

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**204275Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**NDA:** 204275

**Submission date:** 7/12/2012

**Drug:** Fluticasone furoate and vilanterol trifenate

**Sponsor:** Glaxo Group LTD

**Indication:** Chronic obstructive pulmonary disease

**Reviewing Division:** Division of Pulmonary, Allergy and Rheumatology Products

### **Background Comments:**

The pharmacology/toxicology reviewer and team leader in the Division of Pulmonary, Allergy, and Rheumatology Products reviewed the nonclinical information for fluticasone and vilanterol and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above. This product is a combination of fluticasone furoate and the new molecular entity, vilanterol trifenate. Vilanterol is a  $\beta$ 2-adrenergic receptor agonist.

### **Discussion:**

#### Carcinogenicity

The carcinogenicity of vilanterol was assessed in 2-year carcinogenicity studies in rats and mice by the inhalation route. The Executive Carcinogenicity Assessment Committee found these studies to be acceptable. The following neoplasms were considered to be clearly drug-related in the rat: adenomas of the pituitary gland in males and females and leiomyomas of mesovarian ligaments in females. Tubulostromal adenomas in the ovaries were considered to be drug-related in mice. These findings are described in proposed labeling.

#### Developmental and Reproductive Toxicity

The only apparent effect of vilanterol in development and reproductive toxicity studies was an increase in skeletal variations in rabbits at doses that produced exposures much higher than the maximum human exposures. These findings are described in the proposed labeling.

### **Conclusions:**

I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. No additional nonclinical studies are recommended. Use of a pregnancy category of C seems warranted and is consistent with other drugs in these classes.

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/s/  
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PAUL C BROWN  
05/08/2013

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: NDA 204-275

Supporting document/s: Sequences 0000

Applicant's letter date: July 12, 2012

CDER stamp date: July 12, 2012

Product: Breo Ellipta (Fluticasone furoate /Vilanterol  
trifenatate) Dry Powder Inhaler

Indication: Chronic obstructive pulmonary disease (COPD)

Applicant: GSK

Review Division: Division of Pulmonary, Allergy, and Rheumatology  
Products

Reviewer: Luqi Pei, Ph.D.

Supervisor (acting): Marcie Wood, Ph.D.

Division Director: Badrul Chowdhury, M.D., Ph.D.

Project Manager: Angela Ramsey

*Template Version: September 1, 2010*

**Disclaimer**

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## LABELING REVIEW

Edits to nonclinical sections of the proposed labeling of Breo Ellipta are recommended. Edits are made to ensure labeling consistence between products of the same class. The nonclinical sections included Sections 8.1 Pregnancy, 12.1 Mechanism of Action, and 13 Nonclinical Toxicology. Section 13.2 Animal Toxicology and Pharmacology is deleted to comply with the current labeling format. Below is the recommended text for the nonclinical sections of the Breo Ellipta label. See discussions following recommended labeling for rationale and justification for the recommendations.

### LABELING RECOMMENDATION

#### 8.1 Pregnancy

**Teratogenic Effects:** Pregnancy Category C: There are no adequate and well-controlled trials of BREO ELLIPTA in pregnant women. Corticosteroids and beta<sub>2</sub>-agonists have been shown to be teratogenic in laboratory animals when administered systemically at relatively low dosage levels. Because animal (b) (4) studies are not always predictive of human response, BREO ELLIPTA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking BREO ELLIPTA.

**Fluticasone furoate and Vilanterol:** There was no evidence of teratogenic interactions between fluticasone furoate and vilanterol in rats at approximately 9 and 40 times, respectively, the maximum recommended human daily inhalation dose (MRHDID) in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses of fluticasone and vilanterol, alone or in combination, up to approximately 95-mcg/kg/day each).

**Fluticasone furoate:** There were no teratogenic effects in rats and rabbits at approximately 9 and 2 times, respectively, the MRHDID in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses up to 91 and 8 mcg/kg/day in rats and rabbits, respectively). There were no effects on perinatal and post-natal development in rats at approximately 3 times the MRHDID in adults (on a mcg/m<sup>2</sup> at maternal doses up to 27 mcg/kg/day).

**Vilanterol:** There were no teratogenic effects in rats and rabbits at approximately 13,000 and 160 times, respectively, the MRHDID in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses up to 33,700 mcg/kg/day in rats and on an AUC basis at maternal inhaled doses up to 591 mcg/kg/day in rabbits). However, fetal skeletal variations were observed in rabbits at approximately 1,000 times the MRHDID in adults (on an AUC basis at maternal inhaled or subcutaneous doses of 5,740 or 300 mcg/kg/day, respectively). The skeletal variations included decreased or absent ossification in cervical vertebral centrum and metacarpals. There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHDID in adults (on a mcg/m<sup>2</sup> basis at maternal oral doses up to 10,000 mg/kg/day).

**Non-teratogenic Effects:** Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be monitored.

## 12.1 Mechanism of Action

BREO ELLIPTA: Since BREO ELLIPTA contains both vilanterol and fluticasone furoate; therefore, the mechanisms of actions described below for the individual components apply to BREO ELLIPTA. These drugs represent 2 different classes of medications (a synthetic corticosteroid and a LABA).

Fluticasone Furoate: Fluticasone furoate is a synthetic trifluorinated corticosteroid with (b) (4) anti-inflammatory activity. The precise mechanism through which fluticasone furoate affects COPD symptoms is not known. Corticosteroids have been shown to have a wide range of actions on multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, cytokines) involved in inflammation. Specific effects of fluticasone furoate demonstrated in in vitro and in vivo models included activation of the glucocorticoid response element, inhibition of pro-inflammatory transcription factors such as NFκB, and inhibition of antigen-induced lung eosinophilia in sensitized rats.

Fluticasone furoate has been shown in vitro to exhibit a binding affinity for the human glucocorticoid receptor that is approximately 29.9 times that of dexamethasone and 1.7 times that of fluticasone propionate. The clinical relevance of these in vitro findings is unknown.

Vilanterol: Vilanterol is a LABA. In vitro tests have shown the functional selectivity of vilanterol was similar to salmeterol (b) (4)

Although beta<sub>2</sub>-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta<sub>1</sub>-receptors are the predominant receptors in the heart, there are also beta<sub>2</sub>-receptors in the human heart comprising 10% to 50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta<sub>2</sub>-agonists may have cardiac effects.

The pharmacologic effects of beta<sub>2</sub>-adrenoceptor agonists, including vilanterol, are at least in part attributable to stimulation of intracellular adenylyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3',5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.

## 13.1 Mutagenesis, Carcinogenesis and Impairment on Fertility

BREO ELLIPTA: No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with BREO ELLIPTA; however, studies are available for fluticasone furoate and vilanterol, as described below.

Fluticasone Furoate: Fluticasone furoate produced no treatment-related increases in the incidence of tumors in 2-year inhalation studies in rats and mice at inhaled doses up to 9 and 19 mcg/kg/day in rats and rabbits, respectively (approximately equal to the MRHDID in adults on a mcg/m<sup>2</sup> basis).

Fluticasone furoate did not induce gene mutation in bacteria or chromosomal damage in a mammalian cell mutation test in mouse lymphoma L5178Y cells in vitro. There was also no evidence of genotoxicity in the in vivo micronucleus test in rats.

No evidence of impairment of fertility was observed in male and female rats at inhaled doses up to 29 and 91 mcg/kg/day in males and females, respectively (approximately 3 and 9 times, respectively, the MRHDID in adults on a mcg/m<sup>2</sup> basis).

Vilanterol: In a 2-year carcinogenicity study in mice, vilanterol caused a statistically significant increase in ovarian tubulostromal adenomas in females at an inhalation dose of 29,500 mcg/kg/day (approximately 8,750 times the MRHDID in adults on an AUC basis). No tumors were seen at inhalation doses up to 615 mcg/kg/day (approximately 530 times the MRHDID in adults on an AUC basis).

In a 2-year carcinogenicity study in rats, vilanterol caused statistically significant increases in mesovarian leiomyomas in females and shortening of the latency of pituitary tumors at inhalation doses greater than or equal to 84.4 mcg/kg/day (greater than or equal to approximately 45 times the MRHDID in adults on an AUC basis). No tumors were seen at an inhalation dose of 10.5 mcg/kg/day (approximately 2 times the MRHDID in adults on an AUC basis).

These tumor findings in rodents are similar to those reported previously for other beta-adrenergic agonist drugs. The relevance of these findings to human use is unknown.

Vilanterol tested negative in the following genotoxicity assays: the in vitro Ames assay, in vivo rat bone marrow micronucleus assay, in vivo UDS assay, and in vitro SHE cell assay. Vilanterol tested equivocal in the in vitro mouse lymphoma assay.

No evidence of impairment of fertility was observed in rats at inhalation doses from 31,500 to 37,100 mcg/kg/day in males and females, respectively (approximately 12,000 to 14,000 times, respectively, the MRHDID in adults on a mg/m<sup>2</sup> basis).

## INTRODUCTION

The review evaluates the nonclinical sections of Breo Ellipta labeling that GSK proposed on October 12, 2012 and April 2, 2013. The review recommends editing both the proposed text and dose ratios between animals and humans. These edits were made to reflect not only the current labeling format but also the Agency's findings of the animal data.

Breo Ellipta is a dry powder inhaler that contains fluticasone furoate and vilanterol trifenate as active pharmaceutical ingredients (API). Each actuation of Breo Ellipta releases 100-µg fluticasone furoate and 25-µg vilanterol trifenate.<sup>1</sup> For the convenience of discussion, the review refers to the APIs as fluticasone and vilanterol, respectively. Breo Ellipta is indicated for maintenance treatment of chronic obstructive pulmonary disease (COPD). The maximum recommended human daily inhalation dose (MRHDID) is one actuation of the device. The MRHDID corresponds to exposures of 61.67-µg/m<sup>2</sup> fluticasone and 15.42-µg/m<sup>2</sup> vilanterol, respectively, on a mg/m<sup>2</sup> basis for a 60-kg patient. The mean plasma fluticasone and vilanterol AUC<sub>0-24h</sub> values in humans at the MRHDID are 0.182-

<sup>1</sup> The proposed label states that each actuation of the device delivers a 92-µg fluticasone furoate and 22-µg of vilanterol from the mouth piece under the testing condition (i.e., 60-L/min flow rate for 4 seconds).

ng.h/mL and 0.266-ng.h/mL, respectively.<sup>2</sup> The review uses exposure ratios on both a mg/m<sup>2</sup> and AUC basis. AUC ratios were preferred when exposure data from toxicological studies were available.

The Breo Ellipta label will describe relevant nonclinical findings of both APIs, alone or in combination, but the review focuses on the vilanterol studies. The review did not discuss the fluticasone data because the Agency had reviewed all the relevant nonclinical data previously in NDA 22-051. Discussions of vilanterol data were based on nonclinical reviews completed by Dr. Luqi Pei on February 21, 2013 (DARRTS Reference ID #3260556) and March 21, 2013 (DARRTS Reference ID #3274683) in the current application.

GSK submitted several versions of the proposed label for Breo Ellipta. This review comments on two versions only. These versions were submitted on 12-OCT-2012 and 02-APR-2013, respectively. The 12-OCT-2012 version was the applicant's originally proposed version while the 02-APR-2013 was the applicant's response to the Division's preliminary labeling comments that were sent on March 26, 2013.

## DOSE RATIOS

This review uses exposure ratios on both mg/m<sup>2</sup> and AUC bases for the labeling review. AUC ratios were preferred when exposure data from toxicology studies were available. Briefly, all fluticasone ratios were expressed on a mcg/m<sup>2</sup> basis. Vilanterol ratios were expressed on a mcg/m<sup>2</sup> and AUC basis. Specifically, AUC ratios were used for carcinogenicity studies in rats and mice and teratology studies in rabbits. The remaining vilanterol ratios were on a mcg/m<sup>2</sup> basis.

Dose ratios on a body surface area basis were derived as follows: Animal doses in inhalation toxicity studies were expressed in mcg/m<sup>2</sup> and were converted from the delivered doses using appropriate conversion factors: 3, 6, 12, and 37 for mice, rats, rabbits, and humans, respectively. Human doses were from the MRHDID of each API in a 60-kg patient. Specifically, the MRHDID of 100-µg/kg/day fluticasone and 25-µg/kg/day vilanterol correspond to doses of 61.67 and 15.42 µg/m<sup>2</sup>, respectively.

Table 1 (next page) summarizes the dose ratios used for the labeling review. For example, a vilanterol daily dose of 31,500 µg/kg in rats (male fertility study, Report CD2006/01166) provides a dose ratio of 12,000 between rats and humans. The rat nominal vilanterol dose was converted to 189,000 µg/m<sup>2</sup> (31,500 µg/kg x 6 = 189,000 µg/m<sup>2</sup>), and then divided by 15.42 (189,000 ÷ 15.42 = 12,257). The yield of 12,257 was then rounded to 12,000.

This review did not evaluate fluticasone data in support of the Breo Ellipta label because the Agency had reviewed the data previously in the Veramyst Nasal Spray application (NDA 22-051). See Dr. Huiqing Hao's review completed on March 2, 2007 in the reference NDA. Nonclinical sections of the Veramyst label (Ref: DARRTS ID #3177618) are provided below for easy reference.

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<sup>2</sup> Source: Dr. Jianmeng Chen's email dated March 20, 2013. Dr. Chen is the clinical pharmacology reviewer for the application.

**Table 1: Dose Ratios between Animals and Humans**

API	Section	Species	ROA	Dose		AUC (ng.h/ mL)	Dose Ratio		Reference
				$\mu\text{g}/\text{kg}/\text{day}$	$\mu\text{g}/\text{m}^2/\text{day}$		Calculated <sup>a</sup>	Rounded to	
FF	Pregnancy	Rat	IH	91	546		9	9	NDA 22-051
		Rat	IH	95	566	6.36	9	9	CD2007/00973
		Rabbit	IH	8	96		2	2	NDA 22-051
	Post-natal <sup>b</sup>	Rat	IH	27	162		3	3	NDA 22-051
		Fertility	Rat, M	IH	29	144		3	3
	Rat, F		IH	91	546		9	9	NDA 22-051
	Carcinog.	Mouse	IH	19	57		1	1	NDA 22-051
		Rat	IH	9	54		1	1	NDA 22-051
	VI	Pregnancy	Rat	IH	98	588	13.3	50 <sup>c</sup>	50
Rat			IH	33,700	202,200		13,113	13,000	CD2006/01166
Rabbit			IH	5,740	68,800	276	1038 <sup>c</sup>	1000	WD2006/02439
Rabbit			IH	591	7,092	42.6	160 <sup>c</sup>	160	WD2006/02439
Rabbit			SC	30	360	18.4	69 <sup>c</sup>	70	CD2006/02047
Rabbit			SC	300	3,600	306	1150 <sup>c</sup>	1150	CD2006/02047
Post-natal		Rat	PO	10,000	60,000		3,891	3,900	CD2010/00109
Fertility		Rat, M	IH	31,500	189,000		12,257	12,000	CD2007/00581
		Rat, F	IH	37,100	22,260		14,436	14,000	CD2006/01165
Carcinog.		Mouse	IH	615	1,845	141	530 <sup>c</sup>	530	2011N119325
		Mouse	IH	29,500	88,500	2329	8756 <sup>c</sup>	10900	2011N119325
		Rat, M	IH	84.4	506.4	12	45 <sup>c</sup>	45	2011N109253
		Rat, M	IH	10.5	63	0.63	3 <sup>c</sup>	4	2011N109253

a. On a  $\mu\text{g}/\text{m}^2$  basis unless specified.

b. P. natal d. = post natal development.

c. On an AUC basis.

## FLUTICASONE

The evaluation of sections of fluticasone furoate only is based on the Veramyst label approved on August 21, 2012 (DARRTS Reference ID# 317761). As alluded to earlier, no discussions of the nonclinical sections of fluticasone furoate only are needed because the application contained no new data. Please refer to the nonclinical review completed by Dr. Huiqing Hao on March 7, 2007 in NDA 22-051 for evaluation of the fluticasone data. Relevant sections of the Veramyst label are provided below for reference.

### “8.1 Pregnancy

**Teratogenic Effects:** Pregnancy Category C. Corticosteroids have been shown to be teratogenic in laboratory animals when administered systemically at relatively low dosage levels.

There were no teratogenic effects in rats and rabbits at inhaled fluticasone furoate dosages of up to 91 and 8 mcg/kg/day, respectively (approximately 7 and 1 times, respectively, the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis). There was also no effect on pre- or post-natal development in rats treated with up to 27 mcg/kg/day by inhalation during gestation and lactation (approximately 2 times the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis).

There are no adequate and well-controlled studies in pregnant women. VERAMYST Nasal Spray should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully monitored.”

#### “12.1 Mechanism of Action

Fluticasone furoate is a synthetic trifluorinated corticosteroid with potent anti-inflammatory activity. The precise mechanism through which fluticasone furoate affects rhinitis symptoms is not known. Corticosteroids have been shown to have a wide range of actions on multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, cytokines) involved in inflammation. Specific effects of fluticasone furoate demonstrated in in vitro and in vivo models included activation of the glucocorticoid response element, inhibition of pro-inflammatory transcription factors such as NFκB, and inhibition of antigen-induced lung eosinophilia in sensitized rats.

Fluticasone furoate has been shown in vitro to exhibit a binding affinity for the human glucocorticoid receptor that is approximately 29.9 times that of dexamethasone and 1.7 times that of fluticasone propionate. The clinical relevance of these findings is unknown.”

#### “13 NONCLINICAL TOXICOLOGY

##### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Fluticasone furoate produced no treatment-related increases in the incidence of tumors in 2-year inhalation studies in rats and mice at doses of up to 9 and 19 mcg/kg/day, respectively (less than the maximum recommended daily intranasal dose in adults and children on a mcg/m<sup>2</sup> basis).

Fluticasone furoate did not induce gene mutation in bacteria or chromosomal damage in a mammalian cell mutation test in mouse lymphoma L5178Y cells in vitro. There was also no evidence of genotoxicity in the in vivo micronucleus test in rats.

No evidence of impairment of fertility was observed in (b) (4) male and female rats at inhaled fluticasone furoate doses of up to 29 and 91 mcg/kg/day, respectively (approximately 2 and 7 times, respectively, the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis).”

This review recommends edits to the fluticasone furoate text from the Veramyst label. The edits were in two areas: dose ratios and text. New dose ratios between animals and humans were used because Breo Ellipta and Veramyst have a different maximum recommended human dose. Minor text edits were made to comply with the current labeling format.

GSK proposed rewrites to the fluticasone portions of the Breo Ellipta label. Table 2 (next page) provides the text of teratogenic effects section of the approved and proposed label based on the same nonclinical data. The new proposal not only was wordy but also lacked clarity. The review recommends rejecting the proposed changes and retaining the previous label format with necessary edits. See Recommended Labeling Edits section.

**Table 2: Approved and Proposed Text for Teratogenic Effects of Fluticasone**

<b>Veramyst (NDA 22-051)</b> <b>Approved on 21-AUG-2012</b> <b>DARRTS ID# 3177618</b>	<b>Breo Ellipta (NDA 204-275)</b> <b>Proposed</b>
<p>There were no teratogenic effects in rats and rabbits at inhaled fluticasone furoate dosages of up to 91 and 8 mcg/kg/day, respectively (approximately 7 and 1 times, respectively, the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis). There was also no effect on pre- or post-natal development in rats treated with up to 27 mcg/kg/day by inhalation during gestation and lactation (approximately 2 times the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis).</p>	<p>(b) (4)</p>

GSK also proposed to add statements below

(b) (4)

Both the clinical and nonclinical pharmacology teams recommend rejection of the proposal.

(b) (4)

## VILANTEROL

This section discusses the nonclinical information relevant to the vilanterol label, including nonclinical findings that should be included in the Breo Ellipta label. Nonclinical data include the reproductive and development toxicity, mechanism of action, mutagenicity, and carcinogenicity. The reproductive and development toxicity include teratogenicity, impairment of fertility, and post-natal development of juvenile animals.

### Reproductive and Development Toxicity

A battery of reproductive and developmental toxicity studies of vilanterol were completed in rats and rabbits. Another study was completed to evaluate the effect of vilanterol and fluticasone on embryofetal development in rats. The battery of vilanterol studies evaluated the effects of vilanterol on fertility in rats (Reports CD2007/00581 and CD2006/01165),

teratogenicity in rats and rabbits (Reports CD2006/01166, WD2006/02439, CD2006/02047), and peri- and post-natal development in rats (Report CD2010/00109). The studies used the following routes of administration: inhalation (rats and rabbits), subcutaneous (rabbits), and oral (rabbits). Results showed that vilanterol did not affect fertility in rats, but caused dose-dependent, statistically non-significant increases in the incidence of malformations at high doses in rabbits and dose-dependent, statistically significant increases in the incidence of skeletal variations in rats and rabbits. Table 3 provides an overview of the reproductive and developmental toxicity studies of vilanterol.

**Table 3: Reproductive and Developmental Toxicity Studies of Vilanterol**

Segment	Species	ROA	Treatment period	Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Major Findings	Report #
I	Rat, M	IH	8 weeks	0, 62, 824, 31,500 <sup>a</sup>	No effect on male fertility	CD2007/00581
I	Rat, F	IH	14 D before - 6 D after mating	0, 49, 664, 37,100	No effect on female fertility	CD2006/01165
II	Rat, F	IH	GD 6 - 17	0, 45, 613, 33,700	Statistically significant $\uparrow$ in skeletal variations at $\geq 61 \mu\text{g}/\text{kg}/\text{day}$	CD2006/01166
II	Rabbit F	IH	GD 7 - 19	0, 61, 591, 5,740	Statistically non-significant $\uparrow$ in malformations (cleft palate & open eyelids) at HD and $\uparrow$ in skeletal variations in all doses	WD2006/02439
II	Rabbit F	SC	GD 7 - 19	0, 3, 7, 30, 300	Skeletal variations <sup>b</sup> at 300 $\mu\text{g}/\text{kg}/\text{day}$	CD2006/02047
III	Rat	PO	GD 7 – LAD 20	0, 300, 3,000, 10,000	No significant effects on pup postnatal development	CD2010/00109

a. Delivered dose in inhalation toxicity studies. The table lists delivered doses because they were used to calculate dose ratios between animals and humans.

b. The skeletal variations included unossified or incomplete ossification of 5<sup>th</sup> sternebra and xiphisternum.

### Impairment of Fertility

Vilanterol did not affect fertility in male or female rats. The effect of vilanterol on male and female fertility was studied separately (Documents CD2007/00581 and CD2006/01165). Males and females were dosed by the inhalation route of administration with vilanterol up to 31,500 and 37,100  $\mu\text{g}/\text{kg}/\text{day}$  in males and females, respectively. The results showed that vilanterol did not affect fertility parameters in either sex. These findings will be described in Section 13.1 of the vilanterol label. See Recommended Labeling Edits and Justification section for line editing.

### Teratogenicity

Vilanterol was not teratogenic in rats or rabbits, although fetal skeletal variations were observed at a high dose (300  $\mu\text{g}/\text{kg}/\text{day}$ ) by the subcutaneous route of administration in rabbits. Reproductive and embryofetal developmental effects of vilanterol were evaluated in

three studies by the inhalation or oral route of administration in rats and rabbits. Reports of the studies were numbered CD2006/01166, WD2006/02349, and CD2006/02047. Only Report WD2006/02349 showed that vilanterol treatment during the organogenesis period in pregnant rabbits caused dose-dependent but statistically non-significant increases in fetal malformations. Only report CD2006/02047 showed statistically significant increases in skeletal variations at high dose of vilanterol.

Report WD2006/02349 is an embryofetal developmental study in rabbits. Pregnant rabbits were dosed with 0, 63, 591 or 5,740  $\mu\text{g}/\text{kg}/\text{day}$  of vilanterol during the organogenesis period. Table 4 summarizes noticeable findings of the study. Statistically non-significant increases in the incidence of malformations and variations were observed in the vilanterol treatment groups. The malformations included mostly limb flexure or mal-rotation and open or partially opened eyelids in the HD group. The respective incidence was 15% of litters and 6.7% of fetuses for limb abnormality and 10% of litters and 6.3% of fetuses for eyelid abnormality.

**Table 4: Fetal Malformations and Variations in Rabbits (Report WD2006/02349)**

<b>Vilanterol (<math>\mu\text{g}/\text{kg}/\text{day}</math>, delivered dose)</b>	<b>0</b>	<b>63</b>	<b>591</b>	<b>5,740</b>
<b>Vilanterol AUC (ng.h/mL)</b>	<b>-</b>	<b>3.76</b>	<b>42.6</b>	<b>276</b>
No. of litters evaluated	21	16	18	20
<b>Malformations</b>	184	143	155	174
Cleft palate, # of fetuses (%)	0	1 (0.7)	0	8 (4.6)
# of litter (%)	0	1 (6.3)	0	1 (5.0)
Fore/hind limb flexure/mal-rotation, # fetuses (%)	2 (1.1)	2 (1.4)	2 (1.3)	10 (6.7)
# of litter (%)	2 (9.5)	1 (6.3)	2 (11.1)	3 (15.0)
Open/partially open eyelids, # of fetuses (%)	0	10 (7)	0	11 (6.3)
# of litter (%)	0	1 (6.3)	0	2 (10.0)
<b>Variations</b>				
Cranial bone malformation, # of fetuses (%) <sup>a</sup>	0	0	0	8 (4.6)
# of litter (%)	0	0	0	1 (5.0)
Bipartite/misshaped interparietal bone, # of fetuses (%)	0	1 (0.7)	0	7 (4.0)
# of litter (%)	0	1 (6.3)	0	3 (5.0)
Elongated/fissured anterior fontanelle, # of fetuses (%)	0	3 (2.1)	2 (1.3)	4 (2.3)
# of litter (%)	0	1 (6.3)	1 (5.6)	2 (10.0)
Bridge of ossification/fused sternebral center, # of fetuses (%)	5 (2.7)	2 (1.4)	1 (0.6))	13 (7.5)
# of litter (%)	5 (23.8)	2 (12.5)	1 (5.6)	6 (30)
Digit malformation, # of fetuses (%)	0	0	0	9 (5.2)
# of litter (%)	0	0	0	1 (5.0)

a. Misshapen/small frontals, partially fused/fused parietal to squamosal, misshapen zygomatic arch/mandibles, short lower jaw/snout, absent lower incisor sockets.

Report WD2006/02047 is also an embryofetal developmental study in rabbits. The study used different route of administration (i.e, subcutaneous injection) and doses from the previous study. Report WD2006/02047 showed that a subcutaneous dose of 300  $\mu\text{g}/\text{kg}/\text{day}$  vilanterol during the organogenesis period in pregnant rabbits caused a statistically significant increase in fetal skeletal variations (Table 5, next page). The variations included incomplete ossification of cervical ventral centrum, paw bones, and the skull. The review recommends the Breo Ellipta label describe the findings in the Pregnancy section based on the following: 1) the variation reached statistical significance ( $p < 0.05$ ), and 2) the mean

AUC of this study (306 ng.h/mL) was higher than the inhalation toxicity study in which the variation did not reach statistical significance. See Recommended Labeling Edits section for line editing of the Teratogenic Effects.

The review recommends against

(b)(4)

**Table 5: Fetal Malformations and Variations in Rabbits**

<b>Vilanterol (µg/kg/day, subcutaneous dosing)</b>	<b>0</b>	<b>3</b>	<b>7</b>	<b>30</b>	<b>300</b>
<b>Vilanterol AUC (ng.h/mL)</b>	<b>-</b>	<b>1.37</b>	<b>4.06</b>	<b>22.4</b>	<b>306</b>
No. of litters evaluated	20	20	22	11	21
No. of fetus evaluated	163	166	169	185	172
External anomalies: Open eyelid: # fetus (# litter)	0	0	0	0	1 (1)
Skeletal anomalies:					
Cervical vertical centrum not ossified, % fetus (incidence)	0	0	0	0	1.8 * (4/171)
# litter affected	0	0	0	0	3
Forepaw: metacarpal – less than expected number ossified, % of fetus (incidence, litter affected)	1.9 (3, 2)	1.3 (2, 2)	5.7 (11, 6)	0	15.9 * (31, 9)
Hindpaw: talus not ossified % of fetus (incidence, litter affected)	0	0	0	0	3.5 * (7/171, 4)
Skull: incomplete ossified hyoid: % fetus (# fetus)	0.8 (1/86)	0	0.9 (1/92)	3.2 (3/98)	9.4 * (10/92)
# of litters affected	1	0	1	3	22

The above recommendations differed from the original GSK proposal submitted on October 12, 2012. GSK proposed the following text to describe the teratogenicity findings in rabbits:

(b)(4)

The nonclinical review team recommended revising the GSK proposal. The Division sent its preliminary comments to GSK on March 26, 2013. GSK accepted most of the DPARP recommendations in the 02-APR-2013 response. There was a minor difference between the two proposals (Table 6, next page). Specifically, GSK proposed that the Breo Ellipta label reflect statistically non-significant increases in skeletal variations in rabbits at the approximately (b)(4) times (at inhaled dose of (b)(4) mg/kg/day) the MRHDID in Report WD2006/02349. This review concurs with the proposal based on similarities in responses and systemic vilanterol exposures between the two studies.

**Table 6: Most Recent Versions of Proposed and Recommended Label for Breo Ellipta**

DAPRP's 3/26/2013 proposal	GSK's 4/2/2013 Proposal
There were no teratogenic effects in rats and rabbits at approximately 13,000, and (b) (4) times, respectively, the MRHDID in adults (on a mcg/m <sup>2</sup> basis at maternal inhaled doses up to 33,700 in rats and on an AUC basis at maternal inhaled doses up to (b) (4) mcg/kg/day in rabbits, respectively). However, fetal skeletal variations were observed in rabbits at approximately (b) (4) times the MRHDID in adults (on an AUC basis at a maternal subcutaneous dose of 300 mcg/kg/day). The skeletal variations included decreased or absent ossification in cervical vertical centrum and metacarpals.	There were no teratogenic effects in rats and rabbits at approximately 13,000 and 160 times, respectively, the MRHDID in adults (on a mcg/m <sup>2</sup> basis at maternal inhaled doses up to 33,700 mcg/kg/day in rats and on an AUC basis at maternal inhaled doses up to 591 mcg/kg/day in rabbits). However, fetal skeletal variations were observed in rabbits at approximately 1,000 times the MRHDID in adults (on an AUC basis at maternal inhaled or subcutaneous doses of 5,740 or 300 mcg/kg/day, respectively). The skeletal variations included decreased or absent ossification in cervical vertebral centrum and metacarpals.

### Post-natal development

Report CD2010/00109 evaluated effects of vilanterol on parturition and postnatal development of rat pups after dams were dosed during the pregnancy (GD) and lactation (LAD) periods. Dams (F<sub>0</sub>, 24/dose) were dosed with 0, 0.3, 3, and 10-mg/kg/day vilanterol during the period of GD6 – LAD 20. The offspring (F<sub>1</sub>) were evaluated for growth and postnatal development. The MD and HD dose dams showed statistically significant increases in body weight gains (5% - 84%, p < 0.05) during the gestation and lactation periods. The MD and HD F<sub>1</sub> generations showed statistically significant decreases in mean body weights (11% - 12%, P < 0.05) at weaning (Table 7). No abnormalities were observed at ≤ 0.3 mg/kg/day.

**Table 7: F<sub>1</sub> Litter Results in Pre-Weaning Period**

Vilanterol dose in F <sub>0</sub> (mg/kg/day, PO)	0	0.3	3	10
# Viable F <sub>1</sub> litters evaluated	24	23	24	24
# pups delivered (total)	305	299	313	317
Survival index (%; PNDs 1-4)	96.7	99.3**	99.7**	97.7
# pups surviving at PNDs 21	11.4	12.1	12.2	11.8
Mean pup weight at PND 1 <sup>a</sup>	6.5 g	0	↓ 5%	↓ 5%
Mean pup weight at weaning <sup>a</sup>	42.1	↓ 7%	↓ 11%*	↓ 12%*

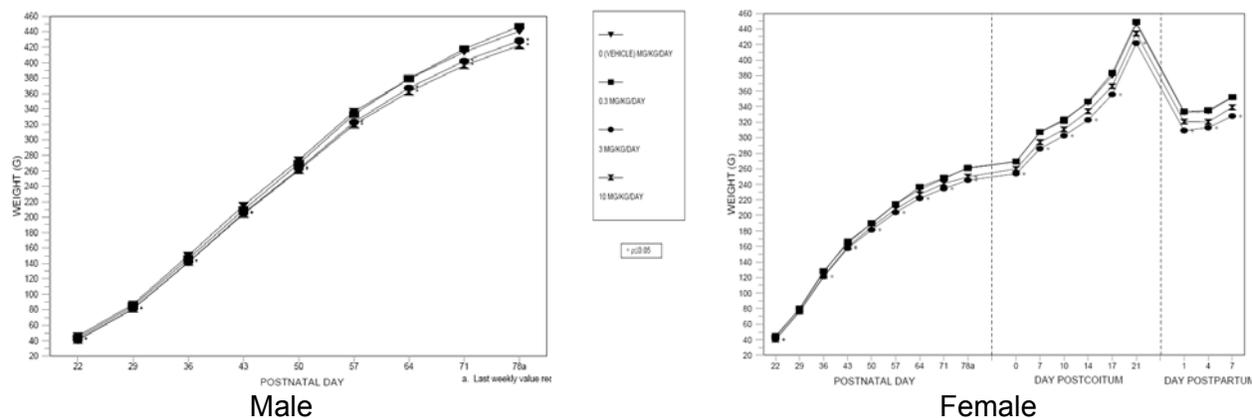
a. The number in treatment groups are changes relative to the control.

\*, p < 0.05; \*\*, p < 0.01.

The decrease in mean body weights of the F<sub>1</sub> generation lasted throughout the lifespan of the animals. Figure 1 (next page) presents the time-course of the F<sub>1</sub> mean body weights after weaning.

The observation of vilanterol effects on mean body weights prompted the review to examine the effects of vilanterol on body weights of pups at birth and in juveniles across the studies. Table 8 summarizes the results of these studies. There was a trend for a dose-dependent and statistically non-significant decrease in mean body weights across studies at the time of birth. The decrease became statistically significant during the lactation period in which the dams were treated with vilanterol continuously until weaning. The finding that vilanterol

treatment was associated with significant decreases in mean body weights in F<sub>1</sub> rats suggests that vilanterol may have some effects on post-natal development in rats, but the significance of this finding to the clinical use of Breo Ellipta is unknown because the vilanterol concentration in both the milk and the pup blood was generally below the detection limit (0.206 ng/mL). Also, the pups tend to have lower mean body weight at birth.



**Figure 1: Body weights in F<sub>1</sub> generation in the Rat segment III study**

The above discussions indicate that vilanterol is non-teratogenic at delivered doses up to 33,700 and 5,740  $\mu\text{g}/\text{kg}/\text{day}$  in rats and rabbits, and subcutaneous doses up to 300  $\mu\text{g}/\text{kg}/\text{day}$  in rabbits, respectively. Vilanterol caused statistically significant increases in fetal skeletal variations when pregnant rabbits were dosed subcutaneously with 300  $\mu\text{g}/\text{kg}/\text{day}$  of the drug, but not with 30  $\mu\text{g}/\text{kg}/\text{day}$ . Vilanterol did not affect pup post-natal development in rats when dams were dosed with up to 10,000- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol during pregnancy and lactation periods. These findings will be described in the label.

**Table 8: Mean Fetal Weights in Dams Treated with Vilanterol**

Section	Species	ROA	Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Mean fetal weight ( $\Delta\%$ change from control)				Report #
				LD	MLD	MHD	HD	
II	Rat	IH	0, 45, 613, 33,700	$\uparrow$ 1.6		$\uparrow$ 1.7	$\downarrow$ 1.7	CD2006/01166
	Rabbit	IH	0, 63, 591, 5740	$\downarrow$ 4.8	-	$\downarrow$ 3.8	$\downarrow$ 10.5	WD2006/02349
				SC	0, 3, 7, 30, 300	$\uparrow$ 0.6	$\uparrow$ 0.8	$\downarrow$ 2.1
III	Rat	PO	0, 300, 3,000, 10,000 (PND 1), PND 21	0	-	$\downarrow$ 5.0	$\downarrow$ 5.0	CD2010/00109
				$\downarrow$ 7.0	-	$\downarrow$ 11*	$\downarrow$ 12*	CD2010/00109

b. The number in treatment groups are changes relative to the control.

\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

## Mechanism of Action

Vilanterol is a long-acting  $\beta_2$  adrenergic receptor agonist (LABA). The mechanism of action of vilanterol mimics salmeterol, formoterol, and indacaterol (three currently marketed LABAs). Binding of LABAs to  $\beta_2$  adrenergic receptors results in subsequent increases in intracellular cAMP levels, relaxation of smooth muscles located in the airways, and decreases in airway resistance.

In vitro studies showed that vilanterol and formoterol possess similar potency on beta-2 receptors. Dr. Huiqing Hao reviewed data determining the selectivity of vilanterol and formoterol to  $\beta_2$  over  $\beta_1$  and  $\beta_3$  receptors in a nonclinical review completed on October 29, 2008. The selectivity was defined as the ratios of EC50s of  $\beta_1$  or  $\beta_3$  over that of  $\beta_2$  in a potency assay (i.e. DX-membrane cAMP potency assay). Table 9 summarizes the results of the selectivity assay (Report SH2003/00036). Vilanterol potency was similar to formoterol (i.e., pEC50s = 9.1 – 9.5) but the beta-2 receptor selectivity was similar to salmeterol. The competitive binding assay showed that the affinity of vilanterol to beta-2 receptors was similar to indacaterol (Report #2012N13541).

**Table 9: pEC50 of  $\beta$ -Adrenergic Agonists and Their Selectivity on  $\beta$ -Receptors**

Drug	pEC50 (Mean $\pm$ SEM) <sup>a</sup>			Selectivity <sup>a</sup>	
	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_2/\beta_1$	$\beta_2/\beta_3$
Isoprenaline	8.84 $\pm$ 0.01	7.6 $\pm$ 0.04	8.24 $\pm$ 0.06	0.05	0.2
Salmeterol	7.29 $\pm$ 0.02	9.6 $\pm$ 0.03	7.28 $\pm$ 0.02	215	219
Formoterol	8.99 $\pm$ 0.02	9.1 $\pm$ 0.03	8.89 $\pm$ 0.01	1.1	1.4
Vilanterol	7.30 $\pm$ 0.08	9.5 $\pm$ 0.15	7.52 $\pm$ 0.10	141	83

a. Extracted from the nonclinical review completed by Dr. Huiqing Hao on October 29, 2008 (p10 – 13). The selectivity was defined as EC50 ratios between different receptor subtypes.

Vilanterol inhibited the contractile response in human bronchus preparations induced by PGF-2 alpha and in guinea pig airway muscles induced by electric stimulation in vitro (Report #SH2003/00037). It also inhibited histamine-induced bronchoconstriction in conscious guinea pigs in vivo (Report #SH2003/00042).

GSK proposed the following text for vilanterol in Section 12.1 of the Breo Ellipta labeling:

“Vilanterol: Vilanterol is a (b) (4) LABA. In vitro tests have shown the functional selectivity of vilanterol was similar to salmeterol (b) (4)

The clinical relevance of these in vitro findings is unknown.

Although beta<sub>2</sub>-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta<sub>1</sub>-receptors are the predominant receptors in the heart, there are also beta<sub>2</sub>-receptors in the human heart comprising 10% to 50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta<sub>2</sub>-agonists may have cardiac effects.

The pharmacologic effects of beta<sub>2</sub>-adrenoceptor agonists, including vilanterol, are at least in part attributable to stimulation of intracellular adenylyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3',5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.”

The above statements are generally acceptable. However, the review recommends deleting the word (b) (4) in the first sentence. This is to ensure label consistency among LABAs. There are three approved LABAs currently on the market. They are salmeterol, formoterol, and indacaterol. Table 10 presents the approved labels of the products.

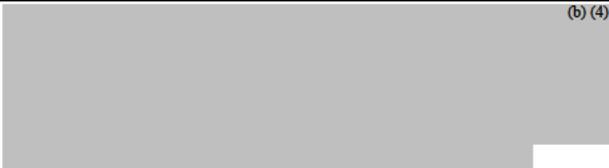
Table 10: Text of Section 12.1 of Labels of Marketed LABAs

Foradil (formoterol) NDA 20-831 Approved on 9/27/2012	Serevent (salmeterol) NDA 20-236, Approved on 9/28/2004	Arcapta (indacaterol) NDA 22-383 approved on July 1, 2011
Formoterol fumarate is a long-acting beta2-adrenergic agonist (beta2-agonist). Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. In vitro studies have shown that formoterol has more than 200-fold greater agonist activity at beta2-receptors than at beta1-receptors. Although beta2-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta1-receptors are the predominant receptors in the heart, there are also beta2-receptors in the human heart comprising 10%-50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta2-agonists may have cardiac effects. DARRTS ID# 3195939	Salmeterol is a long-acting beta2-adrenergic agonist. In vitro studies and in vivo pharmacologic studies demonstrate that salmeterol is selective for beta2-adrenoceptors compared with isoproterenol, which has approximately equal agonist activity on beta1- and beta2-adrenoceptors. In vitro studies show salmeterol to be at least 50 times more selective for beta2-adrenoceptors than albuterol. Although beta2-adrenoceptors are the predominant adrenergic receptors in bronchial smooth muscle and beta1-adrenoceptors are the predominant receptors in the heart, there are also beta2-adrenoceptors in the human heart comprising 10% to 50% of the total beta-adrenoceptors. The precise function of these is not yet established, but they raise the possibility that even highly selective beta2-agonists may have cardiac effects. Drugs @FDA	Indacaterol is a long-acting beta2-adrenergic agonist. When inhaled, indacaterol acts locally in the lung as a bronchodilator. Although beta2-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta1-receptors are the predominant receptors in the heart, there are also beta2-adrenergic receptors in the human heart comprising 10%-50% of the total adrenergic receptors. The precise function of these receptors is not known, but their presence raises the possibility that even highly selective beta2adrenergic agonists may have cardiac effects. The pharmacological effects of beta2-adrenoceptor agonist drugs, including indacaterol, are at least in part attributable to stimulation of intracellular adenylyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic monophosphate). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle. <i>In vitro</i> studies have shown that indacaterol has more than 24-fold greater agonist activity at beta2-receptors compared to beta1-receptors and 20-fold greater agonist activity compared to beta3-receptors. This selectivity profile is similar to formoterol. The clinical significance of these findings is unknown. DARRTS ID# 2968991

## Mutagenesis

Vilanterol tested negative in the following assays: the Ames assay, in vivo rat bone marrow micronucleus assay, in vitro UDS assay, and SHE cell assay. Vilanterol tested equivocal in the mouse lymphoma assay. See nonclinical reviews completed by Dr. Huiqing Hao on June 3 and October 29, 2008 in IND 74,696.

Table 11: Proposed and Recommended Text for Mutagenicity of Vilanterol

Proposed	Recommended
 (b) (4)	Vilanterol tested negative in the following assays: the in vitro Ames assay, in vivo rat bone marrow micronucleus assay, in vivo UDS assay, and in vitro SHE cell assay. Vilanterol tested equivocal in the in vitro mouse lymphoma assay.

GSK's proposal in the mutagenesis section of Breo Ellipta label differs significantly from that of other drugs. It also differs from that of fluticasone in the same section. The review recommends that the label simply describe the conclusion of the each assay in a format similar to fluticasone. See Table 11 (previous page) for the proposed and recommended text for the mutagenicity of vilanterol.

## Carcinogenesis

Vilanterol tested positive for carcinogenicity in rats and mice; however, the tumor findings were typical of beta-agonists in rodents and may not be relevant to humans. The findings should be described in the Breo Ellipta label. Similar to the labels of other approved LABAs, the Breo Ellipta label should state that the relevance of the findings to humans is unknown.

The carcinogenic potential of vilanterol was evaluated by the inhalation route of administration in two traditional 2-year bioassays. Dr. Luqi Pei completed a review of findings of these studies on February 14, 2013. The Executive Carcinogenicity Assessment Committee (ECAC) of the Center evaluated the findings on November 27, 2012. These reviews concluded that vilanterol caused dose-dependent increases in tumor incidences in the female reproductive organs in rats and mice and earlier occurrence of pituitary neoplasms in both sexes in rats. The tumors in females included tubulostromal adenomas in the ovaries in mice at 29,500  $\mu\text{g}/\text{kg}/\text{day}$  ( $p < 0.05$ ) and leiomyomas in mesovarian ligaments in rats at  $\geq 84/28.8 \mu\text{g}/\text{kg}/\text{day}$  ( $P < 0.01$ ). Decreases in the latency period of pituitary adenomas were observed in rats at  $\leq 233 \mu\text{g}/\text{kg}/\text{day}$ . No increases in tumor incidences were observed at 10.5/3.5 and 6150  $\mu\text{g}/\text{kg}/\text{day}$  in rats and mice, respectively.

Vilanterol doses of 84.4/28.2 and 10.5/3.5  $\mu\text{g}/\text{kg}/\text{day}$  in rats reflected dose-adjustments during the studies. Sprague-Dawley rats (60/sex/dose) were scheduled to be exposed by nose-only inhalation to 0 (C, lactose and vehicle), 10.5 (LD), 84.4 (MLD), 223 (MHD), and 657  $\mu\text{g}/\text{kg}/\text{day}$  (HD) of vilanterol (achieved doses) for 104 weeks. Due to excessive mortality during the study, premature terminations and vilanterol dose adjustments were made during the study. Specifically, males were treated with the scheduled dose for 101 weeks. Females were treated with the scheduled dose for 85 weeks and dose adjustments were made from week 86 and onward: Dosing was discontinued in the HLD and HD groups; Vilanterol doses in the LD and MLD groups were reduced to 3.5 and 28.2  $\mu\text{g}/\text{kg}/\text{day}$ , respectively. The three top-dose groups were terminated during weeks 95 – 96 when the number of survivors reached 15/group. The C and LD groups were terminated in week 105.

Due to space limitations in the label and because the general reader is not expected to have an in depth knowledge of carcinogenicity study design and interpretation, it is inappropriate to describe the above dosing scheme in detail. Furthermore, the dose reduction is not considered to have any major impact on the interpretation of the carcinogenicity study findings. The review recommends using the AUC values obtained on Month 6 of the treatment period to estimate vilanterol exposure in animals.

Vilanterol carcinogenicity study findings in rats and mice were similar to that of two currently marketed LABAs: salmeterol and formoterol.<sup>3,4</sup> The interpretation of carcinogenicity findings

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<sup>3</sup> The carcinogenesis section of formoterol (Symbicort, NDA 21-929) states: "Long-term studies were conducted in mice using oral administration and rats using inhalation administration to evaluate the carcinogenic potential of formoterol fumarate.

in the vilanterol label should also be similar to that of salmeterol and formoterol. GSK, however, proposed a significantly different interpretation of the animal findings. See Table 12 (below). The GSK proposal should be rejected. The review recommends that Breo Ellipta use language similar to Advair because they belong to the same NDA holder.

**Table 12: Interpretation of Rodent Carcinogenic Findings of LABAs**

Symbicort (formoterol) NDA 21-929 Approved on 6/25/2010	Advair (salmeterol) NDA 21-077 Approved on 6/25/2010	Breo (Vilanterol) NDA 204,275 Proposed
Other beta-agonist drugs have similarly demonstrated increases in leiomyomas of the genital tract in female rodents. The relevance of these findings to human use is unknown.	These findings in rodents are similar to those reported previously for other beta-adrenergic agonist drugs. The relevance of these findings to human use is unknown.	(b) (4)

The review also recommends edits to the description of the carcinogenicity findings in rats and mice. GSK's proposals included (b) (4)

The review recommends against (b) (4)

Below is the text of the original proposal.

(b) (4)

In a 24-month carcinogenicity study in CD-1 mice, formoterol at oral doses of 0.1 mg/kg and above (approximately 20 times the maximum recommended human daily inhalation dose on a mcg/m<sup>2</sup> basis) caused a dose-related increase in the incidence of uterine leiomyomas.

In a 24-month carcinogenicity study in Sprague-Dawley rats, an increased incidence of mesovarian leiomyoma and uterine leiomyosarcoma were observed at the inhaled dose of 130 mcg/kg (approximately 60 times the maximum recommended human daily inhalation dose on a mcg/m<sup>2</sup> basis). No tumors were seen at 22 mcg/kg (approximately 10 times the maximum recommended human daily inhalation dose on a mcg/m<sup>2</sup> basis)."

<sup>4</sup> The carcinogenesis section of salmeterol (Symbicort, NDA 21-929) states: "In an 18-month carcinogenicity study in CD-mice, salmeterol at oral doses of 1.4 mg/kg and above (approximately 20 times the MRHD for adults and children based on comparison of the plasma AUCs) caused a dose-related increase in the incidence of smooth muscle hyperplasia, cystic glandular hyperplasia, leiomyomas of the uterus, and cysts in the ovaries. No tumors were seen at 0.2 mg/kg (approximately 3 times the MRHD for adults and children based on comparison of the AUCs).

In a 24-month oral and inhalation carcinogenicity study in Sprague Dawley rats, salmeterol caused a dose-related increase in the incidence of mesovarian leiomyomas and ovarian cysts at doses of 0.68 mg/kg and above (approximately 55 and 25 times the MRHD for 1261 adults and children, respectively, on a mg/m<sup>2</sup> basis). No tumors were seen at 0.21 mg/kg (approximately 15 and 8 times the MRHD for adults and children, respectively, on a mg/m<sup>2</sup> basis)."

(b) (4)

DPARP sent its preliminary labeling comments on the original proposal to GSK on March 26, 2013 (Table 13). GSK submitted a counter proposal on April 2, 2013 (Table 13). Briefly, GSK accepted most of DAPRP recommendations, but GSK also insisted to include the phrase of (b) (4)

The review recommends against the proposal for reasons stated previously.

**Table 13: Most Recent Versions of Proposed and Recommended Label for Breo Ellipta**

DAPRP Proposal (3/26/2013)	GSK Proposal (4/2/2013)
<p>In a 2-yr carcinogenicity study in mice, vilanterol caused a statistically significant increase in tubulostromal leiomyomas in females at an inhalation dose of 29,500 mcg/kg/day (approximately 8,750 times the MRHDID in adults on an AUC basis). No tumors were seen at an inhalation dose of 615 mcg/kg/day (approximately 530 times the MRHDID in adults on an AUC basis).</p> <p>In a 2-yr carcinogenicity study in rats, vilanterol caused statistically significant increases in mesovarian leiomyomas in females and shortening of the latency of pituitary tumors at inhalation doses <math>\geq</math> 84.4 mcg/kg/day (approximately <math>\geq</math> 45 times the MRHDID in adults on an AUC basis). No tumors were seen at an inhalation dose of 10.5 mcg/kg/day (approximately 2 times the MRHDID in adults on an AUC basis).</p>	<p>In a 2-year carcinogenicity study in mice, vilanterol caused a statistically significant increase in ovarian tubulostromal adenomas in females at an inhalation dose of 29,500 mcg/kg/day (approximately 8,750 times the MRHDID in adults on an AUC basis) (b) (4)</p> <p>No increase in tumors was seen at an inhalation dose of (b) (4) mcg/kg/day (approximately (b) (4) times the MRHDID in adults on an AUC basis).</p> <p>In a 2-year carcinogenicity study in rats, vilanterol caused statistically significant increases in mesovarian leiomyomas in females and shortening of the latency of pituitary tumors at inhalation doses greater than or equal to 84.4 mcg/kg/day (greater than or equal to approximately 45 times the MRHDID in adults on an AUC basis). No tumors were seen at an inhalation dose of 10.5 mcg/kg/day (approximately 2 times the MRHDID in adults on an AUC basis).</p>

(b) (4)

(b) (4)

## Fluticasone/Vilanterol

Report #CD2007/00973 evaluated the potential teratogenic interactions between fluticasone and vilanterol in rats. Dams were exposed by inhalation up to 94.9- $\mu\text{g}/\text{kg}/\text{day}$  fluticasone and 98.3- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol, alone or in combination during the organogenesis period. The study showed no fetal malformation in any of the groups. The group treated with 94.9- $\mu\text{g}/\text{kg}/\text{day}$  fluticasone and 98.3- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol in combination showed numerically moderate, but statistically significant increases in the incidence of fetal skeletal variations over the groups treated with the individual API alone (Table 14). The variations included unossified/incomplete ossification/semi-bipartite/bipartite in the 5<sup>th</sup> sternebra and xiphisternum. However, these findings were similar to that of vilanterol alone (Report #CD2006/01166).

**Table 14: Findings in Embryofetal Developmental Study of Fluticasone/Vilanterol in Rats**

Group	1	2	3	4	5	6	6
Fluticasone ( $\mu\text{g}/\text{kg}/\text{day}$ )	0	82	94.4	94.9	0	7.9	29.5
Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	0	0	3.5	98.3	86.9	8.3	31.7
Body weight gain (% control) <sup>a</sup>	88.8 g	33 *	28*	29*	110	88	57 *
Food consumption (% control) <sup>a</sup>	336.7g	83*	81*	84*	100	100	91*
Mean fetal body weight (M)	5.82	5.30*	5.27*	5.24*	5.80	5.63	5.63
Mean fetal body weight (F)	5.50	5.01*	5.06*	4.99*	5.52	5.43	5.37
Sternebra 5 and xiphisternum <sup>b</sup>	9.5	15.2	18.1	22.1*	11.4	6.9	9.5

a. GDs 6 – 18.

b. Incidence of unossified/incomplete ossification/semi-bipartite/bipartite.

c. \*, P <0.05.

Data in Table 14 suggest that there might be some potential interactions in the embryofetal skeletal developmental effects of fluticasone and vilanterol, but these effects were manifested as skeletal variations rather than malformations. The review recommends against

(b) (4)

Table 15 presents the proposed and recommended text for the teratogenic interactions between fluticasone and vilanterol.

**Table 15: Most Recent Versions of Proposed and Recommended Label for Breo Ellipta**

GSK Proposal	DAPRP Recommendation
(b) (4)	<p><b>Fluticasone furoate and Vilanterol:</b> There was no evidence of teratogenic interactions between fluticasone furoate and vilanterol in rats at approximately 9 and 40 times, respectively, the maximum recommended human daily inhalation dose (MRHDID) in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses of fluticasone and vilanterol, alone or in combination, up to approximately 95-µg/kg/day each).</p>

## RECOMMENDED LABELING EDITS

Recommended line editing of the proposed text for the nonclinical sections of Breo Ellipta label is provided below. The red highlights indicated recommended editing. Underlined text indicates recommended additions and strikethrough text indicates recommended deletions.

### 8.1 Pregnancy

BREO ELLIPTA: Teratogenic Effects: Pregnancy Category C:

There are no adequate and well-controlled trials of BREO ELLIPTA in pregnant women. Corticosteroids and beta<sub>2</sub>-agonists have been shown to be teratogenic in laboratory animals when administered systemically at relatively low dosage levels. Because animal (b)(4) studies are not always predictive of human response, BREO ELLIPTA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking BREO ELLIPTA.

**Fluticasone furoate and Vilanterol:** There was no evidence of teratogenic interactions between fluticasone furoate and vilanterol in rats at approximately 9 and 40 times, respectively, the maximum recommended human daily inhalation dose (MRHDID) in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses of fluticasone and vilanterol, alone or in combination, up to approximately 95-µg/kg/day each).

**Fluticasone furoate:** There were no teratogenic effects in rats and rabbits at approximately 9 and 2 times, respectively, the MRHDID in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses up to 91 and 8 mcg/kg/day in rats and rabbits, respectively). There were no effects on peri-natal and post-natal development in rats at approximately 3 times the MRHDID in adults (on a mcg/m<sup>2</sup> at maternal doses up to 27 mcg/kg/day).

**Vilanterol:** There were no teratogenic effects in rats and rabbits at approximately 13,000 and 160 times, respectively, the MRHDID in adults (on a mcg/m<sup>2</sup> basis at maternal inhaled doses up to 33,700 mcg/kg/day in rats and on an AUC basis at maternal inhaled doses up to 591 mcg/kg/day in rabbits). However, fetal skeletal variations were observed in rabbits at approximately 1,000 times the MRHDID in adults (on an AUC basis at maternal inhaled or subcutaneous doses of 5,740 or 300 mcg/kg/day, respectively). The skeletal variations

included decreased or absent ossification in cervical vertebral centrum and metacarpals. There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHDID in adults (on a mcg/m<sup>2</sup> basis at maternal oral doses up to 10,000 mg/kg/day).

(b) (4)



Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully monitored.”

## 12.1 Mechanism of Action

**BREO ELLIPTA:** BREO ELLIPTA contains both vilanterol and fluticasone furoate; therefore, the mechanisms of actions described below for the individual components apply to BREO ELLIPTA. These drugs represent two different classes of medications (a synthetic corticosteroid and a LABA).

**Fluticasone Furoate:** Fluticasone furoate is a synthetic trifluorinated corticosteroid with (b)(4) anti-inflammatory activity. The precise mechanism through which fluticasone furoate affects COPD symptoms is not known. Corticosteroids have been shown to have a wide range of actions on multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, cytokines) involved in inflammation. Specific effects of fluticasone furoate demonstrated in in vitro and in vivo models included activation of the glucocorticoid response element, inhibition of pro-inflammatory transcription factors such as NFkB, and inhibition of antigen-induced lung eosinophilia in sensitized rats.

Fluticasone furoate has been shown in vitro to exhibit a binding affinity for the human glucocorticoid receptor that is approximately 29.9 times that of dexamethasone and 1.7 times that of fluticasone propionate.

(b) (4)

**Vilanterol:** Vilanterol is a (b)(4) LABA. In vitro tests have shown the functional selectivity of vilanterol was similar to salmetero (b)(4)

(b)(4) The clinical relevance of these in vitro findings is unknown.

Although beta<sub>2</sub>-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta<sub>1</sub>-receptors are the predominant receptors in the heart, there are also beta<sub>2</sub>-receptors in the human heart comprising 10% to 50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta<sub>2</sub>-agonists may have cardiac effects.

The pharmacologic effects of beta<sub>2</sub>-adrenoceptor agonists, including vilanterol, are at least in part attributable to stimulation of intracellular adenylyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3',5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

**BREO ELLIPTA:** No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with BREO ELLIPTA; however, studies are available for fluticasone furoate and vilanterol, as described below.

**Fluticasone Furoate:** Fluticasone furoate produced no treatment-related increases in the incidence of tumors in 2-year inhalation studies in rats and mice at **inhaled** doses up to 9 and 19 mcg/kg/day in rats and rabbits, respectively (approximately <sup>(b) (4)</sup>—equal to the MRHDID in adults on a mcg/m<sup>2</sup> basis).

Fluticasone furoate did not induce gene mutation in bacteria or chromosomal damage in a mammalian cell mutation test in mouse lymphoma L5178Y cells in vitro. There was also no evidence of genotoxicity in the in vivo micronucleus test in rats.

No evidence of impairment of fertility was observed in <sup>(b) (4)</sup> male and female rats at inhaled doses up to 29 and 91 mcg/kg/day, respectively (approximately 3 and 9 times the MRHDID in adults on a mcg/m<sup>2</sup> basis).

