) which occurred in all dose levels in a dose-related manner (males, 5-8/60 versus 2/120 of control; females, 6-12/60 versus 7/120 of control). There was no associated histopathological finding for the stomach finding.

There were no other treatment related findings in macroscopic examinations.

Histopathology:

Non-neoplastic:

GW685698X related findings included lymphoid atrophy in nasal associated lymphoid tissue (NALT) and bronchiolar associated lymphoid tissues (BALT), reduced eosinophilic inclusion in nasal epithelium, skin changes (inflammation, epidermal ulceration, epidermal hyperplasia, dermal fibrosis and scab formation) and connective tissue hyalinization in the stomach. Most of these findings are typical steroid effects except the findings of reduced eosinophilic inclusion in nasal epithelium and stomach connective tissue hyalinization. The sponsor considered the reduced eosinophilic inclusion a suppression of age-related spontaneous change (incidence and severity of eosinophilic inclusion in the respiratory and olfactory epithelium of the nasal cavity of mice increases with age, Loo 1996; Clair, 1992, Ward, 2001) and the stomach finding being a local effect of swallowed drug. It is **not clear if the sponsor's interpretations are** correct. Nevertheless, for the carcinogenicity evaluation, these findings are not significant.

The following are incidence and severity of the gross lesions.

1. Lungs: There was a clear dose-related reduction in the amount of bronchiolar associated lymphoid tissue (BALT) in all treated groups (see the table below).

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (μg/kg/day)	0	0	2.22	6.09	18.8	0	0	2.22	6.09	18.8
Lungs										
Number examined	60	60	60	60	60	60	60	60	60	60
Cellularity of BALT										
minimal	32	32	32	30	17	28	34	31	30	19
slight	14	19	7	4	5	19	16	12	5	3
moderate	8	4	2	1	3	7	4	4	0	0
marked	0	0	0	0	1	1	0	0	1	0
total	54	55	41b	35b	26b	55	54	47	36b	22b
BALT Absent										
total	6	5	19b	25b	34b	5	6	13	24b	38b

b-$p<0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.

2. Nasal Turbinates: Reduced amount of nasal associated lymphoid tissue (NALT) and reduced incidence of epithelial eosinophilic inclusions were observed in all treated groups (see the table below).

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (µg/kg/day)	0	0	2.22	6.09	18.8	0	0	2.22	6.09	18.8
Nasal Turbinates										
Number examined	60	60	60	60	60	60	60	60	60	60
Cellularity of NALT										
Minimal	9	6	7	15	28	2	6	13	22	20
Slight	33	38	41	23	12	38	38	39	30	36
Moderate	16	13	12	3	1	20	15	7	2	1
Marked	1	0	0	0	0	0	0	0	0	0
Total	59	57	60	41b	41b	60	59	59	54	57
NALT Absent										
Total	1	3	0	19b	18b	0	1	1	6	3
Epithelial Eosinophilic Inclusions										
Minimal	12	15	4	2	4	11	2	13	7	2
Slight	9	13	6	5	3	15	22	9	7	4
Moderate	0	0	0	0	0	2	2	0	0	0
Total	21	28	10a	7b	7b	28	26	22	14a	6b

a-$p<0.05$, b-$p<0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.
Where the significance differs, the lesser degree of significance is indicated (eg a rather than b)

3. Stomach: There was an increased incidence of connective tissue hyalinization in the stomach of males and females at the HD and females at the MD. In the majority of animals it was graded as minimal/slight. The change was not associated with any glandular degeneration and the epithelium retained a normal functional appearance.

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (µg/kg/day)	0	0	2.22	6.09	18.8	0	0	2.22	6.09	18.8
Stomach										
Number examined	60	60	60	60	60	60	60	60	60	59
Hyalinisation										
Minimal	1	1	5	9	24	0	4	13	27	25
Slight	0	0	0	0	8	1	0	0	3	19
Moderate	0	0	0	0	1	0	0	0	0	1
Total	1	1	5	9a	33b	1	4	13a	30b	45b

a-$p<0.05$, b-$p<0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.
Where the significance differs, the lesser degree of significance is indicated (e.g. a rather than b)

4. Skin: There was an overall increase in the incidence of inflammatory changes, including epidermal ulceration, epidermal hyperplasia, dermal fibrosis and scab formation, in males and females at the HD and in males at the MD.