**Vilanterol:** In a 2-year carcinogenicity study in mice, vilanterol caused a statistically significant increase in ovarian tubulostromal adenomas in females at an inhalation dose of 29,500 mcg/kg/day (approximately 8,750 times the MRHDID in adults on an AUC basis). No tumors were seen at inhalation doses up to 615 mcg/kg/day (approximately 530 times the MRHDID in adults on an AUC basis).

In a 2-year carcinogenicity study in rats, vilanterol caused statistically significant increases in mesovarian leiomyomas in females and shortening of the latency of pituitary tumors at inhalation doses greater than or equal to 84.4 mcg/kg/day (greater than or equal to approximately 45 times the MRHDID in adults on an AUC basis). No tumors were seen at an inhalation dose of 10.5 mcg/kg/day (approximately 2 times the MRHDID in adults on an AUC basis).

These tumor findings in rodents are similar to those reported previously for other beta-adrenergic agonist drugs. The relevance of these findings to human use is unknown.

Vilanterol tested negative in the following genotoxicity assays: the in vitro Ames assay, in vivo rat bone marrow micronucleus assay, in vivo UDS assay, and in vitro SHE cell assay. Vilanterol tested equivocal in the in vitro mouse lymphoma assay.

No evidence of impairment of fertility was observed in rats at inhalation doses from 31,500 to 37,100 mcg/kg/day in males and females, respectively (approximately 12,000 to 14,000 times, respectively, the MRHDID in adults on a mg/m<sup>2</sup> basis).

(b) (4)



(b) (4)

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/s/  
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LUQI PEI  
04/22/2013

MARCIE L WOOD  
04/22/2013

## Secondary Pharmacology and Toxicology Review for NDA 204-275

TO: NDA 204-275 (GlaxoSmithKline)

FROM: Marcie Wood, Ph.D.  
Pharmacology and Toxicology Acting Supervisor  
Division of Pulmonary, Allergy, and Rheumatology Drug Products

DATE: March 22, 2013

Overview: I concur with the recommendation of Dr. Luqi Pei (detailed in a nonclinical review dated March 12, 2013) that the pharmacology and toxicology of BREO ELLIPTA (fluticasone furoate and vilanterol trifenate dry powder inhaler) has been adequately studied and the drug product should be approved from a nonclinical perspective. A labeling review is pending.

BREO ELLIPTA is a fixed-dose combination of fluticasone furoate (FF), a glucocorticosteroid, and vilanterol trifenate (VI), a NME long-acting  $\beta_2$ -adrenergic receptor agonist (LABA). It is indicated for chronic, once-daily, oral inhalation treatment of Chronic Obstructive Pulmonary Disease (COPD). The proposed clinical dose is 100  $\mu\text{g}$  FF and 25  $\mu\text{g}$  VI per day (delivered as a single actuation). The nonclinical safety program of BREO ELLIPTA is based upon complete toxicology programs conducted for both individual drugs (FF and VI), as well as a bridging toxicology program with the FF/VI combination to characterize potential toxicological interactions between FF and VI. The pharmacology and toxicology profile of FF was previously well-characterized in NDA 22-051 (Veramyst); therefore, the current review summarizes relevant findings from the VI and FF/VI nonclinical programs.

Pharmacology: Vilanterol is a long-acting,  $\beta_2$ -adrenergic receptor agonist. The pharmacodynamic effects of VI were investigated both *in vitro* and *in vivo*. VI showed preferential binding to  $\beta_2$ -adrenergic receptors ( $K_i = 0.72$  nM) versus  $\beta_1$ - or  $\beta_3$ -adrenergic receptors. VI inhibited the contractile response in human bronchus preparations induced by PGF-2 alpha. In guinea pigs, VI relaxed guinea pig airway muscle contractility induced by electric stimulation, as well as by histamine-induced bronchoconstriction.

Toxicology: General inhalation vilanterol toxicology studies were conducted in the mouse, rat, and dog up to 13-weeks, 26-weeks, and 39-weeks, respectively. These studies identified the following target organs of toxicity: upper airways (mouse, rat, and dog), lung (mouse, rat, and dog), heart (rat and dog), liver (mouse and dog), female reproductive tract (mouse and rat), and testes (dog). Study findings were typical for  $\beta_2$ -adrenergic receptor agonists or inhaled drugs. There were no significant toxicological interactions between FF and VI in 13-week inhalation toxicity studies in rats and dogs.

Genotoxicity: Vilanterol was negative in a complete battery of in vitro (Ames assay, UDS assay, SHE cell assay) and in vivo (rat bone marrow micronucleus assay) assays. VI was equivocal in the mouse lymphoma assay.

Carcinogenicity: Two 2-year carcinogenicity bioassays were conducted with vilanterol in rats and mice at inhalation doses up to 657 and 29,500 µg/kg/day (achieved doses), respectively. Rats of both sexes showed a dose-dependent decreased latency for pituitary neoplasms and females had an increased incidence of leiomyomas in the mesovarian ligaments. Female mice had an increased incidence of tubulostromal carcinomas in the ovaries. The tumor findings in the female rodent reproductive tract, as well pituitary findings in rats, are considered to be a class effect of β<sub>2</sub>-adrenergic receptor agonists and have been observed with other approved drugs of the same class. Currently, there is no clinical evidence to suggest that these tumor findings are relevant to humans. However, the relevance of these findings to human use is still unknown.

Reproductive and Developmental Toxicology: Reproductive and developmental toxicity studies of vilanterol were completed in rats and rabbits via the inhalation (rat and rabbit), oral (rat), and subcutaneous (rabbit) routes of administration. These studies evaluated the effects of VI on fertility in rats, teratogenicity in rats and rabbits, and pre- and post-natal development in rats. In rats, VI had no effects on male or female fertility, was not teratogenic, and had no effects on pre- or post-natal development. In rabbits, a high dose of subcutaneous VI (300 µg/kg/day) resulted in fetal skeletal variations. There were no significant toxicological interactions between FF and VI in an inhalation teratogenicity study in rats.

Labeling: A labeling review is pending. The pharmacology, reproductive toxicology, genotoxicity, and carcinogenicity of the monoproducts were well characterized in Veramyst NDA 22-051 for fluticasone furoate, as well as in the vilanterol and fluticasone/vilanterol nonclinical development programs that were conducted in support of the current NDA. These aspects will be reflected in relevant nonclinical sections of the labeling for each monoproduct, as well as for the combination product (where applicable).

There are no outstanding Pharmacology and Toxicology issues for this product.

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/s/  
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MARCIE L WOOD  
03/22/2013

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

**PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION**

Application number: NDA 204-275  
Supporting document/s: Sequences 0000  
Applicant's letter date: July 12, 2012  
CDER stamp date: July 12, 2012  
Product: Breo Ellipta (Fluticasone furoate and Vilanterol trifenate) Dry Powder Inhaler  
Indication: Chronic Obstructive Pulmonary Disease (COPD)  
Applicant: GlaxoSmithKline (GSK)  
Review Division: Pulmonary, Allergy, and Rheumatology  
Reviewer: Luqi Pei, Ph.D.  
Supervisor (acting): Marcie Wood, Ph.D.  
Division Director: Badrul Chowdhury, M.D., Ph.D.  
Project Manager: Angela Ramsey

*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

This application (NDA 204-275) proposes to register Breo Ellipta for the indication of chronic obstructive pulmonary diseases (COPD) in adult patients. Breo Ellipta is a dry powder inhaler that delivers a mixture of fluticasone furoate and vilanterol trifenate, the active pharmaceutical ingredients. Each actuation of the device delivers 100- $\mu$ g fluticasone and 25- $\mu$ g vilanterol. The maximum recommended clinical dose of the device is one actuation daily. Breo Ellipta contains magnesium stearate and lactose as the excipients. See Section 2.3 Drug Formulation for additional formulation information.

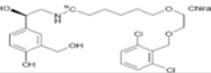
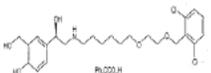
This application contained all nonclinical studies conducted with fluticasone and vilanterol, alone or combination. Some of the studies were previously submitted to three INDs and one NDA and were reviewed by the Agency. The INDs were 70,297 (fluticasone inhalation powder), 74,696 (vilanterol inhalation powder) and 77,855 (fluticasone/vilanterol inhalation powder). The NDA was the Veramyst Nasal Spray application (Fluticasone furoate, NDA 22-051). DPARP has generated a number of written reviews under these applications. See Section 3.3 Previous Reviews Referenced for lists of the reviews. Because the Agency had reviewed previously all fluticasone data in NDA 22-051, the current review focuses on the nonclinical development of vilanterol, alone or in combination with fluticasone.

This review uses fluticasone and vilanterol as the name of the active ingredients. Please note that other names have been used in current and previous submissions, study reports, and regulatory reviews of these two drugs. Also, fluticasone and vilanterol have additional names because each exists in different salt or ester forms. For example, fluticasone has two ester forms: fluticasone propionate and fluticasone furoate. Both forms are approved and currently on the market. Fluticasone propionate is marketed as Flonase (NDA 20-833) and fluticasone furoate as Veramyst (NDA 22-051). Fluticasone in this review refers to fluticasone furoate ester only.

A number of names and codes have been used to describe vilanterol base and its salts. Specifically, two code names (GW642444 or GW642444X) have been used to describe the free base and three salts have been used in the developmental program. The salts were (b)(4) (GW642444A), (b)(4) (GW642444H), and trifenate (or triphenylacetate, GW642444M) salts. Table 1 (next page) lists names of these esters or salts for easy references.

The nonclinical studies of vilanterol used both GW642444H and GW642444M while clinical trials used GW642444M only. Particularly, early pharmacological, pharmacokinetic, and toxicology program of vilanterol used GW642444H, while the pivotal toxicology studies used GW642444M. There was no nonclinical safety concern about the switch, according to the nonclinical review completed by Dr. Quiqing Hao on October 29, 2008. Also, both micronized and un-micronized forms of vilanterol have been used in nonclinical studies. There was no significant difference in toxicity profile between these vilanterol substances. All vilanterol doses referenced in the review were the GW642444 (free base, or parent compound) doses.

**Table 1: Compounds and Their Code Names**

Code name	Chemical Name	Structure	CAS #	Molecular formula	Weight
GW685698, GW285698X	Fluticasone furoate		90566-53-3	397864-44-7	538.6
GW642444X, or GW642444	Vilanterol (VI) base, or parent compound		503068-34-6	C <sub>24</sub> H <sub>33</sub> Cl <sub>2</sub> NO <sub>5</sub>	486.4
GQ642444A GI179710					(b)(4)
GW642444M	VI trifenate, or VI triphenylacetate		503070-58-4	C <sub>24</sub> H <sub>33</sub> Cl <sub>2</sub> NO <sub>5</sub> • C <sub>20</sub> H <sub>16</sub> O <sub>2</sub>	774.8
GW642444H					(b)(4)

This review uses pulmonary deposited doses to convey vilanterol dosimetry in inhalation toxicity studies, with the exception of inhalation carcinogenicity studies. Pulmonary deposited doses were derived by applying (i.e., multiplying) appropriate deposition factors to the achieved dose of each study report. The submitted study reports generally used the formula to calculate the achieved doses:

$$D = (RMV \times T \times C)/(BW), \text{ where}$$

D, RMV, T, C, and BW stand for dose ( $\mu\text{g}/\text{kg}/\text{day}$ ), respiratory minute volume (L/min), exposure duration (min), chamber vilanterol concentration ( $\mu\text{g}/\text{L}$ , analytical results) and body weight (g), respectively. Respiratory minute volume was calculated using formula:  $RMV = 0.499 \times BW \text{ (kg)}^{0.899}$ . The achieved dose generally assumed 100% of deposition to the respiratory system. This review uses pulmonary deposition factors of 0.1, 0.1, 0.25 and 0.25 in mice, rats, dogs and monkeys, respectively, to calculate the pulmonary deposited doses in relevant species.

## 1.2 Brief Discussion of Nonclinical Findings

### 1.2.1 Fluticasone

No new, significant fluticasone nonclinical data were submitted. All fluticasone nonclinical data was previously submitted to and reviewed in NDA 22-051 (Veramyst Nasal Spray) which was approved on April 27, 2007. Briefly, fluticasone possesses a toxicity profile typical of inhaled corticosteroids. The drug is non-genotoxic, non-carcinogenic and non-teratogenic.<sup>1</sup> The target organ of inhaled fluticasone was the respiratory and immune systems.

### 1.2.2 Vilanterol

Vilanterol is a long-acting beta<sub>2</sub> receptor agonist (LABA). Vilanterol binds to and activates beta-2 receptors resulting in subsequent increases in intracellular cAMP levels, relaxation of

<sup>1</sup> See [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2011/022051s007lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022051s007lbl.pdf) for a complete label.

smooth muscles located in the airways, and decreases in airway resistance. Vilanterol relaxed guinea pig airway muscle contractility induced by electric stimulation and inhibited the contractile response in human bronchus preparation induced by PGF-2 alpha in vitro. It also relaxed histamine-induced bronchoconstriction in conscious guinea pig.

Vilanterol was well-absorbed by the inhalation route of administration in rat, dog and human, but the absolute bioavailability of the drug after this route of administration is unknown in animals and 27.3% in humans. Plasma drug concentrations were generally proportional to the inhalation dose in laboratory animals. Peak plasma drug levels were found at the end of inhalation exposure. Vilanterol is highly bound to plasma proteins (92-99%) in animals and humans. Vilanterol is metabolized by CYP 2B2 in rats and CYP 3A4 enzyme in dogs and humans. The elimination half-life was 5.3 and 9.8 hrs in rats and dogs, respectively.

General toxicity of vilanterol was evaluated after the inhalation route of administration in mice, rats, and dogs. Pivotal general toxicity studies were 13-, 26- and 39- weeks in mice, rats, and dogs, respectively. These studies identified the following organs as the target organs of vilanterol toxicity: the upper airways, lung, heart, liver, and testes. Findings are typical of beta agonists or inhaled xenobiotics.

Vilanterol tested negative in the Ames assay, UDS assay in vitro, and SHE cell assay in vitro, and rat bone marrow micronucleus assay in vivo; and equivocal in the mouse lymphoma assay.

The carcinogenicity potential of vilanterol was evaluated via inhalation in two traditional 2-year bioassays. The rat showed a dose-related shortening of latency for pituitary neoplasms at  $\geq 84.4/28.2$   $\mu\text{g}/\text{kg}/\text{day}$  (achieved dose) in both sexes and increases in the incidence of leiomyomas in mesovarian ligaments in females. In mice, females showed increases in the incidence of tubulostromal carcinomas in the ovaries at 29,500  $\mu\text{g}/\text{kg}/\text{day}$ . Nonstatistically significant increases in the leiomyomas and leiomyosarcomas were observed in the uterus in mice exposed to  $\geq 62$ - $\mu\text{g}/\text{kg}/\text{day}$  vilanterol. The findings in these studies were typical of beta agonists in rodents.

Maternal exposure of vilanterol caused dose-dependent increases in fetal malformations in rabbits and variations in both rats and rabbits. The malformations in rabbits included cleft palate, open or partially opened eyelids, and limb flexure or mal-rotations. The variations in rats included unossified or incomplete ossification of 5<sup>th</sup> sternebra and xiphisternum. The variations in rabbits included cranial bone malformations, bipartite/misshaped interparietal bone, elongated/fused anterior fontanelle, bridge of ossification/fused sternebra center, digit malformations, unossified cervical vertical centrum, metacarpal forepaws, and hindpaws, and incompletely ossified hyoid skull. These effects occurred at inhalation doses 3,150 and 575  $\mu\text{g}/\text{kg}/\text{day}$  in rats and rabbits, respectively, and subcutaneous dose of 300  $\mu\text{g}/\text{kg}/\text{day}$  in rabbits. In rabbits, the 575- $\mu\text{g}/\text{kg}/\text{day}$  inhalation dose and the 300- $\mu\text{g}/\text{kg}/\text{day}$  subcutaneous dose yielded similar AUC values (i.e., 276 – 306 ng.h/mL). No treatment-related fetal effects were observed at maternal inhalation doses of  $\leq 56$   $\mu\text{g}/\text{kg}/\text{day}$  in rats and  $\leq 59$   $\mu\text{g}/\text{kg}/\text{day}$  in rabbits, respectively, or  $\leq$  a subcutaneous dose of 30  $\mu\text{g}/\text{kg}/\text{day}$  in rabbits. Vilanterol alone did not affect fertility in either males or females at inhalation doses up to 3,150  $\mu\text{g}/\text{kg}/\text{day}$  in rats.

### **1.2.3 Vilanterol and fluticasone in combination**

There were no significant toxicological interactions between vilanterol and fluticasone after inhalation administration. Potential toxicological interactions between inhaled vilanterol and fluticasone were evaluated in rats and dogs. Animals were exposed by inhalation dusts containing vilanterol and fluticasone daily for up to 13 weeks. The fluticasone:vilanterol ratio in the test formulation ranged from 2:1 to 10:1 in rats and 2:1 to 60:1 in dogs, respectively. Neither study identified significant toxicological interactions between vilanterol and fluticasone. See Section 11.3 for additional information.

## **1.3 Recommendations**

### **1.3.1 Approvability**

Approval of the application is recommended from the nonclinical perspective. The application has conducted adequate nonclinical characterization of the toxicity profile of its active pharmaceutical ingredients (APIs): vilanterol and fluticasone. The characterization included evaluation of the pharmacological, pharmacokinetic and toxicological profiles of the individual APIs and potential toxicological interactions between the APIs. The evaluations did not reveal unknown toxicities associated with drugs in their respective pharmacological classes, neither did they reveal significant toxicological interactions between the APIs. The application has fulfilled nonclinical requirements for the registration of the proposed product, Breo Ellipta.

### **1.3.2 Additional Nonclinical Recommendations**

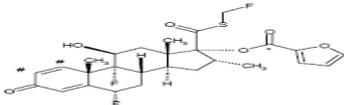
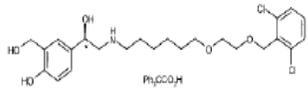
None.

### **1.3.3 Labeling**

A labeling review will be done at a later time.

## 2 Drug Information

### 2.1 Drug

CAS Registry Number:	90566-53-3	503070-58-4
Generic Name:	Fluticasone Furoate	Vilanterol trifenate
Code Name:	GW685698	GW642444M
Chemical Name:	Androsta-1,4-diene-17-carbothioic acid, 6,9-difluoro-11,17-dihydroxy-16-methyl-3-oxo-, S-(fluoromethyl) ester, (6alpha,11beta,16alpha,17alpha)-	4-((1R)-2-((6-(2-(2,6-dichlorophenyl)methoxy)ethoxy)hexyl)amino)-1-hydroxyethyl)-2-(hydroxymethyl)phenol
Molecular Formula/Weight:	C <sub>27</sub> H <sub>29</sub> F <sub>3</sub> O <sub>6</sub> S/538.6	C <sub>24</sub> H <sub>33</sub> C <sub>12</sub> NO <sub>5</sub> .C <sub>20</sub> H <sub>16</sub> O <sub>2</sub> /774.8
Structure:		
Pharmacologic Class:	Corticosteroid	Long acting beta <sub>2</sub> agonist

### 2.2 Relevant INDs, NDAs, and DMFs

IND/NDA	Product	Indication	Date (active since)
IND 48,647	Fluticasone	Rhinitis	10/30/2003
IND (b) (4) <sup>a</sup>	(b) (4)	(b) (4)	06/07/2000
IND 70,297	Fluticasone Furoate (FF)	Asthma (≥ 12 yr)	10/26/2006
IND 74,696	Vilanterol (VI)	COPD + bronchospasm	12/10/2007
IND 77,855	FF/VI	COPD	6/27/2009
IND (b) (4)	(b) (4)	(b) (4)	12/08/2010
IND (b) (4)	VI/GSK573719	(b) (4)	12/16/2009
IND (b) (4)	(b) (4)	(b) (4)	12/18/2011
IND (b) (4)	(b) (4)	(b) (4)	8/10/2012
NDA 22-051	FF	Rhinitis	4/27/2007 <sup>b</sup>
DMF (b) (4)	VI	N/A	N/A

a. This IND contains toxicity studies pivotal to the qualification of magnesium stearate, an excipient.

b. Approval date.

### 2.3 Drug Formulation

Fluticasone and vilanterol are stored in two separate blister strips. Contents of the blisters are released and mixed when the device is actuated. A mixture of dry powder is released from the mouth piece of the device. Table 2 (below) shows the contents of each blister.

Table 2: Composition of Breo Ellipta

Blister strip	Ingredient	Quantity/ blister	Function
FF strip	Fluticasone furoate	100 µg	Active
	Lactose	(b) (4)	(b) (4)
VI strip	Vilanterol	25 µg	Active
	Magnesium stearate	0.125 mg	(b) (4)
	Lactose	(b) (4)	(b) (4)

## 2.4 Comments on Novel Excipients

The product uses magnesium stearate and lactose as inactive ingredients. Each actuation (daily dose) releases 125- $\mu$ g magnesium stearate (b) (4). Neither excipient is novel, but the expected exposure of patients to magnesium (Mg) stearate was higher than levels in approved products. The applicant submitted additional nonclinical data (up to 6 months in rats) to support the proposed use of Mg stearate. The NOAEL in the 6 month toxicity study provided adequate safety margins for the proposed use of the excipients.

**Lactose:** Lactose has been used as an excipient in a number of currently marketed inhalation drug products. The expected daily exposure of lactose from the proposed use of the product is (b) (4).

**Magnesium stearate:** Magnesium stearate is present as an excipient in approved inhalation drug products. Foradil<sup>®</sup> CertiHaler<sup>™</sup> (NDA 21-592, approval date of December 15, 2006) contains (b) (4) magnesium stearate/capsule. Exposure of patients to Mg stearate is approximately (b) (4). The expected exposure of patients to Mg stearate from the use of Breo Ellipta is approximately 125  $\mu$ g/day. Because the expected exposure of patients to Mg stearate from the proposed product (b) (4), the applicant has provided additional nonclinical data to support the safety of the excipient. The data included inhalation toxicity studies of Mg stearate up to 6 months in duration in rats. These studies did not reveal any safety concern about the compound. See the nonclinical review completed by Dr. L. Pei on June 7, 2000 in IND (b) (4) for detailed information about these studies. Below is a brief summary of the 6-month study in rats.

Sprague-Dawley rats were exposed via nose-only inhalation to magnesium stearate at pulmonary deposited doses up to 180  $\mu$ g/kg/day for 26 weeks (GSK Report WD2006/03154; (b) (4) 724353). No treatment-related effects were observed at any dose levels. The NOAEL for inhaled magnesium stearate was 180- $\mu$ g/kg/day in rats.

The expected exposure of patients to magnesium stearate from Breo Ellipta was (b) (4) (b) (4) (or 125  $\mu$ g/day/patient). The rat data provided a safety margin of 90 for the proposed clinical doses. Such a safety margin is considered adequate coverage for proposed use of magnesium stearate.

In summary, the applicant has provided nonclinical data to support the safety of the excipients of the application. The product uses lactose and magnesium stearate as excipients (see Section 2.3 Formulation). There were no safety concerns about either compound as discussed above.

## 2.5 Comments on Impurities/Degradants of Concern

None of the impurities identified in the product had estimated exposure levels (b) (4). There is no safety concern about any of the impurities that may be present in the product.

## 2.6 Proposed Clinical Population and Dosing Regimen

Adults with COPD will use one oral inhalation (one actuation) of Breo Ellipta daily. Each actuation of Breo Ellipta delivers 92- $\mu$ g FF and 22- $\mu$ g VI from its mouth piece, although the dosage strength of the drug is 100- $\mu$ g FF and 25- $\mu$ g VI.

## 2.7 Regulatory Background

The Breo Ellipta NDA (204,275) used an NDA (#22-051) and 3 INDs (#70,297, 74,696 and 77,885) for its nonclinical support. NDA 22-051 (Veramyst Nasal Spray, approved on April 27, 2007) and IND 70,297 contained all nonclinical fluticasone furoate data that GSK resubmitted to the current NDA. Because fluticasone furoate monoproduct data has been previously submitted to and reviewed by the Agency, the following discussions focus on vilanterol (INDs 74,696 and 77855) only. Generally, IND 74,696 contained vilanterol monoproduct data while IND 77,855 contained vilanterol and fluticasone combination data. However, some data were submitted, reviewed, or discussed in both applications.

DPARP and GSK held numerous meetings and written communications to discuss the development of vilanterol alone or in combination with fluticasone in INDs 74,696 and 77,855. Table 3 lists the major interactions discussing the nonclinical issues in the relevant applications. NDA 22-051 is listed for completeness.

**Table 3: Key Regulatory Events in Relevant Applications**

<b>Application</b>	<b>API</b>	<b>Key regulatory events</b>	<b>Dates</b>
N022,051	Fluticasone (FF)	Approval of FF for rhinitis indication	4/27/2007
1074,696	Vilanterol (VI)	Pre-IND meeting	1/31/2007
		IND filing	11/8/2007
		Carcinogenicity study protocol, amendments, and related issues	7/18/2007 – 11/18/2009
1077,855	FF/ VI	Pre-IND meeting	3/19/2008
		IND filing	6/27/2008
		EOP2 meeting	3/31/2009
		Pre-NDA meeting	7/13/2011
		CMC Pre-NDA meeting	9/14/2011
N204,275	FF/VI	Filing of NDA	7/12/2012

Four key meetings were held for IND 77,855. They were the 19-APR-2008 pre-IND meeting, the 31-MAR-2009 EOP2 meeting, the 13-JUL-2011 Pre-NDA meeting, and the 14-SEP-2011 CMC Pre-NDA meeting. The pre-IND meeting discussed nonclinical requirements for opening an IND trial and requirements for juvenile animal studies in support of clinical pediatric trials. The EOP2 meeting discussed qualification of potentially genotoxic impurities (Ref.: Question 5). The Pre-NDA meeting concentrated on the interpretation and evaluation of the 2-year rodent carcinogenicity data. See Questions 22 – 24 (p13-14) of the minutes for additional information. Both Pre-NDA meetings and Pre-NDA CMC also discussed leachables/extractable issues. See Additional Nonclinical Comments in minutes of the 13-JUL-2011 meeting (p15) and Question 16 (p8) in the minutes of the 14-SEP-2011 meeting. Minutes of the meetings are available in DARRTS.

Two meetings were held for IND 74,696. They were the 31-JAN-2007 pre-IND meeting and the 10-23-2007 telecon as a follow-up to the Pre-IND meeting. Also, a number of written communications occurred between 2007 and 2009 discussing the design and conduct of the rodent carcinogenicity assays of vilanterol. The Pre-NDA meeting for IND 77,855 discussed the evaluation and interpretation of the results of 2-year carcinogenicity studies of vilanterol in mice and rats. See the nonclinical review of the carcinogenicity studies completed by Dr. Luqi Pei on February 14 in NDA 204-275 for these communications.

Fluticasone furoate is an approved and currently marketed drug. Veramyst Nasal Spray (NDA 22-051) indicated for rhinitis was approved on April 27, 2007. Fluticasone has two ester forms currently on the market. The other is fluticasone propionate currently marketed alone (e.g., 20-833) or in combination with other drugs (i.e., salmeterol – NDA21-077 or azelastine – NDA 202,236). The active ingredient of the current application is fluticasone furoate ester.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

Table 4 provides a list of pivotal nonclinical studies reviewed in this document.

**Table 4: Pivotal Nonclinical Studies Reviewed**

Report No.	Description	Location
<b><i>Reproductive toxicity</i></b>		
CD2007/00581	Fertility study of VI in male rats	4.2.3.5.1
CD2006/01165	Fertility study of VI in female rats	4.2.3.5.1
CD2006/01166	Teratology study of VI in pregnant rats	4.2.3.5.2
WD2006/02439	Teratology study of VI in pregnant rats	4.2.3.5.2
CD2010/00109	Peri- and post-natal developmental study of VI in rats	4.2.3.5.3
CD2007/00973	Teratology study of VI/FF in rats	4.2.3.5.3

#### 3.2 Studies Not Reviewed

General and genetic toxicity studies of vilanterol and most vilanterol pharmacology and pharmacokinetic studies, as well as fluticasone nonclinical studies, were previously reviewed by the Division and were not reviewed again here. See the nonclinical review completed by Dr. Larry Sancilio on September 30, 2010 in IND 77,855 for the combination studies, and reviews by Dr. Huiqing Hao on June 3, and October 29, 2008 and by Dr. Larry Sancilio on March 5, 2010 in IND 74,686 for the vilanterol studies. See the nonclinical review completed by Dr. Huiqing Hao on March 2, 2007 in NDA 22-051 (Veramyst, Nasal Spray) for lists of the fluticasone furoate studies.

Also, none of the nonclinical toxicity studies of vilanterol in combinations with cholinergic antagonists were reviewed. Specifically, GSK submitted toxicity studies of vilanterol in combination with two cholinergic antagonists: (b)(4) and GSK573719A (IND (b)(4)). These studies were not reviewed because they were not pivotal to the safety evaluation of the current NDA. Table 5 lists the studies of vilanterol with cholinergic antagonists.

**Table 5: Studies of Vilanterol in Combination with Anticholinergic Agents**

Report No.	Description	Location
FD2008/00365	Safety pharmacol.: CVS effects of VI and GSK573719A in dogs	4.2.1.
FD2008/00031	Safety pharmacol.: (b)(4)	
FD2009/00345	(b)(4)	4.2.3.2
FD2009/00345	13-wk IH study of VI and GSK573719A in dogs	4.2.3.2
WD2008/01317	4-wk IN study of VI and GSK1004723E in dogs	4.2.3.2
WD2004/00488	4-wk IH study of magnesium stearate in dogs	4.2.3.2
FD2009/00017	IH Segment II study of VI and GSK573719A in rabbits	4.2.3.5.2
CD2009/00970	SC and IH dose-ranging Segment II study of VI and GSK573719A in rabbits	4.2.3.5.2

Some studies of vilanterol and other chemicals were not reviewed either because they do not provide information pivotal to the safety evaluation of the current NDA. Table 6 lists these studies.

**Table 6: Non-clinical Studies of Vilanterol Not Reviewed**

Report No.	Description	Location
ED2007/00138	In vitro eye irritation test of IV in reconstituted human cornea tissue (treatment period: 4 – 24 hr)	4.2.3.6
ED2007/00139	In vitro eye irritation test of IV in reconstituted human cornea tissue (treatment: 60 minutes)	4.2.3.6
ED2008/00006	Ames test of GW844166X	4.2.3.7.2
ED2008/00006	Ames test of 2,6-dichlorobenzyl chloride	4.2.3.7.2
WD2006/00235	In vitro hemolytic potential of VI in rat and human blood	4.2.3.7.7

### 3.3 Previous Reviews Referenced

This review references a number of nonclinical reviews completed previously by DPARP staff in related IND and NDA applications. See Table 7 for a list of these reviews.

**Table 7: Previous Reviews Referenced**

Application No. *	Author	Review Content	Date of Completion
(b)(4)	L. Pei	26-wk IH toxicity study of Mg Stearate in rats	6/7/2000
I077,855	L. Sancilio	13-week toxicity study of FF/VI in rats and dogs	9/30/2010
I074,696	H. Hao	Genetic toxicity data & 13-wk IH studies of VI in mice & rats	6/3/2008
	H. Hao	Original IND review	10/29/2008
	L. Sancilio	26-week IH study in rats	3/5/2010
N022,051	H. Hao	Original NDA review	3/2/2007
N204-275	L. Pei	Review of carcinogenicity study reports	2/14/2013

\* Most of the reviews were written for multiple applications because these applications used vilanterol as the only or as one of the active ingredients. The table lists the review once only to keep the table concise.

## 4 Pharmacology

Vilanterol is a long-acting  $\beta_2$  adrenergic receptor agonist (LABA). The mechanism of action of vilanterol mimics formoterol and salmeterol, two currently marketed LABAs. Binding of LABAs to  $\beta_2$  adrenergic receptor results in subsequent increases in intracellular cAMP levels, relaxation of smooth muscles located in the airways, and decreases in airway resistance.

Nonclinical studies were completed to evaluate the primary, secondary, and safety pharmacology of vilanterol. Primary pharmacodynamics of vilanterol were studied in vitro and in vivo. The in vitro assays assessed potency of vilanterol and selectivity of the drug to receptor subtypes. The in vivo studies determined efficacy of vilanterol. Secondary pharmacodynamic studies evaluated the binding and potency of vilanterol on a broad panel of receptors, ion channels, and enzymes. Safety pharmacology studies assessed the potential effects on cardiovascular, respiratory, and CNS systems in rats, mice, and monkeys.

### 4.1 Primary Pharmacology

The primary pharmacodynamics of vilanterol have been studied in vitro and in vivo. The in vitro studies assessed the receptor binding kinetics and specificity of vilanterol to  $\beta_2$ -,  $\beta_1$ - or  $\beta_3$ -receptors. The in vivo studies assessed anti-bronchoconstrictor effects of vilanterol using whole body plethysmography in conscious guinea pigs.

Binding kinetics of vilanterol at recombinant human  $\beta_2$ -receptors expressed in Chinese Hamster Ovary cells were assessed in the presence and absence of 100- $\mu$ M Gpp(NH)p, anon-hydrolysable analogue of the nucleotide guanosine triphosphate in vitro (Report #2012N13541). Vilanterol showed high affinity to  $\beta_2$ -receptors (i.e.,  $pK_D = 9.4 - 10.8$ , Table 8). The dissociation of vilanterol-receptor complex was biphasic because of the presence of low and high affinity receptor populations. The  $t_{1/2}$  was approximately 3.0 and 47 minutes for the fast- and slow-dissociation phases. The high affinity receptors had a larger  $t_{1/2}$ . This data, however, may not be reflective of the clinical situation because the affinity of vilanterol to  $\beta_2$ -receptors from the membrane of human lung parenchyma ( $pK_D = 8.8 \pm 0.3$ ) was lower than that of the recombinant receptors.

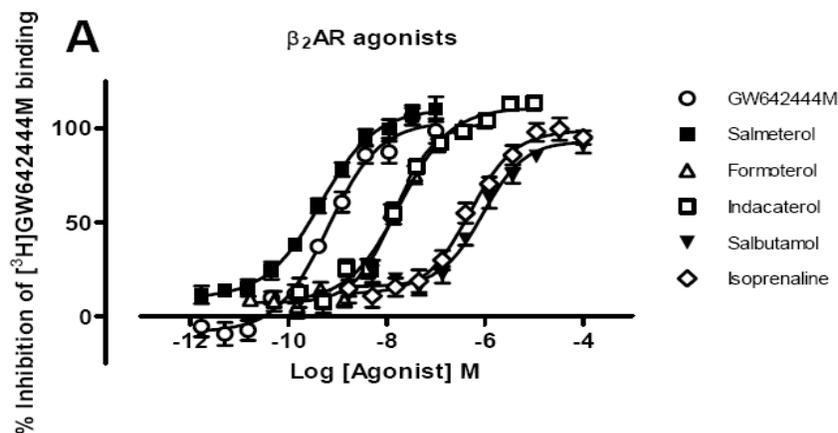
**Table 8: Binding Kinetics of Vilanterol at Recombinant Human  $\beta_2$ -Receptors**

Condition	$k_{on} M^{-1} \cdot min^{-1}$ , <sup>a</sup>	Fast $k_{on} M^{-1}$	Slow $k_{off} M^{-1}$	Low Affinity $pK_D$	High Affinity $pK_D$
+Gpp(NH)p (20°C)	$3.8 \pm 0.5 \times 10^8$	$0.20 \pm 0.02$	ND <sup>a</sup>	$9.44 \pm 0.07$	ND
-Gpp(NH)p (20°C)	$3.2 \pm 0.7 \times 10^8$	$0.27 \pm 0.03$	$0.015 \pm 0.001$	$9.47 \pm 0.17$	$10.8 \pm 0.12$
-Gpp(NH)p (37°C)	ND	$0.23 \pm 0.04$	ND	$9.52 \pm 0.24$	ND

a. ND, Not determined.

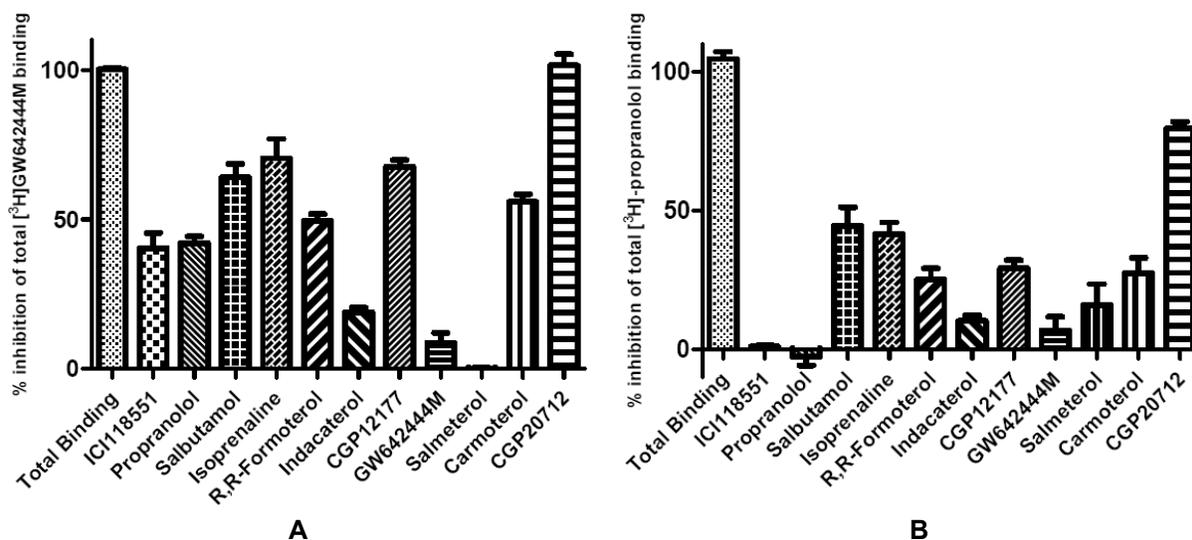
b.  $k_{on}$ , association rate;  $k_{off}$ , dissociation rate;  $pK_D$ , negative log 10 of  $K_D$  (dissociation constant).

Vilanterol is a competitive  $\beta_2$ -receptor agonist. The binding of vilanterol to  $\beta_2$ -receptors can be displaced (or inhibited) by the presence of other  $\beta$ -agonists and antagonists. Figure 1 presents the competitive displacement of vilanterol binding to the recombinant  $\beta_2$ -receptors by other agonists. Similar effects were observed with beta-antagonists (i.e., IC1118551, CGP12177, propranolol and sotalol).



**Figure 1: Competitive displacement bind curves for  $\beta$ -agonists against vilanterol (GW642444M).**

The ability of beta-agonists and antagonists to replace the binding of vilanterol to recombinant  $\beta_2$ -receptors differed with the compounds. Figure 2 shows the degree of inhibition of the binding of vilanterol to beta-receptors in the membrane of human lung parenchyma by some beta agonists.



**Figure 2: Competitive displacement of  $10\ \mu\text{M}$   $^3\text{H}$ -vilanterol (Panel A) or  $^3\text{H}$ -Propranolol (Panel B) by different compounds in human lung parenchyma membrane.**

Data were normalized to total binding at either  $10\text{-}\mu\text{M}$  salmeterol for  $^3\text{H}$ -vilanterol (GW642444M) or  $10\text{-}\mu\text{M}$  ICI118551 for  $^3\text{H}$ -propranolol. Data shown are the mean  $\pm$  SEM of at least 4 individual experiments carried out in quadruplicate. Among the compounds studied, salmeterol, formoterol, indacaterol, carmoterol and ICI118551 are  $\beta_2$ -receptor agonists. Isoprenaline is non-selective beta-agonist. CGP12177 is a partial non-selective beta-agonist. Salbutamol is short-acting  $\beta_2$ -receptor agonist while CGP20712 is  $\beta_1$ -receptor agonist.

Functionally, binding of vilanterol to  $\beta$ -receptors resulted in increases in cAMP levels in target cells. In vitro studies were completed to evaluate the potency of vilanterol on increasing cAMP levels in CHO cells expressing  $\beta$ -receptors. Results showed that vilanterol activated all three  $\beta$ -receptor subtypes (i.e.,  $\beta_2$ -,  $\beta_1$ - or  $\beta_3$ ), as indicated by increases in cAMP levels in both Discovery X membrane and TR-FRET LANCE™ assays (Table 9).

**Table 9: pEC50 of  $\beta$ -Adrenergic Agonists and Their Selectivity on  $\beta$ -Receptors**

Assay	Compound	pEC50 (Mean $\pm$ SEM) of cAMP Assay			Selectivity	
		$\beta_1$	$\beta_2$	$\beta_3$	$\beta_2/\beta_1$	$\beta_2/\beta_3$
Discovery X membrane <sup>a</sup>	Isoprenaline	8.84 $\pm$ 0.01	7.6 $\pm$ 0.04	8.24 $\pm$ 0.06	0.05	0.2
	Salmeterol	7.29 $\pm$ 0.02	9.6 $\pm$ 0.03	7.28 $\pm$ 0.02	215	219
	Formoterol	8.99 $\pm$ 0.02	9.1 $\pm$ 0.03	8.89 $\pm$ 0.01	1.1	1.4
	Vilanterol	7.30 $\pm$ 0.08	9.5 $\pm$ 0.15	7.52 $\pm$ 0.10	141	83
TR-FRET LANCE <sup>TM, b</sup>	Indacaterol	8.3 $\pm$ 0.04	9.5 $\pm$ 0.08	8.2 $\pm$ 0.03	16	20
	Salmeterol	6.3 $\pm$ 0.12	9.8 $\pm$ 0.1	6.5 $\pm$ 0.13	2,996	2,073
	Formoterol	8.0 $\pm$ 0.03	10.1 $\pm$ 0.08	8.4 $\pm$ 0.03	149	59
	Vilanterol	7.0 $\pm$ 0.03	10.4 $\pm$ 0.05	7.4 $\pm$ 0.03	2,425	1,027

a. Data was extracted from the nonclinical review completed by Dr. Huiqing Hao on October 29, 2008 (p10 – 13). The electivity was defined as EC50 ratios between different receptor subtypes.

b. Data were extracted from GSK Reference Report No. 2012N135141 (p32).

Differences in the pEC50s for these receptor subtypes indicated that vilanterol was more selective to  $\beta_2$ -receptors than  $\beta_1$ - or  $\beta_3$ -receptors. Specifically, the TR-FRET LANCE<sup>TM</sup> assay showed differences of more than 1000 fold in selectivity of vilanterol to the  $\beta_2$ -receptor over  $\beta_1$ - or  $\beta_3$ -receptors; however, vilanterol and salmeterol have the same selectivity on receptor subtypes in both assays. This observation may have labeling implications.

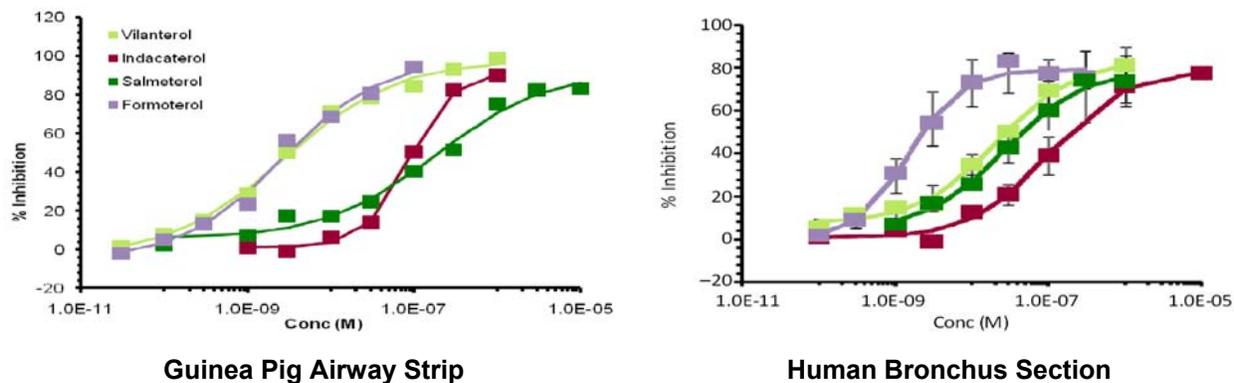
Additional functional assays were completed to study the efficacy of vilanterol on activity of  $\beta_2$ -receptors expressed in CHO cells or isolated frog melanophores. Table 10 summarizes the results of these assays.

**Table 10: Efficacy of Selective  $\beta$ -Agonists on  $\beta_2$  Receptor Activity**

	pEC50 + SEM <sup>a</sup>			
	Melanophore	FP cAMP	DX Membrane	DX Whole cell cAMP
Vilanterol	9.3 $\pm$ 0.08	9.4 $\pm$ 0.08	9.54 $\pm$ 0.15	9.4 $\pm$ 0.04
Isoprenaline	9.1 $\pm$ 0.01	7.3 $\pm$ 0.03	7.6 $\pm$ 0.04	7.2 $\pm$ 0.02
Salmeterol	8.8 $\pm$ 0.02	9.5 $\pm$ 0.04	9.6 $\pm$ 0.03	9.3 $\pm$ 0.04
Formoterol	9.4 $\pm$ 0.09	8.7 $\pm$ 0.03	9.1 $\pm$ 0.03	9.0 $\pm$ 0.03

a. N ranged 7 – 19, 26 – 517, 38 – 517, and 30 – 87 for vilanterol, isoprenaline, salmeterol and formoterol, respectively.

Vilanterol inhibited contractions of smooth muscles in airways of guinea pigs and humans in vitro (GSK Document # 2012N135141). Contractions of the smooth muscle strips of guinea pig airways were elicited by electromagnet field stimulation (EFS). Contractions of human bronchus were induced by 10- $\mu$ M methacholine. Vilanterol and other beta-agonists inhibited contractions of smooth muscles from human and guinea pig tissues in a dose-dependent manner (Figure 3). Other beta agonists included salmeterol, indacaterol and formoterol. Vilanterol mimicked formoterol in the guinea pig tissue and salmeterol in the human tissue. It is unclear at the present time whether these differences in dose-response curves were effects of the assay conditions or tissue origins, or both.



**Figure 3: Inhibition of EFS-contraction of guinea pig trachea by vilanterol and other  $\beta_2$ -agonists.**

Other reports also showed that vilanterol inhibited contractions of airway smooth muscles in vitro and in vivo. GSK Document# SH2003/00037 showed that vilanterol inhibited contraction of guinea pig airway strips induced by ESF and contraction of human bronchus rings induced by prostaglandin 2. GSK Document #SH2003/00042 showed that daily inhalation of vilanterol aerosols for 4 days attenuated histamine-induced bronchoconstriction in guinea pigs. See nonclinical review completed by Dr. Huiqing Hao on October 29, 2008 in IND 74,696 for additional information.

A study was performed to compare efficacy of the S-enantiomer (GSK907117) and metabolites of vilanterol on recombinant beta 1 and 2 receptors (Report HR2008/00016). The efficacy endpoints included  $PEC_{50}$ s and intrinsic activity. Results showed that these compounds were less active than vilanterol on both beta 1 and 2 receptors (Table 11).

**Table 11: Efficacy of Vilanterol and Metabolites on Human Beta Receptors**

	Beta <sub>2</sub>		Beta <sub>1</sub>	
	Mean $pEC_{50}$ (n) [SD]	Intrinsic Activity Mean % [SD%]	Mean $pEC_{50}$ (n) [SD]	Intrinsic Activity Mean % [SD%]
GW642444	10.4 (24) [0.2]	98 [3]	7.0 (22) [0.2]	88 [2]
GSK907117 (S-enantiomer)	8.6 (12) [0.2]	97 [4]	5.4 (9) [0.3]	65 [13]
GSK932009 [M33] (metabolite)	6.9 (11) [0.2]	102 [1]	5.0 (8) [0.4]	71 [32]
GW630200 [M29] (metabolite)	7.0 (12) [0.2]	99 [1]	5.7 (9) [0.2]	73 [8]
GSK1676112 [M20] (metabolite)	5.0 (8) [0.2]	69 [10]	5.3 (7) [0.2]	72 [20]
GW875428 [M40] (metabolite)	5.9 (7) [NR]	29 [NR]	<4.5 (10) [NR]	NR
GW853734 (potential metabolite)	5.4 (8) [0.4]	46 [9]	<4.5 (10) [NR]	NR
Salbutamol (comparator)	7.0 (28) [0.2]	94 [5]	5.9 (24) [0.1]	90 [7]

## 4.2 Secondary Pharmacology

Non-specific binding of vilanterol to a panel of receptors and ion transporters was assessed in vitro (GSK document# 2003/00036). The panel referred as a "receptogram" included

receptors and subtypes of adenosine, angiotensin, cannabanoid, dopamine, endothelin, GABA, NMDA, histamine, muscurine, tachykinin, serotonin and etc. Vilanterol was tested at a concentration of 1  $\mu$ M. Vilanterol showed significant inhibition ( $\downarrow >50\%$ ) of ligand binding at the following receptors:  $\beta_1$  ( $\downarrow 68\%$ ),  $\beta_2$  ( $\downarrow 98\%$ ), 5HT1A ( $\downarrow 57\%$ ), serotonin transporter ( $\downarrow 74\%$ ), and non-selective Sigma receptors ( $\downarrow 74\%$ ). Table 12 lists degree of inhibition of individual receptors screened in the assay.

**Table 12: Inhibition of 1- $\mu$ M Vilanterol to the Binding of Agonists to Their Receptors**

Species	Receptor/Transporter	Inhibition (%)	Species	Receptor/Transporter	Inhibition (%)
Human	Adenosine A1	-12	Rat	Glycine sensitive	0
Human	Adenosine A2A	6	G. pig	Histamine H1, central	17
Human	Adenosine A2B	18	G. pig	Histamine H2	3
Human	Adenosine A3	-2	Human	Leukotriene B4	-17
G. pig	Adenosine A transporter	-3	G. pig	Leukotriene D4	-9
Rat	$\alpha$ 1 adrenergic	7	Human	Muscarinic M1	8
Rat	$\alpha$ 2 adrenergic	37	Human	Muscarinic M2	10
Human	$\beta_1$ adrenergic	62	Human	Muscarinic M3	9
Human	$\beta_2$ adrenergic	98	Human	Muscarinic M4	11
Human	$\beta$ Norepinerdric transorper	14	Human	Muscarinic M5	22
Human	Angiotensin AT1	3	Human	Tachykinin NK1	13
Human	Angiotensin AT2	-4	Human	Tachykinin NK2	2
Human	Bradykinin B2	2	Human	Tachykinin NK3	1
Human	CGRP	0	Human	Neuropeptide Y1	3
Human	Cannabinoid CB1	0	Mouse	Neurotensin	2
Human	Cannabinoid CB2	-14	Rat	Nicotinic, central	6
Human	Dopamine D1	14	Rat	Opiate. Non-selective	1
Human	Dopamine D2L	16	Rabbit	PAF	5
Human	Dopamine D3	10	Rabbit	Purinergic P2X	2
Human	Dopamine D4	9	Rat	Purinergic P2Y	-6
Human	Dopamine D5	-1	Human	Serotonin 5-HT <sub>1A</sub>	57
Human	Dopamine transporter	30	Human	Serotonin 5-HT <sub>1B</sub>	13
Human	Endothelin ETA	10	Human	Serotonin 5-HT <sub>2A</sub>	1
Human	Endothelin ETB	3	Human	Serotonin 5-HT <sub>3</sub>	19
Rat	GABA transporter	7	G. pig	Serotonin 5-HT <sub>4</sub>	10
Rat	GABA A agonist site	0	Human	Serotonin 5-HT <sub>7</sub>	41
Rat	GABA B	-3	Human	Serotonin transporter	74
Rat	Glutemate, NMBA	-18	G. pig	Sigma, non-selective	74
Rat	Glutemate, NMBA, glycine	6	Rabbit	Throboxane A2	3
Rat	Glutemate, NMBA, glycine phencycline	3			

### 4.3 Safety Pharmacology

Safety pharmacology studies in vitro and in vivo evaluated the effects of vilanterol on the central nervous (CNS), cardiovascular (CVS), and respiratory systems (RS). In vitro studies evaluated the effect of vilanterol on hERG potassium channels in HEK923 cells and action potential in dog Purkinjie fibers (GSK Document# FD2003/00330 and FD2003/00323). Effects of vilanterol on CNS activity and body temperature after IV administration were studied in rats (GSK Documents VD2003/00131 and VD2005/00527). The effect on respiratory system after inhalation (IH) administration was studied in rats (GSK Documents CD2003/00833 and CD2005/01091). The effect on CVS after IV administration was studied

in rats and dogs (GSK Documents CD2003/00833 and CD2005/01091). Table 13 provides an overview of these studies. Briefly, vilanterol caused dose-dependent decreases in body temperature and decreases in locomotor activity in rats. It decreased in mean blood pressure and PR and QTc intervals, and increases in heart rates in dogs. Vilanterol inhibited hERG channel current in HEK923 cells ( $IC_{50} = 4.8 \mu M$ ). These effects were typical of beta-agonists. See Nonclinical review completed by Dr. Huiqing Hao on October 28, 2008 for additional information.

**Table 13: Safety Pharmacology Studies of Vilanterol**

Study Type	Species	#/ group	ROA	Dose ( $\mu g/kg$ )	Findings	Study #
CNS & temperature	Rat	8 M	IV	0, 25, 100, 400	↓ Body temp. (0.6 - 1.2°C) at $\geq 100 \mu g/kg$	VD2003/00131
CNS & temperature	Rat	8 M	IH	0, 36, 612, 34,399	↓ Body temp. (1.6°C) at HD	VD2005/00527
Respiratory effects	Rat	6 M	IH	0, 61, 241, 666	No significant findings	CD2003/00833
Respiratory effects	Rat	6 M	IH	0, 36, 718, 36.3	No significant findings	CD2005/01091
hERG assay	-	-	In vitro	0.15 – 14.9 $\mu g/mL$	$IC_{50} = 4.8 \mu M$ (2.3 $\mu g/mL$ )	FD2003/00330
Purkinjie Fibrer assay	-	-	In vitro	0 – 100 $\mu M$	↓ RMP, UA, & MRD @ $\geq 10 \mu M^a$	FD2003/00323
Cardiovascular effects	Dog	4 M	IV	0, 100, 300, 1000	↑ HR @ $\geq 0.3 mg/kg$	FD2003/00275
Cardiovascular effects	Dog	4 M	IV	0, 100, 300, 1000	↓ in PR, RR, QTc @ $\geq 0.3 mg/kg$	FD2005/00097

a. RMP = Resting membrane potential, UA = upstroke amplitude, MRD = maximum rate of depolarization, PR = PR interval, QTc = corrected Q-T interval.

## 5 Pharmacokinetics and Toxicokinetics

### 5.1 PK/ADME

Vilanterol was well-absorbed by the inhalation route of administration in rat, dog and human, but the absolute bioavailability of the drug after this route of administration is unknown in animals and 27.3% in humans, respectively. The oral bioavailability of vilanterol was variable among species. It ranged from 0.1% in mice, 0.9% in rats, <2% in humans, to 34% in dogs, respectively. Plasma drug concentrations were generally proportional to the inhalation dose in laboratory animals. Peak plasma drug levels were found at the end of inhalation exposure. Vilanterol is highly bound to plasma proteins (92-99%) in animals and humans. Vilanterol is metabolized by CYP 2B2 in rats and CYP 3A4 enzyme in dogs and humans. Hepatocytes metabolize the drug into a number of metabolites. These metabolites possessed little or significantly reduced  $\beta_1$ - and  $\beta_2$ -agonist activity. The elimination half-life was 5.3 and 9.8 hrs in rats and dogs, respectively. Table 13 summarizes the pharmacokinetic profile of vilanterol in animals.

**Absorption:** Vilanterol is readily absorbed after inhalation administration, but the bioavailability of vilanterol after inhalation administration is unknown in animals. Clinical data showed that the absolute bioavailability of inhaled vilanterol was 27.3% in humans. The oral bioavailability of vilanterol was variable, ranging from 0.1% in mice, 0.9% in rats, <2% in humans, and 33.7% in dogs, respectively (Table 14). Plasma drug levels generally increased with increasing inhalation doses in animals. The fraction of vilanterol binding to

proteins in plasma was 94.3%, 92.3%, 98.9%, 93.4%, 98.7% and 97.79% in mouse, rat, guinea pig, rabbit, dog, and human plasma (from proposed labeling), respectively.<sup>2</sup>

**Table 14: Pharmacokinetics Profiles of Vilanterol in Laboratory Animals**

ROA	Species	VI (µg/kg)	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>0-inf</sub> (ng.h/mL)	T <sub>1/2</sub> (hr)	CL (mL/h/kg)	F (%)	GSK Document #
PO	Mouse	1000	- <sup>a</sup>	1.2	5.1	-	80.1	0.07	WD2007/01613
	Rat (SD)	500	0.5	0.51	2.03	-	-	0.91	SH2009/00322
	Dog (M)	250	0.25	267	402	-	-	33.7	WD2009/00334
IH	Rat (SD)	470	-	-	380	8	-	-	SH2003/00040
	Dog (M)	135	1.12	13.3	52.0	-	-	-	WD2003/01081
IV	Rat (SD)	500	0.25	295	283	9.8	30.3	-	SH2009/00322
	Dog (M)	50	0.25	348	180	5.3	4.5	-	WD2009/00334

a. Data not available.

**Distribution:** Vilanterol is widely distributed throughout the body. Tissue vilanterol concentrations were determined at various times after intravenous injection in rats (Report FD2003/00261). Male rats (Lester Hooded-pigmented) were given a bolus injection of 350-µg/kg <sup>14</sup>C-vilanterol (b)(4) in saline over a period of 30 seconds. Tissue drug concentrations were measured at hours 0.25 and 6; and days 1, 3 and 10 post-dosing. Table 15 (next page) summarizes the results. Blood rich organs and secretory organs had highest vilanterol concentrations 15 minutes after the injection. These organs include kidneys, adrenal glands, pancreas, thyroid glands, salivary glands and gonads. At 24 hrs after dosing, the highest drug concentration was found in the choroid plexus, pituitary and preputial glands, and pancreas. At 3 days after dosing, little drug was present in most organs except for uveal tract/retina and the preputial glands. In fact, these two organs continued to show very high levels even 10 days after dosing. Specifically, the respective vilanterol concentrations in the uveal tract/retina and preputial gland was 0.866 and 0.422 µg/g tissue at 0.25 hr post dosing, and 0.312 and 0.251 µg/g on 10 days post dosing. The significance of these findings is unknown at the present time.

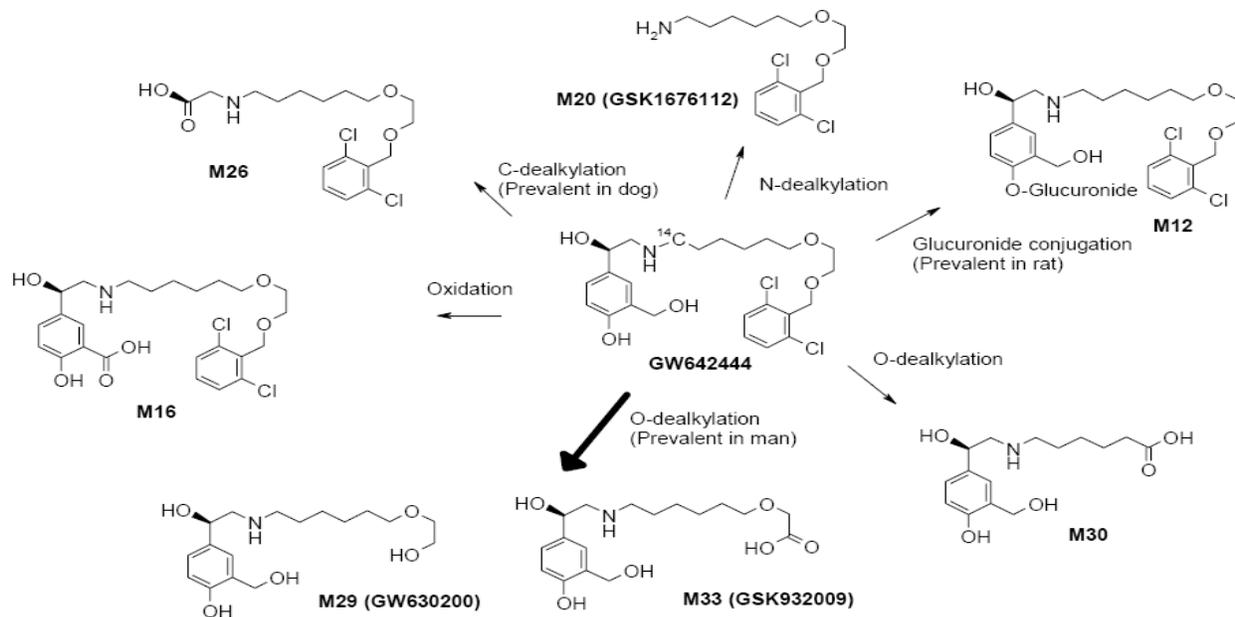
**Metabolism:** Vilanterol trifenate is first metabolized to the vilanterol base and trifenate ions. The base (also referred as vilanterol in this section) is further metabolized by the liver enzymes CYC2B2 in rats and CYP 3A4 enzymes in dog and humans, respectively, to other compounds. Vilanterol was metabolized by dealkylation, glucuronidation, and oxidation. Figure 4 (p 22) presents major metabolic pathways of vilanterol. There were at least 3-dealkylation pathways (O-, C-, and N-positions), but O-dealkylation was the predominant pathway in humans. O-dealkylation resulted in GW630200 and GSK932009. Both metabolites were present in animals. At least one animal species in pivotal toxicity studies had higher plasma concentrations of each metabolite than humans. Also, none of these metabolites possessed significant beta-agonist activity.

<sup>2</sup> Incubation of GW642444 with plasma at 37°C for 10 minutes followed by equilibrium dialysis for 8 hours with concentrations determined by HPLC-MS/MS (GSK Document# WD2006/02044).

**Table 15: Tissue Vilanterol Concentrations after Oral Administration in Rats**

		Concentration (mcg equivalents /g tissue). One animal per timepoint					
Sampling Times:		15 minutes	6 hours	1 day	3 days	10 days	35 days
Tissue Type	Tissue						
Vascular/ lymphatic	Blood	0.061	0.022	0.016	BLQ	BLQ	BLQ
	Aorta	0.340	0.073	0.021	BLQ	BLQ	BLQ
	Mandibular lymph nodes	0.351	0.156	0.027	BLQ	BLQ	BLQ
Metabolic/ excretory	Kidney cortex (inner)	1.62	0.190	0.102	0.029	BLQ	BLQ
	Kidney cortex (outer)	2.63	0.187	0.066	0.015	0.006	BLQ
	Kidney medulla	0.991	0.160	0.042	0.006	0.004	BLQ
	Liver	0.776	0.291	0.119	0.019	0.004	BLQ
CNS	Brain	0.006	0.005	BLQ	BLQ	BLQ	BLQ
	Choroid plexus	1.03	0.401	0.391	0.078	BLQ	BLQ
	Meninges	0.161	0.063	0.052	BLQ	0.035	BLQ
	Pineal body	0.710	0.194	0.020	BLQ	BLQ	BLQ
	Spinal cord	0.008	0.006	BLQ	BLQ	BLQ	BLQ
Endocrine	Adrenal cortex	1.56	0.188	0.048	0.016	BLQ	BLQ
	Adrenal medulla	1.47	0.215	0.048	BLQ	BLQ	BLQ
	Pituitary	0.578	0.522	0.270	0.063	BLQ	BLQ
	Thymus	0.136	0.089	0.042	BLQ	BLQ	BLQ
Secretory	Thyroid	0.970	0.072	0.016	BLQ	BLQ	BLQ
	Exorbital lachrymal gland	0.470	0.162	0.018	BLQ	BLQ	BLQ
	Harderian gland	0.220	0.311	0.089	BLQ	BLQ	BLQ
	Intra-orbital lachrymal gland	0.465	0.148	0.019	0.004	BLQ	BLQ
Fatty	Salivary glands	0.681	0.100	0.014	BLQ	BLQ	BLQ
	Brown fat	0.133	0.149	0.044	0.004	BLQ	BLQ
Gonads	White fat	0.009	0.021	0.010	BLQ	BLQ	BLQ
	Bulbo-urethral gland	0.894	0.046	0.026	BLQ	BLQ	BLQ
Muscular	Epididymis	0.069	0.048	0.038	0.004	BLQ	BLQ
	Preputial gland	0.422	0.483	0.442	0.284	0.251	BLQ
	Prostate	0.206	0.064	0.020	BLQ	BLQ	BLQ
	Seminal vesicles	0.090	0.139	0.035	BLQ	BLQ	BLQ
	Testis	0.017	0.012	0.015	0.008	0.007	0.007
Unclassified	Muscle	0.324	0.096	0.009	BLQ	BLQ	BLQ
	Myocardium	0.750	0.058	0.013	BLQ	BLQ	BLQ
	Tongue	0.506	0.213	0.021	BLQ	BLQ	BLQ
Gastrointestinal	Bone marrow	0.445	0.103	0.015	BLQ	BLQ	BLQ
	Lung	0.387	0.143	0.035	0.007	BLQ	BLQ
	Nasal mucosa	0.081	0.028	0.011	BLQ	BLQ	BLQ
	Non-pigmented skin	0.108	0.047	0.018	BLQ	BLQ	BLQ
	Pancreas	0.838	0.850	0.415	0.009	BLQ	BLQ
	Peridontal membrane	0.167	0.108	0.114	BLQ	BLQ	BLQ
	Pigmented skin	0.219	0.073	0.025	0.006	BLQ	BLQ
	Spleen	0.347	0.119	0.038	0.006	BLQ	BLQ
	Tooth pulp	0.463	0.090	0.016	BLQ	BLQ	BLQ
	Ileal tract/retina	0.866	0.808	0.680	0.424	0.312	0.342
	Stomach mucosa (fundus)	0.554	0.256	0.042	BLQ	BLQ	BLQ
	Stomach mucosa (non-fundic)	0.040	0.120	0.022	BLQ	BLQ	BLQ
	Small intestine mucosa	0.228	0.105	0.047	BLQ	BLQ	BLQ
Caecum mucosa	0.293	0.145*	0.055	0.020	BLQ	BLQ	
Large intestine mucosa	0.436	0.048	0.130*	BLQ	BLQ	BLQ	
Rectum mucosa	0.154	0.094	0.104*	BLQ	BLQ	BLQ	

Vilanterol metabolism was studied both in vitro and in vivo. The in vitro studies used primary hepatocytes or perfused livers while the in vivo studies used intact subjects.



**Figure 4: Major metabolic pathways of vilanterol.**

The thick arrow indicates the major pathways in man.

**Metabolism in vitro:** The in vitro studies used hepatocytes from several species including humans and perfused livers from rats. These studies identified a number of metabolites. Table 16 presents qualitative assessments of metabolite formation when vilanterol was incubated with hepatocytes from mouse, rat, rabbit, dog, and human in vitro.

**Table 16: Qualitative Assessment of Vilanterol Metabolism by Hepatocytes in vitro<sup>a</sup>**

Species (conc of $^{14}\text{C}$ -GW642444 [mcM])	Metabolite <sup>a</sup> - presence (Y) or absence (N)																					
	M1	M3	M4	M30	M7	M9	M12	M13	M16	M20	M26	M28	M29	M31	M32	M33	M38	M39	M40	M42	M43	M44
Mouse (10)	Y	N	N	Y	N	Y	Y	N	N	Y	Y	N	Y	N	N	N	Y	Y	Y	N	N	N
(50)	Y	Y	N	Y	N	Y	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	Y	N	N	N
Rat (10)	Y	Y	Y <sup>c</sup>	N	Y	Y	N	N	Y	Y	N	Y	N	N	Y	Y	Y	Y	N	N	N	N
(50)	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	N	Y	N	Y	Y	Y	N	N	N	N	N
Rabbit (10)	N	N	N	Y	N	N	Y	N	Y	Y	Y	N	Y	N	N	Y	N	N	N	Y	N	N
(50)	N	N	N	Y	N	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	N	N	N	Y	Y	N
Dog (10)	N	N	N	Y	N	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	Y	N	Y	Y	N	N
(50)	N	N	N	Y	N	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	N	N	N	Y	Y	N
Human (10)	N	N	N	Y	N	Y	N	N	N	N	N	N	Y	N	N	Y	Y	Y	Y	N	N	Y
(50)	N	N	N	Y	N	Y	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	N	N
B <sup>b</sup>	N	N	N	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N	N	Y

a. Taken from Report 2012N137904 (p187). The incubation condition was 10- $\mu\text{M}$  vilanterol for 24 hours (10) or 50- $\mu\text{M}$  vilanterol for 4 hours (50). See Report WD2006/02574 of the submission for details.

Table 17 presents quantitative assessments of vilanterol by rat, dog, and human hepatocytes in vitro. Compounds M29 (GW630200) and M33 (GSK932009) were identified as the major metabolites in humans because each of them accounted for more than 10% of the total drug-related material. Each of the human metabolites was identified in at least one

animal species. Dogs were most similar to humans in terms of the kind and quantity of metabolites produced. Each quantifiable human metabolite was present in dogs.

**Table 17: Vilanterol Metabolism by Hepatocytes in vitro <sup>a</sup>**

Metabolite detected	% of Total metabolism			Metabolite detected	% of Total metabolism		
	Rat	Dog	Human		Rat	Dog	Human
M1	1.1	-	-	M26	See M14	26.3	2.9
M4	6	-	-	M29	3.3	8.5	24.1
M9	3.6	2.5	-	M30/31	-	1.5	6.3
M10	4.4	-	-	M32	-	6.5	2.9
M11/M13	6.2	-	BLQ	M33	4.3	7.9	11.5
M12	24.3	-	-	A	2.1	-	-
M14	4.3 <sup>b</sup>	-	-	B	-	2.3	-
M16	-	1.6	BLQ	C	-	3.6	-
M20	1.5	-	BLQ	D	-	-	BLQ
Total					61.2	60.7	47.7

a. Taken from Report 2012N137904 (p186). Hepatocytes were incubated in the presence of 10- $\mu$ M <sup>14</sup>C-vilanterol (b)(4) for 4 hours. See Report WD2003/01248 of the submission for details.

b. The sum of M14 and M26 values for the assay, as these two compounds could not be separated.

**Metabolism in vivo:** Plasma levels of major metabolites of vilanterol were monitored in animals and humans. Table 18 summarizes the plasma levels of vilanterol and its metabolites in animals and humans. Animal data represented steady state values at 26, 26, and 39 weeks in mice, rats, and dogs, respectively. Note that vilanterol doses in animals cited in these tables were the HD groups in the respective studies. These doses were used because lower dose groups showed no consistent metabolite results. Human data were from clinical trials using unlabelled or labeled vilanterol. Dogs showed significantly higher levels of vilanterol and its metabolites.

**Table 18: Mean Plasma AUCs and Percentage of Vilanterol and its Metabolites**

Vilanterol dose ( $\mu$ g/kg/day)	Mean plasma AUCs (ng.h/mL) <sup>a</sup>				Percentage (%) of Total <sup>b</sup>			
	Mice	Rat	Dog	Human	Mice	Rat	Dog	Human <sup>c</sup>
	2950	66	128	25 $\mu$ g/pt	2950	66	128	200 $\mu$ g/pt
Vilanterol	4853	70.2	319.5	0.266 <sup>d</sup>	11.2	33.5	60.6	17.7
GSK932009 (M33)	125.1	1.2	2.42	0.488 <sup>e</sup>	0.3	0.6	0.5	32.5
GW630200 (M29)	4.9	-	16.8		0.0	-	3.2	
GI179710	38350	138	188.5		88.5	65.9	35.8	
Total	43333	209.4	527.2		100.0	100.0	100.0	100.0

a. Mean plasma levels from inhalation toxicity studies in males and females. Vilanterol doses were the pulmonary deposited dose in each species. The AUC values were calculated based on results in Reports 2011/119325 (wk 26), 2010N209253 (wk 26), and CD2007/01006 (wk 39) in mice, rats, and dogs, respectively.

b. Derived from the AUC values in the Table.

c. From Report 2012N137904 (p184). Healthy subjects were given orally 200- $\mu$ g <sup>14</sup>C-Vilanterol. Radioactivity in plasma, urine, and feces was monitored for 24 hours post administration. A total of 64% of administered radioactivity was found in plasma at 0.5 hrs. GSK632009 and GSK630200 accounted for 32.5% of the total plasma radioactivity.

d. From Section 2.5 Clinical Overview (p18).

e. Calculated using the vilanterol level of 0.266  $\mu$ g/mL and the ratio between the levels of vilanterol and the sum of GW630200 and GSK932009 derived from the far right column of the table.

Cytochrome P450 enzyme CYP2B2 appeared primarily responsible for vilanterol metabolism in rats, as it was the only the inducible isoenzyme in females of this species.

Report WD2004/01331 assessed the mRNA levels in hepatocytes in a 28-day toxicity study in rats (Report CD2003/01111). Table 19 presents the summary results. Only changes in CYP2B2 in females were considered noticeable.

**Table 19: Changes in mRNA levels in Hepatocytes from Rats treated with Vilanterol**

Estimated Achieved Dose CYP	mRNA level (fold-increase)							
	0 mcg/kg/day		45.1 mcg/kg/day		261.1 mcg/kg/day		708.7 mcg/kg/day	
	Male	Female	Male	Female	Male	Female	Male	Female
CYP1A1 <sup>a</sup>	1.00 ± 0.39	1.00 ± 0.66	0.79 ± 0.21	4.50 ± 2.92	0.56 ± 0.28	1.89 ± 0.58	0.40 ± 0.03	3.39 ± 0.54
CYP1A2 <sup>b</sup>	1.00 ± 0.21	1.00 ± 0.12	1.09 ± 0.20	1.35 ± 0.39	1.15 ± 0.20	0.97 ± 0.21	0.99 ± 0.17	0.99 ± 0.28
CYP2B1 <sup>a</sup>	1.00 ± 0.54	1.00 ± 0.08	1.04 ± 0.44	1.76 ± 0.20	3.07 ± 1.96	2.41 ± 1.83	1.32 ± 0.27	2.28 ± 1.01
CYP2B2 <sup>a</sup>	1.00 ± 0.31	1.00 ± 0.72	0.95 ± 0.42	7.17 ± 0.53	0.90 ± 0.53	5.69 ± 5.22	0.50 ± 0.09	6.13 ± 1.30
CYP2E1 <sup>b</sup>	1.00 ± 0.10	1.00 ± 0.29	1.41 ± 0.18	1.13 ± 0.07	1.16 ± 0.35	0.92 ± 0.11	1.17 ± 0.09	0.86 ± 0.07
CYP3A2 <sup>b</sup>	1.00 ± 0.46	1.00 ± 0.31	0.99 ± 0.91	0.43 ± 0.08	1.15 ± 0.36	0.17 ± 0.03	1.14 ± 0.23	0.23 ± 0.03
CYP3A23 <sup>b</sup>	1.00 ± 0.32	1.00 ± 0.15	0.80 ± 0.03	1.11 ± 0.22	0.76 ± 0.17	1.10 ± 0.26	0.56 ± 0.21	1.32 ± 0.78
CYP4A1 <sup>b</sup>	1.00 ± 0.05	1.00 ± 0.08	0.95 ± 0.23	1.42 ± 0.30	1.21 ± 0.85	0.97 ± 0.14	0.52 ± 0.11	0.95 ± 0.05

a = Increase in mRNA level of  $\geq 5$ -fold is considered notable.

b = Increase in mRNA level of  $\geq 2$ -fold is considered notable.

**Elimination:** Vilanterol was excreted primarily through feces, although a considerable amount was excreted in the urine. Studies were completed to evaluate the route of excretion after oral and intravenous administration of vilanterol in rats (Report WD2002/01882) and dogs (Report FD2003/00324). The feces were the major route of excretion in animals and humans (Table 20). After intravenous administration, the respective vilanterol-related radioactivity recovered was in the urine and feces at 18.3% and 69.5% in rats and 38.8% and 47.9% in dogs. Higher amounts of radioactivity were found in feces after oral administration of vilanterol in both rats and dogs. Approximately 8.8% of intravenously administered vilanterol was excreted into bile (Report WD2006/02955). The elimination half live was 5.3 and 9.8 hrs in rats and dogs, respectively.

**Table 20: Excretion of Vilanterol in Animals and Humans**

Species/dose	Time (Hr)	Percentage (%) of administered vilanterol dose recovered <sup>a</sup>							
		IV				Oral			
		Urine	Feces	Cage <sup>b</sup>	Total	Urine	Feces	Cage <sup>b</sup>	Total
Rat 350 mg/kg	0 - 24	17.1	55.4	3.0		4.4	81.4	0.8	
	24 - 48	1.2	11.8	0.4		0.2	4.2	0.1	
	0 - 96	18.3	69.5	3.5	93.3	4.7	86.1	0.9	91.8
Dog 35 mg/kg	0 - 24	36.1	34.0	0.4		19.8	50.7	0.6	
	24 - 48	2.0	9.6	0.2		1.4	3.4	0.1	
	0 - 96	38.8	47.9	1.1	88.8	21.7	56.1	1.0	78.7
Human <sup>c</sup> 200 µg/subj.	0 - 120	-	-	-	-	21.2	50.4	-	71.6

a. Extracted from Report 2012N137904 (p202-204). The test material was GW642444H <sup>(b) (4)</sup>

b. Results of Cage Wash A. The cage was washed twice in each study. These washes were named wash A and B, respectively. The most overwhelming amount of activity was found in Wash A.

c. Extracted from Report 2011N115614 (p13).

There is no clear evidence that vilanterol is secreted into milk in animals. No vilanterol was detected in the plasma of pups when their mothers received up to 10,000 µg/kg/day

vilanterol orally during gestation and lactation periods (Report# CD2010/00109). Vilanterol concentrations were measured on postnatal day 10; however, pups at the 10,000- $\mu\text{g}/\text{kg}/\text{day}$  groups showed decreases in mean body weights.

GI179710 was eliminated in the rat and dog both by metabolism and biliary secretion of unchanged drug. Acyl glucuronidation was the only major route of metabolism in human hepatocytes and in all nonclinical species investigated (mouse, rat, dog, rabbit hepatocytes and in vivo in rat and dog). GI179710 was present at high levels in pivotal toxicity studies, including chronic toxicity, carcinogenicity studies, and reproductive and developmental toxicity studies.

## 5.2 Toxicokinetics

Vilanterol did not accumulate in vivo after repeat dosing in animals. There were no significant differences in AUCs between the first dose exposure and steady-state values. Table 21 presents steady-state AUCs of vilanterol and its major metabolites in animals. These values reflected AUC values in weeks 26, 26, and 39 in mice, rats, and dogs, respectively. The values in mice and rats were taken from 2-yr inhalation carcinogenicity studies (Reports 2011N119325 and Report 2010N109253) and the dog value from a 39-week inhalation study (Report WD2007/01006). These studies were chosen because they were ones with the longest treatment duration and may be necessary for the labeling review. For AUC values in general toxicity studies in mice and rats and subchronic toxicity studies in dogs, please see nonclinical reviews completed by Dr. Huiqing Hao on June 3 and October 29, 2008 in IND 74,696 or Table B3 of Report 2012N140307 (p92 - 94) of the original submission.

**Table 21: Steady State Plasma AUCs of Vilanterol and Metabolites in Animals**

Vilanterol <sup>a</sup> ( $\mu\text{g}/\text{kg}/\text{day}$ )	Plasma AUC (ng.h/mL) <sup>b</sup>										
	Vilanterol			GW932009			GI179710			GW632200	
	Mouse	Rat	Dog	Mouse	Rat	Dog	Mouse	Rat	Dog	Mouse	Dog
2.4			7.73								
6.4	13.6										
10.5		0.63									
15.6			36.9			0.83					
62	49.2										
84.4		12.0									
128			319.5			16.8			188.5		2.4
223		19.1			0.46			44.2			
615	141			1.9			422				
657		70.2			0.9			138			
6150	815			20.4			4459			1.20	
29,500	2,329			125			38350			4.9	

a. Vilanterol doses were pulmonary deposited doses in dogs and the achieved doses in mice and rats.

b. These values are the average of males and females at Weeks 26, 26, and 39 in mice, rats, and dogs, respectively. See review of the 2-yr carcinogenicity studies in mice (Report 2011N119325) and rats (Report 2010N109253), and the 39-week inhalation study in dogs (Report WD2007/01006), respectively, for group means in each sex.

## 6 General Toxicology

General toxicity studies of vilanterol, alone or in combination with fluticasone furoate, have been reviewed previously by DPARP staff. See nonclinical reviews completed by Dr.

Huiqing Hao on June 3 and October 29, 2008; and by Dr. Lawrence Sancilio on March 5 and September 30, 2010.

## 7 Genetic Toxicology

Genetic toxicity studies were completed to evaluate the genotoxicity profile of vilanterol and GI179710. Vilanterol tested negative in the following assays: the Ames assay, in vivo rat bone marrow micronucleus assay, in vitro UDS assay, and SHE cell assay. Vilanterol tested equivocal in the mouse lymphoma assay. GI179710 tested negative in the following assays: the Ames test and in the L5178Y mammalian cell gene mutation assay. See nonclinical reviews completed by Dr. Huiqing Hao on June 3 and October 29, 2008 in IND 74,696 for results and evaluation of these assays.

## 8 Carcinogenicity

The carcinogenic potential of vilanterol was evaluated by the inhalation route of administration in two traditional 2-year bioassays in rats and mice (one each). Vilanterol tested positive in both rats and mice for its carcinogenicity. The rat showed a shortened latency of pituitary neoplasms and increases in tumor-related mortalities in both sexes and leiomyomas in mesovarian ligaments in females. In mice, females showed increases in the incidence of tubulostromal carcinomas in the ovaries. The findings in these studies were typical of beta agonists in rodents. See the nonclinical review completed by Dr. Luqi Pei on February 14, 2013 in this NDA for details.

## 9 Reproductive and Developmental Toxicology

A battery of studies was completed to evaluate the reproductive and developmental toxicity of vilanterol alone or in combination with fluticasone furoate in rats and rabbits. These studies evaluated the effects of vilanterol on male and female fertility in rats (Reports CD2007/00581 and CD2006/01165), the teratogenicity of vilanterol in rats and rabbits (Reports CD2006/01166, WD2007/02439 and CD2006/02047), the teratogenicity of vilanterol and fluticasone in combination in rabbits (CD2007/00973), and peri- and post-natal development of vilanterol in rats (Report CD2010/00109). Results showed that vilanterol caused dose-dependent statistically non-significant increases in the incidence of cleft palate and opened/partially opened eyelids, and statistically significant increases in the incident of skeletal variations at high doses in rabbit fetuses. The drug caused dose-dependent, statistically significant decreases in fetal weights at high doses in rats. It had no effects on fertility in rats.

### 9.1 Fertility and Early Developmental Toxicity

#### 9.1.1 Male Fertility

##### Study Title: GW642444: Inhalation Male Fertility Study in Rats

Study number: 901125 (b) (4) GSK Report #CD2007/00581;  
GSK Reference #G06352

Study report location: eCTD 4.3.2.5.1

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 14, 2006

Study termination date: January 19, 2007

Report date: August 31, 2007

GLP compliance: Yes, with a signed statement

QA statement: Yes, with a signed statement

Drug lot # & purity: Batch R251081, Lot 061126650, purity 99.3%

### Key Study Findings

- Male rats dosed by nose-only inhalation with up to 3,150- $\mu$ g/kg/day vilanterol (pulmonary deposited doses) for 2 weeks did not show any treatment-related effects on fertility.
- These rats dosed at 82 and 3,150- $\mu$ g/kg/day vilanterol (pulmonary deposited doses) for 8 weeks, however, showed statistically significant decreases ( $p < 0.05$ ) in organ weights of male reproductive system: epididymis, prostate, and seminal vesicle.

### Study design:

Vilanterol-treated males were allowed to mate with untreated-females. Males (25/dose) were evaluated by their mating behaviors, fertility parameters, and necropsy of the reproductive organs. Females were evaluated by their uterine contents on day 20 post coitus (CT). Males were dosed with vilanterol by nose-only inhalation for 14 days before they were allowed to mate with females (1:1 ratio for mating). The mating duration was up to 7 days. Vilanterol treatment in the males continued during mating and for 5 weeks after mating. The males were sacrificed after the last treatment for necropsy. Males received pulmonary deposited vilanterol doses of 0.006, 0.082, or 3.15 mg/kg/day in LD, MD, and HD groups, respectively. The duration of exposure was 60 minutes/day. Table 22 presents the study design.

**Table 22: Design of the Male Fertility Study in Rats**

	Group			
	1	2	3	4
Vilanterol (mg/kg/day), Pulmonary <sup>a</sup>	0	0.006	0.082	3.15
Achieved	0	0.062	0.824	31.5
Target	0	0.05	0.73	32.9
Vilanterol chamber concentration ( $\mu$ g/L)	0	1.8	24	917
MMAD ( $\mu$ m) $\pm$ GSD		4.1 $\pm$ 2.0	3.3 $\pm$ 1.9	3.7 $\pm$ 1.8
Number of male rats	25	25	25	25

a. Obtained by multiplying the reported achieved dose by 0.1.

**Methods:**

- Doses:* Pulmonary deposited doses of 0, 0.006, 0.082, and 3.15 mg/kg/day and achieved doses of 0, 0.062, 0.824, and 31.5 mg/kg/day, respectively
- Frequency of dosing:* One episode of 60 minutes daily
- Dose volume:* Not applicable
- Species/strain:* Rats, Crl:CD (SD)
- Route of administration:* Inhalation (nose-only)
- Formulation/vehicle:* Vilanterol (4 and 40%) in lactose
- #/sex/group:* 25 males/dose
- Unique study design:* Treated males mated with untreated females.
- Age:* 12 weeks at the start of dosing
- Weight:* 363 - 475 g at the start of dosing
- Housing:* Individually caged, except for the mating period
- Treatment duration:* 14 days prior to mating, through mating, and 5 weeks after mating. The total duration of treatment was 54 - 57 days.
- Aerosol characterization:* Aerosol samples at the breathing zone were collected daily using a cascade impactor. Analyses of particle size distribution and vilanterol concentrations in the cascade impactor were done weekly.
- Protocol deviations:* A major deviation occurred during the study. Dose orders between the MD and HD groups were reversed during the period of days 17 – 21. This deviation, however, did not affect the overall interpretation of the study results because essentially all males had successfully mated before day 17.

**Rationale for dose selection:** Results of 14-day and 13-week inhalation toxicity studies in the same species (GSK Document# WD2005/00844 and WD2006/01716). Respective vilanterol doses (pulmonary deposits) were 0.005, 0.073, and 3.29 mg/kg/day in the 14-day study and 0.006, 0.066, 1.04, and 3.88 mg/kg/day in the 13-week study. Significant irritation to the respiratory tract was seen at  $\geq 0.073$  mg/kg/day in the males.

**Observations and Results:**

**Mortality:** Mortality was observed twice daily. No death occurred in any groups.

**Clinical Signs:** Clinical signs were observed twice daily. The MD and HD groups showed periodic fur staining and salivation post dosing period.

**Body Weight:** Body weights were measured daily. There were dose-dependent changes in mean body weight gains. Specifically, mean weight gains were increased prior to mating and decreased during and after mating. Table 23 summarizes the weight changes during the entire study. Figure 1 presents the time-course of mean body weight of the males during the study. Body weight gains were increased 44% - 145% in the pre-mating period (days 1 – 14), but decreased up to 41% during days 28-56. However, there was no difference in the final body weight among groups.

**Table 23: Body Weight Gain in the Males (Report 2007/0581)**

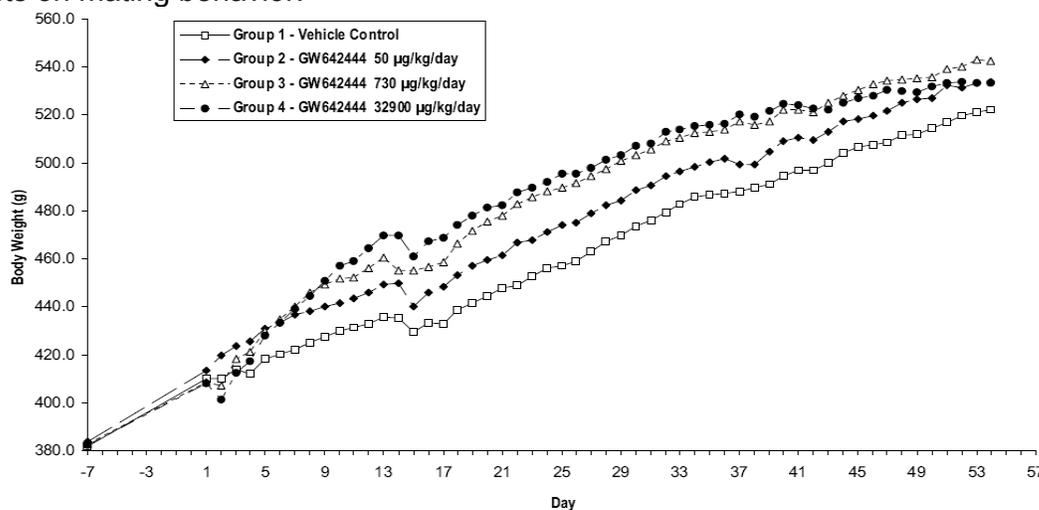
Parameter	Time (days)	Control	Fold (x) of control		
		(g)	LD	MD	HD
Body Weight gain	1 - 14	25.2	1.44*	1.86*	2.45*
Body Weight gain	14 - 28	32.3	1.00	1.30*	0.97
Body Weight gain	28 - 56	54.7	0.94	0.83	0.59*
Body weight	54	522.2	1.02	1.04	1.02

\*,  $p < 0.05$ .

**Food Consumption:** Food consumption was measured weekly. No treatment-related effects were observed. The vilanterol treatment groups showed statistically non-significant increases (1 - 3%) in food consumption.

**Male reproductive parameters:** No treatment-related effects were observed. There were no differences in mating index, fertility index and mating frequencies among groups.

**Mating:** Males and females (1:1 ratio) cohabited for a maximum period of 7 days. Successful mating was determined by the presence of sperm in vaginal smears (daily). Males were allowed to cohabit with another untreated female for 7 days if there was no evidence of mating in the initial mating period. No treatment-related effects were observed. Four females (No. 1514-C, 2509-LD, 2518-LD and 4525-HD) failed to show evidence of mating. They were replaced by Rats No. 1614, 2609, 2618 and 4625. Each of these replacement rats became pregnant during the first cohabitation period. The fact that each group of males had a mating index of 100% indicated that there were no treatment-related effects on mating behavior.



**Figure 5: Body Weight-time course in the female fertility study.**

Legends represent the reported target doses of vilanterol (GW642444). The pulmonary deposited dose was 0.006, 0.08, and 3.15 mg/k/day for Groups 2, 3 and 4, respectively.

**Fertility index:** No treatment related effects were observed. The fertility index was 96%, 100%, 96% and 96% in C, LD, MD and HD groups, respectively.

**Female reproductive parameters:** No treatment-related effects were observed. There were no differences among groups in following parameters: numbers of corpora lutea, implantation sites, live and dead fetuses, resorption, and pre-and post-implantation losses. There were also no differences in sex ratios or gravid uterine weights.

Note: The laboratory failed to examine the uterine contents of the females that failed to show evidence of mating. This did not affect the overall conclusion of the study because of the random distribution of the rats that failed to become pregnant.

**Organ weights:** Weights of the male reproductive organs (epididymis, prostate, and seminal vesicle) were measured at the necropsy. The MD and HD males showed statistically significant decreases (up to 18%,  $P < 0.05$ ) in the organ weights of epididymis, prostate, and seminal vesicle (Table 24). The decreases were apparent in both absolute and/or relative organ weight.

**Table 24: Mean Organ Weights in the Males (Report 2007/0581)**

Organ	Weight type	Mean Weight			
		Control (g)	Fold (x) of control		
			LD	MD	HD
Epididymides, left	Absolute	0.692	0.99	0.93*	0.89*
	Relative	0.135	0.97	0.90*	0.88*
Prostate, ventral	Absolute	0.765	0.91	0.82*	0.84*
	Relative	0.148	0.89	0.80*	0.83*
Seminal vesicle	Absolute	1.66	1.02	0.92	0.83
	Relative	0.322	1.00	0.89	0.81*

a. Relative to body weight.

\*,  $p < 0.05$ .

**Evaluation:** This study was adequate and acceptable. The study design, the duration of treatment, and vilanterol dose selection were acceptable. Significant drug effects were observed. These effects included the changes in mean body weight gains and the weights of male reproductive organs. The study showed that vilanterol did not affect male fertility at the highest tested dose (3.15 mg/kg/day); however, weights of several organs in the male reproductive system (i.e., epididymides, prostate and seminal vesicles) were decreased at vilanterol doses  $\geq 0.082$  mg/kg/day. Although there were no significant differences in mean body weight among groups at the end of the study, there were dose-related and statistically significant increases (44% - 145%) in body weights during the pre-mating period.

### 9.1.2 Female Fertility

#### Study Title: GW642444: Inhalation Female Fertility and Early Embryonic Development Study in Rats

Study number: 900087 (b) (4) GSK Report #CD2006/01165;  
GSK Reference #R26490

Study report location: eCTD 4.3.2.5.1

Conducting laboratory and (b) (4)

location: [REDACTED] (b) (4)

Date of study initiation: February 6, 2006

Study termination date: March 25, 2006

Report date: November 7, 2006

GLP compliance: Yes, with a signed statement

QA statement: Yes, with a signed statement

Drug lot # & purity: Batch R204768, Lot 051114265, purity 98%

## Key Study Findings

- Vilanterol did not affect female fertility at doses up to 3.71 mg/kg/day (pulmonary deposited dose) in rats.
- Vilanterol treatment at doses  $\geq 0.066$  mg/kg/day from 2 weeks prior to mating to gestation day (GD) 6 caused statistically significant increases ( $p < 0.05$ ) in mean body weights and body weight gains in dams. Increases in body weights were effects typical of beta agonists in rats.

## Study design:

Vilanterol-treated females were allowed to mate with untreated-males. Females (25/dose) were dosed with vilanterol for 14 days before mating, through mating, and until 6 days after mating. They were sacrificed on GD 20 for examination of uterine contents. Vilanterol was given by nose-only inhalation for 60 minutes/day. Females received pulmonary deposited doses of 0.005, 0.066, or 3.71 mg/kg/day vilanterol. Table 25 presents the study design.

**Table 25: Design of the Female Fertility Study in Rats**

	Group			
	1	2	3	4
Vilanterol (mg/kg/day), Pulmonary <sup>a</sup>	0	0.005	0.066	3.71
Achieved <sup>b</sup>	0	0.049	0.664	37.1
Target	0	0.05	0.73	32.9
Vilanterol chamber concentration ( $\mu\text{g/L}$ )	0	1.28	17.4	973
MMAD ( $\mu\text{m}$ ) $\pm$ GSD		4.2 $\pm$ 2.0	3.6 $\pm$ 1.9	3.3 $\pm$ 1.9
Number of male rats	25	25	25	25

a. Obtained by multiplying the reported achieved dose by 0.1.

b. The reported achieved dose (p28). These doses were derived using the following parameters: RMV of 0.171 – 0.179 L/min and weight weights of 0.267 – 0.282 kg. See the report for exact numbers used for each group.

## Methods:

Doses: Pulmonary deposited doses of 0, 0.005, 0.066, and 3.71 mg/kg/day; and achieved doses of 0.049, 0.664, and 37.1 mg/kg/day, respectively

Frequency of dosing: One episode of 60 minutes daily

Dose volume: Not applicable

- Species/strain:* Rats, Crl:CD (SD)  
*Route of administration:* Inhalation (nose-only)  
*Formulation/vehicle:* Vilanterol (4%-LD and MD groups and 40%-HD) in lactose  
*#/sex/group):* 25 females/dose  
*Unique study design:* Treated females mated with untreated males  
*Age:* 11 weeks at the start of dosing  
*Weight:* 227 - 271 g at the start of dosing  
*Housing:* Individually caged, except for the mating period  
*Treatment duration:* 14 days prior to mating, through mating and 6 days after PC.  
*Mating:* A female cohabitated with an untreated male during the 7-day mating period. The male was removed when successful mating occurred. A female which had failed to mate with the original male was allowed to cohabit with another untreated male for 7 days.  
*Aerosol characterization:* Aerosol samples at the breathing zone were collected daily using a cascade impactor. Analyses of particle size distribution and vilanterol concentrations in the cascade impactor were done weekly.  
*Protocol deviations:* No major deviations occurred during the study.

**Rationale for dose selection:** The target doses were the same target doses used in a 14-day study in male and female rats [GSK Document Number WD2005/00844/00]. All doses were associated with increased body weight gain, and respiratory tract irritancy was seen at the mid and high doses.<sup>3</sup> The report also argues that “the high dose was a maximum achievable dose in that study”.

### Observations and Results:

**Mortality:** Mortality was observed twice daily. No treatment-related effects were observed or no deaths occurred during the study.

**Clinical Signs:** Clinical signs were observed twice daily. The HD group showed periodic increased salivation during the first 10-day period.

**Body Weight:** Body weights were measured daily during the treatment period and GDs 7, 10, 14, 17, and 20 after treatment. The MD and HD groups showed significant increases in body weight gains (↑ 120 – 156%,  $p < 0.05$ , Table 26) prior to mating. Figure 6 presents the time-course of the mean body weight of females during the study.

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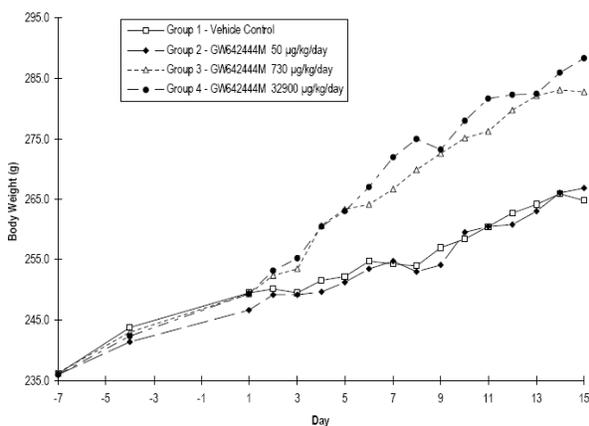
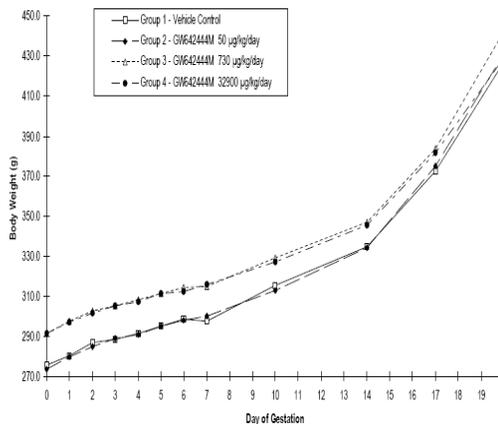
<sup>3</sup> The report also argues that “the high dose was a maximum achievable dose in that study”. Such an argument is flawed because the study has not used the maximum treatment duration of 3 hours that the Agency requires for inhalation toxicity studies. The treatment duration in the current study was 60 minutes.

**Table 26: Body Weight Gain in the Females (Report 2006/01166)**

Treatment period	Body weight gain			
	Control (g)	Change from control		
		LD	MD	HD
Pre-mating days 1 – 15	15.2	↑ 32.9%	↑ 119.7%*	↑ 155.9*
Gestation days 0 - 6	23.0	↑ 7.4%	0	↓ 9.1%

\*,  $p < 0.05$ .

**Food Consumption:** Food consumption was measured weekly. No treatment-related effects were observed. The vilanterol treatment groups showed statistically non-significant increases (1 - 3%) in food consumption.

**Pre-mating Period****Gestation period****Figure 6: Body Weight-time course in the female fertility study.**

Legends represent the reported target doses of vilanterol (GW642444). The pulmonary deposited dose was 0.006, 0.08, and 3.71 mg/k/day for Groups 2, 3, and 4, respectively.

**Female reproductive parameters:** No treatment-related effects were observed. There were no differences in mating index and fertility index and mating frequencies among groups.

**Estrus cycles:** Estrus cycles were determined by daily vaginal cytology during the period of pre-treatment day 14 to the mating day. No treatment-related effects were observed. The average cycle length was 3.9 – 4.2 days. The rats experienced 3.2 – 3.6 cycles during the pre-mating period.

**Mating:** Males and females (1:1 ratio) cohabited for a maximum period of 7 days. Successful mating was determined by the presence of sperm in vaginal smears (daily). No treatment-related effects were observed. The mating index was 100% in each group. The mean days to mate was 2.2 – 2.9 days.

**Fertility index:** No treatment related effects were observed. The fertility index was 92%, 100%, 96%, and 100% in C, LD, MD, and HD groups, respectively.

**Litter parameters:** No treatment-related effects were observed. There were no differences among groups in the following parameters: numbers of corpora lutea, implantation sites, live and dead fetuses, resorption, and pre-and post-implantation losses. There were also no differences in sex ratios or gravid uterine weights.

**Evaluation:** This study was adequately performed. The study design, the duration of the treatment, and vilanterol dose selection were acceptable. Significant drug effects on body weights were observed in the MD and HD groups. The result showed that vilanterol did not affect female fertility in rats.

## 9.2 Teratology Studies

### 9.2.1 Teratology Study of Vilanterol

#### 9.2.1.1 Teratology Study of Vilanterol in rats

##### Study Title: GW642444M: Inhalation Embryo-fetal Development Study in Rats

*Study number:* 900874 (b) (4) GSK Report #CD2006/01166;  
GSK Reference #R26491

*Study report location:* eCTD 4.3.2.5.1

*Conducting laboratory and location:* (b) (4)

*Date of study initiation:* February 6, 2006

*Study termination date:* March 6, 2006

*Report date:* May 8, 2006

*GLP compliance:* Yes, with a signed statement

*QA statement:* Yes, with a signed statement

*Drug lot # & purity:* Batch R204768, Lot 051114265, purity 98%

#### Key Study Findings

- Vilanterol by nose only inhalation exposure at pulmonary doses up to 3.37 mg/kg/day was not teratogenic in pregnant rats.
- Vilanterol at pulmonary doses of 3.37 mg/kg/day caused a statistically significant increase in the incidence of skeletal variations. The variations included unossified or incomplete ossification of 5<sup>th</sup> sternebra and xiphisternum. The percentage of fetuses affected was  $17.3 \pm 4.5\%$ ,  $22 \pm 4.8\%$ ,  $35.3 \pm 5.7\%$ , and  $39.2 \pm 6.3\%$  in the C, LD, MD, and HD groups, respectively.
- The MD and HD group dams showed statistically significant increases in body weight gains during the treatment period (22% and 30% in the MD and HD group, respectively).
- The NOAEL for both maternal and fetal effects was 5  $\mu\text{g}/\text{kg}/\text{day}$ .

#### Methods:

Pregnant female rats (18-22/dose) were dosed by nose-only inhalation with vilanterol during the period of gestation days (GD) 6 - 17. They were sacrificed on GD 21 for the examination of uterine contents. The duration of vilanterol exposure was 60 minutes/day. The pulmonary

deposited doses of vilanterol were 0.005, 0.066, or 3.71 mg/kg/day vilanterol. See Table 27 for study design.

**Table 27: Design of Teratology Study in Rats (Report 2006/01166)**

	Group			
	1	2	3	4
Vilanterol (mg/kg/day), Pulmonary <sup>a</sup>	0	0.005	0.061	3.37
Achieved <sup>b</sup>	0	0.045	0.613	33.7
Target	0	0.05	0.73	32.9
Vilanterol chamber concentration (µg/L)	0	1.21	16.4	905
MMAD (µm) ± GSD		4.9 ± 2.1	4.2 ± 2.0	3.4 ± 1.9
Number of mated female rats dosed	22	22	22	22
Number of pregnant females	18	22	20	22

a. Obtained by multiplying the reported achieved dose by 0.1.

b. The reported achieved dose (p27). These doses were derived using the following parameters: RMV of 0.191 – 0.196 L/min and weight weights of 0.304 – 0.316 kg. See the report for exact numbers used for each group.

## Methods:

- Doses:* Pulmonary deposited doses of 0, 0.005, 0.061, and 3.37 mg/kg/day; and achieved doses of 0.045, 0.613, and 33.7 mg/kg/day, respectively
- Frequency of dosing:* One episode of 60 minutes daily
- Dose volume:* Not applicable
- Species/strain:* Pregnant rats, Crl:CD (SD)
- Route of administration:* Inhalation (nose-only)
- Formulation/vehicle:* Vilanterol (4%-LD and MD groups and 40%-HD) in lactose
- #/sex/group:* 22 mated females/dose, 18 – 22 pregnant females
- Unique study design:* None
  - Age:* 11 weeks at the start of dosing
  - Weight:* 227 - 271 g at the start of dosing
  - Housing:* Individually caged
  - Treatment duration:* GD 6 - 17
- Aerosol characterization:* Aerosol samples at the breathing zone were collected daily using a cascade impactor. Analyses of particle size distribution and vilanterol concentrations in the cascade impactor were done weekly.
- Protocol deviations:* No major deviations occurred during the study.

**Rationale for dose selection:** The target doses were the same target doses used in a 14-day study in male and female rats [GSK Document Number WD2005/00844/00]. All doses were associated with increased body weight gain, and respiratory tract irritancy was seen at the mid and high doses.<sup>4</sup>

<sup>4</sup> The report also argues that “the high dose was a maximum achievable dose in that study”. Such an argument is flawed because the study has not used the maximum treatment duration of 3 hours that the

## Results and Observations:

**Mortality:** Mortality was observed twice daily. No treatment-related effects were observed. No deaths occurred during the study.

**Clinical signs:** Clinical signs were observed twice daily. The HD rats (up to 17/22) showed salivations after dosing.

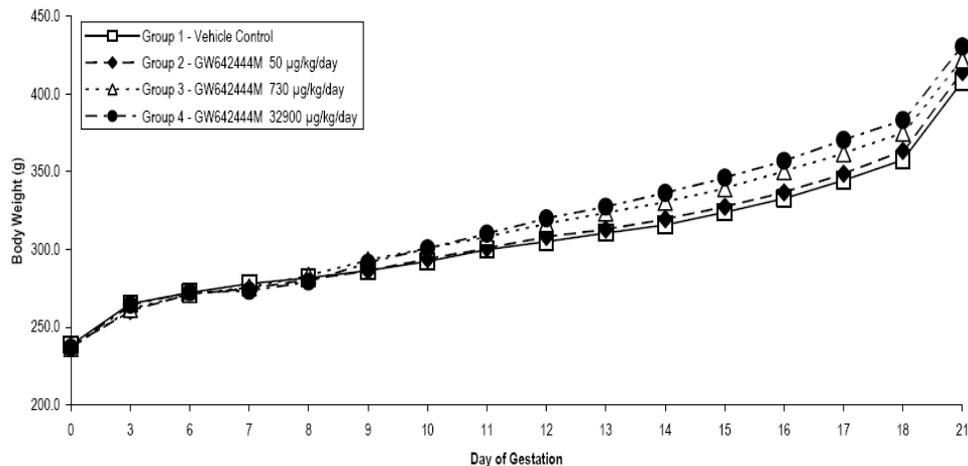
**Body weight:** Body weights were measured daily. The MD and HD group dams showed statistically significant increases in body weight gains (22% - 30%) during the treatment period (Table 28). Figure 7 shows the body weight-time course during the gestation period of the study.

**Table 28: Body Weight Gain in the Males (Report 2006/01166)**

	Body weight gain			
	Control (g)	LD	MD	HD
Body weight gain (GD 6 – 18)	85.3	↑8.0	↑ 21.7%*	↑29.9%*
Food consumption (GD6 – 18)	316	↑3.8%	↑ 5.1%	↑7.3%*

\*,  $p < 0.05$ .

**Food consumption:** Food consumption was measured every 3 days. The HD group dams showed statistically significant increases (↑ 7.3%,  $P < 0.05$ ) in mean food consumption during the treatment period.



**Figure 7: Mean body weights as a function of time in rat teratology study.**

Legends reflect the reported target doses of vilanterol (GW642444). The pulmonary deposited dose was 0.005, 0.061, and 3.37 mg/k/day for Groups 2, 3 and 4, respectively.

**Stability and homogeneity:** The testing formulation (dry powder in lactose) was stable in room temperature. The coefficient variation (CV%) of chamber drug concentration homogeneity ranged from 7.3 to 23.1%.

**Necropsy:** Necropsy was performed on GD 21. No treatment-related effects were observed.

Agency requires for inhalation toxicity studies. The treatment duration in the current study was 60 minutes.

**C-Section and litter observations:** No treatment-related effects were observed.

**Fetal Malformations:** No treatment-related effects were observed.

**Fetal Variations:** The MD and HD groups showed increases ( $P < 0.05$ ) in the incidence of unossified or incomplete ossification of 5<sup>th</sup> sternebra and xiphisternum. The percentage of fetuses affected was  $17.3 \pm 4.5\%$ ,  $22 \pm 4.8\%$ ,  $35.3 \pm 5.7\%$ , and  $39.2 \pm 6.3\%$  in the C, LD, MD, and HD groups, respectively.

**Evaluations:** This study was adequate in evaluating the teratogenicity of vilanterol in rats. Dosing selection of vilanterol and parameters of evaluations were acceptable. Pharmacologically active doses were used. No malformations were observed at any dose. Skeletal variations were observed at 60 and 3,370- $\mu\text{g}/\text{kg}/\text{day}$ . The NOAEL for both maternal and fetal effects was 5  $\mu\text{g}/\text{day}$ .

### 9.2.1.2 Teratology Study of Vilanterol in Rabbits

#### Study Title: GW642444M: Inhaled Embryo-fetal Development Study in Rabbits

*Study number:* L26578 (b) (4) GSK Report #WD2006/02349; GSK Reference# L26578 and BVR0876/062331

*Study report location:* eCTD 4.3.2.5.1

*Conducting laboratory and location:* (b) (4)

*Date of study initiation:* January 31, 2006

*Study termination date:* May 4, 2006

*Report date:* November 16, 2006

*GLP compliance:* Yes, with a signed statement

*QA statement:* Yes, with a signed statement

*Drug lot # & purity:* Batch R204768, purity 97.5%

#### Key Study Findings

- Vilanterol was not teratogenic at up to 0.574 mg/kg/day (pulmonary deposited dose) in pregnant rabbits.
- The rabbits were treated during gestation days 7 – 19 and sacrificed on GD 29.
- The MD and HD group (0.059 and 0.574 mg/kg/day, respectively) showed numeric increases in the incidence of malformations and variations, but none of the increases reached a statistically significant level ( $p = 0.05$ ).

#### Study Design:

Pregnant female rabbits (22/dose) were dosed with inhaled vilanterol during the period of gestation days (GD) 7 - 19. They were sacrificed on GD 29 for the examination of uterine contents. Vilanterol was given by snout-only inhalation for 60 minutes/day. Rabbits received pulmonary deposited doses of vilanterol of 0.006, 0.059, or 0.574 mg/kg/day (Table 29).

**Table 29: Design of Teratology Study in Rabbits (Report 2006/02349)**

	Group			
	1	2	3	4
Vilanterol, pulmonary dose (mg/kg/day) <sup>a</sup>	0	0.006	0.056	0.57
Achieved dose (mg/kg/day) <sup>b</sup>	0	0.063	0.591	5.74
Target dose (mg/kg/day)	0	0.075	0.64	5.4
Chamber concentration (µg/L)	0	2.71	25.5	248
MMAD (µm) ± GSD	-	1.7 ± 2.4	2.2 ± 2.5	2.7 ± 2.1
Number of mated female rats	22	22	22	22

a. Obtained by multiplying the reported achieved dose by 0.1.

b. The reported achieved dose (p68). These doses were derived using the following parameters: RMV of 0.191 – 0.196 L/min and weight weights of 3.83 – 3.95 kg. See the report for exact numbers used for each group.

### Methods:

*Doses:* Pulmonary deposited doses of 0, 0.006, 0.059, and 0.574 mg/kg/day; and achieved doses of 0, 0.063, 0.591, and 5.74 mg/kg/day, respectively

*Frequency of dosing:* One episode of 60 minutes daily

*Dose volume:* Not applicable

*Species/strain:* Pregnant rabbits, New Zealand white

*Route of administration:* Inhalation (nose-only)

*Formulation/vehicle:* Vilanterol (7%) in lactose

*#/sex/group:* 22 mated females/dose, 16 – 22 pregnant females on GD 29

*Unique study design:* None

*Age:* 21 - 26 weeks at the start of dosing (GD 7)

*Weight:* 3.24 – 4.50 kg at the start of dosing

*Housing:* Individually caged

*Treatment duration:* GD 7 - 19

*Aerosol characterization:* Aerosol samples at the breathing zone were collected daily using a cascade impactor. Analyses of particle size distribution and vilanterol concentrations in the cascade impactor were done weekly.

*Protocol deviations:* No major deviations occurred during the study.

**Rationale for dose selection:** The vilanterol doses were selected based on the results of a dose-ranging study (GSK Document Number WD2004/01583]. Pregnant rabbits (5/dose) were given by nose-only inhalation 0, 5.33, or 18.8-mg/kg/day vilanterol (achieved doses) during the period of GD 7- 19. The HD rabbits showed a 52% decrease in mean food consumption in the first 2 days of the treatment and a high rate of post-implantation loss (44.2% vs 22% in control). Also, both the LD and HD group had signs of abortion (one in each group).

## Results and Observations:

**Mortality:** Mortality was observed twice daily. No treatment-related effects were observed. Four rabbits in the treatment groups (incidence: 1-LD, 2-MD, and 1-HD) died or were euthanized during the study. These deaths were not considered treatment-related because of the lack of a dose-response relationship.

Three abortions (incidence: 2-MD and 1-HD) occurred. The abortions occurred on GDs 16 (#57-MD), 24 (#58-MD), and 28 (#73-HD). No fetal abnormalities were observed in any of the aborted fetuses. The abortions were considered unrelated to the treatment and were excluded from the fetal evaluation data.

**Clinical signs:** Clinical signs were observed twice daily. No treatment-related effects were observed.

Eight rabbits were found not pregnant. The number of non-pregnant rabbits was 1 (#13), 5 (#24, 27, 35, 37 and 39), 2 (#59 and 60), and 1 (#73) in the C, LD, MD, and HD groups, respectively. None of these rabbits showed any abnormalities in their ovaries upon necropsy examination.

**Body weight:** Dam's body weights were measured daily during the treatment (GD 7 – 19) and on GDs 1, 2, 4, 20, 25, and 29. No treatment-related effects on dam body weights were observed. There were no significant differences in mean body weights among groups on any days, especially during the treatment period (i.e., GD 7 – 19).

**Food consumption:** Food consumption was measured daily starting from GD 4. No significant, treatment-related effects were observed. The mean food consumption of the LD, MD, and HD groups during the period of GD 7 – 19 was decreased by 28%, 10%, and 11% in comparison to the vehicle control (110 g/rabbit/day). None of the decreases reached statistically significant levels ( $p < 0.05$ ). The changes in food consumption were not considered a treatment effect because of the lack of a dose-response relationship.

**Stability and homogeneity:** The testing formulation (dry powder in lactose) had a content uniformity of at least 93.8% during the study.

**Necropsy:** Necropsy of dams was performed on GD 29. No treatment-related effects were observed.

**C-Section and litter observations:** C-sections were done on GD 29. The respective numbers evaluated in the C, LD, MD, and HD group was 21, 16, 18, and 20 in the number of litters and 184, 143, 155, and 174 in the number of fetuses. Treatment-related effects were observed in the fetal weights, fetal malformations, and variations as discussed below. Also, none of the observations reached a statistically significant level of  $p = 0.05$ .

**Fetal body weights:** The HD group showed a decrease of approximately 10.5% in fetal weight (both group mean and individual fetal body weights). The LD and MD groups also showed decreases in body weights, but to a lesser degree. Table 30 summarizes the mean fetal weights among groups.

**Table 30: Fetal Body Weights in Rabbits (Report WD2006/02349)**

Vilanterol (mg/kg/day, pulmonary)	Absolute Body weight				Δ% (Relative to Control)		
	0	0.006	0.059	0.57	0.006	0.059	0.57
No. of litters evaluated	21	16	18	20	-	-	-
No. of fetus evaluated	184	143	155	174	-	-	-
Mean litter fetal body weight (g)	362.1	344.9	340.2	324.8	↓4.8	↓6.0	↓10.3
Mean individual fetal body (g)	42.0	40.0	40.4	37.6*	↓4.8	↓3.8	↓10.5

\*, P &lt; 0.05.

**Fetal Malformations:** The HD group showed statistically non-significant increases in the incidence of malformations: cleft palate (4.6%), open or partially open eyelids (6.3%), and limb flexure and mal-rotation (6.7%, Table 31). The malformations in the HD group, however, concentrated in one litter (Dam # 72). The LD group showed slight increases in some similar findings. The findings in the LD group were probably not treatment-related because of the low incidence and lack of a dose-response relationship.

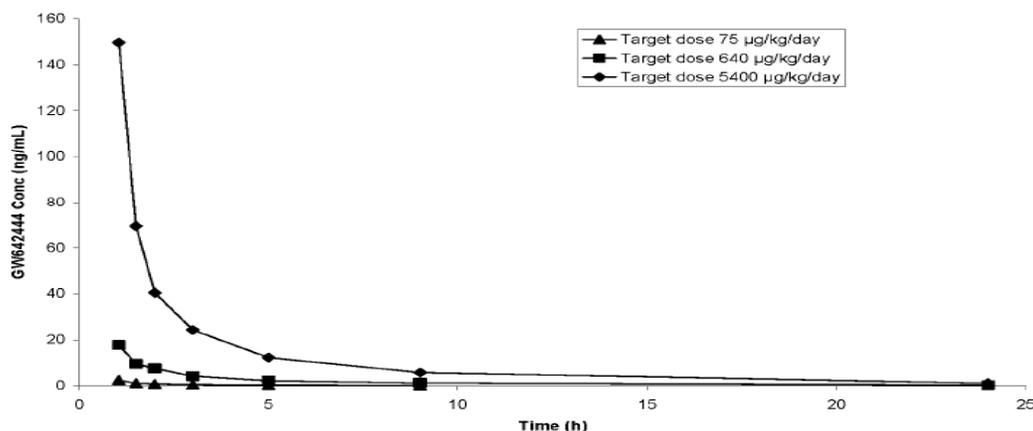
**Fetal Variations:** Statistically non-significant increases in skeletal variations were observed in the head bones, sternebra, and digits in all treated groups (Table 31). Head bone abnormalities included cranial malformation (misshapen/small frontals, partially fused/fused parietal to squamosal, misshapen zygomatic arch/mandibles, short lower jaw/snout, absent lower incisor sockets), misshapen interparietal bones, and elongated/fissured anterior fontanelle. Digit malformations (ectrodactyl/brachydactyly, absent digit/claw, fused metacarpals, metatarsals, misshapen clavicles and scapula) were observed in the HD group only.