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (µg/kg/day)	0	0	2.22	6.09	18.8	0	0	2.22	6.09	18.8
Skin										
Number examined	60	60	60	60	60	60	60	60	60	60
Epidermal Ulceration										
Minimal	2	0	3	1	1	0	0	0	0	1
Slight	1	1	3	1	6	1	0	0	1	3
Moderate	1	1	1	1	4	2	0	1	0	9
Marked	0	1	0	2	0	0	1	0	2	0
Severe	0	0	1	0	0	0	0	0	1	0
Total	4	3	8	5	11	3	1	1	4	13a
Epidermal Hyperplasia										
Minimal	1	3	3	1	8	0	0	1	1	1
Slight	4	2	8	13	7	5	0	2	6	14
Moderate	1	1	0	3	2	0	1	0	1	3
Total	6	6	11	17a	17a	5	1	3	8	18b
Dermal Fibrosis										
Minimal	2	1	1	0	1	0	0	2	0	2
Slight	8	2	10	15	13	5	7	3	6	7
Moderate	1	2	0	1	6	0	1	0	0	0
Total	11	5	11	16	20	5	8	5	6	9
Scab(s)										
Total	5	3	5	10	13	4	0	2	4	12

a-$p<0.05$, b-$p<0.01$ with Fisher's Exact Test, on total incidences only
Statistical significance only reported if incidence significant compared with each control group separately.

Neoplastic:
There was no treatment related increase of tumor incidence. The following table displays the comparisons that would be statistically significant at the 0.05 level if one does not adjust for the multiplicity of comparisons. The Haseman-Lin-Rahman rules for adjusting for the large number of comparisons specify that those tumor-organ combinations with one or fewer tumors in the pooled control group would be classified as rare tumors, the remainder as common. Then, using these rules, no pairwise comparison or test of trend would be considered as statistically significant, although the test of trend in pooled pars distalis and pars intermedia in the pituitary gland of male mice is close to statistical significance (p=0.0281 versus the 0.0250 specified by the Haseman-Lin-Rahman rules). Note that the corresponding asymptotic test would be statistically significant using these rules (p=0.0127), although with only five tumors it seems apparent that the statistical significance would be inflated (i.e., smaller p-value).

Summary of potentially statistically significant tumorogenicity results in mice

Organ / Tumor	Con-trol1	Con-trol2	Low	Med-ium	High	p-values: Trend	Hi vs Cntrl
Males							
LUNGS & BRONCHI							
BRONCHIOLOALVEOLAR ADENOMA	12	8	11	21	15	0.0637	0.0425
Bronch. Adenoma/Carcinoma	14	10	12	23	18	0.0343	0.0316
PITUITARY							
ADENOMA - PARS INTERMEDIA	0	0	0	0	2	0.0412	0.1095
Adenoma Pars Dist./Inter.	0	1	0	1	3	0.0281	0.1068
Females							
MUSCLE							
RHABDOMYOSARCOMA	0	0	0	0	2	0.0391	0.1176
UTERUS							
ENDOMETRIAL POLYP	3	3	1	4	6	0.0340	0.0945
Uterus/cervix							
ENDOMETRIAL POLYP	3	3	2	4	6	0.0481	0.0919

(Source: Dr. Steve Thomson's review on 11/15/2006)

The same conclusion was made by the sponsor that this drug is non-tumorigenic under the study condition. Additionally, the sponsor concluded that there was a decreased incidence of malignant lymphoma in the HD females (control 1, 1/60; control 2, 0/60; LD, 0/60; MD, 1/60; HD, 0/60). There is no confirmation provided by the review statistician, and this reviewer considers this finding not biologically significant.

Toxicokinetics:

Plasma drug levels in the control males were below limit of quantification (20 pg/mL) and not measured in control females due to early deaths. Mice in each of the dose groups demonstrated systemic exposure to GW685698X following 39 weeks of inhalation administration. There was no gender related differences noted in TK parameters. Systemic exposure (AUC) to GW685698X was approximately proportional to the doses. There were insufficient data to determine the AUC(0-t) at the lowest dose of 2.22 µg/kg/day. The Tmax was observed right after the end of the exposure period at every dose group.

Sex	Target Dose (µg/kg/day)	$AUC_{(0-t)}$ (pg.h/mL)	Observed C_{max} (pg/mL)	Observed T_{max} [1] (h)
Male	2	NC	55.4	1.08
	6	168	109	1.05
	18	604	316	1.12
Female	2	NC	128	1.13
	6	207	134	1.09
	18	750	390	1.09

1. Allowing for a 1 hour exposure period
NC: Not calculated, insufficient data to define an AUC

2.6.6.6 Reproductive and developmental toxicology

No new studies were submitted to this NDA. Previously submitted studies were reviewed under IND 48,647 (reviews 4 and 5).

2.6.6.7 Local tolerance

An eye irritation study was conducted in three male New Zealand White rabbits (Study Report No. RD2005/00377/01). In this study, 0.1 mL of 0.05% w/w aqueous nasal suspension of GW685698X was placed into the everted lower lid of the right eye. The left eye served as the untreated control. The eyes of the rabbits remained unflushed for approximately 24 hours following instillation of the test article. Eye irritation was evaluated and scored following the standardized Draize scoring technique at approximately 1, 4, 24, 48, and 72 hours after instillation. Mortality, clinical signs and body weights were also recorded.