**Table 31: Fetal Malformations and Variations in Rabbits (Report WD2006/02349)**

Vilanterol (mg/kg/day, pulmonary deposit)	0	0.006	0.059	0.57
No. of litters evaluated	21	16	18	20
<b>Malformations</b>	184	143	155	174
Cleft palate, # of fetuses (%)	0	1 (0.7)	0	8 (4.6)
# of litter (%)	0	1 (6.3)	0	1 (5.0)
Fore/hind limb flexure/mal-rotation, # fetuses (%)	2 (1.1)	2 (1.4)	2 (1.3)	10 (6.7)
# of litter (%)	2 (9.5)	1 (6.3)	2 (11.1)	3 (15.0)
Open/partially open eyelids, # of fetuses (%)	0	10 (7)	0	11 (6.3)
# of litter (%)	0	1 (6.3)	0	2 (10.0)
<b>Variations</b>				
Cranial bone malformation, # of fetuses (%) <sup>a</sup>	0	0	0	8 (4.6)
# of litter (%)	0	0	0	1 (5.0)
Bipartite/misshaped interparietal bone, # of fetuses (%)	0	1 (0.7)	0	7 (4.0)
# of litter (%)	0	1 (6.3)	0	3 (5.0)
Elongated/fissured anterior fontanelle, # of fetuses (%)	0	3 (2.1)	2 (1.3)	4 (2.3)
# of litter (%)	0	1 (6.3)	1 (5.6)	2 (10.0)
Bridge of ossification/fused sternebra center, # of fetuses (%)	5 (2.7)	2 (1.4)	1 (0.6)	13 (7.5)
# of litter (%)	5 (23.8)	2 (12.5)	1 (5.6)	6 (30)
Digit malformation, # of fetuses (%)	0	0	0	9 (5.2)
# of litter (%)	0	0	0	1 (5.0)

a. Misshapen/small frontals, partially fused/fused parietal to squamosal, misshapen zygomatic arch/mandibles, short lower jaw/snout, absent lower incisor sockets.

**Plasma drug concentrations:** Plasma concentrations of vilanterol and DI179710 (b)(4) were determined at 0, 0.5, 2, 4, 8, and 23 hours after completion of dosing on Day 5 of the treatment (Day 11 pc, 6 rabbits/group). Figure 8 presents the time course of mean plasma vilanterol concentration. Table 32 presents the summary results.



**Figure 8: Mean plasma vilanterol concentrations on GD 11 in Pregnant Rabbits.**

The vilanterol dose levels in the graph are the target achieved dose. See the review for the pulmonary deposited doses.

**Table 32: Plasma Vilanterol and GI179710 levels in Pregnant Rabbits (IH Study)**

Compound	Vilanterol			GI179710		
Vilanterol dose (mg/kg)	0.006	0.059	0.57	0.006	0.059	0.57
AUC <sub>0-t</sub> (ng.h/mL)	3.76	42.6	276	NC	147	1240
C <sub>max</sub> (ng/mL)	2.07	16.5	144	8.84	68.0	459

**Evaluations:** This study was adequate for evaluating the teratogenicity of vilanterol in rabbits. Dosing selection of vilanterol and parameters of evaluations were acceptable. Pharmacologically active doses were used. The HD (575 µg/kg/day) group showed numerical (although statistically non-significant) increases in the incidence of fetal malformations and variations. The malformations include cleft palate (4.6%), open or partially opened eyelids (6.3%), and limb flexure and mal-rotation (6.7%). The variations included cranial bone malformations (4.6%), bipartite/misshaped interparietal bone (4.0%), elongated/fused anterior fontanelle (2.3%), bridge of ossification/fused sternebral center (7.5%), and digit malformations (5.2%). The HD group also showed a statistically significant decrease in mean individual fetal body weight (↓ 10.5%, p < 0.05). The LD and MD groups showed slight and statistically non-significant increases (P < 0.05) in the incidence of elongated/fused anterior fontanelle (1.3% - 2.1%). The NOAEL of the study was the LD group (3.76 ng.h/mL) based on the low incidence of findings in the current study and the lack of similar findings at higher AUC (22.4 ng.h/mL) in the subcutaneous study in the same species (Document CD2010/00109, below).

**Study Title: GW642444M: Subcutaneous Embryo-fetal Development Study in Rabbits**

*Study number:* G06285 (GSK), GSK Report #CD2006/02047  
*Study report location:* eCTD 4.3.2.5.1  
*Conducting laboratory and location:* GSK US Research and Development, Safety Assessment, 709 Swedeland Rd. King of Prussia, PA 10406  
*Date of study initiation:* January 5, 2007  
*Study termination date:* March 6, 2007  
*Report date:* July 27, 2007  
*GLP compliance:* Yes, with a signed statement  
*QA statement:* Yes, with a signed statement  
*Drug lot # & purity:* Lot 61126655, purity 99.2%

**Key Study Findings**

- Vilanterol caused statistically significant increases in the fetal skeletal variations when pregnant rabbits were dosed with a subcutaneous dose of 300 µg/kg/day during the period of GDs 7 – 19.
- Variations included un-ossified cervical vertical centrum (1.8%), metacarpal forepaws (15.9%) and hind-paws (3.5%), and incompletely ossified hyoid skull (9.4%).
- No variations were found at ≤ 30 µg/kg/day.
- The AUC was 22.4 and 306 ng.h/mL in the 30 and 300 µg/kg/day dose groups, respectively.

**Methods:**

*Doses:* 0, 3, 7, 30, and 300 µg/kg/day  
*Frequency of dosing:* Once a day  
*Dose volume:* 2 mL/kg  
*Species/strain:* Pregnant rabbits, New Zealand white  
*Route of administration:* Oral gavage  
*Formulation/vehicle:* 0, 1.5, 3.5, 15, and 150 µg/mL in vehicle (20:80 PEG 400/8% 2-hydroxypropyl beta cyclodextrin (HPBCD))  
*#/sex/group:* 22 mated females/dose, 16 – 22 pregnant females on GD 29  
*Unique study design:* None  
*Age:* 26 weeks at the start of dosing (GD 7)  
*Weight:* 2.63 – 2.93 kg at the start of dosing  
*Housing:* Individually caged  
*Treatment duration:* GD 7 - 19  
*Aerosol characterization:* Aerosol samples at the breathing zone were collected daily using a cascade impactor. Analyses of particle size distribution and vilanterol concentrations in the cascade impactor were done weekly.  
*Protocol deviations:* No major deviations occurred during the study.

**Rationale for dose selection:** The vilanterol doses were selected based on the results of an inhalation segment-II toxicity study (GSK Document Number WD2006/02439) in which the HD group showed treatment-related but statistically non-significant increases in malformations and variations. The high doses of the current study (300 µg/kg/day) and the inhalation segment II study (570 µg/kg/day, pulmonary deposit) showed comparable systemic exposures of vilanterol (approximately 300 ng.h/mL).

## Results and Observations:

**Mortality:** No treatment-related effects were observed. No deaths occurred during the study. One 3-µg/kg/day dam (#H07F7197) was euthanized on GD 24 after the rabbit aborted. This rabbit consumed little food for 10 days prior to abortion. This pre-scheduled sacrificed was not considered treatment-related because of the lack of a dose-response relationship.

**Clinical signs:** Clinical signs were observed twice daily. No treatment-related effects were observed.

**Body weight:** Dam's body weights were measured daily during treatment (GD 7 – 19) and on GDs 1, 2, 4, 20, 25, and 29. Dose-related increases in body weight gains were observed (Table 33); only the increase (43%) in the HD group reached a statistically significant level ( $p < 0.05$ ).

**Table 33: Dam Body Weights & Food Consumption in Rabbits (PO Study)**

Vilanterol (µg/kg/day, pulmonary)	Change from Control				
	0	3	7	30	300
Dam body weight gain, GD 7 - 20	0.23 kg	↑ 13%	↑ 22%	↑ 30%	↑ 43%*
Dam food consumption, GD 7 - 20	121 g/day	↑ 1%	↑ 2	↓ 4%	↓ 13%*

\*,  $P < 0.05$ .

**Food consumption:** Food consumption was measured daily starting from GD 4. The HD group showed a statistically significant decrease (↓ 13%,  $p < 0.05$ ) in food consumption (Table 33).

**Stability and homogeneity:** The testing formulation (dry powder in lactose) had a content uniformity ( $\pm 10\%$ ) during the study.

**Necropsy:** Necropsy of dams was performed on GD 29. No treatment-related effects were observed.

**C-Section and litter observations:** C-sections were done on GD 29. The respective numbers evaluated in the C, LD, MD, and HD groups were 21, 16, 18, and 20 in the number of litters and 184, 143, 155, and 174 in the number of fetuses. Treatment-related effects were observed in the fetal weights, fetal malformations, and variations as discussed below.

**Fetal body weights:** No significant effects were observed. The HD group showed approximately a 5% decrease in mean fetal weight (Table 34). The decrease, however, did not reach statistical significance of  $p < 0.05$ .

**Table 34: Fetal Body Weights in Rabbits (Report CD2006/02047)**

	Mean fetal Weight (g)					Δ % from Control			
	0	3	7	30	300	3	7	30	300
Male	41.14	41.63	42.74	40.76	39.13	↑ 1.2	↑ 3.9	↓ 0.9	↓ 4.9
Female	41.11	41.12	41.40	39.79	38.66	0	↑ 0.7	↓ 3.2	6.0↓

\*, P < 0.05.

**Fetal Malformations:** One 300-μg/kg/day group fetus had open eyelids. This finding could be treatment-related because the same finding was reported in another study (CD2006/02349) at similar levels of plasma vilanterol levels. However, the findings did not reach statistical significance in either studies.

**Fetal Variations:** The HD group fetuses showed increases in the incidence of incomplete or lack of ossification of some bones, including the following: skull (9.4%), limbs (3.5 – 15.9%), and cervixes (1.8%). The incidence in the control groups was 0 – 1.9%. See Table 35 for incidences among groups.

**Table 35: Fetal Malformations and Variations in Rabbits**

Vilanterol (mg/kg/day, pulmonary deposit)	0	3	7	30	300
No. of litters evaluated	20	20	22	11	21
No. of fetus evaluated	163	166	169	185	172
External anomalies: Open eyelid: # fetus (# litter)	0	0	0	0	1 (1)
Skeletal anomalies:					
Cervical vertical centrum not ossified, % fetus (incidence)	0	0	0	0	1.8 * (4/171)
# litter affected	0	0	0	0	3
Forepaw: metacarpal – less than expected number ossified, % of fetus (incidence, litter affected)	1.9 (3, 2)	1.3 (2, 2)	5.7 (11, 6)	0	15.9 * (31, 9)
Hindpaw: talus not ossified % of fetus (incidence, litter affected)	0	0	0	0	3.5 * (7/171, 4)
Skull: incomplete ossified hyoid: % fetus (# fetus)	0.8 (1/86)	0	0.9 (1/92)	3.2 (3/98)	9.4 * (10/92)
# of litters affected	1	0	1	3	22

a. Misshapen/small frontals, partially fused/fused parietal to squamosal, misshapen zygomatic arch/mandibles, short lower jaw/snout, absent lower incisor sockets.

**Plasma drug concentrations:** Plasma concentrations of vilanterol and D1179710 <sup>(b)(4)</sup> were determined at 0, 0.5, 1, 2, 4, 8, and 23 hours after completion of dosing on Day 5 of treatment (Day 11 pc, 3 rabbits/time point). Table 36 presents the summary results.

**Table 36: Plasma Vilanterol and GI179710 levels in Pregnant Rabbits (PO study)**

Compound	Vilanterol				GI179710			
	3	7	30	300	3	7	30	300
Vilanterol dose (mg/kg)	3	7	30	300	3	7	30	300
AUC <sub>0-t</sub> (ng.h/mL)	1.37	4.06	22.4	306	NC	NC	18.4	300
C <sub>max</sub> (ng/mL)	0.53	0.95	6.26	59.1	NQ	NQ	12.4	103

**Evaluations:** This study was adequate in evaluating the teratogenicity of vilanterol in rabbits. Dosing selection of vilanterol and parameters of evaluations were acceptable. Pharmacologically active doses were used.

## 9.2.2 Teratology Study of Vilanterol and Fluticasone in Combination

### Study Title: GW685698X and GW642444M: Inhalation Embryo-fetal Development Study in Rats

*Study number:* 901290 (b) (4) GSK Report #CD2007/00973;  
GSK Reference #G07046

*Study report location:* eCTD 4.3.2.5.1

*Conducting laboratory and location:* (b) (4)

*Date of study initiation:* April 12, 2006

*Study termination date:* June 22, 2006

*Report date:* November 15, 2007

*GLP compliance:* Yes, with a signed statement

*QA statement:* Yes, with a signed statement

*Drug lot # & purity:* Batches R232326 (Lot 061120928) and R251082 (Lot 061126655) for fluticasone and vilanterol, respectively. The purity was 99.6% and 99.2% for fluticasone and vilanterol, respectively.

### Key Study Findings

- Pregnant rats were dosed by nose-only inhalation with various doses of vilanterol and fluticasone, alone or in combination, during gestation days 6 – 17. The pulmonary deposited doses ranged 0 – 9.5 µg/kg/day and 0 – 9.8 µg/kg/day for fluticasone and vilanterol, respectively. Specifically, the pulmonary deposited doses of fluticasone/vilanterol were 0/0, 8.2/0, 9.4/0.4, 9.5/9.8, 0/8.7, 0.8/0.8, and 3.0/3.2 µg/kg/day.
- No malformations were observed in any of the treatment groups.
- The 9.5/9.8 group showed a statistically significant increase ( $p < 0.05$ ) in the incidence of skeletal variations, including the following: unossified, incomplete ossification, and semi-bipartite/bipartite in the 5<sup>th</sup> sternebrae and xiphisternum.
- The study did not show any significant interactions in the effects of fluticasone and vilanterol on embryofetal development.

### Methods:

Pregnant rats (18-22/dose) were dosed by nose-only inhalation with different doses and ratios of vilanterol and fluticasone, alone or in combination, during the period of gestation days (GD) 6 - 17. They were sacrificed on GD 21 for the examination of uterine contents. The duration of exposure was 60 minutes/day. The pulmonary deposited doses of fluticasone/vilanterol were 0/0, 8.2/0, 9.4/0.4, 9.5/9.8, 0/8.7, 0.8/0.8, and 3.0/3.2 µg/kg/day

in Groups 1, 2, 3, 4, 5, 6, and 7, respectively. The FF/VI ratios ranged from 30:1 to 1:1. Table 37 presents the dosimetry of the study.

**Table 37: Dosimetry of Teratologic Interaction Study of Fluticasone/Vilanterol in Rats**

Group	Treatment	Formulation (FF/VI ratio)	Aerosol Concentration ( $\mu\text{g/L}$ , achieved) <sup>a</sup>		Dose ( $\mu\text{g/kg/day}$ )				
			FF <sup>b</sup>	VI	Achieved <sup>a</sup>		Pulmonary <sup>c</sup>		
					FF	VI	FF	VI	MgS
1	Lactose	-	0	0	0	0	0	0	3.3
2	FF	-	2.14	0	82	0	8.2	0	1.7
3	FF/VI	30:1	2.46	0.09	94.4	3.5	9.4	0.4	2.0
4	FF/VI	1:1	2.48	2.57	94.9	98.3	9.5	9.8	2.3
5	VI	-	0	2.33	0	86.9	0	8.7	1.9
6	FF/VI	1:1	0.21	0.22	7.9	8.3	0.8	0.8	1.9
7	FF/VI	1:1	0.78	0.83	29.5	31.7	3.0	3.2	2.6

a. FF, fluticasone furoate; VI, vilanterol; MgS, Magnesium stearate.

b. Extracted from page 35 for the study report.

c. Calculated by multiplying the achieved dose by 0.1.

*Doses:* Pulmonary deposited doses of fluticasone/vilanterol: 0/0, 8.2/0, 9.4/0.4, 9.5/9.8, 0/8.7, 0.8/0.8, and 3.0/3.2  $\mu\text{g/kg/day}$  in groups 1, 2, 3, 4, 5, 6, and 7, respectively

*Frequency of dosing:* One episode of 60 minutes daily

*Dose volume:* Not applicable

*Species/strain:* Pregnant rats, CrI:CD (SD)

*Route of administration:* Inhalation (nose-only)

*Formulation/vehicle:* Formulation: FF/VI in lactose containing 1.0% magnesium stearate. The FF/VI concentrations (in %) were 0/0, 3/0, 3/0.1, 3/3, 0/3, 0.3/0.3, and 1/1 in Groups 1, 2, 3, 4, 5, 6 and 7, respectively.

*#/sex/group:* 22 mated females/dose, 22/group mated females; 19 – 22 pregnant females

*# in Satellite group:* 9 pregnant females/group

*Unique study design:* None

*Age:* 10 - 11 weeks at the start of dosing

*Weight:* 233 - 317 g at the start of dosing

*Housing:* Individually caged

*Treatment duration:* GD 6 - 17

*Aerosol characterization:* Aerosol drug concentrations at the breathing zone were determined daily (Groups 2 and 6) or twice weekly (Groups 3 – 5 and 7). Analyses of particle size distribution and vilanterol concentrations in the cascade impactor were done weekly.

*Protocol deviations:* No major deviations occurred during the study.

**Rationale for dose selection:** Not given.

## Results and Observations:

**Aerosol Characterization:** The MMAD ranged 2.2 – 3.6  $\mu\text{m}$  and 2.8 – 3.6  $\mu\text{m}$  for fluticasone and vilanterol, respectively. Table 38 presents the MMADs and GSDs in each group.

**Table 38: Aerosol Particle Characteristics of Fluticasone/vilanterol Segment II Study in Rats**

Group	Treatment	FF/VI ( $\mu\text{g}/\text{kg}/\text{day}$ )	MMAD $\pm$ GSD		
			Fluticasone	Vilanterol	Mg Stearate
1	Vehicle	-	-	-	1.9 $\pm$ 2.8
2	FF	8.2/0	3.1 $\pm$ 2.2	-	2.7 $\pm$ 2.5
3	FF/VI	9.4/0.4	2.9 $\pm$ 2.5	2.9 $\pm$ 2.5	2.1 $\pm$ 2.2
4	FF/VI	9.5/9.8	3.6 $\pm$ 2.1	3.6 $\pm$ 2.1	2.4 $\pm$ 2.4
5	VI	0/8.7	-	3.3 $\pm$ 2.0	2.8 $\pm$ 2.3
6	FF/VI	0.8/0.8	3.0 $\pm$ 2.2	2.8 $\pm$ 2.4	2.2 $\pm$ 2.6
7	FF/VI	3.0/3.2	2.2 $\pm$ 1.7	2.9 $\pm$ 2.2	2.6 $\pm$ 2.4

**Mortality:** Mortality was observed twice daily. No treatment-related effects were observed. Two deaths occurred during the study, but the deaths were not considered treatment related. Deaths occurred in the vehicle group (Rabbit #1529) and Group 4 (Rabbit #4513, 9.5- $\mu\text{g}/\text{kg}/\text{day}$  fluticasone/9.8- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol) on treatment days 4 (Rabbit #1529) and 5 (Rabbit #4512). No specific cause of death was determined in each rabbit. The deaths were considered non-treatment related because of the low incidence.

**Clinical signs:** Clinical signs were observed twice daily. No treatment-related effects were observed.

**Body weight:** Body weights were measured daily. Dams receiving  $\geq$  3.0- $\mu\text{g}/\text{kg}/\text{day}$  fluticasone showed statistically significant decreases in body weight gains (43% - 72%) during the treatment period (Table 39). Figure 9 shows the body weight-time course during the gestation period of the study.

**Table 39: Findings in Teratologic Interaction Study of Fluticasone/Vilanterol in Rats**

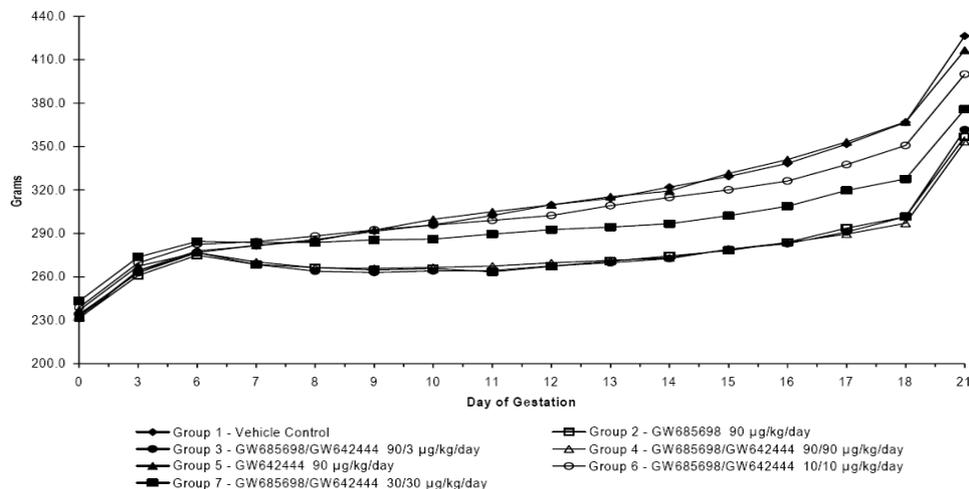
Group	1	2	3	4	5	6	6
Fluticasone ( $\mu\text{g}/\text{kg}/\text{day}$ )	0	8.2	9.4	9.5	0	0.8	3.0
Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	0	0	0.4	9.8	8.7	0.8	3.2
Body weight gain (% control) <sup>a</sup>	88.8 g	33 *	28*	29*	110	88	57 *
Food consumption (% control) <sup>a</sup>	336.7g	83*	81*	84*	100	100	91*
Mean fetal body weight (M)	5.82	5.30*	5.27*	5.24*	5.80	5.63	5.63
Mean fetal body weight (F)	5.50	5.01*	5.06*	4.99*	5.52	5.43	5.37
Sternebra 5 and xiphisternum <sup>b</sup>	9.5	15.2	18.1	22.1*	11.4	6.9	9.5

a. GDs 6 – 18.

b. Incidence of unossified/incomplete ossification/semi-bipartite/bipartite.

c. \*, P <0.05.

**Food consumption:** Food consumption was measured every 3 days. Dams receiving  $\geq$  3.0- $\mu\text{g}/\text{kg}/\text{day}$  fluticasone showed statistically significant decreases in body weight gains (9% - 19%) during the treatment period (Table 39).



**Figure 9: Mean body weight as a function of time in fluticasone/vilanterol teratology study.**

GW 685698 and GW642444 were code names for fluticasone and vilanterol, respectively. Doses in the legends were reported target achieved doses.

**Stability and homogeneity:** The testing formulation (dry powder in lactose) was stable at room temperature. The coefficient variation (CV%) in the chamber drug concentration homogeneity ranged from 5% - 21%.

**Necropsy:** Necropsy was performed on GD 21. No treatment-related effects were observed.

**C-Section and litter observations:** Groups receiving high doses of fluticasone (i.e., 8.2 – 9.5 µg/kg/day) showed statistically significant decreases in mean fetal weights ( $P < 0.05$ , Table 18).

**Fetal Malformations:** No treatment-related effects were observed.

**Fetal Variations:** The group receiving 9.5-µg/kg/day fluticasone and 9.8-µg/kg/day vilanterol showed a statistically significant increase in the incidence of abnormal bone ossifications in the 5<sup>th</sup> sternebrae ( $p < 0.05$ , Table 39) and xiphisternum. Abnormal bone ossification included unossified, incompletely ossification, and semi-bipartite/bipartite observations.

**Toxicokinetics:** Blood fluticasone and vilanterol levels were determined at hours 0.08, 0.5, 1, 2, 4, 8, and 23 hours on treatment day 5 (GD 11). Plasma levels of both fluticasone and vilanterol generally rose in a dose-dependent manner. Table 40 (next page) presents the AUC and  $C_{max}$  values of the study.

**Evaluations:** This study adequately evaluated the potential interaction of teratogenicity of fluticasone and vilanterol. Pregnant rats were dosed by nose-only inhalation with various doses of vilanterol and fluticasone, alone or in combination, during gestation days 6 – 17. The pulmonary deposited doses ranged from 0 – 9.5 µg/kg/day and 0 – 9.8 µg/kg/day for fluticasone and vilanterol, respectively.

**Table 40: Toxicokinetic Parameters of Fluticasone and Vilanterol in Pregnant Rats.**

Group	Treatment	FF/VI ( $\mu\text{g}/\text{kg}/\text{day}$ )	Plasma PK Parameters <sup>a</sup>			
			$C_{\text{max}}$ (ng/mL)		AUC <sub>0-23h</sub> (ng.h/mL)	
			Fluticasone	Vilanterol	Fluticasone	Vilanterol
1	Vehicle	-	-	-	-	-
2	FF	8.2/0	2.05	-	4.67	-
3	FF/VI	9.4/0.4	3.30	-	7.93	-
4	FF/VI	9.5/9.8	3.80	7.37	6.36	13.3
5	VI	0/8.7	-	4.69	-	9.21
6	FF/VI	0.8/0.8	0.14	0.20	0.174	-
7	FF/VI	3.0/32	0.89	1.49	1.36	3.36

a. Extracted from page 14 of the study report.

### 9.3 Peri- and Post Natal Developmental Toxicity

#### Study Title: GW642444M: Oral Pre- and Postnatal Developmental Study in Rats

<i>Study number:</i>	GSK Document No. CD2010/00109, GSK reference No. G10018, (b) (4) Study No. AFA00860
<i>Location in electronic submission:</i>	4.2.3.5.3.1
<i>Conducting laboratory and location:</i>	(b) (4)
<i>Date of study initiation:</i>	April 4, 2010
<i>Study termination date:</i>	June 10, 2010
<i>Report date:</i>	March 14, 2011
<i>GLP compliance:</i>	Yes, with a signed statement
<i>QA reports:</i>	Yes, with a signed statement
<i>Drug lot # &amp; purity:</i>	Batch No. 071137021, purity 98.4%
<i>Formulation/vehicle:</i>	1.0% methylcellulose aqueous solution

#### Key Study Findings:

- Pregnant and lactating dams were dosed by oral gavage with 0, 0.3, 3, or 10 mg/kg/day of vilanterol from gestation day 6 to lactation day 20.
- The MD and HD dams showed statistically significant increases in body weight gains (5% - 84%,  $p < 0.05$ ) during the gestation and lactation periods.
- The MD and HD F1 generations showed statistically significant decreases (4% - 12%,  $P < 0.05$ ) in mean body weight at PNDs 21 and 78.
- No abnormalities were observed at 0.3 mg/kg/day dose. The plasma vilanterol and G1179710 levels were below the detection limit of 0.206 ng.h/mL.

**Methods:**

<i>Doses</i>	0, 0.3, 3, and 10 mg/kg/day
<i>Frequency of dosing:</i>	Daily
<i>Dosing volume:</i>	10 mL/kg body weight
<i>Route of administration:</i>	Oral gavage
<i>Vehicle/formulation:</i>	0, 0.03, 0.3, and 1.0 mg/mL vilanterol in vehicle which is 1.0% methylcellulose aqueous solution.
<i>#/sex/group (main study):</i>	24/sex/dose
<i>Species/strain:</i>	Female rats (pregnant and lactation), Crl:CD (SD)
<i>Study design:</i>	See study design section below
<i>Age:</i>	14 weeks at commencement of treatment
<i>Weight:</i>	238 - 290g
<i>Treatment duration:</i>	From gestation day 7 (day 6 postcoitum) to lactation day 20 (or day 24 if the dam did not deliver)
<i>Protocol deviations:</i>	No major deviations

**Study Design:** Effects of vilanterol on parturition and postnatal development of pups were evaluated by dosing the dams during the pregnancy (GD) and lactation (LAD) periods in rats.<sup>5</sup> Dams (F<sub>0</sub>, 24/dose) were dosed with 0, 0.3, 3, or 10-mg/kg/day vilanterol during the period of GD6 – LAD 20.<sup>6</sup> The dams were allowed to deliver naturally. They were evaluated for pregnancy and delivery parameters. The offspring (F<sub>1</sub>) were evaluated for growth and postnatal development. The postnatal development parameters included body weight, motor activity, sexual development, auditory startle habituation, learning and memory, and reproductive performance. The reproductive performance of F<sub>1</sub> offspring was evaluated by examining mating behavior, reproductive parameters, and their litter parameters and the survival of their offspring (F<sub>2</sub>) during the period of the first week after birth. All parameters obtained on F<sub>1</sub> pups older than PND 21 were made on selected F<sub>1</sub> pups that were culled to 2 rats/sex/litter (46 – 48/sex/dose).

**Results and Observations:****F0 Generation:**

**Survival:** No treatment-related effects were observed. Mortality was checked twice daily. No death occurred during the study.

**Clinical signs:** Clinical observations were made twice daily. No treatment-related effects were observed.

**Body weight:** Body weights were measured daily. The MD and HD groups showed dose-dependent and statistically significant increases ( $p < 0.05$ ) in body weights and body weight gains during the gestation and lactation periods (Table 41). Figure 10 presents the time-course of F<sub>0</sub> body weight during the study. The MD and HD groups showed increases that ranged from 5 – 13% in mean body weights and 18 – 84% in mean body weight gains.

<sup>5</sup> The report described the gestation and lactation days as days post coitus and post parturition, respectively. The days of mating and parturition were referred as days 0 pc and pp, respectively.

<sup>6</sup> During pregnancy, dams were dosed until delivery or GD 24 for those that did not deliver pups. The average length of the pregnancy period was 22.2 – 22.8 days.

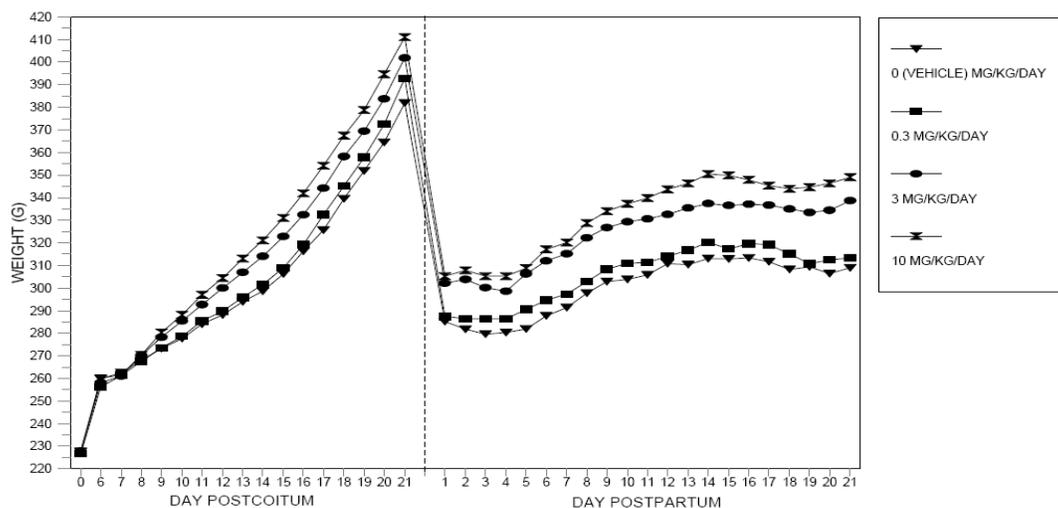
**Table 41: Results of F<sub>0</sub> Females Generation**

Vilanterol (mg/kg/day)	0	0.3	3	10
# Female rats tested	24	24	24	24
# Pregnant rats	24	23	24	24
Body weight at GD 21 <sup>a</sup>	381.9 g	↑ 3%	↑ 5%*	↑ 8%*
Body weight gain (GDs 6 – 21) <sup>a</sup>	122.2 g	↑ 12%*	↑ 18%*	↑ 24%*
Body weight (LAD 21) <sup>a</sup>	309.0 g	↑ 2%	↑ 10%*	↑ 13%*
Body weight gain (LADs 1 – 21) <sup>a</sup>	23.2 g	↑ 12%	↑ 57%*	↑ 84%*
Food consumption (GDs 19- 21) <sup>a</sup>	21.5 g/d	↑ 2%	↑ 11%*	↑ 15%*
Food consumption (GDs 6- 21) <sup>a</sup>	22.2 g/d	↑ 1%	↑ 5%	↑ 6%
Food consumption (LADs 1 – 14) <sup>a</sup>	44.7 g/d	↑ 2%	↑ 7%	↑ 8%
Gestation Index (%)	100	100	100	100
# Dams with stillborn (%)	1 (4.2)	0	1 (4.2)	4 (16.7)
Average pup delivery time (Min)	10	17.6	12.6	21.5

a. The number in treatment groups are changes relative to the control.

\*, p < 0.05; \*\*, p < 0.01.

**Food consumption:** Food consumption was measured during the following periods: GDs 0 - 6, 6 - 10, 10-14, 14 - 19, and 19 - 21, and LADs 1 - 4, 4 - 7, 7 - 10, and 10 - 14. Dose-related, but statistically non-significant increases (up to 8%) in food consumption were observed (Table 16).

**Figure 10: Body weights in F<sub>0</sub> generation in the Segment III Study in Rats**

**Stability and homogeneity:** Stability and homogeneity data showed the testing formulation was stable. Vilanterol concentrations in the formulations were within an acceptable range ( $\pm 15\%$ ).

**Maternal behavior:** Maternal behaviors during the lactation period were evaluated on post parturition days 1, 4, 7, 14, 18, and 21. No treatment-related effects were observed.

**F<sub>0</sub> parturition:** Time and behavior of parturition were observed from GD 20 – 24. The HD vilanterol groups had a numerically longer mean delivery time. There was no difference in the duration of pregnancy among groups (22.4 – 22.8 days). The mean delivery time of the

HD group was twice as long as the control group (duration: 10, 17.6, 12.6, and 21.5 minutes in the C, LD, MD, and HD groups, respectively).

## F1 Generation

**Litter sizes and survival:** Litter sizes were determined at the time of delivery. Pup viability was checked daily. No treatment-related effects on either litter sizes or survival rates were observed. See Table 42 for summary results.

**Table 42: F<sub>1</sub> Litter Results in Pre-Weaning Period**

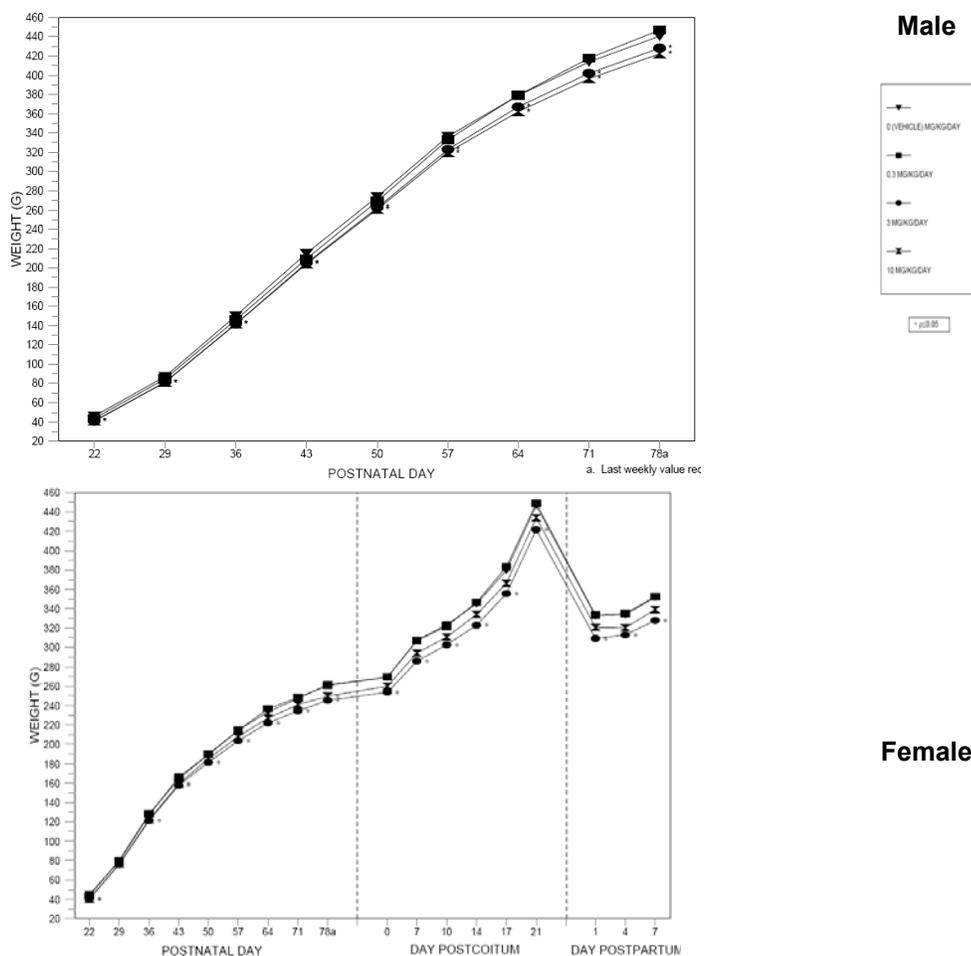
Vilanterol dose in F <sub>0</sub> (mg/kg/day)	0	0.3	3	10
# Viable F <sub>1</sub> litters evaluated	24	23	24	24
# pups delivered (total)	3.5	299	313	317
Mean # live pups/litter at birth	12.6	13.0	13.0	13.0
Stillborn: Total # (%)	3/305 (1.0)	0/299 (0)	1/303 (0.3)	6/317 (1.9)
Total litter # (%)	1 (4.2)	0	1 (4.2)	4 (16.7)
Pup sex ratio at PND 1 (% male)	48.4	50.7	47.7	52.0
Survival index (% , PNDs 1-4)	96.7	99.3**	99.7**	97.7
# pups surviving at PNDs 21	11.4	12.1	12.2	11.8
Mean pup weight at PND 1 <sup>a</sup>	6.5 g	0	↓ 5%	↓ 5%
Mean pup weight at weaning <sup>a</sup>	42.1	↓ 7%	↓ 11%*	↓ 12%*

a. The number in treatment groups are changes relative to the control.

\*, p < 0.05; \*\*, p < 0.01.

**Clinical signs:** Clinical signs in pups were checked daily. No treatment-related effects were observed.

**Body weight:** Body weights were measured weekly. The MD and HD pups showed decreases in mean body weights throughout the study. The decreases generally reached statistical significance ( $P < 0.05$ ) except for PND1. The respective decreases in mean body weights in the MD and HD males were 5% and 5% on PND 1, 11% and 12% on PND 21, and 3% and 4% on PND 78. In the females, the respective decreases in mean body weights in the MD and HD groups were 5% and 5% on PND1, 7% and 6% on PND 21, and 6% and 4% on PND 78. Figure 11 presents the body weight-time course of F1 generation during the study.



**Figure 11: Body weights in F<sub>1</sub> generation in the Rat segment III study**

**Behavioral and sexual developments and reproductive performance:** Pup development and reproductive performance post weaning were evaluated in selected pups. Pups were culled to  $\leq 2$ /sex/litter (46 – 48/sex/dose) on PND 21. The survivors were divided into 2 subsets for assessments of learning and memory, sexual development, and reproductive performance as following:

Subset 1: This subset (23-24/sex/dose) was used to assess the effect of vilanterol on sexual maturation, auditory startling habituation, and reproductive performance. Sexual maturation (i.e., developments) was evaluated by comparing the time for vaginal opening and preputial separation in females and males, respectively. The monitoring for sexual maturation started from PND 28 and 39 in females and males, respectively. Auditory startling habituation was evaluated on approximately PND 77 (range of PNDs 76 – 79). Reproductive performance was assessed by allowing the F<sub>1</sub> males and females to mate at an age of approximately 12 weeks.

Subset 2: This subset (23-24/sex/dose) was used to evaluate the effect of vilanterol on auditory startling habituation, motor activity, learning and memory, and sexual maturation. Auditory startling habituation was assessed during PNDs 43 and 45. Motor activity was assessed during the period of PNDs 57 and

63 using passive infrared sensor. Learning and memory was assessed using the Morris watermaze test during the period of PNDs 65 – 87.

**Motor Activity:** No treatment-related effects were observed. Motor activity was evaluated once between PNDs 57 and 63. The movements of each F<sub>1</sub> rat were monitored by a passive infrared sensor mounted outside a cage. Each test session was one hour in 10-minute increments with the number of movements. There were no dose-response patterns in either sex (Figure 12).

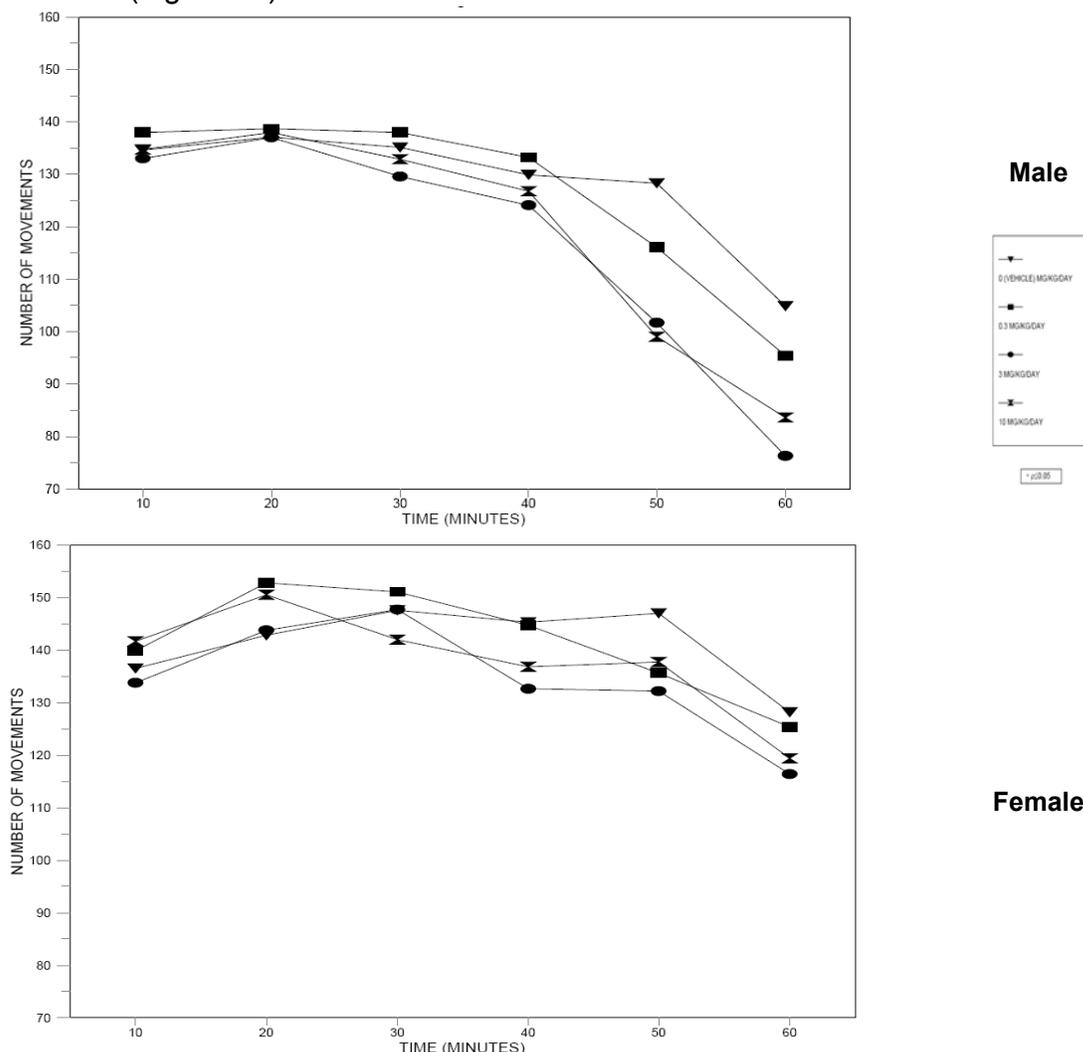


Figure 12: Motor activity on PND 63 ( $\pm 3$ ) in F<sub>1</sub> generation

**Sexual development:** No treatment-related effects were observed in age of sexual maturation (preputial separation in males and vaginal opening in females). The preputial separation occurred at approximately PND 45. Vaginal opening occurred at approximately PND 32. As reflected in the body weight section, both sexes in the HD group and the MD females ( $n = 46 - 48/\text{sex}/\text{dose}$ ) showed statistically significant decreases in mean body weight at the time of sexual maturation (respective weights in g in C, LD, MD, and HD groups were  $233.4 \pm 21.6$ ,  $232.6 \pm 21.7$ ,  $224.5 \pm 25.6$ , and  $221.9 \pm 18.9$  [ $p < 0.05$ ] in

males and  $99.1 \pm 9.8$ ,  $100.3 \pm 9.8$ ,  $94.1 \pm 9.2$  ( $p < 0.05$ ), and  $94.3 \pm 10.2$  ( $p < 0.05$ ) in females].

**Table 43: F<sub>1</sub> Generation Results in the Post Weaning Peirod**

Vilanterol dose in F <sub>0</sub> (mg/kg/day)	Male				Female			
	0	0.3	3	10	0	0.3	3	10
# F <sub>1</sub> litters evaluated on PND 78	47	46	48	48	47	46	48	47
Mean weight (g/ Δ) at PND 78 <sup>a</sup>	441g	↑ 1%	↓ 3%*	↓ 4%*	261g	0%	↓ 6%*	↓ 4%*
Mean age at sexual maturation (PNDs) <sup>b</sup>	45.3	45.7	45.5	45.5	32.1	32.2	32.0	32.0
# Estrous cycles (10 days pre-mating)	-	-	-	-	2.5	2.4	2.3	2.1
# Rats in mating study	24	23	24	24	24	23	24	24
# Rats that mated	24	22	23	24	24	23	23	24
# days prior to mating (mean)	2.4	2.7	3.2	3.2	2.5	2.4	2.3	2.1
# Pregnant female rats	-	-	-	-	22	22	24	20
Fertility index (%)	91.7	95.4	95.8	91.7	91.7	95.6	100	83.3
Mean duration of gestation (days)	-	-	-	-	22.4	22.3	22.3	22.3
Average pup delivery time (min)	-	-	-	-	9.6	13.2	9.8	11.0
# Rats delivered live pups	-	-	-	-	22	22	24	19
# Pup delivered (total)	-	-	-	-	339	363	365	307
# Pup deliver/litter	-	-	-	-	15.4	16.5	15.2	16.2
Mean # of implantations	-	-	-	-	16.5	17.2	16.1	16.5

a. The number in treatment groups are changes relative to the control.

b. Sexual maturation was measured by the time of vaginal opening and preputial separation in males and females, respectively.

**Estrous cycles:** No significant effects were observed. The number of estrous cycles during the periods of 10 days prior to cohabitation and during cohabitation (up to 14 days) that began on PNDs 79 – 84 was recorded. There were dose-related decreases in the number of estrous cycles during the period prior to cohabitation (# cycles:  $2.5 \pm 0.7$ ,  $2.4 \pm 0.9$ ,  $2.3 \pm 0.6$ , and  $2.1 \pm 0.9$  in C, LD, MD, and HD groups,  $n = 23 - 24$ /dose, Table 43), but none of the decreases reached statistical significance ( $p < 0.05$ ).

**Neurological assessment:** No treatment-related effects were observed in the auditory startling test and a watermaze test. The auditory startling test was performed during the period of PNDs 43 – 45 (subset 1) and 76 – 79 (subset 2). The watermaze test was performed on three consecutive days with 12 trials in each session. There were no differences among groups in any of the parameters tested.

**Toxicokinetics:** Plasma vilanterol and GI179710 levels were determined using HPLC-MS-MS on PND 11 (3, 8, and 22 hours post maternal dosing,  $n = 3$ /sex/time point) in pups. The lower limit of quantitation (LOQ) was 0.206 ng/mL. No vilanterol or GI179710 was detected in all but one sample (a 3-mg/kg female pup).

**Reproduction:** The following parameters were assessed: the number of days in cohabitation, mating and fertility indices, length of pregnancy, parturition, etc. Cohabitation of F<sub>1</sub> males and females (23 - 24/sex/dose, 1:1 ratio/cage) started at 79 – 84 days of age. There were two cohabitation periods that lasted for a maximum duration of 7 days each. Females that did not mate with the first male in 7 days were allowed to cohabitate with another male of the same group for 7 additional days.

## F2 Generation

F<sub>2</sub> pups were examined for body weights, clinical signs, sex, and viability for up to 7 days. Pups that died during this period were examined for visceral defects. Table 44 summarizes the results in the F<sub>2</sub> generation. No treatment-related effects were observed. It was noted that the vilanterol-treated groups had lower mean fetal weights on both PNDs 1 and 7; however, none of the decreases reached statistical significance of  $p < 0.05$ .

**Table 44: F<sub>2</sub> Litter Results**

Vilanterol dose in F <sub>0</sub> (mg/kg/day)	0	0.3	3	10
Number of F <sub>2</sub> litters evaluated	22	22	24	19
Mean # live pups/litter	15.1	16.0	14.8	15.9
Mean # stillborn pup/litter	0.3	0.4	0.4	0.3
Survival index (% , PNDs 1-4)	99.1	98.9	96.6	98.0
Pup sex ratio at PND 1 (% male)	51.7	48.3	51.5	53.5
Mean pup weight at PND 1 <sup>a</sup>	6.2 g	↓ 3%	↓ 5%	↓ 5%
Mean pup weight at PND 7 <sup>a</sup>	12.4 g	↓ 2%	↓ 4%	↓ 4%

a. The number in treatment groups are changes relative to the control.

## 10 Special Toxicology Studies

No special studies were reviewed because they were not pivotal to the safety evaluation of the current application.

## 11 Integrated Summary and Safety Evaluation

This review recommends the approval of the Breo Ellipta application (NDA 204-275), pending labeling review. Breo Ellipta is a fixed combination, dry powder inhaler of vilanterol and fluticasone furoate. The application has conducted adequate nonclinical safety evaluations of active pharmaceutical ingredients (APIs, alone and in combination), their metabolites, excipients, impurities, and extractables of the device product. There are no outstanding nonclinical issues at the present time. The application has met nonclinical requirements for product approval; therefore, the review recommends approval of the product from the nonclinical perspective.

Breo Ellipta is a dry powder inhaler that delivers a dry powder mixture of fluticasone furoate and vilanterol trifenate. Each actuation delivers 100- $\mu$ g fluticasone and 25- $\mu$ g vilanterol. Breo Ellipta contains magnesium stearate and lactose as excipients. The application proposes to register Breo Ellipta for chronic obstructive pulmonary diseases (COPD) in adult patients. The maximum recommended human dose of Breo Ellipta is 100- $\mu$ g FF and 25- $\mu$ g VI once daily.

For clarification, this section is divided the following topics: vilanterol, fluticasone, vilanterol and fluticasone in combination, and metabolites.

### 11.1 Vilanterol

The nonclinical data submitted in this application assessed adequately the pharmacology, pharmacokinetics, and toxicology of vilanterol in laboratory animals. The animal species

were primarily mice, rats, and dogs. The following summary is based on the current as well as the previous reviews completed by DPARP staff.

**Pharmacology:** Vilanterol is a LABA. Similarly to formoterol, a currently marketed LABA, vilanterol binds to and activates beta2 receptors resulting in subsequent increases in intracellular cAMP levels, relaxation of smooth muscles located in the airways, and decreases in airway resistance. The potency of vilanterol is comparable to that of formoterol in pEC50s (~ 9.4). In vivo studies showed that vilanterol relaxed guinea pig airway muscle contractility induced by electric stimulation. Similarly, vilanterol inhibited the contractile response in human bronchus preparations induced by PGF-2 alpha. It also relaxed histamine-induced bronchoconstriction in conscious guinea pigs.

**Pharmacokinetics:** Vilanterol was well-absorbed following inhalation administration in rat, dog, and human, but the absolute bioavailability of the drug after this route of administration is unknown in animals and 27.3% in humans. The oral bioavailability of vilanterol was variable among species, ranging from 0.1% in mice, 0.9% in rats, <2% in humans, to 34% in dogs. Plasma drug concentrations were generally proportional to the inhalation dose in laboratory animals. Peak plasma drug levels were found at the end of inhalation exposure. Vilanterol is highly bound to plasma proteins (92-99%) in animals and humans. Vilanterol is metabolized by CYP 2B2 in rats and CYP 3A4 enzyme in dogs and humans. The elimination half life was 5.3 and 9.8 hrs in rats and dogs, respectively.

**Toxicology:** The nonclinical characterization of the toxicity profile of vilanterol included general, reproductive and developmental, and genetic toxicity, as well as carcinogenicity. The results of these studies showed that vilanterol possess a toxicity profile typical of beta 2 agonists.

**General toxicity:** General toxicity of vilanterol was evaluated after inhalation administration in mice, rats, and dogs. Pivotal general toxicity studies were 13-, 26-, and 39- weeks in mice, rats, and dogs, respectively. The exposure conditions entailed administration of various aerosol vilanterol concentrations for 60 minute/day across species. These studies identified the following organs (Table 45) as the target organs of vilanterol toxicity. Table 45 also provides an overview of pivotal general toxicity studies of vilanterol.

**Table 45: Pivotal Toxicity Studies of Vilanterol**

Species	Duration (wk)	Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ ) <sup>b</sup>	Target organs	Report No.	Reference <sup>a</sup>
Mouse	13	0, 5.9, 102, 649, 6360/3820	Nose, liver, & uterus	WD2006/01713	A
Rat	13	0, <b>5.6</b> , <sup>c</sup> 65.8, 1039, 3885	Airways & lung	WD2006/01716	A
Rat	26	0, <b>5.7</b> , 53.7, 267, 1025	Airways and lung	WD2008/00903	C
Dog	13	0, <b>2.3</b> , 16.5, 125 <sup>d</sup>	liver, heart, & nose	WD2006/01711	B
Dog	39	0, 2.4, <b>15.6</b> , 128 <sup>d</sup>	heart, lung, liver, kidneys and testes	CD2007/01006	C

a. Nonclinical reviews in which the study report were evaluated. References A, B, and C were nonclinical reviews completed by Dr. Huiqing Hao on 03-JUN-2008 and 29-OCT-2008, and by Dr. Larry Sancilio on 05-MAR-2010, respectively, in IND 74,796.

b. Pulmonary deposited doses.

c. Bold face indicates the NOAEL value.

d. The HD group was dosed with 30- $\mu\text{g}/\text{kg}/\text{day}$  for 3 days prior to being treated at these doses.

## Mouse

A 13-week inhalation toxicity study of vilanterol in mice was completed as a dose-ranging study for a 2-yr carcinogenicity bioassay (Report WD2006/01713). CD-1 mice (12/sex/dose) were dosed with 0 (C), 5.9 (LD), 102 (MD), 649 (MHD), or 6360/3820 (HD)- $\mu\text{g}/\text{kg}/\text{day}$  (pulmonary deposits) vilanterol for 13 weeks.<sup>7</sup> Dose-related changes in organ weights and microscopic findings in the respiratory tract were observed in the treatment groups. Changes in organs weights were limited to ovaries and lungs. The increases in mean ovary weights (29 – 104%) were observed in all vilanterol groups while increases in mean lung weights (9 – 15%) were observed in the MHD and HD groups. Microscopic changes were observed in the nasal cavity, nose, liver, and uterus. In the nasal cavity, eosinophilic inclusions in the respiratory and olfactory epithelial cells were observed in all dose groups; the HD group also showed degeneration and regeneration of respiratory epithelial cystic glands. Epithelial squamous cell metaplasia in the larynx and myometrial hyperplasia in the uterus was observed in the  $\geq$ MD groups. The HD groups also showed decreases in generalized hepatocyte cytoplasm rarefaction. The study did not establish an NOAEL. See nonclinical review completed by Dr. Huiqing Hao on June 3, 2008 in IND 74,696 (p5 – 12) for additional information.

## Rat

Inhalation toxicity studies of vilanterol up to 26 weeks in treatment duration were completed in rats. Report WD2006/01716 was a 13-week inhalation toxicity rat study. Sprague-Dawley rats (12 sex/dose) were dosed with 0 (C), 5.6 (LD), 66 (MD), 1039 (MHD), or 3885 (HD)- $\mu\text{g}/\text{kg}/\text{day}$  (pulmonary deposits) vilanterol for 13 weeks. Dose-related changes were observed in body weights and microscopic findings in the respiratory tract. The HD females showed a 19% increase in mean body weights. Microscopic findings in the respiratory tract were observed in the  $\geq$  MD groups. The location in the respiratory tract included the nose, nasopharynx, larynx, trachea, tracheal bifurcation, and bronchi. Specifically, the MD group showed transitional epithelial degeneration and regeneration in the nasal cavity, metaplasia in the larynx, and respiratory epithelial degeneration/regeneration in the trachea. The MHD and HD groups showed additional changes, including the following: squamous metaplasia, olfactory epithelial ulceration, degeneration and regeneration in the nasal cavity; squamous metaplasia, respiratory epithelial ulceration, inflammation and goblet cell hyperplasia in the nasopharynx; epithelial hyperplasia in the larynx; and squamous metaplasia in the tracheal bifurcation. The HD group also showed low incidences of squamous metaplasia (male only) and epithelial hyperplasia in the bronchi. The NOAEL of the study was 5.6  $\mu\text{g}/\text{kg}/\text{day}$ . See the nonclinical review completed by Dr. Huiqing Hao on June 3, 2008 in IND 74,696 (p13 – 19) for additional information.

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<sup>7</sup> The HD group received a vilanterol dose of 6360- $\mu\text{g}/\text{kg}/\text{day}$  for the first 8 days and 3820  $\mu\text{g}/\text{kg}/\text{day}$  for the rest of treatment period. The vilanterol dose was reduced because of excessive deaths observed in the first 8 days. A total of 11 deaths (2/24 and 9/12 in the main study and TK section mice, respectively) occurred in the 8-day period. Additional deaths (10) occurred after day 8 (b) (4). Five mice that died in the first days showed nasal irritation (epithelial degeneration or necrosis), but the nasal findings were not considered the cause of deaths. (b) (4)

Report WD2006/01716 was a 26-week inhalation toxicity rat study. Sprague-Dawley rats (12 sex/dose) were dosed with 0 (C), 5.7 (LD), 54 (MD), 267 (MHD), or 1025 (HD)- $\mu\text{g}/\text{kg}/\text{day}$  (pulmonary deposits) vilanterol for 26 weeks. Additional dogs (6/sex/dose) were included in the C, MHD, and HD groups to evaluate the recovery process of any lesions after a recovery period of 8 weeks. The MHD and HD groups showed up to 20% increases in body weight gains and food consumption during the first half of the treatment, but there were no significant differences in either absolute body weights or food consumption by the end of the 26-week treatment period. Microscopic changes were observed in the upper airways, heart, lung, and ovaries. In the upper airways, the HD group showed degeneration and metaplasia of the olfactory epithelium in the nasal cavity in both sexes and squamous metaplasia in the larynx. In the heart, myocardial degeneration/fibrosis was observed in MHD and HD groups in both sexes. In the lung, increased incidence of macrophage accumulation was observed in the MHD and HD males and hemorrhage was observed in the tracheobronchial lymph node in the HD females. In the ovaries, cystic follicular dilation was observed in the  $\geq$  MD groups. The NOAEL was 267.4 mcg/kg ( $\text{AUC}_{0-t}$  of 334 ng.h/mL) in males and 5.77 mcg/kg ( $\text{AUC}_{0-t}$ , 3.7ng.h/ml) in females, respectively. See the nonclinical review completed by Dr. Larry Sancilio on March 5, 2010 in IND 74,696 (p5 – 14) for additional information.

### Dog

Inhalation toxicity studies of vilanterol up to 26 weeks in treatment duration were completed in dogs. In the 13-week inhalation toxicity study in dogs (Report WD2006/01711), beagle dogs (4 sex/dose) were dosed with 0 (C), 2.3 (LD), 16.5 (MD), or 125 (HD)- $\mu\text{g}/\text{kg}/\text{day}$  (pulmonary deposits) vilanterol for 13 weeks.<sup>8</sup> Additional dogs (2/sex/group) were included in the C and HD groups to evaluate the recovery process of any lesions after a recovery period of 4 weeks. The HD group showed a 0.7-kg increase in mean body weight. Dose-related decreases (17% – 37%) in mean heart rates prior to dosing and increases (24% - 86%) after dosing were observed in all treated groups. The MD and HD groups also showed increases in plasma Troponin I levels after vilanterol treatment. The highest troponin level was up to 0.59, 2.97, and 3.89  $\mu\text{g}/\text{mL}$  in the C, MD, and HD groups, respectively. The target organs of toxicity included the liver, heart, and nose. The liver showed decreased glycogen deposition in all treatment groups. The heart showed ventricular papillary muscle fibrosis in the MD and HD groups. In the nasal cavity, the nasal cavity turbinates showed lymphoid infiltration in the lamina propria of olfactory and respiratory epithelium. In the recovery period, both HD females showed myocardial fibrosis in the ventricular papillary muscles. The NOAEL was 2.3  $\mu\text{g}/\text{kg}/\text{day}$  (7 ng.h/mL in plasma drug AUC). See nonclinical review completed by Dr. Huiqing Hao on October 29, 2008 in IND 74,696 (p31 – 37) for additional information.

In the 39-week inhalation toxicity dog study (Report WD2007/01006), beagle dogs (4 sex/dose) were dosed with 0 (C), 2.4 (LD), 15.6 (MD), or 128 (HD)- $\mu\text{g}/\text{kg}/\text{day}$  (pulmonary deposits) vilanterol for 39 weeks. Treatment-related effects were observed in clinical signs, heart rates, body weights, plasma Troponin I levels, and microscopic examinations. Clinical signs included vasodilation (ears and gums) in the MD and HD groups. Changes in heart rates were observed in both pulse rates and ECG. Heart rates increased in a dose-dependent fashion in all dose groups. The magnitude of the increase (up to 70%) was

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<sup>8</sup> The HD group dogs were dosed with 30- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol for 3 days before the treatment day 1.

greater in the early phase than the end of the treatment period. Plasma troponin I levels were increased in the MD and HD groups. Microscopic changes were observed in the heart, lung, liver, kidneys, and testes. Most of the changes were limited to the HD group except for the liver findings which showed drug effects in all treatment groups. Myocardial fibrosis and mesothelial hyperplasia were observed in HD females. Squamous metaplasia was observed in the tracheal bifurcation. Tubular atrophy and cortex fibrosis were observed in the kidneys. Hypospermatogenesis was observed in the testes. The NOAEL was 15.6  $\mu\text{g}/\text{kg}/\text{day}$  (AUCs of 34.8 and 39  $\text{ng}\cdot\text{h}/\text{mL}$  in males and females, respectively). See nonclinical review completed by Dr. Larry Sancilio on March 3, 2010 in IND 74,696 (p15 – 25) for additional information.

**Genetic toxicity:** Vilanterol tested negative in the Ames assay, UDS assay in vitro, and SHE cell assay in vitro; and in an in vivo rat bone marrow micronucleus assay. The drug tested equivocal in the mouse lymphoma assay.

**Carcinogenicity:** The carcinogenic potential of vilanterol was evaluated via inhalation in two traditional 2-year bioassays. Vilanterol tested positive in both rats and mice for evidence of carcinogenicity. The rat showed a dose-related shortening of latency for pituitary neoplasms at  $\geq 84.4/28.2$   $\mu\text{g}/\text{kg}/\text{day}$  (achieved dose) in both sexes and increases in the incidence of leiomyomas in mesovarian ligaments in females at  $\geq 84.4/28.2$   $\mu\text{g}/\text{kg}/\text{day}$ . In mice, females showed increases in the incidence of tubulostromal carcinomas in the ovaries at 29,500  $\mu\text{g}/\text{kg}/\text{day}$ . These findings, however, were typical of beta agonists in rodents.

Sprague-Dawley rats (60/sex/dose) were exposed by nose-only inhalation to vehicle (C), which was lactose powder, low-dose (LD), mid dose (MD), mid-high dose (MHD), or high dose (HD) of vilanterol for up to 104 weeks. Specifically, males were treated with 0, 10.5, 84.4, 223, or 657- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol (achieved doses) for 101 weeks. Females were exposed to the same doses for 85 weeks and dose adjustments were made subsequently due to excessive mortalities in the vilanterol-treated groups. The dose adjustments consisted of the following: dosing was discontinued in the MHD and HD groups and vilanterol doses in the LD and MD groups were reduced to 3.5 and 28.2  $\mu\text{g}/\text{kg}/\text{day}$ , respectively. The three top-dose groups ( $\geq 84.4/28.2$ ) were terminated during weeks 95 – 96 when the number of survivors reached 15/group. Both male and female rats showed dose-related increases in mortality ( $P < 0.01$ ) and shortening in latency to pituitary neoplasms. The pituitary tumors were the cause of death in majority of the premature deaths, although the increases in overall tumor incidence did not reach the statistically significant level of 0.01 for this common tumor. (Note: Control incidences were 70% for males and 90% for females.) Specifically, by week 78, the respective deaths attributed to pituitary neoplasms in the C, LD, MD, MHD, and HD was 2, 2, 5, 12, and 15 in males and 7, 10, 19, 24, and 23 in females. The three top-dose female groups also showed statistically significant increases in the incidence of mesovarian leiomyomas. The incidence was 0, 0, 5 ( $P < 0.007$ ), 4 ( $p < 0.020$ ), and 4 ( $p < 0.020$ ) in the C, LD, MD, MHD, and HD groups, respectively.

Mice (84/sex/dose, CD-1) were treated by nose-only inhalation with 0 (C), 6 (LD), 62 (MLD), 615 (MD), 6,150 (MHD), or 29,500 (HD)- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol (achieved doses) for 101 – 104 weeks. HD females showed a statistically significant increase in ovarian tubulostromal adenoma ( $p = 0.014$ ) (incidence: 0/84, 0/83, 1/84, 0/84, 2/84, and 6/83 in the C, LD, MLD, MD, MHD, and HD, respectively). The four top-dose groups in females also showed numerical increases in the incidence of leiomyomas and leiomyosarcomas in the uterus, alone or in combination, but none of the increases reached the statistically significant level

of  $p < 0.01$ . Specifically, the respective tumor incidence in the C, LD, MLD, MD, MHD, and HD was 2, 2, 5, 5, 1, and 2 for leiomyomas; 0, 1, 2, 4, 6, and 4 for leiomyosarcomas; and 2, 3, 7, 9, 7, and 6 for the leiomyoma and leiomyosarcoma combination.

**Reproductive and developmental toxicity:** Reproductive and developmental toxicity of Breo Ellipta was evaluated in batteries of studies with the active pharmaceutical ingredients, fluticasone and vilanterol, alone, as well as in a teratology study of the two compounds in combination. Results of the battery of fluticasone studies have been summarized in the Labeling of Veramyst Nasal Spray (NDA 22-051).<sup>9</sup> Below are summaries of effects of vilanterol alone or in combination with fluticasone furoate.

A battery of reproductive and developmental toxicity studies of vilanterol were completed in rats and rabbits. Another study was completed to evaluate the effect of vilanterol and fluticasone on embryofetal development in rats. The battery of vilanterol studies evaluated the effects of vilanterol on fertility in rats (Reports CD2007/00581 and CD2006/01165), teratogenicity in rats and rabbits (Reports CD2006/01166, WD2006/02439, CD2006/02047), and peri- and post-natal development in rats (Report CD2010/00109). The studies used the following routes of administration: inhalation (rats and rabbits), subcutaneous (rabbits), and oral (rabbits). Results showed that vilanterol did not affect fertility in rats, but caused dose-dependent, statistically non-significant increases in the incidence of malformations at high doses in rabbits and dose-dependent, statistically significant increases in the incidence of skeletal variations in rats and rabbits. The vilanterol and fluticasone combination study did not reveal significant interactions. Table 46 (next page) provides an overview of the reproductive and developmental toxicity studies of vilanterol.

**Fertility:** The effect of vilanterol on fertility was studied in rats. The effect of vilanterol on male and female fertility was studied separately (Documents CD2007/00581 and CD2006/01165). Males and females were dosed with vilanterol by inhalation administration. They were allowed to mate with their untreated partners. Routine examinations of fertility parameters were carried out. The results showed the vilanterol did not affect fertility parameters in males or females.

Report CD2007/00581 evaluated the effect of vilanterol on male fertility in rats. Vilanterol-treated males were allowed to mate with untreated females. Males (25/sex/dose, SD) were dosed by nose-only inhalation with 0, 6, 82 or 3,150- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol (pulmonary deposited doses) from 14 days prior to mating, during mating, and to 5 weeks after mating. Males were sacrificed at the end of treatment. Females were sacrificed on GD 7. Fertility parameters were evaluated in both sexes. Males were also examined for organ weights and gross abnormalities of reproductive organs. Results showed that vilanterol did not affect male fertility at any dose, but the MD and HD groups showed statistically significant

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<sup>9</sup> Veramyst label states: "Teratogenic Effects: Pregnancy Category C. ... There were no teratogenic effects in rats and rabbits at inhaled fluticasone furoate dosages of up to 91 and 8 mcg/kg/day, respectively (approximately 7 and 1 times, respectively, the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis). There was also no effect on pre- or post-natal development in rats treated with up to 27 mcg/kg/day by inhalation during gestation and lactation (approximately 2 times the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis)... No evidence of impairment of fertility was observed in reproductive studies conducted in male and female rats at inhaled fluticasone furoate doses of up to 24 and 91 mcg/kg/day, respectively (approximately 2 and 7 times, respectively, the maximum recommended daily intranasal dose in adults on a mcg/m<sup>2</sup> basis)."

decreases (up to 18%,  $p < 0.05$ ) in organ weights of epididymis, prostate and/or seminal vesicles.

**Table 46: Reproductive and Developmental Toxicity Studies of Vilanterol**

Segment	Species	ROA	Time of Treatment	Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Major Findings	Reference #
I	Rat, M	IH	8 weeks, mated with untreated females in week 3	0 <sup>a</sup> , 6, 82, 3,150	No effect on fertility, ↓ weights of testes, epididymides, and seminal vesicles at $\geq 0.082$ mg/kg/day	CD2007/00581
I	Rat, F	IH	From 14 days before, to 6 days after mating	0, 5, 66, 3,710	No effect on female fertility at any doses.	CD2006/01165
II	Rat, F	IH	GD 6 - 17	0, 5, 61, 3,370	Statistically non-significant ↑ in skeletal variations at $\geq 0.061$ mg/kg/day	CD2006/01166
II	Rabbit F	IH	GD 7 - 19	0, 6, 59, 575	Statistically non-significant ↑ in malformations (cleft palate & open eyelids) at HD; skeletal variations in all doses	WD2006/02439
II	Rabbit F	SC	GD 7 - 19	0 <sup>b</sup> , 3, 7, 30, 300	Skeletal variations at 300 mg/kg/day	CD2006/02047
III	Rat	PO	GD 7 – LAD 20	0 <sup>b</sup> , 300, 3,000, 10,000	No significant effects on pup postnatal development	CD2010/00109

a. The vehicle for all inhalation toxicity studies was lactose powder.

b. The vehicle was 20:80 PEG400/8% 2-hydroxypropyl beta cyclodextrin.

c. The vehicle was an aqueous solution containing 0.5% sodium lauryl sulfate, 0.5% methylcellulose and 0.01% simethicone.

Report CD2006/01165 evaluated the effect of vilanterol on female fertility in rats. Vilanterol-treated females were allowed to mate with untreated males. Females (25/sex/dose, SD) were dosed by nose-only inhalation with 0, 5, 66, or 3,710 mg/kg/day of vilanterol (pulmonary deposited doses) from 14 days before mating, during mating, and 6 days after the period. They were sacrificed on GD 20 for evaluations of ovaries and uterine contents and fertility parameters. The results showed that vilanterol did not affect female fertility at any doses. The HD group showed a 156% increase in mean body weight gains during the pre-mating period.

**Teratogenicity:** Reports CD2006/01166, CD2006/02439, and CD2006/02047 evaluated the teratogenicity of vilanterol in rats and rabbits. Pregnant dams were dosed by the inhalation and subcutaneous routes of administration with vilanterol up to the maximal tolerated doses during the organogenesis period. Results showed that vilanterol caused dose-dependent, statistically non-significant increases in the incidence of malformations at high doses in rabbits and dose-dependent, statistically significant increases in the incidence of skeletal variations in rats and rabbits.

Report CD2006/01166 evaluated the effect of vilanterol on early embryofetal development in rats. Pregnant rats were dosed by nose-only inhalation with 0, 5, 66, or 3,370- $\mu\text{g}/\text{kg}/\text{day}$

vilanterol during the period of gestation days 6 – 17. They were sacrificed on GD 21 for the examination of uterine contents. The MD and HD groups showed statistically significant increases ( $p < 0.05$ ) in mean body weight gains (22% and 33% in MD and HD groups, respectively) during the period of GDs 6 - 18. These groups also showed increases ( $P < 0.05$ ) in the incidence of unossified or incomplete ossification of 5<sup>th</sup> sternebra and xiphisternum. The percentage of fetuses affected was  $17.3 \pm 4.5\%$ ,  $22 \pm 4.8\%$ ,  $35.3 \pm 5.7\%$ , and  $39.2 \pm 6.3\%$  in the C, LD, MD, and HD groups, respectively. There was no increase in the incidence of malformations in the vilanterol treatment groups. The results showed that vilanterol is non-teratogenic in rats. The vilanterol AUC<sub>0-t</sub> was 3.76, 42.6, and 276 ng.h/mL in the LD, MD, and HD groups respectively. The NOAEL was 5  $\mu\text{g}/\text{kg}/\text{day}$  or 3.76 ng.h/mL on a nominal dose or AUC basis, respectively.

Document CD2006/02439 evaluated the effect of vilanterol on early embryofetal development in rabbits. Pregnant rats were dosed by nose-only inhalation with 0, 6, 59, or 575- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol during the period of gestation days 7 – 19. They were sacrificed on GD 29 for the examinations of uterine contents. The HD group fetuses showed statistically non-significant increases in the incidence of malformations and variations. The malformations included cleft palate (4.6%), open or partially opened eyelids (6.3%), and limb flexure and mal-rotation (6.7%). The variations included cranial bone malformations (4.6%), bipartite/misshaped interparietal bone (4.0%), elongated/fused anterior fontanelle (2.3%), bridge of ossification/fused sternbral center (7.5%), and digit malformations (5.2%). These malformations concentrated in one or two litters. The HD group also showed a statistically significant decrease in mean individual fetal body weight ( $\downarrow 10.5\%$ ,  $p < 0.05$ ). The LD and MD groups showed slight and statistically non-significant increases ( $P < 0.05$ ) in the incidence of elongated/fused anterior fontanelle (1.3% - 2.1%). The NOAEL of the study was the LD group (3.76 ng.h/mL) based on the low incidence of the finding in the current study and the lack of similar findings at higher AUC (22.4 ng.h/mL) in the subcutaneous study in the same species (Document CD2010/00109, below).

Document CD2010/02047 evaluated the effect of vilanterol by the subcutaneous route of administration on early embryofetal development in rabbits. Pregnant rats were dosed subcutaneously with 0, 3, 30 or 300- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol during the period of gestation days 7 – 19. They were sacrificed on GD 29 for the examinations of uterine contents. The HD group fetuses showed statistically non-significant increases in the incidence of malformations and statistically significant increases in the incidence of variations. The malformation was open eyelid in 1.0% fetuses. The variations included unossified cervical vertebra (1.8%), metacarpal forepaws (15.9%) and hindpaws (3.5%), and incompletely ossified hyoid skull (9.4%). No malformations or variations were found at the  $\leq 30\text{-}\mu\text{g}/\text{kg}/\text{day}$ . The AUC was 22.4 and 306 ng.h/mL in the 30 and 300  $\mu\text{g}/\text{kg}/\text{day}$  groups, respectively.

Report CD2010/00109 evaluated effects of vilanterol on parturition and postnatal development of rat pups by dosing the dams during the pregnancy (GD) and lactation (LAD) periods. Dams (F<sub>0</sub>, 24/dose) were dosed with 0, 0.3, 3, or 10-mg/kg/day vilanterol during the period of GD6 – LAD 20. The pregnant dams were allowed to deliver naturally. The offspring (F<sub>1</sub>) were evaluated for growth and postnatal development. The postnatal development parameters included body weights, motor activity, sexual development, auditory startle habituation, learning and memory, and reproductive performance. The reproductive performance of F<sub>1</sub> (24/sex/dose) was evaluated by evaluating their mating behavior, reproductive parameters, and the litter and survival parameters of F<sub>2</sub>. The

MD and HD dose dams showed statistically significant increases in body weight gains (5% - 84%,  $p < 0.05$ ) during the gestation and lactation periods. The MD and HD F<sub>1</sub> generations showed statistically significant decreases (4% - 12%,  $P < 0.05$ ) in mean body weight at PNDs 21 and 78. No abnormalities were observed at  $\leq 30 \mu\text{g}/\text{kg}/\text{day}$ . The plasma vilanterol and GI179710 levels were below the detection limit of 0.206 ng.h/mL.

Interaction between fluticasone and vilanterol on embryo-fetal development: A Segment II study was completed to evaluate potential interactions of fluticasone and vilanterol on embryofetal development in rats. Pregnant rats (18-22/dose, SD) were dosed by nose-only inhalation with different doses and ratios of vilanterol and fluticasone, alone or in combination, during the period of gestation days (GD) 6 - 17. The pulmonary deposited doses ranged from 0 – 9.5  $\mu\text{g}/\text{kg}/\text{day}$  and 0 – 9.8  $\mu\text{g}/\text{kg}/\text{day}$  for fluticasone and vilanterol, respectively. Specifically, the pulmonary deposited doses of fluticasone/vilanterol were 0/0, 8.2/0, 9.4/0.4, 9.5/9.8, 0/8.7, 0.8/0.8 and 3.0/3.2  $\mu\text{g}/\text{kg}/\text{day}$ . The dams were sacrificed on day ? for examination of uterine content. Dams receiving 8.2 – 9.5- $\mu\text{g}/\text{kg}/\text{day}$  fluticasone showed statistically significant decreases ( $p < 0.05$ ) in body weight gains and food consumption. The mean fetal weights in these groups also showed statistically significant decreases in mean fetal weights. The 9.5/9.8 group showed a statistically significant increase ( $p < 0.05$ ) in the incidence of skeletal variations, including the following: unossified, incomplete ossification, and semi-bipartite/bipartite in the 5<sup>th</sup> sternebrae and xiphisternum. No malformations were observed in any of the treatment groups. The study did not show any significant interactions in the effects of fluticasone and vilanterol on embryofetal development.

Summary of Reproductive and Developmental Toxicity of Vilanterol: The reproductive and developmental toxicity of vilanterol, alone or in combination with fluticasone furoate, was evaluated by inhalation, oral, and subcutaneous routes of administration in rats and rabbits. Vilanterol alone did not affect fertility in either males or females at inhalation doses up to 3150  $\mu\text{g}/\text{kg}/\text{day}$  in rats. Vilanterol caused dose-dependent increases in fetal malformations in rabbits and variations in both rats and rabbits. The malformations in rabbits included cleft palate, open or partially opened eyelids, and limb flexure or mal-rotations. The variations in rats included unossified or incomplete ossification of 5<sup>th</sup> sternebra and xiphisternum. The variations in rabbits included cranial bone malformations, bipartite/misshaped interparietal bone, elongated/fused anterior fontanelle, bridge of ossification/fused sternebra center, digit malformations, unossified cervical vertebra centrum, metacarpal forepaws, and hindpaws, and incompletely ossified hyoid skull. These effects occurred at inhalation doses of 3,150 and 575  $\mu\text{g}/\text{kg}/\text{day}$  in rats and rabbits, respectively, and a subcutaneous dose of 300  $\mu\text{g}/\text{kg}/\text{day}$  in rabbits. In rabbits, the 575- $\mu\text{g}/\text{kg}/\text{day}$  inhalation dose and the 300- $\mu\text{g}/\text{kg}/\text{day}$  subcutaneous dose yielded similar AUC values (i.e., 276 – 306 ng.h/mL). No treatment-related fetal effects were observed at maternal inhalation doses of  $\leq 56 \mu\text{g}/\text{kg}/\text{day}$  in rats and  $\leq 59 \mu\text{g}/\text{kg}/\text{day}$  in rabbits, respectively, or  $\leq$  subcutaneous doses of 30  $\mu\text{g}/\text{kg}/\text{day}$  in rabbits.

## 11.2 Fluticasone Furoate

The toxicological profile of fluticasone furoate has been characterized previously. Briefly, fluticasone furoate is non-mutagenic, non-carcinogenic, and non-teratogenic. The effect of

fluticasone furoate on the respiratory system has also been evaluated previously in NDA 22-051. According to the nonclinical review completed by Dr. H. Hao on March 7, 2007, inhalation toxicity studies up to 6 and 9 months in treatment duration were completed in rats and dogs, respectively. These studies showed that fluticasone furoate possessed a toxicity profile typical of glucocorticosteroids, with findings that included the following: 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, and epiphyseal plate retention.

### 11.3 Vilanterol and Fluticasone in combination

There were no significant toxicological interactions between vilanterol and fluticasone after inhalation administration. Potential toxicological interactions between inhaled vilanterol and fluticasone were evaluated in rats and dogs. Animals were exposed by inhalation to dusts containing vilanterol and fluticasone daily for up to 13 weeks. The fluticasone:vilanterol ratio in the test formulation ranged from 2:1 to 10:1 in rats and 2:1 to 60:1 in dogs, respectively. The following is a summary of the pivotal (13 week) toxicological interaction studies in rats and dogs. See the nonclinical review completed by Dr. L. Sancilio on September 30, 2010 in IND 77,855 for additional information.

Report FD2008/00342 evaluated the potential interaction of inhaled vilanterol and fluticasone in combination in rats. Sprague-Dawley rats (12/sex/dose) were dosed via nose-only inhalation with the following doses of fluticasone/vilanterol (pulmonary deposits in  $\mu\text{g}/\text{kg}/\text{day}$ ): 0/0 (lactose), 5.6/0, 0/2.5, 0.79/0.52, 2.0/1.2, 5.4/3.1, or 5.3/0.58. Results showed effects of typical of fluticasone: immunosuppression (lymphoid depletion of the thymus, spleen and lymph nodes) and decreased cellularity in the bone marrow. The results did not indicate any significant toxicological interactions between fluticasone and vilanterol.

Report FD2008/01441 evaluated the potential interaction of inhaled vilanterol and fluticasone in combination in dogs. Beagle dogs (4/sex/dose) were dosed via nose-only inhalation with the following doses of fluticasone/vilanterol (pulmonary deposits in  $\mu\text{g}/\text{kg}/\text{day}$ ): 0/0 (lactose + 1% magnesium stearate), 14.0/0, 0/8.4, 1.73/0.95, 5.1/2.9, 16.0/8.8, or 15.3/0.29. Results showed effects of typical of fluticasone and vilanterol: immunosuppression (lymphoid depletion of the thymus, spleen and lymph nodes), decreased cellularity in the bone marrow, atrophy of the adrenal cortex, and decreases in glycogen in the liver. The results did not indicate any significant toxicological interactions between fluticasone and vilanterol.

### 11.4 Metabolites

Three compounds were identified as major metabolites of vilanterol in humans. They were GW630200 (or M29), GSK932009 (or M33), and GI179720. Each of the human metabolites was found in animals. Specifically, the respective levels at the steady state in the mice, rats, and dogs was 125.1, 1.2, and 16.8  $\mu\text{g}\cdot\text{h}/\text{mL}$  for GSK932009; 4.9, incalculable, and 16.8  $\text{ng}\cdot\text{h}/\text{mL}$  for GW630200; and 38,350, 138, and 188.5  $\text{ng}\cdot\text{h}/\text{mL}$  for GI179710. The human metabolite data at the clinical dose is not as complete as in animals. An estimate only for the sum of GSK932009 and GW630200 (0.488  $\mu\text{L}\cdot\text{h}/\text{mL}$ ) was available.

Animals apparently had significantly higher plasma levels (1.2 – 19.2 µg.h/mL) of GSK932009 and GW630200 than humans (0.488 µg.h/mL). The level of GI179710 in humans is unknown, but it appears unlikely that the humans would have GI179710 levels similar to that of animals. The applicant has adequately addressed metabolite issues.

In summary, the applicant has provided sufficient nonclinical data to characterize the toxicity profile of fluticasone and vilanterol, alone or combination, in animals.

### 11.5 Recommendation:

The nonclinical safety program for fluticasone furoate and vilanterol trifenate inhalation powder is complete.

From a nonclinical perspective, the application is recommended for approval.

No additional nonclinical studies are required.

Luqi Pei, Ph.D.

Pharmacologist/Toxicologist

Marcie Wood, Ph.D.

Pharmacology Supervisor (Acting)

## 12 Appendices

- A. Nonclinical review completed by Dr. L. Sancilio on September 30, 2010 in IND 77,855
- B. Nonclinical review completed by Dr. L. Sancilio on March 5, 2010 in IND 74,696
- C. Nonclinical review completed by Dr. H. Hao on October 29, 2008 in IND 74,696
- D. Nonclinical review completed by Dr. H. Hao on June 3, 2008 in IND 74,696
- E. Nonclinical review completed by Dr. L. Pei on June 7, 2008 in IND (b) (4)

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

**PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION**

Application number: IND 48,647, 70,297, 74,696, 77,855, (b) (4)

Supporting document/s: SDN 35

Sponsor's letter date: 2/19/09

CDER stamp date: 2/20/09

Product: Combination of Fluticasone furoate and  
GW642444

Indication: Treatment of COPD

Sponsor: GlaxoSmithKline

Review Division: Pulmonary, Allergy and Rheumatology Products

Reviewer: Lawrence F. Sancilio, Ph.D.

Supervisor/Team Leader: Molly Topper, Ph.D.

Division Director: Badrul Chowdhury, M.D., Ph.D.

Project Manager: Angela Ramsey

*Template Version: December 7, 2009*

## **Executive Summary**

### **Recommendation**

In the 13-week inhalation combination toxicity studies of GW642444 and fluticasone furoate in rats and dogs with magnesium stearate as an excipient, there was no indication of a drug interaction resulting in new toxicity giving preclinical support to the proposed Phase III clinical combination studies.

### **Brief Discussion of Nonclinical Findings**

Thirteen week combination inhalation toxicity studies involving fluticasone furoate (GW685698) and GW642444 in rats and dogs were submitted for review.

In the 13-week toxicity study in rats, the deposited doses were : Group 1 (control, lactose +1% magnesium stearate), Group 2 (5.64 mcg/ kg of fluticasone furoate alone), Group 3 (2.49 mcg/ kg of GW642444 alone), Group 4 (0.785 mcg/kg of fluticasone furoate +0.524 mcg/kg of GW642444 ), Group 5 (1.98 mcg/ kg of fluticasone furoate +1.17 mcg/kg of GW642444 ), Group 6 (5.38 mcg/kg of fluticasone furoate +3.07 mcg/kg of GW642444) and Group 7 (5.26 mcg/kg of fluticasone furoate + 0.582 mcg/kg of GW642444). For drug interaction evaluation, focus was on the results with Groups 2, 3 and 6.

Fluticasone furoate alone produced the characteristic effects of a glucocorticoid: hair loss, decreased body weight gained and food consumption, decreased white blood cells, lymphocytes, eosinophils, basophils and large unstained cells. These changes were not affected when fluticasone furoate was combined with GW642444.

GW642444 alone accumulated in both sexes; this accumulation did not change when combined with fluticasone furoate. For fluticasone furoate, with and without the presence of GW642444, accumulation occurred in males. In females, fluticasone furoate alone did not accumulate; however, accumulation did occur (1.3 fold increase in AUC vs. a 2.7 fold increase in AUC) when combined with GW642444 indicating that GW642444 altered the pharmacokinetics of the steroid.

The high dose of GW642444 when combined with the high dose of fluticasone furoate and HD of GW642444 modulated the effects of fluticasone furoate (Groups 6 and 3 vs. Group 2) where there was an increase or decrease in the incidence of the histopathology. In males, histopathological changes resulting from combination were increased incidence bronchiolar eosinophilic inclusions in the lungs, increased incidence of epidermal hyperplasia and scabs in the skin, increased incidence of lymphoid depletion of the mesenteric lymph node and decreased incidence of lymphoid depletion of the bronchial lymph node. In females, the changes were: increased incidence of mammary gland secretory activity, increased incidence of decreased BALT in lungs, decreased incidence of lymphoid depletion of the mesenteric and mandibular lymph nodes, decreased

incidence of decreased cellularity of the sternum bone marrow and decrease incidence of dermal inflammation.

No new toxicities emerged with the combination of fluticasone furoate and GW642444 as the changes observed were related to steroid activity. There was no evidence of additive or synergistic toxicity with doses of the combination up to 5.38 mcg/kg of fluticasone furoate +3.07 mcg/kg of GW642444.

In the 13-week toxicity study, dogs were exposed by inhalation (oronasal face mask) to deposited doses of 14 mcg/ kg of fluticasone fuorate (Group 2), 8.38 mcg/ kg of GW642444 (Group 3), 1.73 mcg/kg of fluticasone fuorate +0.95 mcg/kg of GW642444(Group 4), 5.15 mcg/ kg of fluticasone furoate +2.93 mcg/kg of GW642444 (Group 5), 15.98 mcg/kg of fluticasone furoate +8.75 mcg/kg of GW642444 (Group 6), and 15.25 mcg/kg of fluticasone furoate + 0.29 mcg/kg of GW642444 (Group 7). The vehicle (control) was lactose +1% magnesium stearate.

In the combination study, GW642444 did not effect the increased food consumption and changes in the hematology and clinical chemistry (increased cholesterol and triglyceride levels) due to fluticasone furoate nor did the fluticasone furoate affect the cardiac effects of GW642444.

Accumulation occurred with fluticasone furoate and GW642444 alone. The accumulative effect seen with GW642444 was abolished when combined with fluticasone furoate.

The high dose of GW642444 when combined with the high dose fluticasone furoate modulated the effects of fluticasone furoate (Group 6 vs. Group 2) where there was an increase or decrease in the incidence of the histopathology. In males, there was an increased incidence of alveolitis, an increased incidence of decreased centrilobular periportal hepatocyte rarefaction (glycogen), a decreased incidence of increased incidence of hepatic rarefaction (glycogen), an increased incidence of epithelial hypertrophy of the gall bladder, increased incidence of atrophy of the adrenal zona glomerulosa, a decrease incidence of epidermal atrophy, a decreased incidence of renal perivascular/interstitial lymphoid cells, a decreased incidence of epithelial regenerative hyperplasia and mucosal inflammation of the body of the stomach and a decreased incidence of GALT depletion of the cecum.

In females, there was an increased incidence of inflammatory cells in the bronchi at the tracheal bifurcation, an increased incidence of mucosal inflammation of the bronchi, an increased incidence of alveolitis, an increased incidence of decreased centrilobular/increased periportal hepatocyte rarefaction (glycogen), a decreased incidence of increased hepatocyte rarefaction (glycogen), a decreased incidence of gall bladder hypertrophy, a decreased incidence of renal perivascular/interstitial lymphoid cells, a decreased incidence of myofibre atrophy of the skeletal muscle, a decreased incidence of epidermal atrophy, a decreased incidence of decreased cellularity of the sternum bone marrow, an increased incidence of mucosal hypertrophy/epithelial regenerative hyperplasia of the antrum of the stomach, an increased incidence of GALT

depletion of the cecum and an increased incidence of cell infiltration in the mammary gland. The latter toxicity is new resulting from the combined administration of fluticasone furoate and GW642444. However, this finding is not considered clinically significant since the severity was minimal and there was no evidence of surrounding cellular injury in this gland.

There was no evidence of additive or synergistic toxicity resulting from the combination with doses up to 15.98 mcg/kg of fluticasone furoate +8.75 mcg/kg of GW642444. In both rats and dogs, the changes seen were mainly alterations of the steroid effects of fluticasone furoate.

### Drugs:

Trade name: Vilanterol trivenate (GW642444 M)

Generic names: Fluticasone furoate, GW685698, GW64244 M

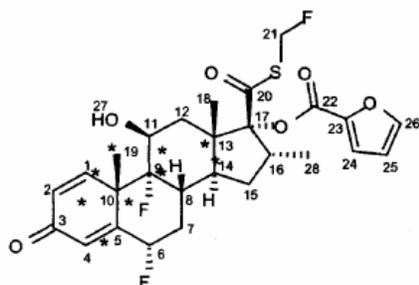
Registry number: GW685698 (fluticasone furoate), 397864-44-7 M

Chemical name: Fluticasone furoate, Androsta-1, 4-diene-17-carbothioic acid, 6, 9-difluoro-17-[(2furanylcarbonyl) oxy]-11-hydroxy-16-methyl-3-oxo-S-(Fluoromethyl) ester, (6a, 11 $\beta$ , 16a, 17a)-(9Cl)

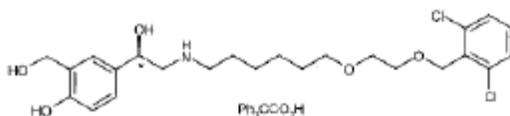
GW642444: Triphenylacetic acid-4-{(1R)-2-[(6-2)2-[2,6-2},dichlorobenzyl)oxy]ethoxy{hexyl)amino]-1-hydroxyethyl{-2-(hydroxymethyl)phenol (1:1).

Chemical structure:

Fluticasone furoate



GW64244 M (salt)



In the toxicity studies, the doses are expressed as the free base (GW642444).

Molecular formula/wt: GW685698, C<sub>27</sub>H<sub>29</sub>F<sub>3</sub>O<sub>6</sub>S/538.58; GW642444, C<sub>24</sub>H<sub>33</sub>C<sub>12</sub>NO<sub>5</sub>.C<sub>20</sub>H<sub>16</sub>O<sub>2</sub>/744.78

Relevant INDs/NDAs/DMFs: IND 70,297, (b)(4), 48,647 and 74,696

Drug class: Fluticasone furoate: Corticosteroid.

GW64244 M: Beta2 agonist.

Indication: Treatment of asthma and COPD.

Clinical formulation: Dry-powder inhaler. The inhaler contains 2 strips. One strip contains a blend of micronized GW685698 (b)(4) and lactose. The second strip contains a blend of micronized GW642444 (40 mcg), magnesium stearate (125 mcg) and lactose.

Proposed route of administration: oral inhalation.

Previous clinical experience: Under IND 70,297, inhalation studies were conducted with GW685698 in adult asthmatics for 8 weeks at a dose of 400 mcg/day. GW642444 at inhalation doses up to 50 mcg/day (AUC 0.33 ng·h/ml) was administered to asthmatic adults for 4 weeks under IND 74,696. A combination (single dose) of 800 mcg of GW685698 and 100 mcg of GW642444 was tested in Australia.

Proposed Clinical Study: Fifty-two weeks 100 mcg of fluticasone furoate and 25 mcg of GW642444 and 200 mcg of fluticasone furoate and 25 mcg of GW642444.

Disclaimer: the reviewer constructs tabular and graphical information unless cited otherwise.

#### **Studies reviewed within this submission:**

Toxicity

Multidose toxicity

13-Week inhalation combination toxicity study of fluticasone furoate and GW642444M in, No. FD2008/00342/00

13-Week inhalation combination toxicity study of fluticasone furoate and GW642444M in dogs, no. FD2008/01441/00

**Studies not reviewed within this submission:** None

#### **Introduction and Drug History**

Fluticasone furoate is an approved drug and is marketed as the active drug in Veramyst nasal spray for the treatment of allergic rhinitis. GW642444, a long acting  $\beta_2$  agonist is being developed alone (IND 74,696) and in combination with fluticasone furoate in this IND for the treatment of asthma and COPD.

In the initial combination study, a clinical 4-week protocol involving the once a day administration of 400 mcg of fluticasone furoate and 25 mcg of GW642444 administered by a novel DPI was approved based on preclinical support for the dose of each monoproduct and combination (5/27/08 Medical review of Anthony Durmowicz).

In the 2/27/09 meeting package, the sponsor proposed Phase III combination studies summarizing the results of the completed 3-month inhalation toxicity studies in rats and dogs and the reproductive toxicity in rats. The Agency indicated that no additional studies were needed to support the proposed combination studies (3/30/10 Fax to the sponsor by Angela Robinson). The 13-week inhalation toxicity studies in rats and dogs are the subject for review in this submission.

## **TOXICOLOGY**

### **Repeat-Dose Toxicity**

#### **Multidose**

**Study title: 13-Week inhalation combination toxicity study of fluticasone furoate (GW685698) and GW642444M in rats**

#### **Key findings:**

- Rats were exposed by nose-only inhalation to deposited doses of 5.64 mcg/ kg of fluticasone fuorate, 2.49 mcg/ kg of GW642444, 0.785 mcg/kg of fluticasone fuorate +0.524 mcg/kg of GW642444, 1.98 mcg/ kg of fluticasone furoate +1.17 mcg/kg of GW642444, 5.38 mcg/kg of fluticasone furoate +3.07 mcg/kg of GW642444) and 5.26 mcg/kg of fluticasone furoate + 0.582 mcg/kg of GW642444. The vehicle (control) was lactose +1% magnesium stearate).
- In females, accumulation developed with fluticasone furoate when combined with GW642444.
- Histopathological changes occurred with fluticasone furoate alone involved the lungs, skin, spleen, thymus, mammary gland, lymph nodes and bone marrow.
- For GW642444 alone, the histopathology involved the lungs, liver, gall bladder, skeletal muscle, kidneys, bronchi and lymph node.
- Combining GW642444 with fluticasone furoate resulted in increasing or decreasing some of the effects of the steroid.
- No new toxicities were observed in rats from combining fluticasone furoate and GW642444.
- No additive or synergistic toxicities resulted from the combination with doses up to 5.38 mcg/kg of fluticasone furoate +3.07 mcg/kg of GW642444.

**Study no:** FD2008/00342/00

**Volume # and page #:** Vol. 1 and page 1.

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 12/6/07

**GLP compliance:** Yes.

**QA report:** yes (X)

**Drug, lot #, and % purity:** The nominal blend concentrations were: 2% w/w

GW685698; 1% W/W GW642444, 2% W/W GW685698 +1% GW642444 and 4% GW685698 +0.4% GW642444 in lactose with 1% magnesium stearate.

**Batch no.** GW685698:061120932; GW642444:071137021.

**Percent purity:** GW685698 and GW642444 ranged from 99.3% to 99.4%

**Control vehicle, Formulation/Vehicle:** 1% magnesium stearate (lot no. 61127066) in lactose (lot no. 071138760). Both lactose and magnesium stearate are approved excipients.

#### Methods

Species/strain: Crl:CD (SD) rats.

#/Sex/group: Main group, 12; toxicokinetics group, 12, 3 were used at each bleeding time.

Weight (range of average weight): M, 199-254 g; F, 139-193 g.

Route: Inhalation; exposure was by ADG nose only snout inhalation chamber for 20, 40 or 60 min/day.

Particle size (range of MMAD and GSD):

GW685698: MMAD, 1.5 to 2.2  $\mu\text{m}$ ; GSD, 2.55-3.05.

GW642444: MMAD, 1.3 to 1.8  $\mu\text{m}$ ; GSD, 2.18-2.63.

Magnesium Stearate: MMAD, 1.5 to 3.5  $\mu\text{m}$ ; GSD, 2.35-3.81.

Doses: The test groups are presented in the following table excerpted from the submission. The HD of 56.4  $\mu\text{g}/\text{kg}$  of GW685698 was selected based on results in the 3 month inhalation toxicity study where 24.3  $\mu\text{g}/\text{kg}$  produced a decrease in body weight gained, and in the 8-week inhalation toxicity study, 72  $\mu\text{g}/\text{kg}$  produced weight loss within the first 2 weeks of dosing. The intermediate and low doses of 20 and 7 mcg/kg of fluticasone propionate were selected since they were similar to the doses used in the rat in the 1 and 6 month studies to explore the dose relationship. At the time of the selection, the final clinical ratios were undecided. The maximum and minimum clinical ratios were anticipated to range from 2:1 to 100:1. The maximum ratio of 56.4  $\mu\text{g}/\text{kg}$  of GW685698 to 5.82  $\mu\text{g}/\text{kg}$  of GW642444 was ultimately selected to achieve the systemic exposure to GW642444 (based on AUC) which was estimated to be comparable to the maximal clinical 25  $\mu\text{g}$  exposure anticipated for the Phase III studies. This provided a dose ratio of 9:1 (Group 7). Other dose ratios of fluticasone furoate to GW642444M tested were: (Group 4, 1.49) and Group 5 (1.69) and Group 6 (1.75).

Group	1	2	3	4	5	6	7
Compound	Control	GW685698	GW642444	GW685698 and GW642444	GW685698 and GW642444	GW685698 and GW642444	GW685698 and GW642444
Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )	0	56.4	24.9	7.85/5.24	19.8/11.7	53.8/30.7	52.6/5.82

The method used to calculate the achieved dose is presented in the following table excerpted from the submission.

Calculation of achieved dosage:<sup>1</sup> The achieved dose of active ingredient (mg/kg/day) for each treatment level was determined as follows:

$$\text{Achieved dose of active ingredient (mg/kg/day)} = \frac{\text{RMV} \times \text{Active Concentration} \times \text{T}}{\text{BW}}$$

Where RMV (L/min) = respiratory minute volume calculated<sup>2</sup>

Active concentration (mg/L) = chamber concentration of active test material determined by chemical analysis

T (min) = treatment time (up to maximum of 120 minutes)

BW (kg) = mean body weight per sex per group from the regular body weight occasions during treatment

<sup>1</sup> Total body dose assuming a deposition fraction of 100%

<sup>2</sup>  $0.499 \times [\text{body weight (kg)}]^{0.809}$  L/min (Bide, R.W. et al 2000). It is assumed that this parameter is unaffected by exposure to the test article

The targeted, achieved and lung deposited doses based on a deposition factor of 10% for the particle size of < 5 µm are presented in the following table.

Group No.	Target Dose µg/kg GW685698	Average Achieved Dose µg/kg GW685698	Deposited Dose µg/kg GW685698	Target Dose µg/kg GW642444	Average Achieved Dose µg/kg GW642444	Deposited Dose µg/kg GW642444
1	0	0	0	0	0	0
2	56	56.4	5.64	0	0	0
3	0	0	0	28	24.9	2.49
4	7	7.85	0.785	3.5	5.24	0.524
5	20	19.8	1.98	10	11.7	1.17
6	56	53.8	5.38	28	30.7	3.07
7	56	52.6	5.26	6	5.82	0.582

Clinical signs: Daily.

Body weights: Day 1, twice weekly and at necropsy.

Food consumption: Prior to and weekly during the treatment.

Ophthalmoscopy: Prior to initiation of treatment and on day 1 of week 13.

Hematology: During week 4 and 13 for male rats and at the scheduled necropsy for female coagulation samples. Blood was collected from the orbital sinus. The analysis was complete.

Clinical chemistry: During week 4 and 13 and at scheduled necropsy. The analysis was complete.

Urinalysis: During week 4 and 13.

Toxicokinetics: Blood (0.5 ml) was collected from the tail vein on day 1 and during weeks 4 and 13 of dosing. Two samples were taken from each rat on sampling occasion, with 3 rats/sex/group being bled immediately after the end of the inhalation exposure and at 0.5, 1, 2, 4, 8, 12 and 23 hours. The plasma was assayed by HPLC/MS/MS analysis. The lower limits of quantification were 100 pg/ml for GW642444 and 40 pg/ml for GW685698.

Gross pathology: Week 13 and at the end of the recovery period. A full necropsy was conducted.

Organs weighed: Relative to body weight was not meaningful in view of the marked decreased body weight gained by fluticasone furoate.

Histopathology: Tissues processed are listed in the following table excerpted from the submission. Tissues from Groups 2, 3 and 6 and all unscheduled deaths and macroscopic abnormalities from the toxicology animals with a marked X were examined. Tissues from Groups 4, 5 and 7 with a marked X<sup>s</sup> were also examined. Administration in this study was by the nose only route; consequently, histopathology of the nasal cavity was not reviewed since this was not relevant to the proposed clinical oral inhalation route.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Pituitary	X
Adrenals	X <sup>s</sup>	Preputial/clitoral gland	
Aorta (thoracic)	X	Prostate	X
Brain	X	Rectum	
Caecum	X	Salivary gland	
Colon	X	submandibular	X
Duodenum	X	sublingual	X
Epididymides	X	parotid	X
Eyes/Optic nerves	X	Sciatic nerve	X
Femur (Femoro-tibial joint)	X <sup>s</sup>	Seminal vesicles	X
Harderian glands		Skeletal muscle (hindlimb)	X <sup>s</sup>
Heart	X	Skin	X <sup>s</sup>
Ileum	X	Spinal cord	
Jejunum	X	cervical	
Kidneys	X <sup>s</sup>	thoracic	
Larynx	X <sup>s</sup>	lumbar	X
Liver (all main lobes)	X	Spleen	X <sup>s</sup>
Lung including bronchi <sup>a</sup>	X <sup>s</sup>	Sternum with bone marrow	X <sup>s</sup>
Lymph node -		Stomach	X
mandibular	X <sup>s</sup>	Teeth <sup>b</sup>	X <sup>s</sup>
mesenteric	X <sup>s</sup>	Testes	X
tracheobronchial	X <sup>s</sup>	Thymus	X <sup>s</sup>
Mammary glands (inguinal area)	X	Thyroids	X
Nasal cavities and nasopharynx with skull	X <sup>s</sup>	Tongue	X
Oesophagus	X	Trachea	X
Ovaries	X	Tracheal Bifurcation (with main stem bronchi)	X
Pancreas	X	Urinary bladder	X
Parathyroids	X	Uterus with cervix	X
		Vagina	X

<sup>a</sup> At least 4 lobes examined, left and right, including proximal and distal area

<sup>b</sup> Tissue processed and examined in response to macroscopic findings at the request of the Sponsor.

Adequate Battery: yes (X), no ( )—explain

Peer review: yes (X), no ( )

## Results

For drug interaction, comparisons were made between Group 2 (high dose of steroid alone) and Group 6 (high dose of steroid and high dose of  $\beta_2$  agonist combination) and Group 3, the  $\beta_2$  agonist alone high dose. This is supported in the incidence in Group 7 (HD of fluticasone furoate and a low dose of GW642444) to be less than that observed in Group 6. For Groups 4, 5 and 6, the combination is a dose related increase; activity is present if there is a dose related change in the incidence.

Mortality: One female animal in the group 2 (HD, GW685698 alone) was found dead on day 74. Examination revealed congested lungs and bronchi, enlarged mandibular lymph node and a pale liver. A relationship of death to treatment cannot be excluded.

Clinical signs: Dose related increased incidence of head hair loss was seen in the HD steroid (fluticasone furoate) treated animals alone and in combination with GW642444 as presented in the following table. GW642444 alone (Group 3) did not cause hair loss and did not affect this increased incidence of hair loss. There was a dose related increase in both sexes (Groups 4, 5 and 6). These effects were attributed to fluticasone furoate and there was no evidence of a drug interaction.

Sex	Incidence , N= 12/Sex/Group/						
	Group1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Male	3	7	3	3	4	11	8
Female	2	11	2	3	4	10	12

Body weights gained: The results are summarized in the following table. The HD of fluticasone furoate produced a marked decrease in body weight gained that was not affected when combined with GW642444 (Group 2 vs. Groups 6 and 7). GW642444 alone had no effect on the body weight gained. The lower doses of fluticasone furoate manifested modest decreases in body weight gained as compared with the HD of the fluticasone furoate alone. There was no indication of a drug interaction between the two compounds.

Group No.	Percent Change from Control, * p<0.05	
	Males	Females
2	-46*	-72*
3	-2	0
4	-16	-19*
5	-24*	-29*
6	-43*	-56*
7	-49*	-70*

\*p <0.05)

Food consumption: The results are summarized in the following table. The HD fluticasone furoate alone (Group 2) in both sexes decreased food consumption while GW642444 alone had no effect. The lower doses of the steroid in combination with GW642444 showed a modest decrease. There was no evidence of a drug interaction.

Group No.	Percent Change from Control, * p<0.05	
	Males	Females
2	-12*	-13*
3	-1	0
4	-6	-4
5	-9	-3
6	-10	-6
7	-13*	-11

Ophthalmoscopy: No effect.

Hematology: The results are presented in the following table. The results on the effect on white blood cells are presented in the following table. GW642444 alone (Group 3) produced a slight increase in leukocytes in males. In both sexes, fluticasone furoate alone (Group 2) significantly decreased to the same degree the levels of white blood cells, lymphocytes, eosinophils, basophils and large unstained cells and in combination at the same dose of fluticasone furoate with GW642444 (groups 6 and 7). In males, the neutrophils response was not dose related (Group 2 vs. Groups 6 and 7). The increased effect on the basophils by GW642444 in females (Groups 6 and 7) was not significant in view of the low number of cells in the group receiving the steroid alone. On the basis of a dose response (Groups 4, 5 and 6), there was a decrease in white blood cells (males and females) and in lymphocytes (males and females).

Sex/Parameter	Percent Change from Control, P<0.05					
	GP2	GP3	GP4	GP5	GP6	GP7
<b><u>Males</u></b>						
White blood cells	-43	+1	0	-23	-41	-58
Neutrophils	0	-6	+15	0	+35	+76
Lymphocytes	-59	+2	0	-31	-56	-59
Eosinophils	-57	+7	0	0	-57	-64
Basophils	-50	0	0	-50	-50	-50
Monocytes	0	0	0	0	-28	0
Large Unstained Cells	-58	+33	0	0	-58	-42
APTT	0	-14	-14	-14	0	0
<b><u>Females</u></b>						
White blood cells	-48	-11	0	0	-51	-56
Neutrophils	0	0	0	0	0	0
Lymphocytes	-58	-9	0	-30	-58	-62
Eosinophils	-46	-8	0	0	-47	-38
Basophils	-50	0	0	0	-100	-100
Monocytes	0	0	0	0	0	0
Large Unstained cells	-50	+10	0	0	-60	-70
APTT	0	0	0	0	0	0

Clinical chemistry: The results are summarized in the following table. The increase in the AST in the combination Group 6 in males may be attributed to an additive effect Group 2 (fluticasone furoate alone) and GW642444M alone (Group 3). However, these increases are not toxicologically significant as they were not > 2.5 times the control values.

Sex/Parameter	Percent Change from Control, P<0.05					
	GP2	GP3	GP4	GP5	GP6	GP7
<b><u>Males</u></b>						
AST	+26	+34	+32	+54	+46	+21
<b><u>Females</u></b>						
AST	NS	NS	NS	NS	+32	NS

**NS, P>0.05**

Urinalysis: The results are summarized in the following table. GW642444 (Group 6) interacted with GW685698 in males by decreasing the increased excretion of total protein and decreased the decreased excretion of TCRR (total creatinine). In females, the high

dose combination group (Group 6) increased the decreased total protein excretion and reduced the decrease in urine volume and decreased excretion of TCRR. These changes were not compound related in view of the lack of a dose response.

Sex/Parameter	Percent Change from Control, P<0.05			
	Gp 2	Gp 3	Gp 6	Gp 7
<b><u>Males</u></b>				
Total protein	+51	0	+2	+33
TCRR	-29	-14	-6	+31
Volume	0	+18	+3	0
<b><u>Females</u></b>				
Total protein	-14	-51	-49	0
Volume	-42	0	-2	0
TCRR	-39	-24	-18	-26

Toxicokinetics: The results are summarized in the following tables excerpted from the submission. The data presented are normalized and not absolute AUC and Cmax values. GW642444M alone (Group 3) showed accumulation (increased AUC) from day 1 to week 13 in both sexes. This accumulative effect decreased when GW642444 was combined with fluticasone furoate (males, Group 3, 3.3 fold increase vs. Group 6, 2.7 fold increase; females, Group 3, 4.7 fold increase vs. Group 6, 2.7 fold increase); however, this was not considered significant since the difference was not decreased two fold, the acceptable criterion for antagonism. There was no accumulation or insufficient data to determine the effect on toxicokinetics of GW642444M and fluticasone furoate in the lower doses groups. For fluticasone furoate alone, there was accumulation in males that did not change when combined with GW642444 (Group 2, 2 fold increase vs. Group 6, 2.4 fold increase). In females, there was an indication of accumulation of fluticasone furoate only when combined with GW642444 (GW642444 (Group 2, 1.3 fold increase vs. Group 6, 2.7 fold increase) indicating that GW642444 altered the pharmacokinetics of fluticasone furoate.

A summary of the plasma toxicokinetic parameter values for GW642444 corrected for the overall estimated achieved doses, are presented below:

Dose-Normalised Parameter		Males				
		Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )				
		24.9	5.24	11.7	30.7	5.82
		Group 3	Group 4	Group 5	Group 6	Group 7
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	1.82	NC	NC	0.706	NC
	Week 4	3.01	1.10	NC	1.72	NC
	Week 13	6.05	NC	0.503	1.90	NC
C <sub>max</sub> (ng/mL)	Day 1	0.697	0.126	0.129	0.461	NC
	Week 4	0.921	0.828	0.374	1.17	0.122
	Week 13	1.67	NC	0.410	0.491	0.157

NC Not calculated due to insufficient data

Dose-Normalised Parameter		Females				
		Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )				
		24.9	5.24	11.7	30.7	5.82
		Group 3	Group 4	Group 5	Group 6	Group 7
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	1.29	NC	NC	0.645	NC
	Week 4	2.04	1.26	NC	1.84	NC
	Week 13	6.05	NC	0.597	2.24	NC
C <sub>max</sub> (ng/mL)	Day 1	0.672	NC	NC	0.461	0.140
	Week 4	0.647	0.943	0.257	1.54	0.192
	Week 13	2.22	NC	0.328	0.829	NC

NC Not calculated due to insufficient data

A summary of the plasma toxicokinetic parameter values for GW685698 corrected for the overall estimated achieved doses, are presented below:

Dose-Normalised Parameter		Males				
		Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )				
		56.4	7.85	19.8	53.8	52.6
		Group 2	Group 4	Group 5	Group 6	Group 7
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	0.959	NC	NC	0.699	1.42
	Week 4	0.620	0.856	0.436	1.40	1.53
	Week 13	1.92	NC	0.297	1.67	1.42
C <sub>max</sub> (ng/mL)	Day 1	0.338	NC	0.0792	0.215	0.368
	Week 4	0.226	0.400	0.139	0.538	0.368
	Week 13	0.508	NC	0.158	0.377	0.316

NC Not calculated due to insufficient data

Dose-Normalised Parameter		Females				
		Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )				
		56.4	7.85	19.8	53.8	52.6
		Group 2	Group 4	Group 5	Group 6	Group 7
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	1.52	NC	0.0990	0.538	1.10
	Week 4	1.52	0.918	0.257	1.35	1.58
	Week 13	1.92	NC	0.475	1.45	1.21
C <sub>max</sub> (ng/mL)	Day 1	0.338	NC	0.0792	0.215	0.263
	Week 4	0.451	0.550	0.119	0.538	0.473
	Week 13	0.508	NC	0.158	0.430	0.316

NC Not calculated due to insufficient data

Organ weight: Not determined as the data were expressed as group mean unadjusted and adjusted values and not adequately described. Data relative to body weight was presented, but not analyzed.

Macroscopic pathology: The results are presented in the following table. Fluticasone furoate alone (Group 2) in both sexes produced hair loss and scabs. GW642444 did not produce this effect. Maximum hair loss incidence occurred with fluticasone furoate alone and in the combination groups (Group 6 and 7) containing the high dose of fluticasone furoate. However, there was an increased incidence of scabs in these two male groups (Groups 6 and 7) that was not dose related. In both sexes, there was a dose related increase in hair loss (Groups 4, 5 and 6).

Organ Observation	Incidence N=12/group						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
<u>Males</u>							
Skin							
Hair loss	0	11	1	5	10	12	12
Scab	0	4	0	2	1	6	7
<u>Females</u>							
Skin							
Hair loss	1	11	2	7	8	12	11
Scab	1	4	0	0	2	4	3

Histopathology: The results are summarized in the following tables. Activity was based on mainly on the incidence and to a lesser degree on the severity score. The following scoring system was used to describe the severity of the pathology: 1, minimal; 2, slight; 3 moderate; 4, marked; 5, severe. Activity was present if there was an increased incidence by two above or below the reference group or if there was a difference in severity.

### Males

Fluticasone furoate alone (Group 1 vs. Group 2)

There were increase incidences of the following: foamy alveolar macrophages (not dose related but related to increased severity), decreased BALT (bronchial associated lymphoid tissue), and bronchiolar eosinophilic inclusions in lungs, epidermal hyperplasia of the skin, dermal scabs, lymphoid depletion of the spleen and thymus and mesenteric, mandibular and bronchial lymph nodes and decreased cellularity of the sternum bone marrow.

GW642444 alone (Group 1 vs. Group 3)

The following changes occurred: decreased incidence of aggregated foamy alveolar macrophages (severity was similar) and increased incidence of macrophages in the lungs and increased incidence of renal interstitial inflammatory cells.

High dose of fluticasone furoate alone and GW642444 alone vs. the high dose combination (Groups 2 and 3 vs. Group 6)

The following changes occurred: increased incidence bronchiolar eosinophilic inclusions in the lungs, increased incidences of epidermal hyperplasia and scabs in the skin,

increased incidence of lymphoid depletion of the mesenteric lymph node and decreased incidence of lymphoid depletion of the bronchial lymph node.

Dose related effect with Groups 4, 5 and 6.

The following dose related effects were observed: increased incidence of decreased BALT, epidermal hyperplasia, scabs, lymphoid depletion of the spleen, thymus and mandibular and mesenteric lymph nodes and decreased incidence of cellularity of the sternum bone marrow. These effects were attributed to the steroid, fluticasone furoate .