Upon eye examinations, there was no residual test article present. All animals survived to the terminal sacrifice at 72 hours post-instillation. Irritation characterized by redness (irritation score of 1) of the conjunctivae was present at 1 and 4 hours post-instillation in 2 of 3 rabbits. All signs of irritation resolved by 24 hours. Corneal injury was evaluated by sodium fluorescein examination at the 24-hour observation period. There was no effect on the cornea or iris in any of the treated eyes. Total mean irritancy score was 1.3. No other clinical findings were observed. No treatment-related body weight changes were noted.

In conclusion, GW685698X 0.05% aqueous nasal suspension was practically nonirritating (total mean irritancy score of 1.3) to the eyes of the rabbits under the conditions of this study.

Reference: Draize, J.H., "Eye Mucosa," In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics- Dermal Toxicity. Association of Food and Drug Officials of the United States, Austin, Texas, pp. 49-50.

Additionally, a rat study demonstrated that single oral gavage of GW685698X at 1.25 mg/kg produced no gastrointestinal tract irritation as evaluated by histopathological examinations at 48 hour after dosing (IND 48647, review 5)

2.6.6.8 Special toxicology studies

Study title: A 5- Day Inhalation Tolerability Study of a Powder Aerosol Formulation in the Guinea Pig Followed by an Immunologic Sensitization Potential Study in Guinea Pigs via Inhalation

Key study findings: Five daily inhalation doses of GW685698X at 70.6 mcg/kg were well tolerated in guinea pigs. Administration of 5 daily doses of GW685698X (70.9 mcg/kg/day) followed by a single inhalation challenge exposure of GW685698X (67.1

mcg/kg) 17 days later, induces no respiratory hypersensitivity reactions in guinea pigs in this study.

Study no.: Report No. CD2005/00215/00; Study No. G05086
Volume #, and page #: e-submission, module 4
Conducting laboratory and location: [redacted]

Date of study initiation: April 4, 2005
GLP compliance: Yes, a signed GLP statement was accompanied with the report
QA reports: yes (X) no ( )
Drug, lot #, and % purity: GW685698X, lot 041053515, purity of 99.9%. GW857238X, lot 041060814; lactose, Batch number 78891-09-03-05-01 were used

Formulation/vehicle: GW685698X was blended with GW857238X (cellobiose octaacetate or COA) in lactose. The nominal concentration was 1% and 7% for GW685698X and GW857238X, respectively. Vehicle was 7% GW857238X in lactose.

Methods

Doses: 0 and 80 mcg/kg (target dose)

Study design:

1. Tolerability study: Male guinea pigs (5/group) were exposed to GW685698X (80 mcg/kg/day) or vehicle by nose only inhalation for 5 days. Observations made in this study are presented in the results section.
2. Immunological potential study: Male guinea pigs (10/group) were exposed to GW685698X (80 mcg/kg/day) by inhalation for 5 days, following by a 17-day off-drug period and an inhalation challenge of GW685698X (80 mcg/kg) on Day 22. Positive control group was treated with ovalbumin inhalation (8 mcg/kg) with the same regimen. Among the 10 Ovalbumin positive control guinea pigs, five animals were protected from acute anaphylaxis by intraperitoneal injection of pyrilamine maleate (10 mg/kg) approximately 30 minutes prior to inhalation challenge exposure. Observations made in this study are presented in the results section.

Groups employed in these two studies are the following:

	Group	n	Days1-5	Day 6-21	Day 22 inhalation
Tolerability	Vehicle	5 M	Vehicle	N/A	
	FF	5 M	FF		
Immunol. potential	Air/Air	10 M	Air	latent	Air
	OVA/saline	10 M	OVA		Saline
	Saline/OVA	10 M	Saline		OVA
	OVA/OVA	10 M	OVA		OVA
	FF/vehicle	10 M	FF		Vehicle
	Vehicle/FF	10 M	Vehicle		FF
	FF/FF	10 M	FF		

FF: fluticasone furoate (GW685698X); OVA, ovalbumin
For FF group and related control groups, each sensitization dose and challenge dose were administered in 60 minutes of inhalation. For positive control and related groups, each sensitization inhalation was completed in 30 minutes and challenge inhalation was completed in 15 minutes.