High dose of fluticasone furoate vs. high dose of fluticasone furoate + low dose of GW642444 (Group 2 vs. Group 7)

There were changes in the following incidences: aggregated foamy macrophages, increase incidence of eosinophilic inclusions in the lungs, increase incidence of epidermal hyperplasia and scabs in the skin, increase incidence of lymphoid depletion of the spleen, and the mesenteric lymph node, decrease incidence of lymphoid depletion of the bronchial lymph node and decrease incidence of decreased cellularity of the sternum bone marrow. These effects were attributed to the steroid, fluticasone furoate

There was no new toxicity in males resulting from the combination of fluticasone furoate and GW642444. Effects were primarily attributed to fluticasone furoate and the combination resulted in the modulation of the effects of the steroid.

### Females

Fluticasone furoate alone (Group 1 vs. Group 2)

The following increased incidences occurred: secretory activity of the mammary gland, aggregates of foamy macrophages, bronchiolar eosinophilic inclusions and decreased BALT in the lungs, lymphoid depletion of the spleen, thymus and mesenteric, mandibular and bronchial lymph nodes, decreased anagen hair follicles, dermal inflammation, epidermal hyperplasia, scabs and decreased cellularity of the sternum and femur joint bone marrow. In many of these changes, there was accompanying increased severity.

GW642444 alone (Group 1 vs. Group 3)

The following changes occurred: increased incidences of aggregates of foamy alveolar macrophages in lungs and decreased incidence of decreased anagen hair follicles.

High dose of fluticasone furoate and high dose of GW642444 vs. the high dose combination (Groups 2 and 3 vs. Group 6).

The following changes occurred: increased incidence of mammary gland secretory activity, an increased incidence of decreased BALT in lungs, decreased incidence of lymphoid depletion of the mesenteric and mandibular lymph nodes, decreased incidence of decreased cellularity of the sternum marrow and decrease incidence of dermal inflammation. These were primarily attributed to the steroid, fluticasone furoate.

Dose related effect with Groups 4, 5 and 6.

There was dose related increased incidence of the following: secretory activity of the mammary gland, aggregated foamy alveolar macrophages, bronchiolar eosinophilic inclusions and decreased BALT in lungs, lymphoid depletion of the spleen, thymus and mandibular and mesenteric lymph nodes, decreased anagen hair follicles, epidermal hyperplasia and decreased cellularity of the sternum and femur bone joint bone marrow. These effects were primarily attributed to a change in the incidence of steroid activity.

Fluticasone furoate vs. Fluticasone furoate + GW642444 (Group 2 vs. Group 7)

There were changes in the following: increased incidences of secretory activity of the mammary gland, increased incidence of decreased BALT, increased incidence of scabs, decreased incidence of lymphoid depletion of mandibular lymph node, increased incidence of lymphoid depletion of the thymus and bronchial lymph node, and increased incidence of decreased cellularity of the femur bone joint bone marrow. These were primarily attributed to the modulation of the steroid effect.

There was no new toxicity in females resulting from the combination of fluticasone furoate and GW642444.

Organ Observation	Males, Incidence and Average Severity Score, N=12/group						
	GP1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Lungs							
Aggregated foamy Alveolar macrophages	8	9	5	11	12	10	12
Avg. severity score	1.0	1.8	1.0	1.3	1.1	1.7	2.4
Alveolar macrophages	4	2	8	4	0	3	1
Avg. severity score	1.0	1.0	1.0	1.0		1.0	1.0
Decreased BALT <sup>a</sup>	1	12	0	6	11	11	12
Avg. severity score	1.0	4.0		2.5	2.8	2.5	4.0
Bronchiolar eosinophilic Inclusions	0	9	0	10	10	11	12
Avg. severity score		1.0		1.0	1.0	1.0	1.0
Kidneys							
Interstitial inflam. cells	1	1	4	1	1	0	0
Avg. severity score	1.0	1.0	1.0	1.0	1.0		
Skin							
Epidermal hyperplasia	0	2	0	2	2	5	7
Avg. severity score		1.5		1.5	1.5	2.2	1.4
Scab(s)	0	2	0	2	2	4	6
Avg. severity score		1.0		1.0	1.0	1.3	1.1
Spleen							
Lymphoid depletion	0	10	0	0	7	10	12
Avg. severity score		2.3			1.6	1.5	2.3
LN Mesenteric							
Lymphoid depletion	0	6	0	0	4	9	8
Avg. severity score		1.5			1.3	1.3	1.3
Thymus							
Lymphoid depletion	0	12	1	5	9	12	12
Avg. severity score		1.6	1.0	1.2	1.1	1.6	2.0
LN Mandibular							
Lymphoid depletion	2	6	0	0	0	5	5
Avg. severity score	1.0	1.1				1.2	1.0
LN Bronchial							
Lymphoid depletion	0	6	0	0	5	4	4
Avg. severity score		1.3			1.4	1.0	1.8
Sternum+ marrow							
Decreased cellularity	0	7	0	2	2	7	5
Avg. severity score		1.0		1.0	1.0	1.0	1.0

<sup>a</sup> Bronchial Associated Lymphoid tissue

Organ Observation	Females, Incidence N=12/group						
	Gp1	Gp2 <sup>a</sup>	Gp3	Gp4	Gp5	Gp6	Gp7
Mammary gland							
Secretory activity	1	7	1	5	7	12	11
Avg. severity score	1.0	1.7	1.0	2.2	1.1	1.5	1.4
Lungs							
Aggregates of foamy macrophages	6	11	8	8	12	11	12
Avg. severity score	1.1	1.5	1.0	1.0	1.3	1.3	1.5
Bronchiolar eosinophilic Inclusions	0	9	0	4	10	9	8
Avg. severity score		1.0		1.0	1.0	1.0	1.0
Decreased BALTB <sup>b</sup>	0	10	0	6	10	12	12
Avg. severity score		4.2		2.3	2.1	3.8	3.3
Spleen							
Lymphoid depletion	0	10	1	0	4	9	9
Avg. severity score		1.5	1.0		2.3	1.3	1.5
LN Mesenteric							
Lymphoid depletion	0	7	1	0	3	5	6
Avg. severity score		1.1	1.0		1.0	2.2	1.7
Thymus							
Lymphoid depletion	1	9	0	1	0	10	11
Avg. severity score	1.0	1.4		1.0		1.0	1.3
LN Mandibular							
Lymphoid depletion	0	6	0	0	1	3	3
Avg. severity score		1.0			1.0	1.7	1.0
Skin							
Decreased anagen hair follicles	5	11	3	5	9	11	11
Dermal inflammation	1	5	0	1	3	2	4
Avg. severity score	1.0	1.2		1.0	1.7	1.0	1.0
Epidermal hyperplasia	1	6	1	4	4	7	6
Avg. severity score	2.0	1.8	1.0	1.3	1.8	1.3	1.7
Scab(s)	1	4	0	6	4	3	7
Avg. severity score	2.0	1.3		1.0	1.7	1.7	1.0
Sternum+ marrow							
Decreased cellularity	0	9	1	1	7	7	9
Avg. severity score		1.1	1.0	1.0	1.0	1.0	1.1
Femur Joint+ marrow							
decreased cellularity	1	8	1	1	5	7	10
Avg. severity score	1.0	1.8	1.0	2.0	1.0	1.1	1.4
LN Bronchial							
Lymphoid depletion	0	6	0	1	0	5	8
Avg. severity score		1.3		1.0		1.2	1.3

<sup>a</sup>Group 2 had 11 animals.

<sup>b</sup> Bronchial Associated Lymphoid tissue

**Study title: 13-Week inhalation combination toxicity study of fluticasone furoate (GW685698) and GW642444 in dogs****Key findings:**

- Dogs were exposed by inhalation (oronasal face mask) to deposited doses of 14 mcg/kg of fluticasone furoate, 8.38 mcg/kg of GW642444, 1.73 mcg/kg of fluticasone furoate +0.95 mcg/kg of GW642444, 5.15 mcg/kg of fluticasone furoate +2.93 mcg/kg of GW642444, 15.98 mcg/kg of fluticasone furoate +8.75 mcg/kg of GW642444 and 15.25 mcg/kg of fluticasone furoate + 0.29 mcg/kg of GW642444. The vehicle (control) was lactose +1% magnesium stearate.
- Accumulation occurred in both sexes with fluticasone furoate alone and in combination with fluticasone furoate. With GW642444 accumulation occurred alone in both sexes and in females in combination with fluticasone furoate, but not in males.
- The histopathology that occurred with fluticasone furoate alone involved the lungs, bronchi, lymph nodes, spleen, liver, gall bladder, kidneys, thymus, skeletal muscle, skin, sternum bone marrow, adrenal glands, stomach, tonsils, cecum, ileum and bronchi.
- For GW642444 alone, the histopathology involved the lungs, liver, gall bladder, skeletal muscle, kidneys, bronchi and lymph nodes.
- In the combination study, there was some modulation of the effects of fluticasone furoate.
- There was no evidence of additive and synergistic toxicities with doses of the combination up to 15.98 mcg/kg of fluticasone furoate +8.75 mcg/kg of GW642444.

**Study no.** WD2008/01441/00

Volume # and page #: 2 and p, 379.

**Conducting laboratory and location:** (b)(4)**Date of study initiation:** 1/24/08**GLP compliance:** Yes.**QA report:** yes (X)

**Drug, batch #, and % purity:** Batch No.:GW642444, 071137021; Fluticasone furoate, 061120932. The nominal blend concentrations were: 4% w/w GW685698, 2%w/w GW642444, 4% w/w GW685698 +2% GW642444 and 24% GW685698 +0.4% GW642444 in lactose with 1% magnesium stearate.

**Percent purity:** GW685698 and GW642444 ranged from 99.3% to 99.4%**Control vehicle,** Formulation/Vehicle : Lactose batch no.000016328 and 1% w/w magnesium stearate, batch no. C611967.

Methods

Species/strain: Beagle dogs

#/Sex/group: 4

Weight: Males 9.9 to 15.9 kg; females, 6.7-12.1 kg.

Age: Males and females, 11-14 months.

Route: Inhalation; exposure was by oronasal face mask for 30 min/day. Aerosols were generated (b) (4)

Particle size (range of MMAD and GSD):

GW685698: MMAD, 2.6 to 3.0 µm; GSD, 2.27-2.87.

GW642444: MMAD, 2.0 to 2.7 µm; GSD, 2.09-2.78.

Magnesium Stearate: MMAD, 2.0 to 2.6 µm; GSD, 2.41-2.91.

Doses: The treated groups are presented in the following table excerpted from the submission. The 60 mcg/kg target dose was selected as the HD alone and in combination with GW642444. In the 3- month inhalation toxicity study in dogs, 65 mcg/kg was well tolerated and in the 9-month inhalation toxicity study, 60 mcg/kg was well tolerated.

Four combination groups, three at the anticipated minimum clinical ratio of 2:1 and one at the maximum feasible ratio that achieved systemic exposure (based on AUC estimates) of at least parity with the clinical ratio.

Doses: The method used to calculate the achieved dose is presented in the following table excerpted from the submission.

Calculation of achieved dosage: <sup>1</sup>	The achieved dose of active ingredient (mg/kg/day) for each treatment level was determined as follows:
	Achieved dose of active ingredient (mg/kg/day) = $\frac{RMV \times \text{Active Concentration} \times T}{BW}$
	Where RMV (L/min) = respiratory minute volume calculated <sup>2</sup>
	Active concentration (mg/L) = chamber concentration of active test material determined by chemical analysis
	T (min) = treatment time (up to maximum of 120 minutes)
	BW (kg) = mean body weight per sex per group from the regular body weight occasions during treatment
	<sup>1</sup> Total body dose assuming a deposition fraction of 100%
	<sup>2</sup> $0.499 \times [\text{body weight (kg)}]^{0.809}$ L/min (Bide, R.W. et al 2000). It is assumed that this parameter is unaffected by exposure to the test article

The targeted, achieved and lung deposited doses based on a deposition factor of 25% are presented in the following table.

Group No.	Target Dose $\mu\text{g}/\text{kg}$ GW685698	Average Achieved Dose $\mu\text{g}/\text{kg}$ GW685698	Deposited Dose $\mu\text{g}/\text{kg}$ GW685698	Target Dose $\mu\text{g}/\text{kg}$ GW642444	Average Achieved Dose $\mu\text{g}/\text{kg}$ GW642444	Deposited Dose $\mu\text{g}/\text{kg}$ GW642444
1	0	0	0	0	0	0
2	60	56.1	14.0	0	0	0
3	0	0	0	30/36 <sup>a</sup>	33.5	8.38
4	7	6.92	1.73	3	3.81	0.95
5	20	20.6	5.15	10	11.7	2.93
6	60	63.9	15.98	30	35.0	8.75
7	60	61.0	15.25	1	1.17	0.29

<sup>a</sup> Increase from week 6 in males and from week 4 for females to be comparable with the achieved GW642444 concentration for group 6.

Clinical signs: Prior to and post dosing.

Body weights: Day 1, weekly and at necropsy.

Food consumption: Prior to and weekly during the treatment.

Ophthalmoscopy: Prior to treatment and during weeks 4 and week 13.

Electrocardiography: Prior to treatment and during weeks 4 and week 13. Recordings were made prior to and between immediately after and 1 hour after dosing.

Femoral pulse: Day 1 and weeks 4 and 13: predose, immediately after the end of the inhalation exposure and at 0.5, 1, 2, 4, 8, 12 and 23 hours; days 2-7, pulse was measured at 0.5, 1, 2, 4, 8 and 23 hours.

Hematology: Blood (1 ml) was collected from the jugular vein prior to initiation of dosing and prior to dosing during weeks 4 and 13.

A complete hematology battery was assessed.

Clinical chemistry: Prior to initiation of dosing and prior to dosing during weeks 4 and 13. A complete clinical chemistry battery was assessed.

Plasma cortisol assay: Pre- and post Synacthen assessment was conducted prior to initiation of the study. In the study, blood cortisol levels were determined prior to and 1.5 hr after administering Synacthen (250 mcg) intravenously. Blood (2 ml) was collected from the jugular or cephalic veins.

Troponin I: Blood (1 ml) was collected prior to dosing, immediately after dosing, 4, 8 and 23.5 hr after dosing on day 1 and during weeks 4 and 13.

Urinalysis: Once prior to initiation of dosing and prior to dosing during weeks 4 and 13.

Toxicokinetics: Blood (1.0 ml) was collected on day 1 and weeks 4 and 13 of dosing. Samples were taken from each dog immediately after the end of the inhalation exposure and at 0.5, 1, 2, 4, 8, 12 and 23 hours. The lower limit of quantification for GW642444 was 100 pg/ml and for GW685698, 40 pg/ml.

Gross pathology: Week 13 and at the end of the recovery period. A full necropsy was conducted.

Organs weighed Adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, testes and thymus.

Histopathology: Tissues processed are listed in the following table excerpted from the submission. All tissues marked X from groups 1, 2, 3 and 6 were examined. Samples of abnormalities, adrenals, cecum, gall bladder, liver, lymph nodes, nasal cavity, skeletal muscle, skin, spleen, sternum, stomach, thymus and tonsils were processed and examined from all animals in groups 4, 5 and 7. Administration in this study was by the oronasal face mask; consequently, histopathology of the nasal cavity was not reviewed since this was not related to the proposed clinical oral inhalation route.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Optic nerves	X
Adrenals	X	Ovaries	X
Animal ID (tattoo)	X	Pancreas	X
Aorta (thoracic)	X	Parathyroids	X
Bone marrow smear (for clinical pathology)		Pituitary	X
Brain	X	Prostate	X
Caecum	X	Rectum	
Cervix	X	Salivary glands -	
Colon	X	mandibular <sup>b</sup>	X
Duodenum	X	parotid <sup>b</sup>	X
Epididymides	X	Sciatic nerve <sup>a</sup>	X
Eyes	X	Skeletal muscle <sup>a</sup>	X
Femur (femoral head)	X	Skin	X
Gall bladder	X	Spinal cord -	
Heart	X	cervical	
Ileum	X	lumbar	X
Jejunum	X	Spleen	X
Kidneys	X	Sternum with bone marrow	X
Larynx	X	Stomach	X
Liver (two lobes)	X	Testes	X
Lung <sup>c</sup>	X	Thymus	X
Lymph node -	X	Thyroids	X
cervical	X	Tongue	X
mesenteric	X	Tonsils	X
popliteal	X	Trachea	X
tracheo-bronchial	X	Tracheal bifurcation (with main stem bronchi)	X
Mammary gland (inguinal)	X	Urinary bladder	X
Nasal cavities and nasopharynx with skull	X	Uterus	X
Oesophagus (distal portion)	X	Vagina	X

a. Only one taken per animal

b. Only one examined

c. All lobes retained, at least 6 lobes examined, preferably left and right, including proximal and distal areas

Adequate Battery: yes (X), no ( )—explain

Peer review: yes (X), no ( )

## Results

Clinical signs: None due to treatment.

Body weight gain: No statistics were conducted. The results are summarized in the following table. Fluticasone furoate alone in females in contrast to males produced an unexpected increase in body weight. In both sexes, GW642444 increased body weight

gained. Based on the mean and standard deviation, there is no drug interaction regarding body weight gained when fluticasone furoate was combined with GW642444.

Sex	Body Weight Gained $\pm$ SD, kg						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Male	0.6 $\pm$ 0.3	-0.2 $\pm$ 1.3	1.4 $\pm$ 0.34	-0.2 $\pm$ 0.54	-0.1 $\pm$ 0.54	0.8 $\pm$ 0.99	0.4 $\pm$ 0.67
Female	1.1 $\pm$ 0.51	2.5 $\pm$ 0.82	2.1 $\pm$ 0.48	0.5 $\pm$ 0.47	0.8 $\pm$ 0.64	1.6 $\pm$ 1.26	2.0 $\pm$ 0.90

Food consumption: The results are presented in the following table. In both sexes, there was no indication of a drug interaction from the combination. Females overall showed greater food consumption than males.

Sex	% Change from Control					
	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Male	+6	+4	+1	+3	+4	+3
Female	+26	+8	+16	+11	+27	+20

Ophthalmoscopy: There was no effect by fluticasone furoate and GW642444 alone and when administered in combination.

Electrocardiography: The following table presents the change in heart rate at 60 minutes post dose from the redoes expressed as times the predose (X Predose) at weeks 4 and 13. There was no drug interaction relative to the tachycardia when fluticasone furoate was combined with GW642444M (Group 2 vs. Group 6) and there was no indication of an effect with the other combination groups (Group 4, 5 and 7).

Group	Change in Heart Rate Expressed as X Predose			
	Week 4		Week 13	
	Male	Female	Male	Female
1	0.9	1.1	1.0	1.1
2	0.9	1.0	1.0	1.0
3	1.3	1.6	1.4	1.7
4	0.9	1.2	1.0	1.1
5	1.1	1.2	1.2	1.1
6	1.2	1.8	1.5	1.6
7	1.0	1.3	0.9	1.2

Femoral pulse: The following two tables excerpted from the submission presents the effect of combining fluticasone furoate with GW642444M on the pulse rate determined on days 1, 2, 3, 4, 5, 6, 7 and weeks 4 and 13. With each group, the pulse rate varied throughout the study. The highest change in pulse rate relative to predose with the highest dose of GW642444M (Group 2) occurred at day 1 in males (1.62X) and on day 7 in females (1.68X). The increase in females was not sustained to indicate a drug interaction. During the 13 weeks, the average pulse rate in males fluctuated but never exceeded this level when the 2 compounds were combined (Group 6) at these doses. By week 13, there

was no indication that the steroid inhibited the GW642444M induced increased pulse rate. Further, there was no indication of an effect with combination Groups 4, 5 and 7. The time for the pulse rate to return to predose values was highly variable making it not possible to draw a conclusion regarding a drug interaction on this parameter.

Dose GW685698/ GW642444 (mg/kg/day)	Average predose pulse rate (bpm)		Maximum pulse rate observed (bpm)		Change in maximum pulse rate (X predose)		Time maximum pulse rate observed (hours after completion of dosing)		Time pulse rate returned to predose values (hours after completion of dosing)	
	male	female	male	female	male	female	male	female	male	female
Day 1										
0/0	105	108	107	110	1.02	1.02	IAD	2	IAD <sup>c</sup>	2 <sup>c</sup>
56.1/0	104	110	120	130	1.15	1.18	IAD	IAD	0.5 <sup>c</sup>	0.5
0/33.5	107	117	173	137	1.62	1.17	IAD	IAD	4	0.5
6.92/3.81	90	96	122	133	1.36	1.39	4	IAD	1	0.5
20.6/11.7	106	102	161	132	1.52	1.29	IAD	IAD	4	0.5
63.9/35.0	88	96	147	137	1.67	1.43	1	IAD	8	0.5
61.0/1.17	92	100	136	131	1.48	1.31	IAD	IAD	0.5	0.5
Day 2										
0/0	98	109	98	105	1.00	0.96	4	8	4 <sup>c</sup>	8 <sup>c</sup>
56.1/0	95	104	118	108	1.24	1.04	IAD	8	0.5	1
0/33.5	108	99	152	129	1.41	1.30	IAD	IAD	0.5	0.5
6.92/3.81	103	107	112	122	1.08	1.14	4	IAD	0.5	0.5
20.6/11.7	100	91	125	126	1.25	1.38	IAD	IAD	0.5	0.5
63.9/35.0	105	105	146	134	1.39	1.28	2	IAD	4	0.5
61.0/1.17	97	110	120	128	1.24	1.16	2	IAD	8	0.5
Day 3										
0/0	89	96	119	106	1.34	1.10	8	IAD	2	0.5
56.1/0	89	88	107	101	1.20	1.15	2	IAD	8	0.5
0/33.5	101	99	121	156	1.20	1.58	4	IAD	8	2
6.92/3.81	97	99	131	124	1.35	1.25	IAD	IAD	0.5	0.5
20.6/11.7	112	97	138	129	1.23	1.33	IAD	IAD	0.5	0.5
63.9/35.0	86	88	128	115	1.49	1.31	1	IAD	23.5	8
61.0/1.17	93	91	110	108	1.18	1.19	2	IAD	23.5	0.5
Day 4										
0/0	90	106	116	101	1.29	0.95	4	23.5	- <sup>a</sup>	23.5 <sup>c</sup>
56.1/0	100	94	107	107	1.07	1.14	2	8	4	23.5
0/33.5	108	98	145	163	1.34	1.66	IAD	IAD	0.5	0.5
6.92/3.81	103	103	111	129	1.08	1.25	0.5	IAD	1	0.5
20.6/11.7	104	85	131	135	1.26	1.59	IAD	IAD	0.5	0.5
63.9/35.0	84	91	131	111	1.56	1.22	4	IAD	8	0.5
61.0/1.17	89	96	115	113	1.29	1.18	23.5	IAD	- <sup>d</sup>	0.5
Day 5										
0/0	<sup>a</sup>	101	115	104	- <sup>b</sup>	1.03	0.5	IAD	- <sup>b</sup>	IAD <sup>c</sup>
56.1/0	90	98	104	96	1.16	0.98	8	IAD	23.5	IAD <sup>c</sup>
0/33.5	95	98	141	152	1.48	1.55	IAD	IAD	2	1
6.92/3.81	91	92	111	128	1.22	1.39	0.5	IAD	2	1
20.6/11.7	113	90	129	131	1.14	1.46	IAD	IAD	0.5	0.5
63.9/35.0	100	82	128	105	1.28	1.28	IAD	0.5	0.5	2
61.0/1.17	115	93	113	109	0.98	1.17	IAD	4	IAD <sup>c</sup>	8

Dose GW685698/ GW642444 (mg/kg/day)	Average predose pulse rate (bpm)		Maximum pulse rate observed (bpm)		Change in maximum pulse rate (X predose)		Time maximum pulse rate observed (hours after completion of dosing)		Time pulse rate returned to predose values (hours after completion of dosing)	
	male	female	male	female	male	female	male	female	male	female
<b>Day 6</b>										
0/0	104	91	115	98	1.11	1.08	8	IAD	23.5	0.5
56.1/0	97	84	106	101	1.09	1.20	IAD	IAD	0.5	0.5
0/33.5	102	98	123	143	1.21	1.46	IAD	IAD	1	0.5
6.92/3.81	100	97	112	121	1.12	1.25	IAD	IAD	0.5	0.5
20.6/11.7	96	93	121	120	1.26	1.29	IAD	IAD	1	0.5
63.9/35.0	93	84	119	100	1.28	1.19	1	0.5	4	4
61.0/1.17	104	81	113	96	1.09	1.19	IAD	IAD	0.5	0.5
<b>Day 7</b>										
0/0	96	94	109	108	1.14	1.15	8	8	23.5	23.5
56.1/0	88	91	102	96	1.16	1.05	0.5	IAD	1	1
0/33.5	96	98	133	165	1.39	1.68	IAD	IAD	2	0.5
6.92/3.81	92	102	97	112	1.05	1.10	8	IAD	23.5	0.5
20.6/11.7	97	87	117	128	1.21	1.47	0.5	IAD	1	0.5
63.9/35.0	91	81	108	121	1.19	1.49	IAD	1	0.5	4
61.0/1.17	107	88	103	111	0.96	1.26	8	2	8 <sup>c</sup>	4
<b>Week 4</b>										
0/0	105	106	119	106	1.13	1.00	IAD	4	0.5	4 <sup>c</sup>
56.1/0	101	106	110	107	1.09	1.01	IAD	IAD	0.5	IAD <sup>c</sup>
0/33.5	102	100	120	138	1.18	1.38	IAD	1	0.5	4
6.92/3.81	105	105	119	113	1.13	1.08	IAD	IAD	0.5	0.5
20.6/11.7	101	114	135	117	1.34	1.03	IAD	IAD	0.5	IAD <sup>c</sup>
63.9/35.0	89	95	135	132	1.52	1.39	IAD	IAD	4	0.5
61.0/1.17	102	99	126	125	1.24	1.26	IAD	IAD	0.5	0.5
<b>Week 13</b>										
0/0	112	98	104	109	0.93	1.11	4	23.5	- <sup>e</sup>	- <sup>d</sup>
56.1/0	87	98	113	108	1.30	1.10	2	0.5	8	4
0/33.5	88	92	121	125	1.38	1.36	0.5	0.5	2	12
6.92/3.81	86	99	103	117	1.20	1.18	1	1	4	4
20.6/11.7	91	87	114	107	1.25	1.23	1	1	4	2
63.9/35.0	85	94	122	116	1.44	1.23	0.5	IAD	12	0.5
61.0/1.17	88	97	112	106	1.27	1.09	0.5	1	2	2

bpm beats per minute  
 IAD Immediately after dosing  
 a Data not recorded in error  
 b Value cannot be calculated, due to lack of predose data  
 c Maximum value similar to predose  
 d Not applicable, maximum pulse rate recorded at 23.5 hours after completion of dosing  
 e Not applicable, all post dose pulse rates were lower than predose

Hematology: No statistics was conducted. As a result, the activity is a judgment call by this reviewer here and other parts of the report where statistics was not conducted. The results for the 13-week analysis are presented in the following table. In males, fluticasone furoate alone reduced the lymphocyte, eosinophilic, basophil counts and increased platelet counts while in females, there was an increase in white blood cells, neutrophils, monocytes and platelet counts and decrease eosinophilic counts. GW642444 alone reduced basophil counts in males and increased lymphocytes and platelet counts in females. When the two compounds were combined (Group 6), in males, there was a reversal of the decreased basophil counts and no marked effect on the other affected

parameters. In females, the increased white blood cell counts were normalized and there was a decrease in the lymphocytes. The other combination groups showed changes that were seen in the individual compounds alone. There was a dose related increase in platelet counts (Groups 4, 5 and 6) in males and in females, a dose related increase in neutrophil, eosinophil and platelet counts.

Parameter	Percent Change From Control					
	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Males						
Lymphocytes	-58	NE	NE	-42	-39	-44
Eosinophils	-93	NE	NE	-88	-88	-88
Basophils	-43	-43	NE	NE	NE	NE
Platelets	+49	NE	NE	+22	+51	+22
Females						
White Blood Cells	+48	NE	NE	NE	NE	NE
Neutrophils	+70	NE	NE	+42	+45	NE
Lymphocytes	NE	+54	NE	NE	-37	-26
Eosinophils	-89	NE	-76	-81	-95	-92
Monocytes	+135	NE	NE	NE	+60	+65
Platelets	+88	+24	+35	+44	+75	+32

NE, No effect

Clinical chemistry: No statistics were conducted, and the data was highly variable. Fluticasone furoate alone (Group 2) in both sexes, increased, ALT, cholesterol, and triglyceride levels and decreased creatinine levels. When combined with GW642444M (Group 6), the ratios relative to fluticasone furoate alone were similar or decreased. In the combination groups (Group 4, 5 and 6), there was a dose related increase in cholesterol, and triglyceride levels in males and in females, a dose related increase in ALT level and like in males a dose related increase in cholesterol and triglyceride levels. The increased levels of ALT and ALP did not correlate with any histological liver findings.

Sex/Parameter	Change Expressed as X Control					
	Gp2	Gp3	Gp4	Gp5	Gp 6	Gp7
<u>Males</u>						
ALP	1.39	0.86	1.08	1.08	1.87	0.84
ALT	4.23	1.23	1.64	1.10	1.98	1.50
Creatinine	0.67	1.27	0.98	0.85	0.68	0.71
Cholesterol	1.61	1.03	1.22	1.42	1.71	1.51
Triglycerides	1.90	0.90	0.76	1.05	1.48	1.48
<u>Females</u>						
ALP	4.48	1.49	2.05	1.67	3.54	1.65
ALT	6.78	1.25	1.68	2.04	5.93	2.29
Creatinine	0.52	1.13	0.86	0.75	0.58	0.64
Cholesterol	2.08	1.41	1.23	1.67	2.35	2.09
Triglycerides	2.07	1.44	1.33	1.56	1.60	1.77

Synacthen Challenge: In male and female control animals, cortisol levels were elevated approximately 10 fold following the intravenous administration of Synacthen on weeks 4, 8 and 13. By week 13, in both sexes, fluticasone furoate alone and in the combination groups suppressed the Synacthen response where the cortisol levels were below the limit of qualification suggesting an approximate 100% suppression. GW642444 alone did not alter the response to Synacthen indicating that there was no drug interaction.

Urinalysis: No treatment related effect.

Toxicokinetics: The results of the AUCs on weeks 1 and 13 for both sexes are summarized in the following tables excerpted from the submission. In both sexes accumulation (increase in the AUC from day 1 and week 13) occurred with fluticasone furoate alone (Group 2, males, 2.2 fold increase; females, 3.5 fold increase). Combining GW642444 with fluticasone furoate did not affect the accumulation (Group 6, males, 3.7 fold increase; females, 3.2 fold increase). Accumulation occurred with GW642444 alone (Group 3, males, 4.9 fold increase; females, 5.0 fold increase); however, when fluticasone furoate was combined with GW642444 (Group 6), there was no accumulation in males while in females, the accumulation persisted (Group 6, 2.6 fold increase) indicating that fluticasone furoate changed the pharmacokinetics of GW642444 in males.

In the combination groups (4, 5 and 7), there was accumulation of fluticasone furoate in both sexes. For GW642444, there was accumulation in males in Group 4 and not in Group 5. There was insufficient data to determine the levels of GW642444 in Group 7. In females, the findings were similar to those observed in males, i.e., accumulation in Group 4 and no accumulation in Group 5. There was insufficient data to determine the levels of GW642444 in Group 7.

**GW685698:**

Parameter+	Occasion	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ GW685698)				
		Group 2	Group 4	Group 5	Group 6	Group 7
		<b>56.1</b>	<b>6.92</b>	<b>20.6</b>	<b>63.9</b>	<b>61.0</b>
		Male				
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	1.18	NC	0.247	1.15	0.305
	Week 4	2.64	0.0969	0.391	0.895	3.11
	Week 13	2.58	0.256	1.26	4.22	2.62
C <sub>max</sub> (ng/mL)	Day 1	0.224	NC	0.124	0.383	0.122
	Week 4	0.505	0.0692	0.165	0.320	0.610
	Week 13	0.561	0.118	0.309	0.959	0.488
		Female				
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	1.40	0.0830	1.01	1.85	0.549
	Week 4	2.47	NC	0.783	2.88	1.46
	Week 13	4.97	0.450	2.27	5.94	3.60
C <sub>max</sub> (ng/mL)	Day 1	0.281	0.0761	0.350	0.383	0.183
	Week 4	0.561	0.0415	0.247	0.575	0.366
	Week 13	1.29	0.228	0.494	1.41	0.671

+ Calculated values derived by multiplying the dose-normalised data by the overall estimated achieved dose.

NC Not calculated due to insufficient data

**GW642444:**

Parameter+	Occasion	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ GW642444)				
		Group 3	Group 4	Group 5	Group 6	Group 7
		<b>33.5</b>	<b>3.81</b>	<b>11.7</b>	<b>35.0</b>	<b>1.17</b>
		Male				
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	10.7	NC	7.48	23.4	NC
	Week 4	42.9	1.26	4.38	8.02	NC
	Week 13	52.3	4.80	13.0	25.2	NC
C <sub>max</sub> (ng/mL)	Day 1	5.29	1.48	2.59	8.79	NC
	Week 4	23.2	0.842	2.18	3.36	0.130
	Week 13	21.7	2.00	4.39	10.3	0.173
		Female				
AUC <sub>(0-t)</sub> (ng.h/mL)	Day 1	10.7	0.712	10.7	14.4	NC
	Week 4	43.6	0.823	5.57	14.4	NC
	Week 13	53.3	5.91	11.8	38.2	NC
C <sub>max</sub> (ng/mL)	Day 1	5.56	0.457	4.82	6.44	0.198
	Week 4	25.8	0.537	3.01	7.53	NC
	Week 13	19.4	3.41	5.18	18.4	0.211

+ Calculated values derived by multiplying the dose-normalised data by the overall estimated achieved dose.

NC Not calculated due to insufficient data

Gross Pathology: The results for males are summarized in the following tables. Fluticasone propionate alone (Group 2) produced an increased incidence of small adrenals, pale livers, small bronchial, cervical and mesenteric lymph nodes, atrophied skeletal muscle, raised stomach antrums and excessive adipose tissues. When GW642444M was combined with fluticasone propionate (Group 6), there was a reduction in the increased incidence of incidence of pale livers, small bronchial and mesenteric lymph nodes, atrophied skeletal muscle raised stomach antrum and excessive adipose tissue. Females did not show the raised stomach antrum but did show a thickened pylorus. GW642444M alone (Group 3) alone produce minimal changes mainly in females, a small cervical lymph node and excessive adipose tissue. When both compounds were combined, there were similar findings in males, but in females, there was no atrophy of the mesenteric lymph node and atrophied skeletal muscle. In the combination Groups 4, 5 and 6, there was a dose related increase in the following: small adrenals, small bronchial and cervical lymph nodes, congested stomach body (this was not seen with either compound alone) in males and small adrenals, and excessive adipose tissue in females. In Group 7, findings seen with fluticasone furoate alone were seen in males except the absence of raised antrum. In females, there was the absence of small cervical lymph node and raised antrum. These findings were attributed to a glucocorticoid effect, which in some instances were modulated by GW642444.

Sex/Organ	Incidence, N=4/Group						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
<u>Male</u>							
Adrenals							
Small	0	4	0	3	4	4	4
Liver							
Pale	0	4	0	0	0	2	3
LN Bronchial							
Small	0	4	0	1	1	3	2
LN Cervical							
Small	0	2	0	1	1	3	1
LN Mesenteric							
Small	0	2	0	1	2	1	4
Skeletal Muscle							
Atrophied	0	3	0	0	0	1	3
Stomach Body							
Congested	0	0	0	0	1	2	1
Pyloric							
Antrum Raised	0	3	0	0	0	1	0
Adipose Tissue							
Excessive	0	3	0	0	0	4	3

Sex/Organ	Incidence, N=4/Group						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
<u>Female</u>							
Adrenals							
Small	0	4	0	1	4	4	4
Liver							
Pale	0	2	0	0	0	3	1
LN Bronchial							
Small	0	1	0	0	1	3	1
LN Cervical							
Small	0	2	1	0	0	3	0
LN Mesenteric							
Small	0	1	0	0	0	0	1
Skeletal Muscle							
Atrophied	0	1	0	0	0	0	1
Stomach Body							
Congested	0	0	0	0	0	0	0
Pyloric							
Antrum Raised	0	0	0	0	0	0	1
Pyloric							
Thickened	0	1	0	0	0	3	1
Adipose Tissue							
Excessive	0	3	1	0	1	2	2

Organ weight: The results are summarized in the following table. The results indicate that the fluticasone furoate effect on the adrenal, liver, and thymus weights were not affected when combined with GW642444.

Sex/Organ	X Times Control						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
<u>Males</u>							
Adrenals							
Absolute	-	0.50	1.02	0.56	0.53	0.58	0.47
Relative to BW	-	0.51	0.96	0.59	0.53	0.53	0.48
Liver							
Absolute	-	1.83	1.03	1.06	1.18	1.98	1.68
Relative to BW	-	1.88	1.00	1.11	1.20	1.81	1.70
Thymus							
Absolute	-	0.14	0.39	0.21	0.38	0.14	0.17
Relative to BW	-	0.15	0.40	0.21	0.38	0.13	0.17
<u>Females</u>							
Adrenals							
Absolute	-	0.65	1.02	0.84	0.66	0.59	0.54
Relative to BW	-	0.59	0.99	0.91	0.67	0.62	0.51
Liver							
Absolute	-	2.41	1.13	1.24	1.37	2.00	1.79
Relative to BW	-	2.13	1.07	1.35	1.37	1.98	1.63
Thymus							
Absolute	-	0.23	1.02	0.22	0.27	0.25	0.31
Relative to BW	-	0.21	1.00	0.24	0.27	0.24	0.29

Histopathology: The results showing the incidence and mean severity are summarized in the two following tables. Some of tissues from groups 4 and 5, the lower dose combinations, were not examined. A severity scoring system was devised by this reviewer. Severity was scored: 1 (minimal), 2 (slight), 3 (moderate), 4 (marked) and 5 (severe). Activity was considered present in there was an increased or decreased in the incidence by 1. In the combination groups, the changes that occurred were those of the steroid effects caused by GW642444.

### Males

#### Fluticasone furoate alone (Group 1 vs. Group 2)

There was an increase in the incidence of the following: mucosal inflammation of the bronchi, lymphoid depletion of the mesenteric, cervical, bronchial and popliteal lymph nodes, lymphoid depletion of the white pulp of the spleen, increased hepatocyte rarefaction (glycogen), epithelial hypertrophy and luminal mucin of the gall bladder, myofibre atrophy of the skeletal muscle, adexal and epidermal atrophy of the skin, decreased cellularity of the sternum bone marrow, atrophy of the vacuolation of the adrenal zona fascicular and zona glomerulosa of the adrenals, mucosal erosion, inflammation and hypertrophy of the antrum of the stomach, mucosal inflammation and epithelial regenerative hyperplasia of the body of the stomach and GALT (gut associated lymphoid tissue) depletion of the cecum and ileum.

Although the incidence of thymus involution was similar to that in the control group, the average severity was markedly higher than the control group (4.5 vs. 1.5) indicating that the thymus was also a target organ.

#### GW642444M alone (Group 1 vs. Group 3)

There was an increase in the incidences of the following: alveolitis in the lung, decreased centrilobular periportal hepatocyte rarefaction (glycogen), luminal mucin in the gall bladder, myofibre atrophy of the skeletal muscle, renal perivascular/interstitial cells and bronchial mucosal inflammation.

#### HD of fluticasone furoate and GW642444 alone vs. HD Combination (Groups 2 and 3 vs. 6)

When fluticasone furoate was combined with GW642444, the following changes of the steroid effects were observed: increased incidence of alveolitis, increased incidence of decreased centrilobular periportal hepatocyte rarefaction (glycogen), decreased the incidence of hepatic rarefaction (glycogen), increased incidence of epithelial hypertrophy of the gall bladder, increased incidence of atrophy of vacuolation of the adrenal zona glomerulosa, decrease incidence of epidermal atrophy, decreased incidence of renal perivascular/interstitial lymphoid cells, decreased incidence of epithelial regenerative hyperplasia and mucosal inflammation of the body of the stomach and decreased incidence of GALT depletion of the cecum.

There were no new toxicity findings from this combination.

#### Dose related effect with Groups 4, 5 and 6)

There was a dose related effect: increased incidence of lymphoid depletion of mesenteric, cervical, popliteal and bronchial lymph nodes, increased incidence of decreased centrilobular periportal hepatocyte rarefaction (glycogen), increased incidence of epithelial hypertrophy and luminal mucin of the gall bladder, increased incidence of adaxial and epidermal atrophy of the skin, increased incidence of decreased incidence of cellularity of the sternum marrow, increased incidence of atrophy of vacuolation of the adrenal zona glomerulosa, increased incidence of mucosal erosion and mucosal hypertrophy of the antrum of the stomach and, incidence of GALT depletion of the cecum.

#### Fluticasone furoate vs. fluticasone furoate + GW642444 (Group 2 vs. Group 7)

There were changes in the following incidences: Increased incidence of decreased centrilobular periportal hepatocyte rarefaction (glycogen), decreased incidence of periportal hepatocyte rarefaction (glycogen) decreased incidence of lymphoid depletion of the cervical and popliteal lymph nodes, decreased incidence of skeletal myofibre atrophy, decreased incidence of epidermal atrophy, increased incidence of atrophy of the vacuolation of the adrenal zona glomerulosa, decreased incidence of mucosal erosion of the antrum of the stomach, decreased incidence of mucosal inflammation of the body of the stomach and decreased incidence of the GALT depletion of the cecum and ileum.

There were no new toxicities observed in males.

Sex/Organ	Males, Incidence, N=4/Group, Mean Severity Score						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Lungs							
Alveolitis							
Severity Score	0	0	2	NE	NE	2	NE
Bronchi			1.0				
Mucosal Inflamm.	0	2	1	NE	NE	1	NE
Severity Score		1.0	1.0			1.0	
Thymus							
Involution/Atrophy	4	4	4	4	4	4	4
Severity Score	1.5	4.5	2.5	4.0	4.3	4.8	4.3
LN Mesenteric							
Lymphoid Depletion	0	4	0	0	4	4	4
Severity Score		4.0			3.0	3.8	3.3
Spleen							
White pulp							
Lymphoid depletion	1	3	1	3	2	3	3
Severity Score	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Liver							
Dec. Centri. / Inc.							
Periportal Hepatocyte							
Rarefaction (Glycogen)	0	0	2	0	2	4	1
Severity Score			1.0		1.0	3.3	2.0
Inc. Hepatocyte							
Rarefaction (Glycogen)	0	4	0	2	0	0	3
Severity Score		3.5		1.0			3.3
Gall Bladder							
Epithelial Hypertrophy	0	1	0	0	2	3	1
Severity Score					1.0	1.0	1.0
Luminal Mucin	0	4	1	2	4	4	4
Severity Score		2.3	1.0	1.0	2.0	3.0	2.3
LN Cervical							
Lymphoid depletion	0	4	0	1	3	4	3
Severity Score		3.3		3.0	3.0	3.5	3.3
Skeletal muscle							
Myofibre atrophy	0	4	1	0	0	4	3
Severity Score		1.3	1.0			1.3	1.7
Skin							
Adaxal atrophy	0	4	0	0	4	4	4
Severity Score		4.0			4.0	4.0	4.0
Epidermal atrophy	0	4	0	0	1	2	2
Severity Score		1.0			1.0	1.0	1.0
Sternum+ marrow							
Decreased cellularity	0	4	0	1	3	4	4
Severity Score		3.5		2.0	3.0	3.3	2.0
LN Bronchial							
Lymphoid depletion	0	4	0	2	3	4	4
Severity Score		3.5		2.5	3.0	2.5	3.0
LN Popliteal							
Lymphoid depletion	0	4	0	0	4	3	3
Severity Score		3.3			3.3	4.0	3.7

Organ	Males, Incidence, N=4/Group, Mean Severity Score						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Kidneys Perivascular/Interstit. Lymphoid Cells Severity Score	0	0	2 1.0	NE	NE	0	0
Adrenals Z.Fasc. Vacuolation/ Atrophy Severity Score	0	4 4.0	0	4 3.0	4 4.0	4 4.0	4 4.0
Z. Glomerulosa/ Vacuolation atrophy Severity Score	1 1.0	3 1.3	1 1.0	2 2.5	2 1.0	4 1.8	4 1.5
Stomach Antrum Mucosal erosion Severity Score	0	3 1.7	0	1 10	1 1.0	3 1.3	1 2.0
Mucosal Hypertrophy Severity Score	0	4 1.8	0	0	1 1.0	4 2.0	4 1.3
Mucosal Inflamm. Severity Score	2 1.0	4 2.0	1 1.0	3 1.0	1 1.0	4 2.0	4 1.5
Body Epithelial Regenerative hyperplasia Severity Score	0	2	0	0	0	0	2 1.5
Mucosal Inflamm. Severity Score	0	2 1.3	0	0	0	1 1.0	1 1.0
Cecum GALT Depletion Severity Score	0	4 4.0	0	1 3.0	1 2.0	2 3.0	3 2.7
Ileum GALT Depletion Severity Score	0	4 3.3	0	0	0	4 2.5	0 2.3

### Females

#### Fluticasone furoate alone (Group 1 vs. Group 2)

There was an increase in the incidence of the following histopathological lesions: inflammatory cells in the bronchi at the tracheal bifurcation, alveolitis in the lung, increased hepatic rarefaction (glycogen), gall bladder hypertrophy, adrenal atrophy of the vacuolation of the zona fascicularis, myofibre atrophy of the skeletal muscle, epidermal atrophy of the skin, decreased cellularity of the sternum bone marrow, mucosal hypertrophy/epithelial regenerative hyperplasia of the antrum of the stomach, GALT lymphoid depletion of the cecum and lymphoid depletion of the tonsils and lymphoid depletion of the bronchial and popliteal lymph nodes.

#### GW642444M alone (Group 1 vs. Group 3)

There was an increase in the incidences of the following: inflammatory cells in the bronchi at the tracheal bifurcation, alveolitis in the lung, mucosal inflammation in the lung, decreased centrilobular periportal hepatocyte rarefaction (glycogen), renal perivascular/interstitial lymphoid cells and lymphoid depletion of the bronchial lymph node.

Fluticasone furoate and GW642444M alone vs. Combination (Groups 2 and 3 vs. 6),  
When fluticasone furoate and GW642444 alone were compared with the combination, the following were observed: increased incidence of inflammatory cells in the bronchi at the tracheal bifurcation, increased incidence of mucosal inflammation of the bronchi, increased incidence of alveolitis, increased incidence of decreased centrilobular/increased periportal hepatocyte rarefaction (glycogen), decreased incidence of increased hepatocyte rarefaction (glycogen), decreased incidence of gall bladder hypertrophy, decreased incidence of renal perivascular/interstitial lymphoid cells, decreased incidence of myofibre atrophy of the skeletal muscle, decreased incidence of epidermal atrophy, decreased incidence of decreased cellularity of the sternum bone marrow, increased incidence of mucosal hypertrophy/epithelial regenerative hyperplasia of the antrum of the stomach, increased incidence of GALT depletion of the cecum, increased incidence of cell infiltration in the mammary gland and increased incidence of erythrocytosis. Although erythrocytosis in the popliteal lymph node was not seen in the fluticasone furoate and GW642444 groups, it was present in the control and treated female groups in the mesenteric and bronchial lymph nodes, it is therefore not considered a treatment related toxicity.

#### Dose related effect with Groups 4, 5 and 6

There was a dose related effect in the following: decreased incidence of hepatocyte rarefaction (glycogen), increased incidence of gall bladder hypertrophy of the gall bladder, decreased incidence of epidermal atrophy, increased incidence of decreased cellularity of the sternum bone marrow, increased incidence of mucosal epithelial regenerative hyperplasia of the stomach antrum, increased incidence of GALT depletion of the cecum, increased incidence of tonsil lymph node depletion and bronchial and popliteal lymph node depletion. These changes reflect mainly those of the steroid.

#### Fluticasone furoate vs. fluticasone furoate +GW642444 (Group 2 vs. Group 7)

There were changes in the following histopathological lesions: decreased incidence of increased hepatocyte rarefaction (glycogen), decreased incidence of epithelial hypertrophy of the gall bladder, decreased incidence of myofibre atrophy of the skeletal muscle, decreased incidence of epidermal atrophy, decreased incidence of decreased cellularity of the sternum marrow, increased incidence of mucosal hypertrophy/epithelial regenerative hyperplasia of the antrum of the stomach and decreased incidence of GALT depletion of the cecum. These changes reflect mainly those of the steroid.

A new toxicity was observed as a result of the combination (Groups 2 and Group 3 vs. Group 6): in females: an inflammatory cell infiltration of the mammary gland. However, this not a clinical concern in view of the severity being minimal and no surrounding cellular injury in this tissue.

Sex/Organ	Females, Incidence, N=4/Group, Mean Severity Score						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
Tracheal Bifur Bronchi							
Inflamm. cells	0	1	1	NE	NE	2	NE
Severity Score		1.0	1.0			1.0	
Lung							
Alveolitis	0	1	3	NE	NE	2	NE
Severity Score		1.0	1.0			1.0	
Bronchi							
Mucosal inflamm.	0	0	1	NE	NE	2	NE
Severity Score			1.0			1.0	
Liver							
Dec. Centri. / Inc. Periportal Hepatocyte							
Rarefaction (Glycogen)	0	0	2	0	0	4	0
Severity Score			1.0			2.8	
Inc. Hepatocyte							
Rarefaction (Glycogen)	0	4	0	2	2	0	3
Severity Score		3.5		2.5	2.0		3.0
Gall Bladder							
Epithelial Hypertrophy	0	4	0	1	1	2	0
Severity Score		1.0		1.0	1.0	1.0	
Kidneys							
Perivascular/Interstit.							
Lymphoid Cells	1	1	3	NE	NE	0	0
Severity Score	1.0	1.0	1.0				
Adrenal							
Z.Fasc. Vacuolation/							
Atrophy	0	4	0	4	4	4	4
Severity Score		4.0		2.5	4.0	4.0	4.0
Skeletal Muscle							
Myofibre Atrophy	1	3	0	0	0	1	2
Severity Score	1.0	1.3				1.0	1.0
Skin							
Epidermal Atrophy	0	3	0	0	2	2	0
Severity Score		1.0			1.0	1.0	
Sternum, Marrow							
Dec. Cellularity	0	4	0	2	3	3	3
Severity Score		4.3		2.0	2.7	2.3	3.0
Stomach							
Antrum							
Mucosal							
Hypertrophy/EpithelialRe generative Hyperplasia	0	1	0	0	2	3	4
Severity Score		1.3			1.0	2.0	1.5
Cecum							
GALT							
Lymphoid Depletion	0	3	0	2	2	4	2
Severity Score		3.0		2.0	2.0	3.3	2.5
Mammary							
Inflam. cell Infiltration	0	0	0	NE	NE	2	0
Severity Score						1.0	

NE, Not examined

Sex/Organ	Females, Incidence, N=4/Group, Mean Severity Score						
	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6	Gp7
LN Bronchial Lymphoid Depletion	0	4	1	3	4	4	4
Severity Score		2.0	2.0	1.7	2.3	2.5	2.3
Erythrocytosis	1	1	1	2	0	0	0
Severity Score	1.0	2.0	3.0	1.0			
Tonsils Lymphoid Depletion	0	4	0	2	4	4	4
Severity Score	0	1.5		1.0	1.5	2.8	2.3
LN Popliteal Erythrocytosis	0	0	0	0	0	2	1
Severity Score						1.5	2.0
LN Mesenteric erythrocytosis	3	4	2	3	4	4	4
Severity Score	1.0	2.5	1.0	2.3	2.3	2.8	1.7

## Overall Discussion and Conclusion

This submission contained the results of 13-week inhalation combination toxicity studies in rats and dogs that were administered fluticasone furoate and GW642444 to determine if there is preclinical support of the Phase III clinical trials for the treatment of asthma and COPD. The proposed clinical studies are fifty-two weeks of treatment with 100 mcg of fluticasone furoate and 25 mcg of GW642444 or 200 mcg of fluticasone furoate and 25 mcg of GW642444, 4:1 and 8:1 ratios, respectively.

In rats, the test groups were :Group 2 (5.64 mcg/ kg of fluticasone furoate alone), Group 3 (2.49 mcg/ kg of GW642444 alone), Group 4 (0.785 mcg/kg of fluticasone furoate +0.524 mcg/kg of GW642444 ), Group 5 (1.98 mcg/ kg of fluticasone furoate +1.17 mcg/kg of GW642444 ), Group 6 (5.38 mcg/kg of fluticasone furoate +3.07 mcg/kg of GW642444) and Group 7 (5.26 mcg/kg of fluticasone furoate + 0.582 mcg/kg of GW642444). The dose ratios of fluticasone furoate to GW642444M were Group 4 (1.49) and Group 5 (1.69), Group 6 (1.75) and Group 7 (9.03).

In the 13-week inhalation toxicity study in dogs, groups and deposited doses were : Group 1 (control, lactose +1% magnesium stearate), Group 2 ( 14.0 mcg/kg of fluticasone furoate alone), Group 3 (8.38 mcg/ kg of GW642444M alone), Group 4 (1.73 mcg/kg of fluticasone furoate +0.95 mcg/kg of GW642444M), Group 5 (5.15 mcg/ kg of fluticasone furoate + 2.93.0 mcg/kg of GW642444M), Group 6 (15.98 mcg/kg of fluticasone furoate +8.75 mcg/kg of GW642444M) and Group 7 (15.25 mcg/kg of fluticasone furoate + 0.29 mcg/kg of GW642444M). The dose ratios of fluticasone furoate to GW642444M were: (Group 4, 1.82) and Group 5 (1.76), Group 6 (1.83 and Group 7 (52.5). For compound interaction, comparisons involved control (Group 1), fluticasone furoate alone (Group 2), GW642444M alone (Group 3) and fluticasone furoate + GW642444M (Group 6).

Comparison was made of the changes between rats and dogs in the combination toxicity studies. In both rats and dogs there was no treatment related deaths. In rats, the characteristics glucocorticoid effects (hair loss, decreased body weight gained, and food consumption and hematological changes) of fluticasone furoate were not affected when combined with GW642444. In dogs, there was no activity by GW642444 on the effect by fluticasone furoate on the food consumption, hematology and clinical chemistry changes nor did fluticasone furoate affect the GW642444 induced tachycardia and increased pulse rate.

In rats, GW642444 alone accumulated in both sexes. This accumulation was not changed when combined with fluticasone furoate. In males, fluticasone furoate accumulated alone and in the presence of GW642444. In females, fluticasone furoate alone did not accumulate but did accumulate when combined with GW642444 indicating that GW642444 affected the pharmacokinetics of the steroid. In dogs, the pharmacokinetics was similar and yet different from rats. Fluticasone furoate alone accumulated in both sexes that persisted when was combined with GW642444. GW642444 alone accumulated in both sexes, which persisted in males when combined with fluticasone furoate. However, in females, the accumulation with GW642444 was absent when combined with fluticasone furoate indicating the steroid altered the pharmacokinetics of GW642444.

Macroscopically, in rats and dogs, the changes seen were related to the steroid effect. In rats, the lower dosed combination groups showed a doses related increase in the incidence of hair loss in both sexes and in dogs, the lower dosed groups in both sexes produces a dose related increase in the incidence of small adrenals. There was no drug interaction when comparing the HD of fluticasone furoate and HD of GW642444 with the HD of the combination of fluticasone furoate and GW642444.

In determining drug interaction in the histopathological evaluation, comparison of the incidences was made between the HD of fluticasone furoate alone (Group 2), the HD of GW642444 alone (Group 3) and the HD of the combination of fluticasone furoate and GW642444 (Group 6). In male rats, receiving the combination showed an increased incidence in the steroid effects in the following organs: lungs, skin and mesenteric lymph node. However, there was a decrease incidence in the steroid effect on the bronchial lymph node. In females, the combination group showed an increased incidence in the steroid effect on the mammary gland and lungs and a decrease by GW642444 in the incidence of the steroid effect in the mesenteric and mandibular lymph nodes, sternum bone marrow and skin. No new toxicity was seen in the combination groups in both sexes in rats. The changes that occurred were those of the steroid.

Male dogs receiving the combined drugs showed an increased incidence in the steroid effects in the following organs: liver, gallbladder and adrenal glands; there was reduction by GW642444 in the incidence of the steroid effects in the following organs: skin, liver, popliteal lymph nodes, kidneys, stomach and cecum. In females, the findings were similar and yet different. The incidence increased by GW642444 of the fluticasone furoate effects occurred in the following organs: bronchi, liver, stomach, cecum,

mammary gland and blood. The incidences decreased by GW642444 of the fluticasone furoate effects occurred in the following organs: liver, gall bladder, kidney, skeletal muscle skin and bone marrow.

The increased cell infiltration of the mammary gland in females was a new toxicity. However, the severity was minimal, and there was no evidence of surrounding cellular injury in the tissue that would be a clinical concern.

Toxicities observed in the 13-week bridging toxicity studies with fluticasone furoate and GW642444 in rats and dogs appeared to be primarily attributable to the pharmacological effects of fluticasone furoate. In addition, tachycardia, attributable to GW642444, was observed in dogs. NOAELs were not identified in nonclinical studies with the combination study given that rats and dogs are known to be significantly more sensitive to the toxic effects of glucocorticoids as compared to humans. These findings are in concordance with previous inhalation toxicology studies conducted with fluticasone furoate in rats and dogs. There was no evidence of additive or synergistic toxic effects with the combination of fluticasone furoate and GW642444. There was no evidence of any interactions on toxicokinetic parameters in rats or dogs. Nonclinical ratios of fluticasone furoate to GW642444 tested in rats and dogs encompassed the clinical ratios. Exposure margins of nonclinical doses compared to clinical doses are shown in the table below.

Exposure margins

Species	NOAEL, mcg/kg, A		Exposure Margin, A/B	
	Fluticasone furoate	GW642444	Fluticasone furoate	GW642444
Rat	5.38	3.07		
Dog	15.98	8.75		
B Human Dose				
Fluticasone furoate 2 mcg/kg (100 mcg)			Rat: 2.7 Dog: 8.0	
4 mcg/kg (200 mcg)			Rat: 1.3 Dog: 4.0	
GW642444 0.5 mcg/kg (25 mcg)				Rat: 6.0 Dog: 18.0

Conclusion

In the 13-week toxicity studies, there was no evidence of additive or synergistic toxicity with the combination at doses up to 5.38 mcg/kg of fluticasone furoate + 3.07 mcg/kg of

GW642444 in rats and 15.98 mcg/kg of fluticasone furoate and 8.75 mcg/kg of GW642444 in dogs.

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/s/

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LAWRENCE F SANCILIO  
09/30/2010

MOLLY E TOPPER  
09/30/2010

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**IND number:** 74,696

**Review number:** 4

**Sequence number/date/type of submission:** Supp.Doc. No. 44, 10/06/08; Supp. Doc. No. 52, 2/5/09

**Information to sponsor:** Yes ( ) No (X)

**Sponsor and/or agent:** GlaxoSmithKline.

**Manufacturer for drug substance:** GlaxoSmithKline.

**Reviewer name:** Lawrence F. Sancilio, Ph.D.

**Division name:** Division of Allergy and Pulmonary Products

**HFD #:** 570

**Review completion date:** 3/5/10

**Drug:**

Trade name: Not stated

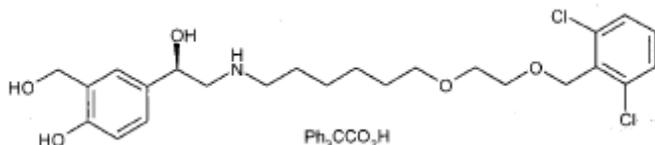
Generic name: Not assigned.