Results:
Achieved doses:

Group		Day 1-5 (mcg/kg)			Day 22 (mcg/kg)		
		FF	COA	OVA	FF	COA	OVA
Tolerability study	FF	70.6	720.3				
Sensitization study	OVA/OVA			7.15			8.44
	FF/Veh	71.2	594.2			638.6	
	Veh./FF		638.0		67.2	500.3	
	FF/FF	70.9	592.4		67.1	499.7	

The blanks indicate not applicable or not obtained.
The achieved doses were delivered doses and calculated as
D = (RMVxTxC) / (BW)
D- Dose (mcg/kg/day)
RMV- 0.499 x BW (kg) 0.809 (L/min) [Bide, 2000]
T- Duration of exposure/day (minutes)
C- Aerosol ovalbumin concentration (mcg/L) as gravimetric
BW Group average body weight for study (kg)

The particle sizes (MMAD) were [redacted] mcm for FF (GW685698X), and [redacted] mcm for COA. Based on these particle sizes, pulmonary deposited doses are approximately 10% of the delivered doses.

Mortality: Mortality was observed twice of each work day. There was no GW685698X related death. Five animals in the positive control group, that were not given pyrilamine

maleate prior to challenge, died of acute anaphylactic response following Ovalbumin challenge. Respiratory distress was noted during challenge exposure in these animals.

Clinical signs: Clinical signs were observed daily throughout the study. Detailed clinical examinations were performed weekly commencing the last week of pre-treatment period. There was no treatment related finding observed.

Body weights: The animals were weighed at least once during the pre-treatment period, prior to each exposure during the treatment period, and on the day of necropsy (fasted body weight). Body weight was unaffected by treatment during either the tolerability or immunologic sensitization potential phase.

Respiratory minute volume (sensitization study animals only): Respiratory minute volume was measured prior to the start and during the challenge exposure. Animals in Ovalbumin positive control that were protected from acute anaphylaxis by intraperitoneal injection of pyrilamine maleate showed a pattern of increased respiratory rate and decreased tidal volume (resulting in an overall decrease in RMV) during challenge exposure. Animals received saline in sensitization phase showed similar pattern of changes in minute volume and tidal volume when challenged with Ovalbumin. However, the large variability suggests that these changes might be incidental. Nevertheless, animals sensitized and/or challenged with W685698X did not exhibit this pattern, neither did the animals in the negative air control (with the exception of one animal which did show an increase in respiratory rate but no corresponding decrease in tidal volume).

Respiratory parameters in each group (mean±SD)

	Minute vol., mL/min		Respire. Rate, breath/min		Tidal volume, mL	
Group (Sensitization/challenge)	Pre-challenge	During challenge	Pre-challenge	During challenge	Pre-challenge	During challenge
Air/Air	441±101	544±226	129±15	132±18	3±0.7	4±1.8
OVA/saline	465±177	450±160	134±15	127±20	3±1.1	4±1.0
Saline/OVA	580±294	501±335	129±15	111±18	5±2.4	4±3.3
OVA/OVA	360±124	298±100	113±16	142±15	3±1.3	2±0.8
FF/vehicle	447±115	473±140	130±16	129±15	4±1.2	4±1.5
Vehicle/FF	488±187	515±244	128±12	127±14	4±1.9	4±2.5
FF/FF	394±90	544±299	126±21	120±13	3±0.6	5±2.5

N=10 for most groups except groups of OVA/OVA for which n=5, and OVA/saline for which n=9.

Macroscopic findings:
Necropsy was performed for the sensitization study only. No GW685698X-related changes were seen during the macroscopic examination of guinea pigs following the sensitization and/or the challenge.

Firm, spongy and/or uncollapsed lung, generally with pale discoloration was seen in positive control animals that died shortly after challenge on Day 22 (4/5 died animals).

Bronchoalveolar lavage:
Bronchoalveolar lavage was performed at 24 hours after the final day of repeat dosing (tolerability study) or the challenge exposure (sensitization study).

Increases of white blood cell (1.5-fold) and eosinophil counts (6-fold) in broncho-alveolar lavage were seen in the Ovalbumin positive control when compared with air-only control. These findings are consistent with a respiratory hypersensitivity reaction. There were no consistent white blood cell count changes in broncho-alveolar lavage of other groups, including GW685698X treated groups.

Group (Sensitization/challenge)	WBC count (10^6/mL)	Eosinophil count (10^6/mL)
Air/Air	4.65±1.2	0.3±0.4
OVA/saline	6.15±2.3	0.4±0.3
Saline/OVA	4.31±2.0	0.4±0.3
OVA/OVA	7.03±2.9	1.8±1.8
FF/vehicle	4.34±1.16	0.4±0.4
Vehicle/FF	6.27±5.7	0.6±0.6
FF/FF	3.07±1.4	0.2±0.1

In conclusion, five daily inhalation doses of GW685698X at 70.6 mcg/kg were well tolerated in guinea pigs. There was no evidence of respiratory hypersensitivity reactions in guinea pigs exposed to 5 daily doses of GW685698X (70.9 mcg/kg/day) followed by a single inhalation challenge exposure of GW685698X (67.1 mcg/kg) 17 days later.

2.6.6.9 Discussion and Conclusions
Preclinical studies submitted under this NDA **satisfy the Division's requirements.** General toxicology studies were mostly conducted by inhalation administration (up to 6 months in rats and up to 9 months in dogs) and intranasal studies (2 weeks in rats, 1 month and 6 months in dogs) serve a bridging purpose for intranasal use. Inhalation studies revealed that most drug-related findings were typical glucocorticoid effects (changes in body weights, clinical pathology, histopathological findings of adrenal atrophy, lymphoid depletion, fatty bone marrow, hair-loss and dermal thinning, liver glycogen deposition, hepatocyte rarefaction and/or hypertrophy, pituitary acidophilic cells, epiphyseal plate retention). All typical glucocorticoid effects were dose and exposure duration dependent. Non-typical glucocorticoid effects include increased eosinophilic inclusions in bronchilar epithelium in rats, and nephropathy, chronic stomach inflammation and biliary tract (bile duct and gall bladder) epithelial vacuolation, and airway inflammatory lesions. The non-typical glucocorticoid effects were mostly seen studies with 3 moths or longer treatment durations, and the findings were mostly in minimum to slight degree. Intranasal studies demonstrated toxicities similar to that observed in the inhalation studies except dog nasal cavity goblet cell hypertrophy that was not seen in inhalation studies.

None of the above drug related findings are of a significant clinical concern because of the extensive clinical experience (typical steroid effects), minimal degree of findings and not clinically significant (eosinophilic inclusions in rat lungs, biliary tract epithelial vacuolation), irrelevancy to the clinical route of administration (airway inflammation in dog inhalation study), clinically monitorable effects (nasal cavity goblet cell hypertrophy) or presence of sufficient safety margins (nephropathy, 29-fold; chronic stomach inflammation, 132-fold for the proposed clinical maximum dose of 110 mcg/day) based on AUC values.

GW685698X is negative in a standard battery of genotoxicity tests and is not carcinogenic in both rat and mouse 2-year carcinogenicity studies.

GW685698X produced no developmental effects in a complete battery of reproductive toxicity studies including fertility and early embryo-fetal development in rats, embryo-fetal development in rabbits and prenatal and postnatal development in rats. However, given the known class effects of glucocorticoids, a Pregnancy Category C is applicable.

In guinea pigs, GW685698X did not induce airway hypersensitivity.

2.6.6.10 Tables and Figures

Summary of toxicity findings in pivotal studies

Species	study	Dose, mcg/kg	Findings	NOAEL#	Safety margin**
Rat	26-w, IH,	3.2, 8.3, 20.3	↓ Body weight/body weight gain, ↑ RBC, ↓WBC/lymphocyte, ↑ cholesterol and triglyceride, lymphoid depletion, fatty bone marrow, hair loss and dermal thinning, ↑eosinophilic inclusion in bronchiolar epithelium.	20.3 mcg/kg (AUC=1238 pg.h/mL)	140
Dog	39-w, IH,	13.3, 30.1, 59.6	↓ Body weight/body weight gain, ↑ cholesterol and triglyceride, adrenal atrophy, lymphoid depletion, ↑liver glycogen deposition and related changes, ↑adipose cell infiltrations in several tissues (bone marrow, heart, pancreas, etc), skin epidermal atrophy, pituitary acidophilic cells, and epiphyseal plate retention. Biliary tract epithelial vacuolation, nephropathy(tubular basophilic) and chronic stomach inflammation	13.3 mcg/kg (AUC=1160 pg.h/mL) based on nephropathy and stomach inflammation	131
	26-w IN	1200, 2400*	Similar to the 39-w inhalation study, without stomach finding, but with additional finding of nasal goblet cell hypertrophy	1200 mcg/day (AUC=258 pg.h/mL) based on nephropathy	29

Note: inhalation doses presented above were delivered doses rather than pulmonary deposited doses; *mcg/day

NOAEL refer to non-monitorable, safety concerning toxicities

**Safety margins are calculated based on AUC comparison, and human intranasal dose of 110 mcg/kg is approximated to AUC value of 8.8 pg.h/mL.