Code name: GW642444M

Chemical name: Triphenylacetic acid-4-{(1R)-2-[(6-{2-(2,6-dichlorobenzyl)oxy}ethoxy)hexyl]amino]-1-hydroxyethyl}-2-(hydroxymethylphenol (1:1)

Chemical formula/molecular weight: C<sub>24</sub>H<sub>33</sub>Cl<sub>2</sub>NO<sub>5</sub>.C<sub>20</sub>H<sub>16</sub>O<sub>2</sub>/ MW: 774.78

Chemical structure



CAS registry number: 503070-58-4

Relevant INDs/NDAs/DMFs: IND 77,855, 48,647, 70,297, (b) (4)

**Drug class:** Long-acting  $\beta_2$  adrenergic agonist (LABA).

**Indication:** Treatment of COPD and asthma.

**Route of administration:** Oral inhalation.

Clinical formulation: Lactose / magnesium stearate GW642444M blend.

**Proposed clinical protocol:** None.

Previous clinical experience: The 28-day inhalation study (Study B2C109575) has been completed with daily doses of 12.5, 25 and 50 mcg of GW642444 in adults and adolescents with persistent asthma.

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

**Studies reviewed within this submission:**

Multidose

A 26-week inhalation toxicity study of a powder aerosol formulation of GW642444M in the rat followed by an 8-week recovery period, CD2008/00903/00, vol. 3 Document No. 52.

Toxicity study by inhalation administration to beagle dogs for 39 weeks, CD2007/01006/01, vol. 3, Document No. 44.

**Studies not reviewed within this submission; they are either not relevant and will not be reviewed or will be reviewed at a later date.**

**Pharmacokinetics/ Toxicokinetics**

Single dose inhalation toxicokinetic Non-GLP study in the male rat, CD2007/01474/00, vol. 1, Document No. 44

Single dose inhalation toxicokinetic Non-GLP study in the male Beagle dog, CD2007/01473/00, vol. 1 Document No. 44

Potential chiral conversion in plasma following inhalation to rats and dogs and in vitro incubation in human, WD2008/00181/01, vol. 1, Document No. 44

Validation of a method for the determination of GSK573719 and GW642444M (range 0.1 to 100 ng/ml) in dog plasma using a HPLC-MS/MS method, WD2008/00560/00, vol. 1, Document No. 44,

Abbreviated validation of a method for the determination of GW642444M (range 0.5 to 500 ng/ml) in human urine using HPLC-MS/MS, No. WD2008/00604/00 vol. 1, Document No. 52. Not relevant since it relates to clinical data.

Validation of a method (b)(4)

Not relevant since it relates to clinical data.

**Toxicology**

**Single Dose**

Single escalating oral dose toxicokinetic and tolerability study in Sprague-Dawley rats, WD2008/1045/00, vol. 1, Document No. 52. (Not relevant since the prescribed route is inhalation)

**Multidose**

A 4-week combination inhalation combination toxicity study in the dog, CD2008//00827/00, vol. 6, Document No. 52.

A 4-week inhalation toxicity study of a powder aerosol formulation and a metered dose inhaler formulation in the Beagle dog, CD2008/00935/00, vol. 8, Document No. 52.

A 28-day inhalation toxicity study of a powder aerosol formulation of GW642444 in the rat, CD2007/01072/00, vol. 1, Document No. 52.

13-Week inhalation toxicity in Beagle dogs to determine the influence of Mg stearate as an excipient on the toxicity and pharmacokinetics, CD2007/0151400, vol. 2, Document No. 44.

**Drug History**

GW642444 is being developed as a monotherapy for treatment of asthma, and as a LABA component of the ICS/ LABA combination product for the treatment of COPD. In the initial clinical trial, a 28-day inhalation study was conducted in persistent asthmatics at doses of 3, 6.25, 12.5, 25 and 50 mcg to evaluate the safety, efficacy and pharmacokinetics. In the clinical review of Study B2C109575 by Dr. Porter dated 9/28/09, the results of the 28-day inhalation study indicated a support of the monotherapy with daily doses of 12.5, 25 and 50 mcg of GW642444 in adults and adolescents with persistent asthma. Based on a communication with the MO (Dr. Porter) for this IND on March 5, 2010, it appears that the clinical dose will not be expected to exceed a single daily dose of 25 mcg GW642444 alone in asthmatic patients.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Brief Summary

Twenty-six and 39-week inhalation toxicity studies were conducted in rats and dogs. In the 26-week rat study with an 8-week recovery group, the lung deposited doses were 5.77 (LD), 53.7 (MD), 267.4 (HD) and 1025.3 (VHD) mcg/kg. There were no treatment related deaths and clinical signs; at week 26, body weight gain in males were not affected by GW642444M while in females, body weight gain increased by 20% and 15% at the HD and VHD, respectively which was reversible. Food consumption increased in all test article treated males which was reversible and in the 3 highest doses females. At the end of the recovery period, the VHD females showed a 15% decrease in food consumption which was not accompanied by a decrease in body weight gain and not considered a toxic effect. In the histopathology evaluation, the nasal cavity/sinuses, larynx, nasopharynx that was targeted organs in both sexes was not clinically relevant since the compound will be administered by oral inhalation. In males, the relevant target organ was the lung (accumulation of macrophages at VHD). In females, the target organs of toxicity included tracheobronchial lymph node (erythrocytosis/hemorrhage at the VHD), the mammary gland (increased secretory activity at  $\geq$  MD, and acinar development at  $\geq$  HD) and ovary (cysts at  $\geq$  HD, absence or decreased corpora lutea at  $\geq$  MD, and increased incidence of estrus at  $\geq$  MD). The cystic follicular dilatation in the ovaries and increased secretory activity in the mammary glands may be attributed to stimulation of the  $\beta_2$  receptors on the ovaries leading to cysts formation and disturbance of the estrus cycle (Lara et al. *Endocrinology* 141: 1059-1072, 2000). The inhalation NOAEL was 267.4 mcg/kg ( $AUC_{0-t}$ , 334 ng.h/ml) in males and 5.77 mcg/kg ( $AUC_{0-t}$ , 3.7ng.h/ml) in females.

In dogs, the deposited doses were 2.38 (LD), 15.6 (MD) and 30.5/127.5 (HD) mcg/kg based on 25% of the estimated achieved doses of 9.55 (LD), 62.5 (MD) and 122/510 (HD) mcg/kg. The test article was formulated as a lactose/nominal 7% GW642444M. There were 2 phases of the study. In Phase 1, the HD was 30.5 mcg/kg administered daily for 3 days. In Phase 2, the control and 3 doses were administered, and the HD was increased from 30.5 mcg/kg to 127.5 mcg/kg. The predominant clinical sign was treatment related vasodilation of the ears at the MD and HD groups in the Phase 2 study based on increased duration. In Phase 1, there was a pronounced increased in pulse rate on all 3 days (the range of increase relative to predose rate pulse rate was: males, 1.31x to 1.62x; females, 1.45x to 1.71x). In Phase 2, at weeks 1.1, 1.4, 4, 13 and 39, there was an inconsistently dose related increase in pulse rates. However, by EKG analysis at week 39, increased heart rate occurred at the MD and HD in both sexes. The tachycardia at the HD in both sexes was lower than the increased heart rate at the MD. Troponin I levels were increased in 7/8 dogs in Phase 1, and 2/8 at the MD and 1/8 in the HD during Phase 2. In females, there was a dose related decrease in the hematocrit and hemoglobin and an increase in platelets, ALP and AST. In males, there was an increase in the absolute and relative prostate weight (HD), and in both sexes, there was a small dose related increase in the absolute and relative liver weight. Histologically, the relevant targeted organs were the heart (myocardial fibrosis, females), lungs and bronchi (alveolar hemorrhage, females), liver (increased periportal hepatocyte rarefaction/ decreased centrilobular rarefaction (glycogen, both sexes), kidneys (cortex, fibrosis and tubular atrophy, females)

and trachea bifurcation (both sexes). The heart and liver findings are characteristic of a  $\beta_2$  agonist. The findings in the nasal turbinates are not considered relevant in a clinical setting. The inhalation NOAEL was MD, 15.6 mcg/kg ( $AUC_{0-t}$ : 34.8 ng.h/ml in males and 39.0 ng.h/ml in females).

#### 2.6.6.3 Repeat-dose toxicity

### **Study Title: A 26-week inhalation toxicity study of a powder aerosol formulation of GW642444M in the rat followed by an 8-week recovery period**

#### **Key Findings:**

- The deposited doses were 5.77 (LD), 53.7 (MD), 267.4 (HD) and 1025.3 (VHD) mcg/kg.
- Body weight gain of the HD and VHD in females at Week 26 was increased up to 20 % (a  $\beta_2$  agonist effect) but comparable to the control at the end of recovery period.
- Food consumption was increased in all test article treated males and in the MD, HD, and VHD females, a  $\beta_2$  agonist effect
- Toxicity finding in the nasal cavity (both sexes) is not clinically relevant since the drug product will be administered by oral inhalation. The larynx (metaplasia) and nasopharynx findings (goblet cell hypertrophy (male) are considered rat specific.
- Histologically, the target organ in males was the lung (accumulated macrophage, VHD), and in females, the target organs were the tracheobronchial lymph node (erythrocytosis/hemorrhage, VHD), mammary gland (secretory gland,  $\geq$  MD; acinar development,  $\geq$ HD) and ovary, (cystic follicular dilation  $\geq$  MD; cysts, HD; decreased corpus lutea,  $\geq$  MD) and estrus cycle (increased estrus,  $\geq$  MD). At the end of the recovery period, the secretory activity in the mammary gland and cystic follicular dilatation in the ovary were still present and the other findings were resolved.
- The inhalation NOAEL was HD, 267.4 mcg/kg ( $AUC_{(0-t)}$  at the end of 26 week: 334 ng.h/ml) in males and LD, 5.8 mcg/kg ( $AUC_{(0-t)}$  at the end of 26 week: 3.7 ng.h/ml) in females.
- The AUC ratios relative to GW642444M were 0.002 and 0.02 for the metabolites, respectively, for GW630200 and GSK932009, and 3.0 for GI179710, (b) (4).

**Study no.:** CD2008/00903/00

**Volume #, and page;** 3 and page 1

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 11/21/06

**GLP compliance:** Yes.

**QA report:** yes (X) no ( )

**Drug, Batch #, and % purity:** RS251083, RS251079, RS251082 and RS251080;

purity, 99.3%

**Formulation:** 4 and 20 % blend of lactose with GW642444M

### Methods

Doses: The estimated achieved dose (D) was determined from the following formula:

$D = (RMV \times T \times C) / BW$ , where

RMV= Respiratory minute volume =  $0.499 \times BW$  (body weight in kg)<sup>0.809</sup> (L/min)

T= Duration of daily exposure (minutes)

C= aerosol concentration ( $\mu$ /L)

Range of MMD: 2.6 to 4.2  $\mu$ m; range of GSD: 1.9 to 2.2.

The achieved doses were:

C, 0; LD, 57.7, MD, 537, HD, 2,674 and 10,253 (VHD) mcg/kg.

Based on 10% deposition factor, the doses determined from the estimated achieved doses were:

C, 0; 5.77 (LD), 53.7 (MD), 267.4 (HD) and 1,025.3 (VHD) mcg/kg.

Species/strain: Crl:CD (SD) rats.

Number/sex/group (main study): 12/sex/group; recovery group (C, HD and VHD), 6/sex/group; toxicokinetics, 6/sex/group.

Route, Inhalation, nose-only; duration: 60 minutes.

Age: 6-weeks

Weight: males, 168-212 g; females, 133-165 g.

Unique study design or methodology (if any): None.

### Parameters

Clinical signs: Daily.

Body weight: Week -1 including day-1, then weekly during treatment and recovery periods.

Food consumption: During the week prior to initial dosing and weekly during treatment and recovery periods.

Ophthalmoscopy: Prior to initiation of dosing and during weeks 13 and 25 (main and recovery animals).

Hematology and Clinical Chemistry: Weeks 4, 13 and 26 and week 32 of recovery animals.

Urinalysis: Weeks 4, 13 and 26 and weeks 32 and 33 of recovery animals.

Toxicokinetics: Blood samples were collected from the toxicokinetics designated animals during weeks 4 and 26. They were bled immediately post dose and then at 0.5, 1, 2, 4, 8 and 23 hrs post dosing. The lower limit of quantification was 0.1 ng/ml for GW642444M, GW630300 and GW630200. The lower limit of quantification for G1179170 was 5 ng/ml. Analysis was by a HPLC/MS/MS method.

### Necropsy

Gross pathology: Full necropsy was done on all animals, scheduled and non-scheduled deaths.

Organ weights: The following organs were weighed, kidney, spleen, brain, heart, liver, adrenals, lungs, thymus, prostate, testes, and ovary.

Estrus cycle: The incidence of proestrus, estrus, early metestrus and late metestrus/diestrus was determined.

Histopathology: The tissues processed and tissues examined are listed in the following table excerpted from the submission. Tissues examined were from all control, HD and VHD treated and recovery group animals, from some LD and MD group animals with gross abnormalities and from animals that died prior to scheduled necropsy.

Adequate Battery: yes (X), no ( )—explain:

Peer review: yes (X), no ( )

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Ovaries	X
Adrenals	X	Pancreas	X
Animal identification		Pituitary	X
Aorta (thoracic)	X	Preputial/Clitoral gland	
Brain -		Prostate	X
cerebrum	X	Rectum	
cerebellum	X	Salivary glands	
medulla	X	mandibular	X
oblongata	X	sublingual	X
midbrain	X	parotid	X
Cecum	X	Sciatic nerve	X
Colon	X	Seminal vesicles	X
Duodenum	X	Skeletal muscle (hindlimb)	X
Epididymides	X	Skin (inguinal)	X
Esophagus	X	Spinal cord	
Eyes	X	cervical	
Femur (femorotibial joint)	X	thoracic	
Harderian glands		lumbar	X
Heart (including section of aorta)	X	Spleen	X
Ileum	X	Sternum (bone marrow)	X
Jejunum	X	Stomach	X
Kidneys	X	Testes	X
Larynx (plus epiglottis)	X	Thymus	X
Liver (two lobes)	X	Thyroids and parathyroids	X
Lungs (all lobes)	X	Tongue	X
Lymph node -		Trachea	X
mandibular	X	Tracheal bifurcation (with mainstem bronchi)	X
mesenteric	X	Urinary bladder	X
tracheo-bronchial	X	Uterus	
Mammary gland (inguinal)	X	horns	X
Nasal turbinates/skull (3 levels)	X	body	X
Nasopharynx	X	cervix	X
Optic nerves	X	Vagina	X

## Results

**Mortality:** Unscheduled deaths were not considered treatment related as stated by the sponsor. There were 4 female unscheduled deaths: In the toxicokinetics group, 1 control and 1 each in the LD and MD group; in the main study, 1 in the LD killed on day 176 due to poor condition which was attributed to a fracture of the muzzle. There were 3 male unscheduled deaths: one control male died in week 25 and 2 VHD males died, one on

day 22 and another on day 87. The 2 VHD male rats that were found dead with no indication as to the cause of death. This reviewer agrees with the sponsor since there were no clinical signs and no significant histopathology to indicate the cause of death.

Clinical signs: No treatment related effect.

Body weight: At week 26 (treatment period) and week 34 (8-week recovery period), there was no treatment related effect on body weight gain in males. In females at week 26, there was a 20 % increase in body weight gain at the HD and 15% increase in the VHD. Although this was not a dose related effect, increased body weight gain is characteristic of a  $\beta_2$  agonist. By week 34, there was no difference in body weight gained between the control and the treated groups.

Food consumption: In Week 26, there was an increase in all treated groups when compared to the control group: for males, LD, +12%; MD, +16%; HD, +25%; VHD, +25%;, in week 34, there was no difference in males in the amount of food consumption between the treated and control groups; for females, there was an increase in food consumption, i.e., MD, +25%; HD, +25%; VHD, +25%; by week 34, the VHD showed a 15% decrease in food consumption when compared to the control group. The increased food consumption in males and females is considered a class effect of  $\beta_2$  agonist and the increased in food consumption were consistent with the increase in body weight gain in the HD and VHD females.

Ophthalmoscopy: No treatment related effect.

Hematology: The results are presented in the following table. A dose related decrease in platelets was observe in males at MD and above at the end of treatment period but was not observed in females; the decrease in males was resolved at the end of recovery period. There was a slight decrease in prothrombin time in VHD males and a minimal dose related decrease in prothrombin time in HD and VHD females. The decrease was not considered toxicologically significant. The classic anticoagulant, warfarin, at 0.1 mg/kg optimally causes a 150% to 200% increase in prothrombin time in rats.

Parameter	% Change from Control, p< 0.05			
	LD	MD	HD	VHD
Males Platelets	NS	-14	-17	-22
Prothrombin Time	NS	NS	NS	-10 <sup>a</sup>
Females Prothrombin Time	NS	NS	-5	-7 <sup>b</sup>

NS, Not significant

<sup>a</sup> Value of 15 sec. was within the historical range of 10-18 sec.

<sup>b</sup> Values of 14.4 and 14.2 were within the historical range of 10-18 sec

Clinical Chemistry: The results are summarized in the following table. In both sexes, there was a similar decrease in glucose levels in all treated groups. This hypoglycemia was in contrast to beta-2 agonists where there is hyperglycemia. The decrease was essentially reversible in MD and VHD males, and in MD females, but partially reversible in VHD females. In females, there was a similar decrease in triglyceride levels at the MD, HD and VHD; the decreased triglyceride levels were reversible. Since there was no dose related response, the slight changes in these clinical chemistry parameters were not considered significant toxic effects. The potassium values in the treated groups, though increased, were within the historical range.

Parameter	% Change from Control, p< 0.05			
	LD	MD	HD	VHD
Males				
Glucose	NS	-25	-27	-30
Recovery period				
Glucose	ND	ND	+10	NS
Females				
Glucose	-25	-30	-33	-30
Triglycerides	NS	-43	-39	-40
Potassium	NS	NS	NS	+9 <sup>a</sup>
Recovery Period				
Glucose	ND	ND	NS	-15
Triglycerides	ND	ND	NS	NS

NS, Not significant

ND, Not determined

<sup>a</sup> Not considered significant since the value of 5.2 was below the historical control value of 5.9 mmol/L

Urinalysis: The results are summarized in the following table. Males showed an increase in the 3 high doses in proteinuria that was not dose related, and were reversible. In females, there was an increase in urinary creatinine that was not dose-dependent, and this change was reversible. There were no associated renal histopathology observations. Hence, these findings were not considered toxicologically significant.

Parameter	% Change from Control, p< 0.05			
	LD	MD	HD	VHD
Males Proteinuria	NS	+66	+50	+74
Recovery period Proteinuria	ND	ND	NS	NS
Females Creatinine	+33	+47	+39	+44
Recovery Period Creatinine	ND	ND	NS	NS

NS, Not significant

ND, Not determined

Toxicokinetics: The results are presented in the following tables, showing the results with GW642444, the two metabolites, GW630200 and GSK932009 and (b)(4) (G1179710) (b)(4). The AUCs and Cmaxs for GW642444 in both sexes were similar dose related and not dose proportional at the 4- and 26- week measurement. There was no accumulation. For the metabolite, GW630200, the AUC and Cmax levels were detected at the HD and VHD at weeks 4 and 26; the second metabolite, GSK932009, was detectable at the MD, HD and VHD during weeks 4 and 26. The AUCs at week 26 for GW630200 were similar in both sexes. For GSK932009, the AUCs were higher at the MD, HD and VHD in males than in females, and accumulation did not occur. For (b)(4) (G1179710) (b)(4), AUCs and Cmaxs levels were detectable at all doses at weeks 4 and 26. However, the female AUCs at week 26 were more than 2 times those of the males. This was also seen in the VHD and Cmax. The report indicated that the AUC ratios relative to GW642444 were 0.002, 0.02 and 3.0, respectively, for GW630200, GSK932009, and G1179710.

GW642444

Parameter+	Week	Male				Female			
		Estimated Achieved Dose				Estimated Achieved Dose			
		57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day	57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day
AUC <sub>(0-1)</sub> (ng.h/mL)	Week 4	6.06	30.9	267	644	6.69	31.0	275	622
	Week 26	4.33	43.4	334	755	3.71	38.4	374	640
C <sub>max</sub> (ng/mL)	Week 4	1.75	11.5	141	269	1.58	8.86	136	250
	Week 26	2.01	13.2	166	292	2.30	15.5	225	248

+ Calculated values derived by multiplying the dose-normalized data (refer to Appendix 4) by the overall estimated achieved dose

GW630200

Parameter+	Week	Male				Female			
		GW642444 Estimated Achieved Dose				GW642444 Estimated Achieved Dose			
		57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day	57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day
AUC <sub>(0-1)</sub> (ng.h/mL)	Week 4	NC	NC	0.586	3.49	NC	NC	NC	0.732
	Week 26	NC	NC	0.495	2.10	NC	NC	0.447	1.57
C <sub>max</sub> (ng/mL)	Week 4	NQ	NQ	0.305	1.07	NQ	NQ	0.134	0.369
	Week 26	NQ	NQ	0.281	0.687	NQ	NQ	0.254	0.636

+ Calculated values derived by multiplying the dose-normalized data (refer to Appendix 4) by the overall estimated achieved dose

NC: Not calculated due to insufficient data

NQ: Not quantifiable, below lower limit of quantification (0.1 ng/mL)

GSK932009

Parameter+	Week	Male				Female			
		GW642444 Estimated Achieved Dose				GW642444 Estimated Achieved Dose			
		57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day	57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day
AUC <sub>(0-1)</sub> (ng.h/mL)	Week 4	NC	0.618	3.66	16.2	NC	0.280	1.18	4.44
	Week 26	NC	0.542	4.79	17.3	NC	0.230	1.52	5.65
C <sub>max</sub> (ng/mL)	Week 4	NQ	0.280	1.18	4.44	NC	0.188	1.11	3.48
	Week 26	NQ	0.230	1.52	5.65	NC	0.278	1.57	6.02

+ Calculated values derived by multiplying the dose-normalized data (refer to Appendix 4) by the overall estimated achieved dose

NC: Not calculated due to insufficient data

NQ: Not quantifiable, below lower limit of quantification (0.1 ng/mL)

GI179710

Parameter+	Week	Male				Female			
		GW642444 Estimated Achieved Dose				GW642444 Estimated Achieved Dose			
		57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day	57.7 µg/kg/day	537 µg/kg/day	2674 µg/kg/day	10253 µg/kg/day
AUC <sub>(0-1)</sub> (ng.h/mL)	Week 4	9.23	65.0	717	2600	9.64	72.0	586	3250
	Week 26	NC	61.8	447	2370	NC	159	1310	6170
C <sub>max</sub> (ng/mL)	Week 4	3.93	19.8	308	656	5.74	30.0	234	824
	Week 26	NQ	34.7	243	667	11.0	44.5	372	2040

+ Calculated values derived by multiplying the dose-normalized data (refer to Appendix 4) by the overall estimated achieved dose

NC: Not calculated due to insufficient data

NQ: Not quantifiable, below lower limit of quantification (0.1 ng/mL)

Organ weight: On absolute weight basis, in males, there was a significant decrease in the heart weight at the MD (14%) and HD (14%) and not at the VHD, and the prostate was significantly decreased at the LD (21%), MD (19%) and HD (19%) and not at the VHD. These changes are not biologically significant since there was no change at the VHD. In the recovery group, there was no change in the organ weights in both sexes. Relative to body weight, the prostate was significantly decreased at the LD (24%), MD (24%) and HD (24%) and not at the VHD, and the heart at the MD showed a 9% decrease in the relative weight. Changes in the prostate and heart were not considered a treatment related effect due to a lack of a dose related response, a small change and/or lack of corresponding histopathologic findings. The recovery group showed no changes based on relative organ weight. Absolute organ weights for recovery animals were not reported.

## Necropsy

Macropathology: Unscheduled deaths: No gross pathology.

Main study: Females: Ovary, cyst: C, 1/11; LD, 0/10; MD, 2/11; HD, 2/12; VHD, 3/12

Recovery group: Females, Pale liver: C1/6; HD, 2/6; VHD, 3/6.

Females, Ovary, cyst, C, 1/6:

Histopathology: The results are presented in the following tables. The following scoring system was used to describe the severity of the pathology: 1, minimal; 2, slight; 3, moderate; 4, marked and 5, severe.

In the main study group, all the organs from the C, HD and VHD and some in the LD and MD were examined. The results from main and recovery groups are presented in the following tables. There were Histopathological changes in the nasal cavity/sinuses in both sexes and nasopharynx and larynx in males, which were not considered clinically relevant. Myocardial fibrosis was observed in the control (9/11), HD (3/12) and VHD (6/10) males. It has been reported that myocardial degeneration/fibrosis was a spontaneous finding in the aged male rats (Peckham, J. C. *Animal Histopathology in Handbook of Toxicology*, 2<sup>nd</sup> edition (pp 649-740), M.J. Derelanko & M.A. Hollinger (eds), Boca Raton: CRA Press LLC, 2002). Since this finding was observed in all group males with relative higher incidence in the control group, it was not considered a treatment related effect. Minimal to slight lung macrophage accumulation was observed in VHD males but not in females, and resolved in the recovery period. Minimal to slight tracheobronchial lymph node erythrocytosis/hemorrhage was observed in VHD females and resolved in recovery period. Increased incidence and severity of secretory activity in the mammary gland was observed in MD, HD and VHD without a clear dose-dependent pattern; this finding was still present in HD and VHD females at the end of recovery period. The cysts follicular dilatation in ovary was observed in the MD, HD and VHD groups. A higher incidence of decreased corpora lutea and estrus cycle occurred at the MD, HD and VHD. The cystic follicular dilatation in the ovaries and increased secretory activity in the mammary glands may be attributed to stimulation of the  $\beta_2$  receptors on the ovaries leading to cysts formation and disturbance of the estrus cycle (Lara et al. *Endocrinology* 141: 1059-1072,

2000). The single finding of mammary gland adenoma in the HD female was not considered a significant test article-related toxicity.

### Males

Sex, Organ, Pathology	Incidence, Mean Severity Score				
	C	LD	MD	HD	VHD
Cavity Nasal/Sinuses Degeneration/Atrophy: Olfactory Epithelium Severity Score	0/11	0/12	0/12	0/12	<b>5/10</b> <b>1.2</b>
Metaplasia Respiratory: Olfactory Epithelium Severity Score	0/11	0/12	0/12	0/12	<b>2/10</b> <b>1.0</b>
Metaplasia Squamous: Olfactory Epithelium Severity Score	0/11	0/12	0/12	0/12	1/10 1.0
Heart Myocardium: Degeneration/ Fibrosis Severity Score	9/11 1.0	NE	NE	3/12 1.0	6/10 1.2
Larynx Metaplasia: Squamous Severity Score	0/11	0/12	0/12	0/12	<b>4/10</b> <b>1.0</b>
Lung Macrophage: Accumulation Severity Score	3/11 1.0	NE	NE	3/12 1.0	<b>5/10</b> <b>1.7</b>
Nasopharynx: Goblet Cells: Hypertrophy Severity Score	0/11	NE	NE	0/12	<b>3/10</b> <b>1.0</b>

NE, Not examined.

**Bolded** values are considered treatment related.

## Females

Organ, Pathology	Incidence, Mean Severity Score				
	C	LD	MD	HD	VHD
Cavity Nasal/Sinuses Olfactory Epithelium Degeneration/Atrophy Severity Score	0/11	0/10	0/11	0/12	2/12 1.5
Goblet Cells: Hypertrophy/ Hyperplasia Severity Score	0/11	0/10	0/11	1/12 1.0	2/12 1.0
Heart Myocardium: Degeneration/ Fibrosis Severity Score	1/11 1.0	NE	NE	2/12 1.0	1/12 1.0
LN Tracheobronchial Erythrocytosis/Hemorrhage Severity Score	1/11 2.0	1/10 1.0	0/11	2/12 1.5	<b>4/10</b> <b>1.5</b>
Mammary Gland Secretory Activity Severity Score	4/11 1.0	4/10 1.0	<b>6/11</b> <b>1.5</b>	<b>5/12</b> <b>1.2</b>	<b>7/12</b> <b>1.3</b>
Acinar development Severity Score	11/11 1.5	10/10 1.2	11/11 1.7	<b>12/12</b> <b>1.8</b>	<b>12/12</b> <b>1.8</b>
Adenoma	0/11	0/10	0/11	<b>1/12</b>	0/12
Ovary Cysts: Follicular Dilatation Severity Score	6/11 1.1	5/10 1.2	<b>9/11</b> <b>1.3</b>	<b>9/12</b> <b>1.6</b>	<b>9/12</b> <b>2.0</b>
Cysts Severity Score	2/11 1.5	3/10 1.0	0/11 0	<b>6/12</b> <b>1.2</b>	<b>4/12</b> <b>1.8</b>
Corpora Lutea: Decreased Number or Absence	2/11	4/10	<b>7/11</b>	<b>8/12</b>	<b>7/12</b>
Estrous Cycle Estrous	3/11	4/10	<b>5/11</b>	<b>8/12</b>	<b>6/12</b>

NE, Not examined.

**Bold** values are considered treatment related.

## Recovery Animals

Organ, Pathology (Females)	Incidence, Mean Severity Score		
	C	HD	VHD
Mammary Gland			
Secretory Activity	2/6	<b>5/6</b>	<b>3/6</b>
Severity Score	1.0	<b>1.2</b>	<b>1.7</b>
Ovary			
Cysts			
Follicular Dilatation	2/6	2/6	<b>3/6</b>
Severity Score	1.5	2.0	<b>1.3</b>

**Bold** values are considered treatment related.

**Study Title:** Toxicity study by inhalation administration to beagle dogs for 39 weeks

**Study No.:** CD2007/01006/01

**Key Findings**

- The deposited doses were 2.38 (LD), 15.6 (MD) and 30.5(Phase 1)/127.5 Phase 2) (HD) µg/kg.
- Vasodilation (ears) was a predominant clinical sign at the MD and HD.
- Pulse rate was increased in the first 3 days of 30.5 µg/kg dose (Phase 1); in Phase 2 where all doses were tested, the increase in pulse rates was observed inconsistently in different time intervals in MD and HD animals;
- In Phase 2, both sexes showed a dose related increased heart rate at the LD and MD. However, the increased heart rate in both sexes at HD was lower than the MD heart rate.
- Clinically relevant target organs of toxicity were the lungs and bronchi (alveolar hemorrhage, HD, Females), heart (myocardial fibrosis associated with mesothelial hyperplasia, HD, Females), kidneys (cortex, fibrosis and tubular atrophy, HD, Females), trachea bifurcation (squamous metaplasia, HD, both sexes) and liver (increased periportal rarefaction/ decreased centrilobular rarefaction, all doses, Females; MD, HD, Males).
- The inhalation NOAEL was 15.6 mcg/kg. The AUC<sub>0-t</sub> at Week 39 was 34.8 ng.h/ml in males and 39.0 ng.h/ml in females.
- The AUC ratios relative to GW642444M were 0.01, 0.06 and 0.6, respectively, for the metabolites, GW630200 and, GSK932009, and for (b)(4) (GI179710).

**Volume No. and page No.:** 3 and page 1.

**Conducting Laboratory and Location:** (b)(4)

**Date of Study Initiation:** 10/23/96

**GLP Compliance:** Yes.

**QA Report:** Yes (X); No ( )

**Drug, Batch No. and % Purity:** R204768; purity not stated.

## Methods

Species/Strain: Beagle dogs.

No./sex/group: 4.

Age: 10-12 months.

Weight: M, 8.5-12.7 kg, F, 6.8-10.6 kg

Dose: The target and estimated doses and concentrations are presented in the following table excerpted from the submission including the method for determining the estimated achieved doses. The mean MMAD ranged from 1.6 to 2.2  $\mu\text{m}$  and the mean GSD (determined by a <sup>(b)(4)</sup> cascade impactor) ranged from 2.22 to 2.28. Based on 25% deposition, the deposited lung doses were: 2.39 (LD), 15.6 (MD) and 30.5/127.5  $\mu\text{g}/\text{kg}$ . On days 1, 2 and 3, the HD was 30.5  $\mu\text{g}/\text{kg}$  (Phase 1), and from day 4 to termination, all the animals were dosed, and the HD dose was increased to 127.5  $\mu\text{g}/\text{kg}$  (Phase 2).

Group Number	Target Dose <sup>1</sup> ( $\mu\text{g}/\text{kg}/\text{day}$ )	Estimated Achieved Dose <sup>1</sup> ( $\mu\text{g}/\text{kg}/\text{day}$ )	Target Mask Aerosol Concentration <sup>1</sup> ( $\mu\text{g}/\text{L}$ )	Achieved Mask Aerosol Concentration <sup>1,2</sup> ( $\mu\text{g}/\text{L}$ )	Number / Sex
1	0	0	0	0	4M+4F
2	10	9.55	1.00	1.04	4M+4F
3	65	62.5	6.75	6.78	4M+4F
4 <sup>a</sup>	130/500	122/510	13.5/52.0	12.5/55.0	4M+4F

1. Doses and concentrations are expressed in terms of GW642444.

2. As determined by chemical analysis

<sup>a</sup> In Group 4, treatment at 510  $\mu\text{g}/\text{kg}/\text{day}$  was preceded by 3 days treatment at 122  $\mu\text{g}/\text{kg}/\text{day}$ .

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:

$$D = (\text{RMV} \times \text{T} \times \text{C}) / (\text{BW})$$

D dose ( $\mu\text{g}/\text{kg}$ )

RMV(L/min)  $0.499 \times \text{BW}(\text{kg})^{0.809}$  (Bide et al, 2000)

T Duration of exposure/day (minutes)

C Aerosol GW642444 concentration ( $\mu\text{g}/\text{L}$ )

BW Group average body weight for study (kg)

RMV Respiratory minute volume (L)

Note: The pretreatment with low dose in Phase 1 followed by Phase 2 treatment for HD animals may have potentially reduced the  $\beta$ -adrenergic toxicity for the HD groups. The pretreatment was not used for the low and mid dose groups. Since the MD was identified as the NOAEL, the impact of the pretreatment phase for HD animals was considered minimal.

Route: Inhalation by way of an oralnasal face mask with a 30 min exposure/day.

Observations and times:

Clinical signs: Daily.

Body weight: Day 1 and weekly thereafter.

Food consumption: Daily.

Ophthalmoscopy: Prior to initiation of study and during week 39. A peer review finding of the results was made in week 40.

EKG: Prior to initiation of study and during week 39.

Femoral pulse rates: Immediately after completion of dosing and at 30 min and 1, 2, 4, 8 and 23.5 hr after completion of dosing on days 1, 2 and 3 in Phase 1. and on days 1, 2, 3, 4, 5, 6 and 7 and 1 day in week 4, 13 and 39 in Phase 2.

Hematology, Clinical Chemistry and Urinalysis: Prior to initiation of dosing and during weeks 4, 13, 26 and 39 in Phase 2.

Urinalysis: Prior to initiation of study and during week 4.

Troponin I: The analysis was carried out on the BayerACS180SE chemiluminescence analyzer. Day 1 (Phases 1, HD, 30.5 mcg/kg; Phase 2, HD, 127.5 mcg/kg), blood samples were collected pre dose, immediately after and at 4, 8 and 23.5 hr after completion of dosing. Day 4 (LD, 2.39, MD, 15.6 mcg/kg) blood samples were collected pre dose immediately after and 4 and, 8 hr after completion of dosing.

Toxicokinetics: Blood was collected during weeks 2, 26 and 39 at 30 min and 1, 2, 4, 8 and 23.5 hr after completion of dosing. Plasma was analyzed for GW642444, GI179710 <sup>(b)(4)</sup> and its metabolites, GW630200 and GSK932009, using a HPLC-MS/MS method. The lower limits of quantification were 0.1 ng/ml for GW642444M and its metabolites and 5 ng/ml for GI179710.

Gross pathology: Detailed external and internal examination.

Organs weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, testes and thymus.

Histopathology: The tissues examined are presented in the following table excerpted from the submission. In the controls and HD, the nasal turbinates were stained with Alcian Blue/PAS and Schmorl's, and the liver was stained for glycogen using PAS. Testes and epididymidis were fixed in Bouin's, and eyes were fixed in Davidson's, transferred to 70% alcohol and processed. Other tissues were stained with hematoxylin and eosin.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Optic nerves <sup>b</sup>	X
Adrenals	X	Ovaries	X
Aorta (thoracic)	X	Pancreas	X
Brain (cerebellum, cerebrum, midbrain, medulla)	X	Pituitary	X
Caecum	X	Prostate	X
Cervix	X	Rectum	
Colon	X	Salivary gland - mandibular <sup>b</sup>	X
Duodenum	X	parotid <sup>b</sup>	X
Epididymides	X	Sciatic nerve <sup>b</sup>	X
Eyes <sup>b</sup>	X	Skeletal muscle <sup>b</sup>	X
Femur (femoral head)	X	Skin	X
Gall bladder	X	Spinal cord - cervical	
Heart (auricles, ventricles and interventricular septum)	X	lumbar	X
Ileum	X	Spleen	X
Jejunum	X	Sternum with bone marrow	X
Kidneys	X	Stomach - cardiac, fundic and pyloric	X
Larynx	X	Testes	X
Liver (left lateral and right medial lobes)	X	Thymus	X
Lung including bronchi <sup>a</sup>	X	Thyroids with parathyroids	X
Lymph node - cervical	X	Tongue	X
mesenteric	X	Tonsils	X
popliteal		Trachea (top, middle and bottom)	X
tracheobronchial	X	Tracheal Bifurcation (with mainstem bronchi)	X
Mammary gland - inguinal	X	Urinary bladder	X
Nasal cavities incorporating nasopharynx and skull	X	Uterus	X
Oesophagus (distal portion)	X	Vagina	X

a. All lobes examined, left and right, to include proximal and distal areas  
 b. Only one examined

Adequate battery of tissues: Yes  
 Peer review: Yes.

**Results**

Mortality: None.

Clinical signs: The results are summarized in the following table. The vasodilation occurred in the Phase 2 study in the MD and HD based on the duration of vasodilation.

Phase / Observation	Incidence, and Mean No. of Days N=4/group							
	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
Phase 1 Vasodilation, Ears	-	-	-	-		-	3, 2	2, 1
Phase 2 Vasodilation, Ears	3, 4	3, 1	3, 6	3, 8	<b>4, 74</b>	<b>4, 77</b>	<b>4, 195</b>	<b>4, 118</b>
Abnormal Gait	1, 2	0	0	0	0	0	2, 8	2, 3

**Bold** values are considered treatment related.

Body weight: There was no treatment related effect observed at the end of week 39. An increase in body weight gain during the first 10 weeks in the MD and HD was observed without a dose related pattern.

Food consumption: No treatment related effect.

Ophthalmoscopy: No treatment related effect.

Femoral pulse rates: Phase 1: In animals receiving the HD (30.5 mcg/kg) daily for 3 days, increased pulse rate occurred on all 3 days. Relative to the predose pulse rate, the daily maximum change in pulse rate for males was 1.62x (day 1), 1.43x (day 2) and 1.31x (day 3) and for females the maximum change in pulse rate was 1.45x (day 1), 1.71x (day 2) and 1.46x (day 3). The results in Phase 2 are summarized in the following table. The HD was increased from 30.5 mcg/kg to 127.5 mcg/kg. In both sexes, the average maximum dose related increased pulse rate on weeks 1.1, 1.7, 4, 13 and 39. The increase in pulse rates was observed inconsistently in different time intervals in MD and HD animals. In males, a dose related maximum increased pulse rate occurred at the MD and HD at weeks 1.1, 1.7, and 13 and not on weeks 4 and 39. In females, a dosed related maximum increased pulse rate occurred at weeks 1.1, 13 and 39 and not on weeks 1.7 and 4.

Dose (µg/kg/day)	Average predose pulse rate (bpm)					Average Maximum pulse rate observed (bpm)					Average Change in maximum pulse rate (X predose)					Average Time maximum pulse rate observed (hours after completion of dosing)				
	Week					Week					Week					Week				
	1.1	1.7	4	13	39	1.1	1.7	4	13	39	1.1	1.7	4	13	39	1.1	1.7	4	13	39
<b>Males</b>																				
0	102	103	95	94	86	114	105	101	105	100	1.12	1.02	1.06	1.12	1.16	23.5	8	0.5	8	23.5
9.55	106	103	99	95	103	117	117	118	116	125	1.10	1.14	1.19	1.22	1.21	2	8	IAD	IAD	0.5
62.5	105	108	101	91	104	150	118	119	117	124	1.43	1.09	1.18	1.29	1.19	0.5	1	8	IAD	IAD
510	105	105	92	94	111	189	135	120	154	128	1.80	1.29	1.30	1.64	1.15	1	IAD	0.5	1	0.5
<b>Females</b>																				
0	98	106	97	97	99	101	106	102	103	99	1.03	1.00	1.05	1.06	1.00	4	PD	2	23.5	PD
9.55	90	102	84	90	96	108	102	100	99	124	1.20	1.00	1.19	1.10	1.29	0.5	PD	IAD	4	1
62.5	86	109	92	96	102	148	124	127	126	121	1.72	1.14	1.38	1.31	1.19	IAD	1	IAD	2	IAD
510	101	98	91	95	110	172	129	124	149	142	1.70	1.32	1.36	1.57	1.29	1	0.5	0.5	1	0.5

bpm Beats per minute  
 IAD Immediately after dosing  
 PD Pre-dose

EKG: The effect on the heart rate in the Phase 2 study by week 39 is summarized in the following table excerpted from the submission. Tachycardia was present at all doses with females showing a greater effect especially at the MD and HD. In both sexes, the response was dose related at the LD and MD. The HD showed a lower tachycardia than the MD. (Note that the pretreatment with low dose in Phase 1 for the HD animals may provide cardiac protection.)

Dose (µg/kg/day)	Group Mean Heart Rate (bpm)			% Predose
	Pre-treatment	Week 39		
		Predose	IAD	
<b>Males</b>				
0	116	110	98	0.89
9.55	110	111	116	1.05
62.5	116	96	117	1.22
510	133	106	119	1.12
<b>Females</b>				
0	101	101	102	1.01
9.55	112	86	95	1.10
62.5	113	77	130	1.69
510	141	96	139	1.45

IAD Immediately after dosing

Hematology/Clinical Chemistry: The results are summarized in the following table. At week 39, in females, there was a decrease in hematocrit and hemoglobin levels and an increase in platelet, ALP and AST levels. All were dose related and are considered clinically monitorable. In both sexes, there was a dose related increase in potassium levels in MD and HD. This was not considered a toxic effect, since the mean values of all the groups were within the normal range (3.8-5.6 mmol/L of dogs, 2008 The Merck Veterinary Manual).

Parameter	% Change from Control					
	LD		MD		HD	
	M	F	M	F	M	F
Hematocrit	NC	-8	NC	-11	NC	-17
Hemoglobin	NC	-8	NC	-11	NC	-17
Platelets	NC	+14	NC	+25	NC	+67
Potassium	-5	+2	+12	+13	+17	+18
ALP	NC	+52	NC	+98	NC	+162
AST	NC	-3	NC	+37	NC	+49

NC, No change from controls.

Urinalysis: No treatment related effect.

Troponin I: The criterion for a positive response was not stated. However, in an earlier 4-week study in dogs conducted by the same laboratory, with the same compound, the criterion for a positive response was levels > 0.15 ng/ml (Hao, 10/29/08 review of IND 74, 696, submission date 11/08//07). This value was used to determine a positive troponin response in this submission. In this study, most of the troponin I levels prior to

administering the compound was 0.03 ng/ml. For those dogs that had baselines above 0.03 ng/ml, a positive response would be an increase of 15 ng/ml above their baseline... The results are summarized in the following table. In the Phase 1, troponin I levels were increased in 3/4 males and 4/4 females in HD. At the end of 23.5 hr following administration, troponin levels were still elevated in 1/4 males and in 1/3 females. In Phase 2, the incidence was 1/4 in MD males and females and 1/4 in HD females. The pretreatment with low dose (Phase 1) in the HD animals may provide certain protection from cardiac injury in the phase 2 HD animals.

Phase	Group	Inh. Dose mcg/kg	Incidence of Increased Troponin Levels	
			Males	Females
1	HD	30.5	3/4	4/4
2	MD	15.6	1/4	1/4
	HD	127.5	0/4	1/4

Toxicokinetics: The following tables present the toxicokinetics of the main compound, GW642444M, the two metabolites, GW630200 and GSK932009 and (b)(4) (G1179710) (b)(4). The AUC<sub>0-t</sub>s and C<sub>max</sub>s of GW642444M in both sexes were similar, dose related and not dose proportional. There was no accumulation. For the metabolite, GW630200, levels were detected only at the HD during weeks 4, 26 and 39; the second metabolite, GSK932009, was detectable at the MD and HD during weeks 4, 26 and 39. The levels at the HD in both sexes were disproportionately higher than the MD. For (b)(4) (G1179710) (b)(4), AUCs were detectable only at the HD. The AUC<sub>0-t</sub>s at the MD was not calculated due to insufficient data. The report indicated that the AUC<sub>0-t</sub> ratios of the metabolites relative to GW642444M were 0.01, 0.06 respectively, for GW630200 and GSK932009 and 0.06 for G1179710, (b)(4) G1179710.

**A summary of the toxicokinetic parameters for GW642444 in male and female dogs is presented below:**

Parameter	Week	Estimated Achieved Dose (µg/kg/day)					
		Male			Female		
		9.55	62.5	510	9.55	62.5	510
AUC <sub>0-t</sub> (ng.h/mL)	4	5.97	34.3	277	3.36	34.6	402
	26	5.39	27.5	248	3.54	27.2	309
	39	8.10	34.8	259	7.36	39.0	380
C <sub>max</sub> (ng/mL)	4	1.79	14.5	93.8	1.84	18.0	190
	26	1.93	11.3	97.9	1.93	13.6	143
	13	2.34	14.7	56.3	3.34	18.2	128

A summary of the toxicokinetic parameters for the metabolite GW630200 in male and female dogs is presented below:

Parameter	Week	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ ) GW642444					
		Male			Female		
		9.55	62.5	510	9.55	62.5	510
AUC <sub>0-t</sub> (ng.h/mL)	4	NC	NC	4.82	NC	NC	2.90
	26	NC	NC	3.45	NC	NC	3.11
	39	NC	NC	2.59	NC	NC	2.25
C <sub>max</sub> (ng/mL)	4	NQ	NQ	0.892	NQ	NQ	0.770
	26	NQ	NQ	0.810	NQ	NQ	0.899
	39	NQ	NQ	0.661	NQ	NQ	0.638

NQ Not quantifiable  
NC Not calculated

A summary of the toxicokinetic parameters for the metabolite GSK932009 in male and female dogs is presented below:

Parameter	Week	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ ) GW642444					
		Male			Female		
		9.55	62.5	510	9.55	62.5	510
AUC <sub>0-t</sub> (ng.h/mL)	4	NC	1.41	25.7	NC	2.84	25.5
	26	NC	0.922	22.3	NC	1.74	22.5
	39	NC	0.603	16.1	NC	1.06	17.5
C <sub>max</sub> (ng/mL)	4	NQ	0.315	3.97	NQ	0.626	3.84
	26	NQ	0.296	3.17	NQ	0.385	3.58
	39	NQ	0.207	2.86	NQ	0.294	2.54

NQ Not quantifiable  
NC Not calculated

A summary of the toxicokinetic parameters for (b)(4) GI179710 in male and female dogs is presented below:

Parameter	Week	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ ) GW642444					
		Male			Female		
		9.55	62.5	510	9.55	62.5	510
AUC <sub>0-t</sub> (ng.h/mL)	4	NC	NC	207	NC	NC	228
	26	NC	NC	153	NC	NC	225
	39	NC	NC	152	NC	NC	141
C <sub>max</sub> (ng/mL)	4	NQ	34.8	259	NQ	39.0	258
	26	NQ	17.7	217	NQ	19.4	238
	39	NQ	19.6	151	NQ	19.3	230

NQ Not quantifiable  
NC Not calculated

Organ weight: The following table shows that in males, there was a dose related small increase in absolute and relative liver weight and at the HD, a decrease in the absolute and relative prostate weight. In females, at the HD there was a small increase in the absolute and relative liver weight. The increased liver weight was accompanied with slightly increased ALP and AST levels.

Organ	% Change from Control					
	LD		MD		HD	
	Absolute	Relative	Absolute	Relative	Absolute	Relative
<b>Male</b>						
Liver	+8	+10	+12	+10	+23	+23
Prostate	-5	+6	-5	-1	-32	-35
<b>Female</b>						
Liver	-8	-10	+9	+8	+27	+21

### Necropsy

Macroscopic pathology: Males, small thymus, C, 2/4; LD, 2/4; MD, 1/4; HD, 4/4.

Histopathology: The results are summarized in the following table. The severity was described as minimal, slight, and moderate and marked. This reviewer used a scoring system of 1, 2, 3 and 4 to describe the severity, and reported the results as the mean severity scores. Targeted organs of toxicity were the lungs (HD, Females), heart (HD, Females), liver (MD and HD, Males; LD, MD and HD, Females), kidneys (HD, Females), and trachea bifurcation (HD, both sexes). The mean severity scores were in the slight and minimal range. The myocardial fibrosis and increased periportal hepatocyte rarefaction/ decreased centrilobular rarefaction (glycogen) are characteristic of a  $\beta_2$  agonist Hornbrook (1970). Mesothelial hyperplasia was associated with inflammation and was present in the dog with myocardial fibrosis, a chronic inflammatory condition is considered a class effect. The low incidence of cardiac toxicity in the HD groups may be attributed to the pretreatment with low dose (Phase 1) in the HD animals which provided protection from cardiac injury in the phase 2 HD animals. The incidence of thymus involution/atrophy was similar in the control and all treated groups males. Although the severity score was higher in HD males when compared to the control males (3.8 vs., 2.5), it was comparable to the control females (3.3). Hence, the thymus involution/atrophy was not considered a significant safety concern for human trials.

In the nasal turbinates, males were more affected than females. The following increased incidence occurred in the: olfactory epithelium- increased nasal associated lymphoid tissue [NALT (HD male)], olfactory epithelium-lymphoid cell infiltrate in the lamina propria cell infiltrate (HD male and female); olfactory epithelium-unilateral epithelial degeneration (HD male); respiratory epithelium-epithelial degeneration/regeneration (all doses in both sexes); respiratory epithelium-mixed inflammatory cell infiltrate in the lamina propria (all doses in males, MD and HD in females); respiratory epithelium-

unilateral erosion (HD male); respiratory epithelium-unilateral submucosal glandular inflammation/degeneration (HD male); respiratory epithelium- unilateral vascular congestion (HD male); squamous epithelium-epithelial degeneration/regeneration (HD male); transitional epithelium-epithelial degeneration/regeneration (HD male) and transitional epithelium-mixed inflammatory cell infiltrate in lamina propria (HD male). These findings indicate a local irritant effect. However, these findings are not clinically relevant since the drug product will be given clinically by inhalation as a DPI; consequently, the drug product will not be in contact with the nasal cavity.

The report indicated that the incidence of hypospermatogenesis seen at the HD was within the background reported for Beagle dogs of this age and strain (Goedken et. al. Toxicologic Pathology 36: 464, 2008). However, supportive data and reference were not submitted by the sponsor. Since the incidence of hypospermatogenesis was 1/4 each in the control, LD and MD groups and 2/4 in the HD males, this small increase in incidence occurred at the HD is not considered a significant clinical concern.

Organ/ Pathology	Incidence/ Mean Severity Score							
	C		LD		MD		HD	
	Male	Female	Male	Female	Male	Female	Male	Female
<b>Heart</b>								
Myocardial Fibrosis Severity Score	0/4	0/4	0/4	0/4	0/4	0/4	0/4	<b>1/4</b> <b>1.0</b>
Mesothelial Hyperplasia Severity Score	0/4	0/4	0/4	0/4	0/4	0/4	0/4	<b>1/4</b> <b>2.0</b>
<b>Lungs and Bronchi</b>								
Alveolar Hemorrhage Severity Score	0/4	1/4 1.0	1/4 1.0	0/4	1/4	0/4	0/4	<b>3/4</b> <b>1.0</b>
<b>Liver<sup>a</sup></b>								
Inc. Periportal Hepatocyte Rarefaction/Dec. Centrilobular Rarefaction Severity Score	0/4	0/4	0/4	<b>2/3</b> <b>1.0</b>	<b>1/4</b> <b>2.0</b>	<b>1/4</b> <b>2.0</b>	<b>3/4</b> <b>1.3</b>	<b>4/4</b> <b>2.0</b>
Pigmented Macro. Severity Score	1/4 1.0	0/4	0/4	0/4	0/4	0/4	1/4 1.0	2/4 1.0
<b>Kidneys</b>								
Cortex-Fibrosis Severity Score	0/4	0/4	0/4	0/4	0/4	0/4	0/4	<b>2/4</b> <b>1.5</b>
Cortex Tubular Atrophy Severity Score	0/4	0/4	0/4	0/4	0/4	0/4	0/4	<b>1/4</b> <b>2.0</b>

<sup>a</sup> PAS staining of selected liver samples indicated that the rarefaction at both sites was due to glycogen.

**Bold** values are considered treatment related.

Organ/ Pathology	Incidence/ Mean Severity Score							
	C		LD		MD		HD	
	Male	Female	Male	Female	Male	Female	Male	Female
<b>Trachea Bifurcation</b>								
Squamous Metaplasia	0/4	1/4	0/4	1/4	0/4	0/4	1/4	2/4
Severity Score		1.0		1.0			1.0	1.0
<b>Thymus</b>								
Atrophy/Involution	4/4	4/4	4/4	4/4	4/4	3/4	4/4	1/4
Severity Score	2.5	3.3	2.5	2.8	1.8	1.3	3.8	3.0
<b>Testes</b>								
Hypospermatogenesis	1/4		1/4		1/4		2/4	
Severity Score	1.0		1.0		2.0		2.0	

**Bold** values are considered treatment related.

## OVERALL SUMMARY, DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

### Summary and Discussion

GW642444 is being developed as a monotherapy for treatment of asthma, and as a LABA component of the ICS/ LABA combination product for the treatment of COPD.

In the 26-week inhalation toxicity study with an 8-week recovery period in rats, the lung deposited doses based 10% of the estimated achieved doses were: 5.77 (LD), 53.7 (MD), 67.4(HD) and 1025.3 (VHD) mcg/kg. There was no treatment related mortality and no treatment related clinical signs. In females, there was a reversible increase in body weight gain at the MD and HD which was attributed to the anabolic effect of  $\beta_2$  agonism (Emery et al. Bioscience Report 4: 986-996, 1984). In males (all doses) and in females (MD, HD and VHD), the increase in food consumption was also characteristic of a  $\beta_2$  agonist (Reeds et al. Br J Nutrition 56: 249-259, 1986). Unlike other  $\beta_2$  agonists, GW642444M decreased serum glucose (both sexes) rather than doing the opposite due to increased gluconeogenesis (Smith et al. L Royal college of Physicians 18 (3):190-194, 1984). However, this effect was small in magnitude and reversible. The AUC ratios relative to GW642444M were 0.002 and 0.02 for the metabolites, respectively, for GW630200 and GSK932009 and 3.0 for GI179710, (b) (4). Histopathology findings included the nasal cavity/sinuses (degeneration/atrophy and squamous metaplasia of the olfactory epithelium), larynx (squamous metaplasia, lung (accumulated macrophages) nasopharynx (goblet cell hypertrophy), tracheobronchial lymph node (erythrocytosis/hemorrhage), mammary gland (secretory activity and acinar development) and ovary (cystic follicular dilatation and cysts). Since GW642444M will be administered as a dry powder inhaler and clinically, the nasal cavity will not be

exposed, there is no concern for the changes seen in the nasal cavity/sinuses and nasopharynx which indicated a local irritant effect. The changes seen in the larynx and lungs at the VHD were attributed to a local irritant effect. The cystic follicular dilatation in the ovaries and increased secretory activity in the mammary glands may be attributed to stimulation of the  $\beta_2$  receptors on the ovaries leading to cysts formation and disturbance of the estrus cycle (Lara et al. *Endocrinology* 141: 1059-1072, 2000). Further, clenbuterol,  $\beta_2$  agonists, in high doses produced in female rats an increase in estrogen-like receptor concentrations and uterine weight accompanied by estrogen-like modifications in the reproductive tract (Re, Badino et al. *Vet.Pharmacol Ther.* 16/3:328-354, 1993 and *Am J Vet Res.* 54: 438-442, 1993). These effects were antagonized when clenbuterol was administered with propranolol, a  $\beta_2$  antagonist, supporting the concept that  $\beta_2$  receptors were present in the ovaries. In this study with clenbuterol, the mammary glands were not investigated. In this 26-week study, at the end of the recovery period, the findings in the mammary gland and the ovary were still evident. The inhalation NOAEL was 267.4 mcg/kg ( $AUC_{0-t}$ : 300.5 ng.h/ml) in males and 5.77 mcg/kg ( $AUC_{0-t}$ : 5.2 ng.h/ml) in females indicating that the females were more sensitive than males due to the effect on the reproductive system.

In the 39-week inhalation toxicity study in the dog, the deposited doses were 2.38 (LD), 15.6 (MD) and 30.5/127.5 (HD) mcg/kg. There were 2 phases of the study. In Phase 1, the HD was 30.5 mcg/kg administered daily for 3 days. In Phase 2, the control and 3 doses were administered with the HD being increased from 30.5 mcg/kg to 127.5 mcg/kg. The only clinical sign was vasodilation seen in the ears at the MD and HD in Phase 2. In addition, there were increased pulse rate and heart rate which are characteristic of  $\beta_2$  agonism. The increased pulse and heart rate was inconsistent with respect to a dose response. Troponin I levels increased in 7/8 animals in Phase 1 HD animals dosed with 30.5 mcg/kg while in the Phase 2 study, 1/8 MD animals and 1/8 HD animals (127.5 mcg/kg) showed an increase in troponin levels indicating some damage to the heart. Hematological changes in females included a dose related decrease in hematocrit and hemoglobin and a dose related increase in platelet, ALP and AST levels. The increase in the ALP and AST levels may be associated with the liver changes (increase in liver weight and histopathology finding) although similar liver changes seen in males were not accompanied by increased ALP and AST levels. Absolute and relative liver weights were increased at the HD (both sexes) and absolute and relative prostate was decreased at the HD. The change in prostate weight was not correlated by histopathology of that organ. The AUC ratios relative to GW642444 were 0.01, 0.06 and 0.6, respectively, for the metabolites, GW630200 and, GSK932009, and for (b)(4) (G1179710), (b)(4). Histologically, the relevant targeted organs were the heart (myocardial fibrosis and mesothelial hyperplasia), lungs and bronchi (alveolar hemorrhage), liver (increased periportal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction), kidneys (cortex- fibrosis and tubular atrophy), and trachea bifurcation (cortex- fibrosis and tubular atrophy). As indicated in the discussion of the rat data, there is no clinical concern for the toxicity observed in the nasal turbinates, administration of the GW642444M dry powder inhaler will be by oral inhalation and not be in contact with the nose. The myocardial fibrosis seen is characteristic of  $\beta_2$  agonist and was only observed in one animal in the HD females in

view of the protective effect of pretreatment with a low dose of GW642444 in the HD animals. The increased periportal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction and increase liver weight seen in both sexes was attributed to a class effect of  $\beta_2$  agonist which is considered to be related to a shift in the distribution of glycogen within the hepatocyte (Hornbrook, Fed. Proc. 29 (4): 1381-1384, 1970). The inhalation NOAEL was pulmonary deposited dose of 15.6 mcg/kg ( $AUC_{0-t}$ : 34.8 ng.h/ml in males and 39.0 ng.h/ml in females due to heart, kidney, lung/bronchi, and trachea findings at the HD.