Safety margins observed in the reproductive toxicity studies and carcinogenicity studies

Study		Dose, mcg/kg	Result	NOAEL, mcg/kg	Safety margin*
Repro. tox	Rat Seg I /II	M: 6.6, 12.9, 29.4 F: 11, 23 and 91	Negative	M: 29.4 F: 91	M: 2 F: 7
	Rabbit Seg II	1.8, 3.2 and 8.1	Negative	8.1	1.2
	Rat seg II/III	5.5, 15.7, 27.2	Negative	27.2	2
Carci.	Rat	1, 3.19, 8.61	Negative	8.61	0.6
	Mouse	2.22, 6.09, 18.8	Negative	18.8	0.7

*Safety margins refer to ratios of NOAEL and human dose (110 mcg/day) on an mg/m^2 basis

2.6.7 TOXICOLOGY TABULATED SUMMARY

N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
GW685698X is a glucocorticoid compound that is being developed for the treatment of allergic rhinitis via intranasal administration for patients aged 2 and above. The recommended daily doses are 55 mcg/day for children up to 12 years old and 110 mcg/day for older patients.

GW685698X, as evidenced by receptor binding, exhibits glucocorticoid receptor agonist effects and typical glucocorticoid effects in animal studies. The oral bioavailability of GW685698X is less than 4% in animals. Systemically absorbed GW685698X is rapidly metabolized and eliminated through bile excretion. The primary metabolic pathway is loss of fluoromethyl carbothioate group to result in M10 which appears to be pharmacologically inactive. A unique human metabolite, a thioester glutathione conjugate (M4) of the acid metabolite M10 (3-9% of the total metabolism), was identified in vitro in human hepatocyte incubations at a GW685698X concentration of 50 mcM. Otherwise, no unique human metabolites were identified. T1/2 of GW685698X is approximately 3 hours in rats and 3-10 hours in dogs.

Adequate preclinical studies were conducted. Toxicities associated with exposure to GW685698X in animals by inhalation and intranasal routes were mostly typical glucocorticoid effects. Non-typical glucocorticoid effects are not of significant clinical concern as most of them are not toxicologically significant or sufficient safety margins for the proposed intranasal doses are present. There is no evidence showing that GW685698X is mutagenic or carcinogenic. GW685698X did not affect fertility, fetal development, and prenatal and postnatal development in rats and/or rabbits. However, given the known developmental effects associated with glucocorticoids, the drug should be given a Category C for Pregnancy. Inhalation administration of GW685698 does not induce hypersensitivity reactions in guinea pigs.

Pharmacology:
GW685698X (fluticasone furoate, FF) is a synthetic glucocorticoid compound with a similar profile of pharmacological activities as compared to fluticasone propionate. FF has a high binding affinity to glucocorticoid receptors (kd=0.3 nM), and a high binding affinity to progesterone receptors (no accurate number can be presented due to the compound lies on the tight binding limit which resulted in an underestimation of the true affinity at both GR and PR). However, for other steroid receptors including androgen receptors, minerocorticoid receptors, and estrogen receptors, FF showed either low binding affinities or negative of receptor agonist effects. In vitro studies showed that several glucocorticoid effects down stream of receptor binding for FF: glucocorticoid nuclear receptor translocation, transrepression of TNFalpha-induced NFKB response, and transactivation of MMTV-luciferase reporter, protected bronchial epithelial cells from protease- and mechanically-induced cellular damage, and inhibited LPS-induced TNF release from PBMCs. In vivo, FF was observed with inhibitory effects on oxazolone-induced ear skin delayed type hypersensitivity (DTH) responses in rats and mice,

ovalbumin induced rat lung eosinophilia, and egg albumin induced allergic symptoms (nasal rubbing and sneezing) in rats.

The primary metabolite of GW685698X, M10 (GW694301) was found with minimum pharmacological effects (in vitro, 6000-fold less potent than the parent in glucocorticoid receptor agonist effects).

Safety pharmacology:
GW685698X has no toxicities in CNS and respiratory functions. In rats, a slight increase of heart rate and a slight decrease of blood pressure were observed within 14 days following a single subcutaneous dose of 4 mg/kg. In dogs, wasting conditions (polyuria and muscle wasting) were observed 2-5 weeks following a subcutaneous doses of 4 and 10 mg/kg.

Pharmacokinetics:
Oral bioavailability is approximately 1-4% in rats and rabbits due to an extensive first-pass effect, and systemic exposures to the parent compound are minimal.

Intravenous administered 3H-GW685698X was distributed rapidly and widely with a volume of distribution of 8L/kg in rats and 10 L/kg in dogs. In rats, radioactive drug related materials in most tissues were higher than corresponding blood levels within 24 h postdosing, and the highest levels at 0.5 h postdosing were seen in the small intestine (the cavity and the wall) demonstrating biliary clearance. At 168 hours post-dosing, levels of radioactivity were only detectable in the liver and kidney cortex. The circulating drug-related materials were predominantly associated with the plasma fraction and plasma protein binding was 99% in humans, rabbits, mice and dogs, and 98% in rats.

The major metabolic route of GW685698X was loss of fluoromethyl carbothioate group to form the carboxylic acid GW694301X (M10). All other metabolites are formed from M10. The second metabolic event often observed is oxidative defluoronation in the C6 position which occurred in M10 to form hydroxylated metabolite at the C-6 position or to form a ketone at C6 position. Oxidative defluoronation of parent compound at C6 position was also seen in hepatocytes of rats and dogs but not in human hepatocytes and accounted for 12-18% of the total metabolism in these species. A unique human metabolite, a thioester glutathione conjugate (M4) of the acid metabolite M10 (3-9% of the total metabolism) was identified in vitro in human hepatocyte incubations at a GW685698X concentration of 50 mcM. Otherwise, no unique human metabolites were identified. Phase II metabolites, M10 conjugates with glucuronide, glutathione, or glycylcysteine were seen in bile or hepatocyte incubations from animals and/or humans.

The primary elimination of GW685698X is via liver metabolism and bile secretion. Rat and dog studies showed that drug-related materials in 0-24-hour bile account for approximately 70-80% of intravenous dose. There is no unchanged parent compound detected in bile from rats and dogs. Elimination T1/2 is 3 hours in rats and 3-13 hours in dogs. Urinary elimination is a minor route accounting for less than 4% of dose in rats, dogs and humans.

Toxicology:
Administered by single intravenous dose, GW685698X is not lethal up to 18 mg/kg in mice, 12 mg/kg in male rats and 18 mg/kg in female rats.

Studies by inhalation administration (up to 6 months in rats and up to 9 months in dogs), and by intranasal administration (2 weeks in rats, one month and 6 months in dogs) showed that effects of FF are mostly typical for glucocorticoids: decrease of body weight gain or body weight loss, changes in clinical pathology (decrease of WBC/lymphocyte counts, increase of RBC count, increase of blood cholesterol and/or triglycerol levels), and histopathology findings of adrenal atrophy (dogs only), lymphoid depletion (thymus, spleen, lymph nodes, and lymphoid tissues of lung and nasal passage), fatty bone marrow, and hair loss and dermal thinning, increased liver glycogen deposition, hepatocyte rarefaction and/or hypertrophy (dogs only). Pituitary acidophilic cells, increased adipose cell infiltrations in heart, pancreas, parotid salivary gland and skeletal muscles, and epiphyseal plate retention were also observed in dogs after 39 weeks of treatment. Based on the extensive clinical experience, these typical glucocorticoid effects are of no clinical concerns.
Non-typical glucocorticoid effects include rat finding of increased eosinophilic inclusions in bronchiolar eipithelium and dog findings of focal nephropathy (tubular basophilia and increased mitotic figures), a slight change in blood chemistry parameters (increase of alkaline phosphatase, gamma glutamyl transferase and/or glutamyl dehydrogenase), biliary tract (bile duct and gall bladder) epithelial vacuolation, airway inflammatory lesions, nasal cavity goblet cell hypertrophy and chronic stomach inflammation. As presented in the following table, none of these non-typical steroid effects was a clinical concern due to minimum degree of changes, clinical monitorable nature, irrelevancy to clinical route of administration or adequate safety margins.

Species	Finding	Description	Evaluation	SM*
Rat	bronchiolar epithel. ↑eos. Inclusions	Found in <0.8% of Clara cells	Not a clinical concern	NA
Dog	biliary tract epi. Vacuole.	Minimum to slight degree, non-progressive from 3 mo to 9 mo, no related inflam. or degeneration	Not a clinical concern	NA
	Blood chem.	2-3X of ALP, GGT, GDH	Clinically monitorable	NA
	Airway infla.	Minimum, inflammation, epi. hyperplasia, pseudogland formation	irrelevant to intranasal administra.	NA
	Nasal goblet cell hypertrophy	Minimum to slight degree	Clinically monitorable	NA
	Focal nephropathy	Min. to slight degree, tubular basophilia, occasional inflammatory cells	NOAEL=258 pg/h/mL, 6-m and 9-m studies	29
	Chron. Stomach infla	Minimum degree	NOAEL,1160 pg.mL/mL, 9-m study	132

SM: Safety margins were calculated based on AUCs of NOAEL and human dose of 110 mcg/day

In a standard battery of genotoxicity tests, GW685698X was negative in Ames test, mouse lymphoma assay and rat micronucleus assay under the conditions tested.

Two-year carcinogenicity studies in rats and mice revealed that GW685698X is not tumorogenic in rats at inhalation doses up to 8.61 mcg/kg and in mice upto18.8 mcg/kg. **CDER's executive CAC concurred that these studies are acceptable and the results were** negative for tumor findings.

Reproductive toxicity studies demonstrated that GW685698X is without effects on fertility and early embryo-fetal development in rats (up to the HD of 29.4 mcg/kg in males and 91 mcg/kg in females), embryo-fetal development in rabbits (up to the HD of 8.1 mcg/kg) or prenatal and postnatal development in rats (up to the HD of 27.2 mcg/kg). Despite the lack of identified effects in the relevant studies, a Category C is still appropriate given the known class effects associated with glucocorticoids.

In Guinea pigs, GW685698X does not induce respiratory hypersensitivity reactions.

Unresolved toxicology issues (if any): None

Recommendations: Fluticasone furoate nasal spray is recommended to be approved from a pharmacology and toxicology perspective.

3 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

✓ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

APPENDIX/ATTACHMENTS

1. Dose ratio calculation table

Drug:	[redacted]							
	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric	2	0.055	1	0.055	12	0.00458	25	0.11
Adult	[redacted]	0.11	1	0.11	50	0.00220	37	0.08

	route	mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
rat	[redacted]		6	0.054	0.7	0.471	1/2	1/2
mouse	[redacted]		3	0.057	0.7	0.497	1/1	1/2
extra			---	---	---	---	---	---
extra			---	---	---	---	---	---
extra			---	---	---	---	---	---
Repro/Fertility:								
rat	[redacted]		6	0.174	2.1	N/A	2	N/A
rat	[redacted]		6	0.546	6.7	N/A	7	N/A
extra			---	---	---	N/A	---	N/A
extra			---	---	---	N/A	---	N/A
Teratogenicity:								
rat	[redacted]		6	0.546	6.7	N/A	7	N/A
rabbit	[redacted]		12	0.096	1.2	N/A	1	N/A
rat	[redacted]		6	0.162	2.0	N/A	2	N/A
extra			---	---	---	N/A	---	N/A
extra			---	---	---	N/A	---	N/A
Overdosage:								
rat	[redacted]		6	12000	147420	104727	147000	105000
mouse	[redacted]		3	6000	73710	52363	74000	52000

2. Executive CAC meeting minutes regarding carcinogenicity studies

APPEARS THIS WAY ON ORIGINAL

Executive CAC
Date of Meeting: January 16, 2007

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D., OND IO, Member
Bayo Laniyonu, Ph.D., DMIHP, Alternate Member
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The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA# 22-051
Drug Name: Fluticasone Furoate
Sponsor: GlaxoSmithKline

Background:
Fluticasone furoate is a glucocorticoid intended to treat allergic rhinitis symptoms. The toxicity profile is typical for glucocorticoids. The drug is negative in the Ames test, mouse lymphoma assay and rat micronucleus assay. The Executive CAC provided no comments on dose selection for the carcinogenicity studies as the study protocols were submitted when the studies were already underway.

Mouse Carcinogenicity Study
Mice (60/sex/dose) in the 2-year carcinogenicity study were exposed to fluticasone furoate at delivered doses of 2.22, 6.09 and 18.8 mcg/kg by inhalation administration. The sponsor selected these doses based on a 13-week dose ranging study in which doses of 7.3, 18.6 and 76.9 mcg/kg were associated with a decrease of body weight gain relative to that of controls or body weight loss relative to pre-treatment values (males, lower body weight gain of 18, 43 and 94% at the LD, MD and HD, respectively; females, lower body weight gain of 27% at the MD; females at the HD had a 3.1% lower body weight compared to pre-study values). The dose of 18.8 mcg/kg was considered by the sponsor to be an appropriate high dose for the 2-year study.

No treatment related increase in neoplasm incidence was observed following inhalation exposure to fluticasone furoate.

Rat Carcinogenicity Study
Rats (60/sex/dose) in the 2-year carcinogenicity study were exposed to fluticasone furoate at delivered doses of 1, 3.1, and 8.16 mcg/kg by inhalation. The sponsor selected these doses based on a 13-week dose ranging study in which doses of 5, 9, and 24 mcg/kg

were associated with lower body weight gains (males, 1, 20 and 33% at the LD, MD and HD; females, 15, 24 and 54% at the LD, MD and HD, respectively) compared to control values. Based on the effect on body weight gain, the sponsor selected 9 mcg/kg as the target high dose.

No treatment related increase in neoplasm incidence was observed following inhalation exposure to fluticasone furoate.

Executive CAC Recommendations and Conclusions:

Mouse:

The Committee concurred that the study was acceptable (based on body weight decrements).

The Committee concurred that there were no drug related neoplasms.

Rat:

The Committee concurred that the study was acceptable (based on body weight decrements and reduced survival in high dose females).

The Committee concurred that there were no drug related neoplasms.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPAP
/Timothy McGovern, DPAP
/Huiqing Hao, DPAP
/Ladan Jafari, DPAP
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/s/

Huiqing Hao
3/2/2007 03:54:29 PM
PHARMACOLOGIST

Timothy McGovern
3/5/2007 07:42:56 AM
PHARMACOLOGIST
I concur.