In comparing the toxicokinetic and toxic profiles in rats and dogs, the metabolism of GW642444 was similar since both produced the metabolites, GW630200 and GSK932009 and both target the lungs.

As indicated by medical review (Dr. Porter) on March 5, 2010, the potential clinical dose may not exceed 25 mcg/day. The safety margins based on this clinical dose was estimated in terms of the lung burden (mcg/g) and AUC comparison, and shown in the following tables. It should be noted that the human AUC value used in the following safety margin calculation was referred to the IND 77, 855 review by Dr. Huiqing Hao dated 6/28/08 in which AUC at 25 mcg/day GW642444 with combination of 400 mcg of fluticasone furoate was expected to be 0.165 ng.h/ml for a proposed 28-day clinical combination study in COPD patients. A more accurate safety margin estimation regarding AUC comparison could be achieved based on AUC values from more relevant clinical study (e.g. asthma patients).

Species	NOAEL				Safety Margin A/B	
	Total Deposited Dose, mcg		Lung Burden mcg/g A			
	Male	Female	Male	Female	Male	Female
Rat	66.8	1.4	45	0.96	1800	38
Dog	156	156	1.4	1.4	57	57
Human dose 25 mcg (0.5 mcg/kg)	25	25	<b>B</b> 0.025	<b>B</b> 0.025		

<sup>a</sup> Lung burden: Total deposited dose (mcg) ÷ Lung weight (Body Weight/Lung Weight for rat, dog and human: 0.250 kg/1.5 g; 10 kg/110 g; 50 kg/1000 g).

Species	NOAEL, mcg/kg				Safety Margin			
	mcg/kg A		AUC <sub>0-t</sub> ng.h/ml C		mcg/kg A/B		AUC <sub>0-t</sub> ng.h/ml C/D	
	Male	Female	Male	Female	Male	Female	Male	Female
Rat	267	5.8	334	3.7	534	11.6	2024	22
Dog	15.6	15.6	34.8	39.0	31	31	211	236
Human dose 25 mcg (0.5 mcg/kg) <b>B</b> AUC: 0.165 <sup>a</sup> ng.h/ml								

<sup>a</sup> Obtained from the 6/26/08 review of IND 77,855 by Huiqing Hao, Ph.D.

**Conclusion:** The target organs of toxicity and the NOAELs were determined in the chronic rat and dog studies of GW642444. With the estimated human exposure of GW642444 from the IND 77,855 review for a combination clinical study at the dose of GW642444 25 mcg per day, the Safety Margins for a clinical dose of 25 mcg/day in terms of AUC or lung burden are well above the acceptable levels based on the NOAELs determined in the rat and dog studies.

**Recommendation:** No action indicated.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-74696	ORIG-1	GLAXOSMITHKLIN E	GW642444 INHALATION POWDER

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/s/

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LAWRENCE F SANCILIO  
03/05/2010

JEAN Q WU  
03/05/2010

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**IND number:** 74,696

**Review number:** 1

**Sequence number/date/type of submission:** 11/8/2007/original submission

**Information to sponsor:** Yes ( ) No (x)

**Sponsor and/or agent:** GlaxoSmithKline

**Manufacturer for drug substance:** GlaxoSmithKline

**Reviewer name:** Huiqing Hao, Ph.D

**Division name:** Pulmonary and Allergy Products

**Review completion date:** 10/29/2008

#### Drug:

Trade name: Not assigned

Generic name: Not assigned

Code name: GW642444X (parent base compound), GW642444M (triphenyl acetate salt), GW642444H (b)(4)

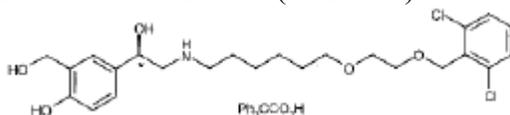
Chemical name: Triphenylacetic acid-4-{(1R)-2-[(6-2{2-[2,6-dichlorobenzyl)oxy]ethoxy{hexyl)amino]-1-hydroxyethyl}{2-(hydroxymethyl)phenol (1:1)

CAS registry number: GW642444X: 503068-34-6; GW642444M;503070-58-4

Molecular formula/molecular weight: C<sub>24</sub>H<sub>33</sub>Cl<sub>2</sub>NO<sub>5</sub> (free base)/MW 486.43; C<sub>24</sub>H<sub>33</sub>Cl<sub>2</sub>NO<sub>5</sub>.C<sub>20</sub>H<sub>16</sub>O<sub>2</sub> (as the triphenylacetate salt, GW642444M)/MW 774.78

Structure:

GW642444M is chira (R-isomer)



**Relevant INDs/NDAs/DMFs:** None

**Drug class:** Orally inhaled agonist of the beta2-adenoceptor

**Intended clinical population:** Asthma and COPD

**Clinical formulation:** GW642444M with strengths of 3, 6.25, 12.5, 25 or 50 mcg per blister (in the novel dual strip dry powder inhaler) will be in a powder blend with magnesium stearate (125 mcg) and lactose (b)(4)

**Route of administration:** Oral inhalation

**Proposed clinical protocol:** B2C109575 (Asthma dose ranging study): 594 subjects, males and females,  $\geq 12$  years of age, with persistent asthma (using an inhaled corticosteroid and have been maintained on a stable dose for 4 weeks prior to Visit 1) will be given GW642444 at 3, 6.25, 12.5, 25 or 50 mcg/day for 28 days.

**Previous clinical experience:** A total of 7 studies have been conducted outside of US. The study with longest treatment duration was a 15 day study which employed doses up to 400 mcg/day of GW642444H.

**Background:**

GW642444 is being developed as the long-acting beta2-agonist (LABA) component of a once-daily inhaled corticosteroid (ICS)/LABA combination product for the treatment of asthmatic patients (b)(4) GW642444 inhalation powder will also be developed as the LABA component of a once-daily long-acting muscarinic antagonist (LAMA)/LABA combination product for the treatment of COPD.

Early nonclinical studies (b)(4) subsequently changed to the triphenylacetate salt of GW642444 (GW642444M). To support clinical studies with this new salt form, 13-week studies in rats and dogs were performed. Nevertheless, there is no toxicity concern with this switch as the salts are expected to be dissociated from the base in body (per discussion with CMC reviewer, Dr. Parasad Peri).

The formulation tested in nonclinical studies contains no magnesium stearate which is planned to be included in commercial formulation. This discrepancy has been evaluated in the preIND phase and no bridging studies are recommended since magnesium stearate is an excipient in an approved inhalation drug product (1/31/07 meeting minutes).

Several nonclinical reviews have been completed previously. These reviews have evaluated some of the nonclinical studies submitted in the current submission. The most important reviews are those for the submissions of 7/18/2007 (contained review of 13-week toxicology studies in rat and mouse, and genotoxicity studies. These studies were used to support the dose selection for carcinogenesis studies. Executive CAC has communicated with the sponsor for the recommended doses to be used) and 11/8/2007 (preliminary review for the original submission which included the dog 4-week and 13-week studies).

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

Note: For IND reviews, unused headings may be deleted.

**Studies reviewed within this submission:**

Study Description	Study No.	Vol.
<b>Pharmacology</b>		
In vitro pharmacological characterization of the beta 2 adrenoceptor	SH2003/00036/00	3

agonist, GW642444		
In vitro pharmacological characterization of the novel beta 2 adrenoceptor agonist, GW642444A, on guinea pig and human isolated airway preparations	SH2003/00037/00	3
The effects of a novel beta2-adrenoceptor agonist, GW642444, on histamine-induced bronchospasm in conscious guinea pigs	SH2003/00042/00	3
<b>Safety Pharmacology</b>		
Single intravenous dose neurobehavioral study in the CD rat	VD2003/00131/00	3
GW642444M: acute neurobehavioral effects following inhalation administration in the conscious CD rat	VD2005/00527/00	3
GW642444H: A single dose respiratory safety pharmacology study in rats	CD20003/00833/00	4
GW642444M: Acute effects on respiratory function following inhalation administration in the conscious CD rat	CD2005/01091/00	4
GW642444H: Effect on acting potential parameters in dog isolated cardiac Purkinje fibers	FD2003/00323/01	5
GW642444H: Single intravenous dose cardiovascular study in dogs	FD2003/00275/00	5
GW642444H: The effects of single intravenous administration, of a range of doses, on cardiovascular function in the conscious beagle dog	FD2005/00097/0	5
<b>Pharmacokinetics</b>		
GW642444: The preliminary pharmacokinetics of GW642444	SH2003/00040/00	6
Investigation of the plasma protein binding of GW642444 and blood cell association of [14C]-GW642444 in mouse, rat, guinea pig, rabbit, dog and human in vitro	WD2006/02044/00	6
An in vitro investigation of both the transport via heterologously expressed human p-glycoprotein and the passive membrane permeability of GW642444 in MDCKII-MDR1 cells	WD2004/00106/00	6
An in vitro investigation of the inhibition by GW642444 of xenobiotic transport via human p-glycoprotein, heterologously expressed in MCKII cells	WD2007/01087/00	6
Quantitative whole-body autoradiography following a single oral or a single intravenous administration of [14C]GW642444 (b)(4) to male pigmented (Lister-Hooded) rats at a target dose level of 0.35 mg/kg	FD2003/00261/01	6
Quantitative whole-body autoradiography following a single oral or a single intravenous administration of [14C]GW642444 (b)(4) to male pigmented (Lister-Hooded) rats at a target dose level of 350 mcg/kg	FD2003/00185/00	6
Quantitative whole-body autoradiography following a single oral or a single intravenous administration of [14C]GI179710 to male pigmented (Lister-Hooded) rats at a target dose levels of 1 and 0.5 mg/kg, respectively	FD2005/00228/01	6
A study to determine the oral absorption and disposition of GW642444 following a single administration of GW642444M (the triphenylacetic acid salt) at a target dose level of 1 mg free base/kg in the FVBn and mdr1a/1b mouse using the short oral absorption model	WD2007/01613/00	6
<b>Toxicology</b>		

GW642444M: Toxicity study by inhalation administration to Beagle dogs for 4 weeks	WD2005/00845/00	22
GW642444M: Toxicity study by inhalation administration to Beagle dogs for 13 weeks followed by a 4 week recovery period	WD2006/01711/00	23

**Studies not reviewed within this submission (reviewed previously in review 1 for the submission of July 18, 2008)8):**

<b>General toxicity</b>		
GW64244M: Preliminary Toxicity study by Inhalation administration to CD-1 mice for 13 weeks	WD2006/01713/00	10
GW64244M: A 13-week Inhalation Toxicity Study of Powder Aerosol Formulation in the rat	WD2006/01716/00	17
<b>Genotoxicity</b>		
GW64244H: Bacterial Reverse Mutation Test	WD2003/01017/00	27
GW64244H: Intravenous Micronucleus Assay in Rats	WD2003/01411/00	27
GW64244H: In Vitro Mutation Assay Using L5178Y Mouse Lymphoma Cells	WD2003/01463/00	27
In Vitro Transformation of Syrian Hamster Cells (SHE) by 7-day exposure	WD2004/00169/00	27
In Vivo DNA Repair (UDS) Test Using Rat Hepatocytes	WD2003/01713/00	27
GI179710X: High Throughput Fluctuation Test	WD2005/00325/00	27
GI179710X: L5178Y Mammalian Cell Mutation Screen	WD2005/00277/00	27

**Studies not reviewed within this submission (deferred to a later time):**

Title	Report No.	Vol.
Investigation of the plasma protein binding of GW642444 and blood cell association of [14C]-GI179710 in animals and human in vitro	WD2006/00125/00	6
A preliminary in vitro investigation into the human oxidative enzymology of GW642444	WD2006/02720/00	6
An in vitro investigation of the metabolism of [14C]GW642444 in mouse, rat, female rabbit, dog and human	WD2006/02574/00	6
A study of the metabolism of [14C]GW642444 in the rat, dog and human in vitro and in the isolated perfused in situ	WD2003/01248/00	7
A study of the metabolism of [14C]- <sup>(b)(4)</sup> in the rat, dog and human in vitro and in the isolated perfused rat liver in situ	WD2003/01413/00	7
An in vitro investigation of the metabolism of [14C]GI179710 in human, mouse, rat, female rabbit and dog	WD2006/00205/00	7
A study to investigate biotransformation of [14C]GI179710 <sup>(b)(4)</sup> in the isolated perfused rat liver model	WD2005/01043/00	7
Quantification and identification of the metabolites of GW642444 in bile duct cannulated Sprague Dawley CD rats following a single intravenous or a single oral administration of [14C]GW642444 free base at 0.5 and 1 mg/kg, respectively	WD2006/02955/00	7
Quantification and identification of the metabolites of GW642444X in male Sprague Dawley rats following a single intravenous or a single oral administration of [14C]GW642444 <sup>(b)(4)</sup> at a dose level of 0.35 mg/kg	WD2003/01680/00	7
Quantification and identification of the metabolites of GI179710 in rats following a single oral or a single intravenous administration of 14C-GI179710 at 1 mg/kg and 0.5 mg/kg free acid, respectively	WD2006/00240/00	7
Quantification and identification of the metabolites of GW642444X in dog following a single oral and intravenous administration of [14C]GW642444 <sup>(b)(4)</sup> at 0.1 and 0.05 mg/kg free base/kg respectively	WD2004/00364/00	7
Quantification and identification of the metabolites of <sup>(b)(4)</sup> in the male dog following a single oral or intravenous administration of [14C] <sup>(b)(4)</sup> at a target dose level of 0.035 mg/kg	WD2003/0168/00	7
Quantification and identification of the metabolites of GI179710 in male beagle dogs following a single oral or a single intravenous administration of 14C-GI179710 at 1 mg/kg and 0.5 mg/kg, respectively	WD2005/01098/00	8
Preliminary characterization of the metabolites of GW642444 in human plasma following repeat dosing by inhalation	WD2006/03024/00	8
The evaluation of the induction potential of GW642444H on the gene expression of cytochrome P450 isoforms in rat livers obtained following inhaled administration at 50, 250, and 730 mcg free base/kg/day for 28 days	WD2004/01331/00	8
GW642444: The effect of GW642444H on hepatic levels of cytochrome P450 and related parameters in male and females Sprague Dawley rats after inhalation administration at 0. 50, 250 an	FD2004/0141/00	8

d730 mcg free base/kg/day for 1 month		
The effect of GW642444M on the mRNA levels of cytochrome P450 genes in rat livers obtained following inhalation administration at target doses of 50, 730 and 32900 mcg/kg/day for 14-days	WD2005/01402/00	8
Elimination of drug-related material following a single oral or a single intravenous administration of [14C]-GW642444 to bile duct cannulated male Sprague Dawley rats at a target dose level of 1 and 0.5 mg/kg free base, respectively	WD2006/01882/00	8
GW642444 (b)(4): elimination of drug derived material following a single oral or a single intravenous administration of [14C]GW642444 (b)(4) to male Sprague Dawley rats at a target dose level of 0.35 mg free base/kg	FD2003/00244/01	8
(b)(4) Elimination of drug-related material following a single oral or a single intravenous administration of [14C]GI179710 to male Sprague Dawley rats at a target dose level of 350 mcg/kg	FD2003/0143/00	8
GI179710: Elimination of drug-related material following a single oral or a single intravenous administration of [14C]GI179710 to male Sprague Dawley rats at a target dose level of 1 and 0.5 mg/kg, respectively	FD2005/00234/00	8
GW642444: elimination of drug derived material following a single oral (target dose level 0.1 mg free base/kg) or a single intravenous (target dose level 0.05 mg free base/kg) administration of [14C]GW642444 (b)(4) to male beagle dogs	FD2003/00324/00	8
(b)(4) Elimination of drug-related material following a single oral or a single intravenous administration of [14C] (b)(4) to male beagle dogs at a target dose level of 35 mcg/kg	FD2003/00217/00	8
GI179710: Elimination of drug-related material following a single oral or a single intravenous administration of [14C]GI179710 to male beagle dogs at target dose levels of 1 mg/kg and 0.5 mg/kg, respectively	FD2005/00186/00	8
<b>General toxicity</b>		
GW642444H: Tolerability study following a single inhalation administration to beagle dogs	WD2003/01081/00	9
GW642444H: A 14 day inhalation toxicity study of a powder aerosol formulation in mouse	WD2005/00839/00	9
GW642444M: preliminary toxicity study by inhalation administration to CD-1 mice for 2 weeks	WD2006/00193/00	10
GW642444M: Single dose and 7-day intravenous irritancy and toxicity study in male Sprague Dawley rats	WD2006/01713/00	12
GW642444H: A 7-day inhalation toxicity study of a powder aerosol formulation in the male Sprague Dawley rat	WD2003/00678/00	12
GW642444M: 7 day inhaled dose range toxicity study Sprague Dawley rats	WD2005/00773/00	13
GW642444H: A 14-day inhalation toxicity study of a powder aerosol formulation in the albino rat	WD2003/01059/01	14
GW642444M: A 14-day inhalation toxicity study of a powder aerosol formulation in the rat	WD2005/00844/00	15

GW642444H: A 28-day inhalation toxicity study of a powder aerosol formulation in the albino rat	CD2003/01111/01	16
GW642444H: A 7-day dose range-finding inhalation toxicity study of a powder aerosol formulation in the Beagle dog	WD2004/01318/00	20
GW642444M: Dose range findings study by inhalation administration to beagle dogs for 7 days	WD2005/00841/00	20
GW642444H: Toxicity study by inhalation administration to Beagle dogs for 2 weeks	WD2003/01082/00	21
GW642444M: A 14-day inhalation toxicity study of a powder aerosol formulation in the rat (magnesium stearate bridging study)	WD2006/02926/00	25
GW642444M: Two week inhalation study to examine the influence of magnesium stearate on the toxicity and toxicokinetics of GW642444M in the Beagle dog	WD206/02929/01	26
GW642444M: Inhalation male fertility study in rats	CD2007/00581/00	28
GW642444M: Inhalation female fertility and early embryonic development study in rats	CD2006/01165/00	31
GW642444M: Inhalation embryo-fetal development study in rats	CD2006/01166/00	33
GW642444M: Inhaled dose range study in non-pregnant and pregnant female rabbits	WD2004/01583/00	34
GW642444M: Inhaled embryo-fetal development study in rabbits	WD2006/02439/00	34
GW642444M: Subcutaneous toxicokinetic study in pregnant rabbits	CD2006/01309/00	35
GW642444M: Subcutaneous embryo-fetal development study in rabbits	CD2006/02047/00	35
GW642444M: In vitro hemolytic potential in rat and human peripheral blood	WD2006/00235/00	36

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## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

GW642444 is a novel long acting beta 2 agonist. The potency of GW642444 agonist activity in human adrenoceptors were similar to that of isoprenaline and R,R-formoterol. Intrinsic activity of GW642444 was lower than isoprenaline and R, R-fomoterol but higher than salmeterol (1.53 versus 2.67, 2.17 and 1.0, sequentially). GW642444 had high selectivity for human beta 2 over beta 1 and 3 adrenoceptors with the selective ratios of 141 and 83, respectively. In vivo studies showed that GW642444 rapidly (onset of action of 6.6 minutes) relaxed guinea pig airway contraction induced by electric stimulation. Similarly, GW642444 showed inhibition of the contractile response induced by PGF-2 alpha in human bronchus preparation, and in relaxation of histamine-induced bronchoconstriction in conscious guinea pig.

Safety pharmacology studies revealed that GW642444 induced decrease of body temperature (0.6-1°C at i.v doses ≥100 mg/kg) and locomotor activity (at i.v doses ≥100 mg/kg). Inhalation administration demonstrated similar effects. To cardiovascular system, GW642444 inhibited hERG channel with the IC50 value of 4.8 mcM. In isolated dog purkinje fibers, this drug inhibited electric activities (reduced upstroke amplitude, maximum rate of depolarization and resting membrane potential). In vivo studies in dogs, however, showed heart rate increase and blood pressure decrease but no QT prolongation. Pulmonary effects studies demonstrated no treatment related findings.

### 2.6.2.2 Primary pharmacodynamics

#### Mechanism of action:

As a beta 2 agonist, GW642444 stimulates beta2-adrenoceptor in the airways and results in bronchodilation by relaxing bronchial smooth muscle cells. The effect of bronchodilation alleviates asthmatic and COPD conditions.

#### Drug activity related to proposed indication:

Note: GW642444A

(b)(4)

1. In vitro: Binding potency and selectivity to beta 2 receptors (SH2003/00036/00)
  - Potency on beta 2 receptors  
Potency of GW642444 on beta 2 receptor activation was evaluated in beta 2 expressing whole cells or membrane based on the down-stream effects of beta 2 adrenoceptors. The measurement endpoints include pigment dispersal in melatonin pretreated R2G1 melanophores expressing beta 2 adrenoceptors, cyclic AMP levels in CHO 6 CRE-LUC beta 2 membrane (cAMP competes with Fluo-cAMP tracer for anti-cAMP, and high fluorescence polarization occurs when there is low level of cAMP, and vice versa), cyclic AMP levels in CHO 6 CRLH-B2-15 beta2 cell membrane and whole cells [DiscoverX cAMP membrane assay and whole cell assay: measurement of turnover of the luminescence substrate in response to beta

galactosidase. Active beta galactosidase is formed by complement of two parts, enzyme donor (ED) and enzyme acceptor (EA). In the reaction system, ED was added in the form of ED-cAMP conjugate. In the presence of anti-cAMP, the complex of anti-cAMP and ED-cAMP conjugate prevents ED from complementing to the EA. Standard or cell lysates containing cAMP peptide compete with the conjugate for the anti-cAMP antibody].

The potency of GW642444 agonist activity at human beta 2 adrenoceptors were slightly greater than salmeterol (pEC50 of 9.3 verses 8.8) and equivalent with isoprenaline (pEC50 of 9.1) and R,R-formoterol (pEC50 of 9.4). The table below presents the details.

Beta 2 adrenoceptor potency of GW642444A compared with isoprenaline, Salmeterol and R, R-formoterol

Compound	Melonophore pEC50±SEM n	FPcAMP pEC50±SEM n	DX Membrane cAMP pEC50±SEM n	DX whole cell cAMP pEC50±SEM n
isoprenaline	9.1±0.01 517	7.3±0.03 66	7.6±0.04 26	7.2±0.02 93
salmeterol	8.8±0.02 517	9.5±0.04 60	9.6±0.03 38	9.3±0.04 109
R,R-formoterol	9.4±0.09 30	8.7±0.03 56	9.1±0.03 33	9.0±0.03 87
GW642444A	9.3±0.08 14	9.4±0.08 7	9.5±0.15 19	9.4±0.04 8

Data are expressed as arithmetic mean±SEM

FP=Fluorescence polarization

DX=DiscoverX

- Intrinsic beta 2 agonist activity

Similar to the measurement of beta 2 agonist activity potency with CHO cell membrane described above, cAMP related fluorescence polarization was measured for relative intrinsic activity of GW642444. A standard cAMP curve was run to enable the cAMP concentrations to be determined. Salmeterol was used as a reference (salmeterol intrinsic activity=1)

Relative intrinsic activity of GW642444A as compared with isoprenaline, Salmeterol and R,R-formoterol in the Human beta 2 FP-cAMP Assay

Compound	Mean Relative Intrinsic Activity Salmeterol=1	SEM	N
Isoprenaline	2.67*	±0.06	115
Salmeterol	1.00*	±0.003	118
GW642444A	1.53	±0.06	12
R,R-Formoterol	2.17*	±0.11	8

\*p<0.05 significantly different from GW642444A; data are expressed as arithmetic mean ±SEM

- Antagonism of GW642444A activity

Studied in CHO DiscoverX cAMP whole cells, GW642444A was antagonized by the beta 2 antagonists including propranolol, ICI 118551 and sotalol in a similar way to salmeterol. The pK<sub>B</sub> (negative log of dissociation constant) values of propranolol, ICI 118551 and sotalol against Salmeterol and GW642444A were obtained as below.

Antagonism of GW642444A and Salmeterol in the CHO DX cAMP whole cell assay (n=4)

	Propranolol		ICI 118551		Sotalol	
	pK <sub>B</sub>	slope	pK <sub>B</sub>	slope	pK <sub>B</sub>	slope
Salmeterol	9.6±0.07	1.0±0.14	9.7±0.19	1.2±0.05	7.3±0.08	0.8±0.19
GW642444	9.7±0.01	1.0±0.04	9.7±0.11	1.2±0.07	7.3±0.06	1.0±0.08

As shown above, the slopes of the Schild plot were not significantly different from unity (all close to 1). There was no significant difference between the pK<sub>B</sub> valued generated for each antagonist GW642444 and Salmeterol. Therefore it was concluded that GW642444 was competitively antagonized by a number of beta 2 adrenoceptor antagoists. Thus, GW642444 was confirmed to act through the beta 2 adrenoceptor.

- Selectivity for beta 2 adrenoceptor over other receptors

In luciferase reporter gene assays, GW642444A demonstrates high selectivity for human beta 2 over human beta 1 and beta 3 adrenoceptors. The selectivity was similar to that of salmeterol, and is higher than that of R, R-formoterol for beta 2 over beta 1 and beta 3 adrenoceptors (see the table below)

Compound	Beta 1			
	Mean pEC <sub>50</sub>	SEM	n	Beta 2 selectivity ratio over Beta 1
isoprenaline	8.84	±0.1	16	0.05
salmeterol	7.29	±0.02	363	215
R,R-formoterol	8.99	±0.02	351	1.1
GW642444A	7.30	±0.08	40	141
	Beta 3			

Compound	Mean pEC <sub>50</sub>	SEM	n	Beta 2 selectivity ratio over Beta 3
isoprenaline	8.24	±0.06	17	0.2
salmeterol	7.28	±0.02	400	219
R,R-formoterol	8.89	±0.01	369	1.4
GW642444A	7.52	±0.10	37	83

Data are expressed as arithmetic mean±SEM.

Selectivity ratio=mean EC<sub>50</sub> beta 1 or beta 3/mean EC<sub>50</sub> beta 2 (DX-membrane cAMP potency assay)

In radioligand binding assays, the selectivity of GW642444A for beta 2 adrenoceptor over other receptors and transporters were further determined. GW642444A at a concentration of 1 µM inhibited more than 50% activity of natural agonists in the following five types of receptors only.

Species	Assay (receptor/transporter)	% inhibition at 1 µM	GW642444 pKi
Human	Adrenergic beta 1	62	NA
Human	Adrenergic beta 2	98	NA
Human	Serotonin 5-HT <sub>1A</sub>	57	6.3
Human	Serotonin transporter	74	7.1
Guinea pig	Sigma, non-selective	74	6.7

2. Ex vivo: GW642444 relaxation effects on isolated guinea pig airway preparation and isolated human bronchus (SH2003/00037/00)

- Guinea pig airway preparation

GW642444 caused a concentration-related inhibition of the contractile response induced by electrical stimulation in guinea pig airway preparation. The pEC<sub>50</sub> values were 7.87, 7.50, 7.68, 7.27 and 9.35 for GW642444A, isoprenaline, salmeterol, salbutamol and formoterol, respectively. Therefore, the potency of GW642444 is similar to that of isoprenaline, sameterol and salbutamol, but is 30-fold weaker than formoterol in this assay.

GW642444A showed a rapid onset of action than salmeterol (Ot<sub>50</sub>, the time taken for an EC<sub>50</sub> concentration to achieve 50% maximum relaxation, of 6.6 min versus 25 min).

The washout profile of GW642444 was similar to that of Salmeterol, there being no recovery of response over 3 hours following removal of the compound.

When sotalol was superfused over guinea pig trachea during the recovery phase, it fully reversed the residual effects of GW642444A and salmeterol. The subsequent withdrawal of sotalol resulted in full reassertion of relaxation, despite no further addition of beta 1 agonist. The reassertion of beta 2 agonist effect suggested retention of the agonist with the vicinity of the receptor or at the receptor exosite.

In histamine-contracted guinea pig trachea, propranolol antagonized the effects of GW642444A in a competitive manner with a pKB of 8.9 and slope not significantly different from unity. This observation is consistent with an effect at beta adrenoceptors.

- Human bronchus preparation

Human bronchus (post mortem) was dissected into 2-3mm wide sections. GW642444 caused a concentration-related inhibition of the contractile response induced by prostaglandin (PGF 2 alpha). The pEC50 value was 7.74 and 8.30 for GW642444 and formoterol, respectively. In this assay, GW642444 was 3-fold weaker than formoterol.

Other characteristics including onset of action and washout profile observed in human bronchus study were similar to that seen in the guinea pig airway preparation.

The following four tables present details.

**Table 1** Guinea Pig Electrically-Stimulated Trachea: Onset, Potency and Duration of Action of GW642444A in Comparison with Isoprenaline, Salmeterol, Salbutamol, and Formoterol.

Agonist	n	OT <sub>50</sub> min (sem)	pEC <sub>50</sub> (sem)	EMR (iso=1)	Curve shift after 60min washout	Curve shift after 180min washout
GW642444A	8	6.6 (±1.5)	7.87 (± 0.12)	0.45	1.1	1.1
Isoprenaline	23	3 (± 2)	7.50 (± 0.05)	1	☆	☆
Salmeterol	6	25 (± 2)	7.68 (± 0.13)	0.43	0.6	0.6
Salbutamol	4	3 (±1)	7.27 (± 0.24)	1.57	☆	☆
Formoterol	6	13 (± 3)	9.35 (± 0.15)	0.01	33	938

☆ - Because isoprenaline/salbutamol are fully recovered within 10 min, a shift value can not be obtained at 60 or 180 min.

OT<sub>50</sub> = the time taken for an EC<sub>50</sub> concentration to achieve 50% maximum relaxant effect. EC<sub>50</sub> = concentration of beta agonist giving 50% inhibition of contraction. EMR = equieffective molar ratio relative to isoprenaline (=1) calculated at the 50% inhibition level. Curve shifts reflect the residual inhibitory effect of the beta agonist following either 60min or 180min of superfusion washout. Shifts are calculated at the 50% inhibition level. The greater the shift value, the greater the washout of agonist.

**Table 2 Human PGF<sub>2α</sub>-Contracted Bronchus: Onset, Potency and Duration of Action of GW642444A in Comparison with Isoprenaline and Formoterol.**

Agonist	n	OT <sub>50</sub> (sem)	pEC <sub>50</sub> (sem)	EMR (iso=1)	Curve shift after 60min washout	Curve shift after 180min washout
GW642444A	2	8 (± 2)	7.74 (± 0.13)	1.28	1.5	1.6
Isoprenaline	24	3 (± 2)	7.07 (± 0.06)	1	☆	☆
Formoterol	4	5 (± 3)	8.30 (± 0.45)	0.06	13.5 (n=2)	≥127 (n=2)

☆ - Because isoprenaline is fully recovered within 10 min, a shift value can not be obtained at 60 or 180 min.

OT<sub>50</sub> = the time taken for an EC<sub>50</sub> concentration to achieve 50% maximum relaxant effect. EC<sub>50</sub> = concentration of beta agonist giving 50% inhibition of contraction. EMR = equieffective molar ratio relative to isoprenaline (=1) calculated at the 50% inhibition level. Curve shifts reflect the residual inhibitory effect of the beta agonist following either 60min or 180min of superfusion washout. Shifts are calculated at the 50% inhibition level. The greater the shift value, the greater the washout of agonist.

**Table 3 Guinea Pig Electrically-Stimulated Trachea: Effects of the beta2 Adrenergic Antagonist Sotalol (10 $\mu$ M) during the Washout Phase of GW642444A and Salmeterol (30nM).**

Agonist	n	Agonist Response (%inhibition)	Sotalol- Induced Reversal (% max)	Mean reassertion response (% max)
GW642444A	4	85 ( $\pm$ 6)	123 ( $\pm$ 15)	92 ( $\pm$ 12)
Salmeterol	3	74 ( $\pm$ 6)	100 ( $\pm$ 2)	94 ( $\pm$ 1)

**Table 4 Guinea Pig Histamine-Contracted Trachea: The Effects of the Beta Adrenoceptor Antagonist Propranolol on the Relaxant Effects of GW642444.**

Agonist	n	pK <sub>B</sub> Propranolol	Slope
GW 642444A	3	8.9 $\pm$ 0.06	0.8 $\pm$ 0.16

3. In vivo: GW642444 relaxed histamine-induced bronchoconstriction in conscious guinea pig (SH2003/00042/00)  
Inhalation administration of GW642444 aerosol for 2 minutes to guinea pigs 30 minutes prior to histamine challenge (histamine diphosphate aerosol) inhibited histamine induced bronchoconstriction. The potency was 2-fold less than Salmeterol when EC<sub>60</sub> levels were compared (see the table below)

Compound (solvent)	EC90, mol/L	EC60, mol/L	EMR*
Salmeterol acetate (DMA/Saline)	5.4 $\times$ 10 <sup>-5</sup>	4.5 $\times$ 10 <sup>-6</sup>	
GW642444 (DMA/Saline)	3 $\times$ 10 <sup>-5</sup>	9 $\times$ 10 <sup>-6</sup>	2

Vehicle was 8% dimethylacetamid (DMA) and 92% saline; EMR (equivalent molar ratio) is expressed as the ratio of [EC<sub>60</sub>] GW642444/[EC<sub>60</sub>] Salmeterol acetate

When dosed at the EC90 (3 $\times$ 10<sup>-5</sup> mol/L), GW642444 showed inhibition of histamine-induced bronchoconstriction above 50% for approximately 10 hours. Salmeterol showed similar duration of action. At 10 $\times$ EC<sub>90</sub>, GW642444 showed >50% protection for 16 hours.

Repeat dosing of GW642444 (once daily inhalation for 4 days at EC<sub>90</sub> dose) resulted in tachyphylaxis, i.e. reduced bronchoprotection. Inhibition of histamine-

induced bronchoconstriction was less than 50% throughout measurement period of 4-24 hours after dosing, whereas single dose exposure group had T1/2 around 10 hours. At 4 hours after dosing, the inhibition was 65% and 35% for the single dose and repeat dose group, respectively.

Finally, the sponsor evaluated therapeutic index based on the ratio of the EC90 at 30 min of inhibition of histamine induced bronchoconstriction and the dose that induced 30% decrease of blood pressure. GW642444 showed less significant effect of decreasing blood pressure compared to Salmeterol. The therapeutic index derived was <10 for salmeterol and >333 for GW642444 (see the table below).

Effects of beta 2-agonists on mean blood pressure in conscious guinea pigs

Compound	Max % fall in MBP	TI (inhalation dose causing a 30% fall in MBP/EC90 at 30 min)
Vehicle	15.2±3.0	
GW642444A	19.16±2.48 at 10 <sup>-2</sup> mM	>10 <sup>-2</sup> mM/3×10 <sup>-5</sup> mM = >333
Salmeterol base	37.43±4.55 at 10 <sup>-3</sup> mM	<10 <sup>-3</sup> mM/10 <sup>-4</sup> mM = <10

### 2.6.2.3 Secondary pharmacodynamics

No study submitted.

### 2.6.2.4 Safety pharmacology

Neurological effects:

- Single i.v dose: Male CD rats (10-11 weeks old) were given GW642444H intravenously at doses of 25, 100 and 400 mg/kg, or vehicle (one part of polyethylene glycol 400 and four parts of 8% w/v aqueous 2-gydropypropyl-beta cyclodextrin) (VD2003/00131/00). Decreased body temperature (0.6-1.2°C relative to control) at 1-2 hours after dosing was observed in the groups given 100 and 400 mcg/kg. These changes were associated decreases of spontaneous locomotor activity and grip strength up to 4 hours postdosing. No effects on neurobehavior parameters or body temperature were noted at 25 mcg/kg. Respective pharmacokinetic parameters at 25, 100 and 400 mcg/kg were 21.4, 113, 492 ng/mL in Cmax values and were 6.36, 30.6 and 126 ng.h/mL AUC0-t values.
- Single inhalation dose: Male CD rats given single inhalation doses of GW642444M (nominal doses of 36, 612, 34299 mcg/kg, or vehicle of lactose (b) (4) at 15% w/w) resulted in a transient (up to 9 hours from the start of dosing) decreases in locomotor activity at all doses. The 34399 mcg/kg dose also a decrease in body temperature (up to 1.6°C) at 1.25 hours post-dosing (VD2005/00527/00).

Cardiovascular effects:

- On hERG channel (FD2003/00330/00): GW642444H inhibited hERG channel stably expressed in HEK293 cells (reduced hERG tail current amplitude) in a

concentration-dependent manner. When tail currents were corrected for the mean vehicle rundown (0.3% DMSO produced a mean decrease in tail current of 19.3%), the estimated nominal IC<sub>25</sub>, IC<sub>50</sub>, IC<sub>75</sub> values for hERG channel inhibition were 2.0, 4.8 and 12.6 mcM (0.99, 2.3, and 6.1 mcg/mL active moiety respectively).

The reference substance, E-4031, inhibited hERG tail current, an effect consistent with its known activity.

- In dog isolated cardiac Purkinje fibers (FD2003/00323/01): In dog isolated Purkinje fibers, exposure to GW642444H, at concentrations of 1 and 10 mcM (Equivalent to 0.49 and 4.9 mcg/mL of GW642444X, respectively) caused concentration dependent decrease in upstroke amplitude (UA), maximum rate of depolarization (MRD) and resting membrane potential (RMP). In the presence of 100 mcM GW642444H (equivalent to 49 mcg/mL of GW642444X) action potentials were abolished in the majority of the fibers tested.
- Single i.v. dose in dogs (FD2003/00275/00): Conscious beagle dogs (4 males) given single i.v. dose of GW642444H at 0.1, 0.3 and 1 mcg/kg showed only a transient increase in heart rate at 0.3 mcg/kg (increase up to 26 bpm for 10 min) and 1 mcg/kg (increase up to 60 bpm for 20-25 min, along with small decreases in blood pressure, PR- and QT-intervals detected 5 minutes post dose) compared to predose heart rate of 60-72 bpm. The doses of 0.3 and 1 mcg/kg were associated with C<sub>max</sub> values of 4.0 and 11.0 ng/mL, and AUC values of 0.5 and 1.5 ng.h/mL, respectively.
- Single i.v. dose in dogs (FD2005/00097/00): Conscious dogs (4 males/group) were given intravenous doses of GW642444M at 0.1, 0.3 and 1 mcg/kg. Decreases of blood pressure (decreased 10 mm Hg from predose value of 101 mm Hg), at 1 mcg/kg dose, and increases of heart rate at 0.3- and 1 mcg/kg (predose, 70 bpm; 0.3 mcg/kg, 107 bpm; 1 mcg/kg, 137 bpm), and reduction of PR, RR, QT and QTcL interval were observed at 0.3 and 1 mcg/kg were observed. QTcL was increased by approximately 6 msec (the predose value was 243 msec), the values returned to predose levels at approximately 40 minutes following the end of infusion. The doses of 0.3 and 1 mcg/kg were associated with AUC<sub>0-t</sub> values of 0.4 and 1.4 ng.h/mL, and C<sub>max</sub> values of 2.3 and 8.3 ng/mL, respectively.

#### Pulmonary effects:

Male SD rats given a single inhalation dose of GW642444H at nominal dose (GW642444H salt) of 61, 241 and 666 mcg/kg (CD2003/00833/00) or GW642444M (triphenyl acetate salt of GW642444X) at nominal doses (expressed as the active moiety GW642444X) of 36.02, 718.13 or 36327.03 mcg/kg (CD2005/01091/00) did not produce any adverse effects on ventilatory function (tidal volume, respiratory rate, and minute volume).

Renal effects: Not done

Gastrointestinal effects: Not done

Abuse liability: Not done

Other: Not done

### **2.6.2.5 Pharmacodynamic drug interactions**

No studies were conducted.

### **2.6.3 PHARMACOLOGY TABULATED SUMMARY**

Not applicable

### **2.6.4 PHARMACOKINETICS/TOXICOKINETICS**

#### **2.6.4.1 Brief summary**

Deferred to a later review due to limited studies were reviewed at this time

#### **2.6.4.2 Methods of Analysis**

[see under individual study reviews]

#### **2.6.4.3 Absorption**

Oral administration to male rats showed that  $F < 5\%$  (SH2003/00040/00).

#### **2.6.4.4 Distribution**

Two studies (FD2003/00261/01 and FD2003/00185/00) of quantitative whole-body autoradiography were performed using male pigmented rats. Both studies used [ $^{14}\text{C}$ ]GW642444H 0.35 mg free base /kg by i.v and oral administrations, and measured tissue radioactivity at designed time points.

1. Tissue Tmax: following intravenous and oral administration, highest concentrations for the majority tissues were observed at the first sampling time point 15 min and 5 minutes after dosing in two i.v. studies, respectively, and 30 min after oral dosing in the second study (FD2003/00185/00). The first oral study (FD2003/0026/01) showed only limited number of tissues with quantifiable radioactivity but the first sampling time point of the study was 1 hour after oral dosing
2. Tissue and blood concentration comparison: Drug movement from blood to tissue occurred quickly. Concentrations in the majority tissues were less than that of blood at 5 minutes after the i.v. dosing (FD2003/00261/01), and were more than that observed in blood at 15 min after i.v. dosing (FD2003/0026/01). The blood drug concentrations were 1.03 and 0.061 mcg equiv/g at 5 and 15 minutes post dosing, respectively.
3. The highest concentrations of radioactivities were associated with the kidney, adrenal, choroid plexus and thyroid at 15 min after i.v. dosing in the study FD2003/00261/01; with the liver, blood, lung, myocardium and kidney at 5 minutes after i.v. dosing in the study FD2003/00185/00. For some tissues, including the harderian gland, brown and white fat, preputial gland, seminal

- vesicles and pancreas did not occur until 6 hours after i.v. dosing (FD2003/00261/01).
4. Concentrations of radioactivity declined from the earlier time points, with the majority of tissues containing concentrations of radioactivity below the limit of quantification (<0.004 mcg equivalents of GW642444X/g) by 3 days after i.v. dosing (FD2003/00261/01). At 35 days no tissues (FD2003/00185/00) or only the uveal tract/retina and testis contained quantifiable radioactivity (FD2003/00261/01).

(b)(4) Following oral or intravenous administration of [<sup>14</sup>C] GI179710 (b)(4) 1 mg/kg orally and 0.5 mg/kg intravenously), the tissue distribution of total radioactivity was similar. Radioactivity was widely distributed, with highest concentrations associated with the liver and kidney cortex. Following either route of administration, the highest concentrations of radioactivity occurred in the vast majority of tissues at the first sampling time (0.5 hours for oral dosing and 5 minutes for i.v. dosing). By 3 day post-dose, concentrations of radioactivities were generally below or close to the limit of quantification (0.003 mcg equiv. of GI179710/g). Following either route of administration, no tissue contained quantifiable concentrations of radioactivity at 35 days (FD2005/00228/01).

Plasma protein binding for GW642444 was 94.3, 92.3, 98.9, 93.4, 98.7 and 97.2% in the mouse, rat, guinea pig, female rabbit, dog and human respectively, with concentration of GW642444H (b)(4) tested at range of 5-625 ng/mL. The extent of blood cell association appeared to be low to moderate and there was no evidence of any concentration dependence on association. The mean blood to plasma ratios of [<sup>14</sup>C] were 1.0, 1.1, 0.73, 1.0, 0.5 and 0.76 in the mouse, rat, guinea pig, female rabbit, dog and human respectively. Blood association (% of compound associated with blood cells among the compound found in the whole blood) were 41.3, 55.9, 15.6, 41.4, 10.7 and 36.1%, in these species, respectively (WD2006/02044/00).

#### 2.6.4.5 Metabolism

#### 2.6.4.6 Excretion

#### 2.6.4.7 Pharmacokinetic drug interactions

In a study with P-glycoprotein (P-gp) transfected MDCKII-MDR1 cells, GW642444 was reported being a substrate for human P-glycoprotein and having low passive membrane permeability (average  $P_{7.4}$  of  $34 \pm 13$  nm/s). However, poor mass balance was observed for GW642444 and the interpretation of the results from the assay become difficult (WD2004/00106/00).

With the same cells, MDCKII-MDR1, GW642444 (0.1 to 100 mcM) was studied for the potential inhibition on P-gp-mediated transport of digoxin (30 nM). GW642444 at 100 mcM exhibited 26% inhibition on digoxin transport. There was no IC50 obtained as the

degree of inhibition over the concentration range tested was too low (WD2007/01087/00).

Following a single oral dose of GW642444M at 1 mg/kg, GW642444 exposures (AUC<sub>0-t</sub> vales) in hepatic portal vein (HPV) plasma were similar between P-gp knockout and wild type mice. Therefore, P-gp does not appear to play a major role in the absorption of GW642444 (WD2007/01613/00)

#### **2.6.4.8 Other Pharmacokinetic Studies**

None

#### **2.6.4.9 Discussion and Conclusions**

Deferred to a later time

#### **2.6.4.10 Tables and figures to include comparative TK summary**

### **2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

### **2.6.6 TOXICOLOGY**

Note: all doses of GW642444 (in H or M salt form) were expressed as free base of GW642444X.

#### **2.6.6.1 Overall toxicology summary**

##### General toxicology:

This study is not reviewed here.

Toxicology study reports reviewed in this document include two studies – a 4-week and a 13-week inhalation study with GW642444 in dogs.

The doses used in these two studies are the following:

- 4-Week study: delivered doses of 10, 97, 135-2010 mcg/kg; pulmonary deposited doses of 2.53, 24 and 33.75-503 mcg/kg. Of note, the HD group received delivered dose of 132 mcg/kg for the three days namely phase I and 2010, 135, 1220 and 571 mcg/kg for Days 1-2, Days 3-4, Days 5-15 and Days 16-28 of phase II, respectively. The average dose for the phase 2 was 891 mcg/kg. The MD group received delivered doses of 123 mcg/kg for the first 15 days and 64.2 mg/kg for additional 13 days. The average dose for the MD was 97 mcg/kg.
- 13-week study: delivered doses of 9.3, 66, 120/501 mcg/kg; pulmonary deposited doses of 2.32, 16.5, 30/125.3 mcg/kg. Of note, the HD group was given 120 mcg/kg for 3 days and followed by 501 mcg/kg for additional 13 weeks.

Systemic toxicities in these two studies are similar and include liver findings (hepatocyte rarefaction, glycogen deposition, increased in periportal region and decreased in

centrilobular region) and heart findings of left ventricle papillary muscle mineralization and/or fibrosis. Plasma troponin I assessed in Week 4 of the 13-w study showed increased troponin I in animals at the MD and HD. The troponin I levels in LD animals were similar to that of the controls. In addition to the systemic toxicities, nasal epithelial lymphoid infiltration was seen in the HD animals in the 13-week study. The 4-week recovery animals in the 13-week study showed only finding of myocardial fibrosis. Therefore, the liver and nasal findings are reversible.

The NOAEL for 4-week study is considered to be 10 mcg/kg (AUC of 5 ng.h/mL) as the heart lesion was not reproduced in the 13-week study. The NOAEL for the 13-week study is defined as 9.3 mcg/kg (AUC of 7 ng.h/mL) based on a minimal concern for the single incidence (1/4 male) of liver finding.

Genetic toxicology:

GW642444 was negative in the Ames assay, rat bone marrow micronucleus assay, in vitro UDS assay and SHE cell assay and equivocal in the mouse lymphoma assay

Carcinogenicity: not conducted

Reproductive toxicology: review of reproductive toxicology is deferred to a later time

Special toxicology: Not conducted

**2.6.6.2 Single-dose toxicity**

Deferred to a later time.

**2.6.6.3 Repeat-dose toxicity**

**Study title:** Toxicity study by Inhalation administration to beagle dogs for 4 weeks

**Key study findings:** Dogs given GW642444M by inhalation for 4 weeks at variable doses (see the method section) showed treatment related findings as increased liver glycogen, myocardial mineralization or fibrosis and thymic atrophy. The liver finding is not of safety concern and heart finding is typical for this class of drug which is considered clinically monitorable based on the heart rate. NOAEL for the thymic finding is 10 mcg/kg (delivered dose).

**Study no.:** [REDACTED]<sup>(b) (4)</sup> Study No. BVR/783; GSK reference No. D26082; report No. WD2005/00845/00

**Volume #, and page #:** Vol 22, page 1

**Conducting laboratory and location:** [REDACTED]<sup>(b) (4)</sup>

**Date of study initiation:** 7/1/2005

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:** GW642444M, Batch No. R11407/182/1, Purity 98.7%, expiry dated 3/9/2006; GW857238X [REDACTED]<sup>(b) (4)</sup>, batch MPD06647/R2-7, purity 99.7%, expiry 4/30/2006; lactose, GSK lot No, 041023421, expiry date 10/7/2005

### Methods

Doses: Delivered doses of 10.1, 130/65, 130/2000/130/1220/571 mcg/kg (see details under unique study design or methodology)

Species/strain: Beagle dog

Number/sex/group or time point (main study): 3/sex/dose

Route, formulation, volume, and infusion rate: oronasal inhalation, Micronized GW642444 was blended in lactose at a nominal concentration of 4% w/w with micronized GW857238X at a nominal concentration of 15% w/w. GW857238X [REDACTED]<sup>(b) (4)</sup> was included [REDACTED]<sup>(b) (4)</sup>

Satellite groups used for toxicokinetics or recovery: None

Age: 12-13 month old

Weight: males, 10.4-14.9 kg; females, 7.1-9.6 kg

Sampling times: see "Observation and Times" below

Unique study design or methodology (if any): The HD groups were designed to use an escalating dose regimen, first 3 days at 30 mcg/kg and additional 28 days at 2000 mcg/kg. However, marked clinical signs seen in intermediate and high dose animals during dosing, typified by violent struggling, aggressive behavior, changes in breathing pattern and general attempts to avoid exposure to the test full administration of the target dose to individual animals on occasions and resulted in one high dose male being killed on Day6. The table below presents doses animals received.

**GW642444M**

Phase	Group name	Group number	Exposure number	Target dose <sup>1</sup> (µg/kg/day)	Estimated achieved dose <sup>1</sup> (µg/kg/day)	Target mask aerosol concentration <sup>1</sup> (µg/L)	Achieved mask aerosol concentration <sup>1,2</sup> (µg/L)	Number/sex
1	High	4	1-3	130	132	6.85	6.93	3
2	Vehicle control	1 <sup>3</sup>	1-28	0	0	0	0	3
	Low	2	1-28	10	10.1	0.53	0.527	3
	Inter-mediate	3	1-15	130	123	6.85	6.45	3
			16-28	65	64.2	6.85	6.73	
			1-28	-	96.7 <sup>4</sup>	-	6.60 <sup>4</sup>	
	High	4	1-2	2000	2010	106	105	3
			3-4	130	135	6.85	7.06	
5-15			1000	1220	53	63.8		
16-28			500	571	53	60.2		
		1-28	-	891 <sup>4</sup>	-	61.0 <sup>4</sup>		

- Not applicable

1. Doses and aerosol concentrations are expressed in terms of pure active moiety, GW642444X

2. As determined by chemical analysis

3. Controls received vehicle (15% w/w GW857238X in lactose) at a feed rate that targeted the GW857238X concentration to be similar to that given to the high dose group (Group 4)

4. Time-weighted average: sum of 'weighted aerosol concentrations' (aerosol concentrations weighted for time) calculated for each target dose level as follows:

$$TWA_1 = (C_1 \times T_1) / 28$$

TWA<sub>1</sub> Time weighted average concentration (µg/L)

C<sub>1</sub> Average aerosol concentration at the specified target dose level (µg/L)

T<sub>1</sub> Number of exposure at the specified target dose level

The sum of TWA<sub>1</sub>, TWA<sub>2</sub>,... is used to calculate the overall estimated achieved aerosol concentration. The overall estimated achieved aerosol concentration is used to calculate the overall estimated achieved dose.

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:

$$D = (RMV \times T \times C) / (BW)$$

D Dose (µg/kg/day)

RMV(L/min) 0.499xBW(kg)<sup>0.809</sup> [Bide et al, 2000]

T Duration of exposure/day (minutes)

C Aerosol GW642444X concentration (µg/L)

BW Group average body weight for study (kg) for Days 0 to 28

The exposure levels of GW857238X (b) (4) in dogs are the following:

**GW857238X**

Phase	Group name	Group number	Exposure number	Estimated achieved dose <sup>1</sup> (µg/kg/day)	Achieved mask aerosol concentration <sup>1,2</sup> (µg/L)
1	High	4	1-3	614	32.3
2	Vehicle control	1 <sup>3</sup>	1-2	9300	481
			3-4	783	40.5
			5-28	4840	251
			Low	2	1-28
	Inter-mediate	3	1-15	601	31.4
			16-28	334	35.0
			1-28	488 <sup>4</sup>	33.3 <sup>4</sup>
	High	4	1-2	6810	356
			3-4	640	33.5
			5-15	5540	290
16-28			2610	275	
1-28			3936 <sup>4</sup>	269 <sup>4</sup>	

1. Doses and aerosol concentrations are expressed in terms of pure active moiety, GW857238X
2. As determined by chemical analysis
3. Controls received vehicle (15% w/w GW857238X in lactose) at a feed rate that targeted the GW857238X concentration to be similar to that of the high dose group (Group 4)
4. Time-weighted average: sum of 'weighted aerosol concentrations' (aerosol concentrations weighted for time) calculated for each target dose level as follows:  
 $TWA_1 = (C_1 \times T_1) / 28$   
 TWA<sub>1</sub> Time weighted average concentration (µg/L)  
 C<sub>1</sub> Average aerosol concentration at the specified target dose level (µg/L)  
 T<sub>1</sub> Number of exposure at the specified target dose level  
 The sum of TWA<sub>1</sub>, TWA<sub>2</sub>,... is used to calculate the overall estimated achieved aerosol concentration. The overall estimated achieved aerosol concentration is used to calculate the overall estimated achieved dose.

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:

$$D = (RMV \times T \times C) / (BW)$$

D Dose (µg/kg/day)

RMV(L/min) 0.499xBW(kg)<sup>0.809</sup> [Bide et al, 2000]

T Duration of exposure/day (minutes)

C Aerosol GW857238X concentration (µg/L)

BW Group average body weight for study (kg) for Days 0 to 28

**Observation and Times: (this information can be provided in a separate section OR evaluation times can be described for each parameter in the results section).**

Clinical signs: immediately before dosing, during dosing, between half an hour and 2 hours after completion of dosing

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: once each of prior to initiation of dosing and during Week 4 of treatment

EKG: Once prior to initiation of dosing and during week 4 of treatment.

Hematology: Blood samples were collected once prior to initiation of dosing, and before dosing during Week 4.

Clinical chemistry: At the same time as that for hematology study

Urinalysis: 16 hour urine samples were collected prior to study and during week 4.

Gross pathology: At necropsy

Organ weights: Adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, testes and thymus. All paired organs were weighted together.

Histopathology: A complete battery of tissues from all dogs was examined (see histopathology inventory). HE staining was used for most of tissue slides. Bone marrow smears were air-dried and subsequently stained using a Romanowski procedure. The additional samples of liver taken for glycogen analysis were stained with PAS (periodic Acid-Schiff)

The histopathology tissue battery is adequate. A peer review of selected microscopic tissue sections and pathology data interpretation was completed. A further peer-review by the sponsor's pathologist was conducted.

### **Results:**

Pulmonary deposited doses: The pulmonary deposited doses are 25% of the delivered doses based on the measured MMAD of 1.5-2.1  $\mu\text{m}$ . Therefore, averaged Day 1-28 pulmonary doses are 2.53, 24, and 222.8 mcg/kg (see the table of drug exposure under methodology part)

Mortality: No toxicity related death. One HD male dog was sacrificed on Day 6 of phase 2 due to aggressive behavior. The dog showed no apparent morphological changes in the heart upon histological examinations.

Clinical signs: Vasodilation of whole body/gum/muzzle/ears and salivation and violent struggling, fast/shallow breathing were observed in all dose levels, in a dose-related. Due to the degree of struggling and aggressive behavior associated with dosing the dogs at 2010 mcg/kg, the dosage for Group 4 was reduced to 135 mcg/kg on day 3 and 4 of phase 2 before increasing it to 1220 mcg/kg on Day 6. Due to the same reasons, the dose of 1220 mcg/kg was further reduced to 571 mcg/kg from Day 6.

Body weights: A slight increase in body weight gain was observed for high dose females (0.7 kg versus 0.0 kg in control)

Food consumption: No treatment related findings

Ophthalmoscopy: No treatment related finding

EKG: Increased heart rate was seen in all treated males and MD and HD females immediately after dosing during week 4 when compared to predose (by 51% to 102%)

and control (by 34% -74%) values and was dose related in both sexes when compared to pre-dose values.

Decreased mean hear rates were observed for all groups before the start of dosing in Week 4 compared to pre-treatment (11-47%) and control (9-48%).

Hematology: Platelet counts were increased for both sexes at the HD (7-30%) when compared to pre-treatment values

Clinical chemistry: Compared to pre-treatment values, alkaline phosphate levels were increased in drug treatment groups (up to 2 fold) in a dose related manner, and creatinine levels were slightly increased at the HD dogs (16-19%). The table below presents the summary data.

			Vehicle	LD	MD	HD
D 28 dose*				10.1/51.1	64.2/488	571/2610
ALP, U/L	Pre-treatment	M	56	54	57	62
		F	41	63	56	42
	Day 28	M	57	71	84	122
		F	34	68	88	68
Creat, mcM/L	Pre-treatment	M	77	72	71	69
		F	73	77	78	69
	Day 28	M	79	77	78	82
		F	72	80	79	80

\* The doses administered on Day 28 (mcg/kg) for GW642444/GW857238x

Urinalysis: No treatment related finding

Gross pathology: No treatment related finding

Organ weights: Reduced thymus weights were observed in treatment groups (all treated male groups, 15-32%; HD females, 11%)

Histopathology:

Findings in the HD dog sacrifice on Day 6 of phase 2 due to aggressive behavior: Minimal increased peripotal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction.

Findings in the terminal sacrifice:

Liver: Hepatocyte rarefaction was increase in peripotal region and was decrease in the centrilobular region. The changes were seen in the MD and HD groups and of minimal to slight degree. PAS staining indicated that the hepatocyte rarefaction was due to the presence of glycogen in these cells.

Heart: Myocardial mineralization of the papillary muscle was seen in a single LD male and myocardial fibrosis of the papillary muscle was seen in a single MD male.

Thymus: Thymic involution/atrophy was seen in the MD and HD groups, mostly in a minimal to slight degree.

Incidence (severity) of histopathological findings in the dog 4-W IH study (3/sex/dose)

		vehicle	LD	MD	HD
<b>Liver</b>					
Increased periportal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction	M	0	0	1 (1)	2 (1.5)
	F	0	0	3 (1)	3 (1.7)
PAS staining					
Presence of centrilobular glycogen	M	2	3	0	0
	F	3	0	0	0
Presence of periportal glycogen	M	0	0	3	2
	F	0	1	3	3
Generalized glycogen	M	1	0	0	0
	F	0	2	0	0
<b>Heart</b>					
Papillary muscle mineralization	M	0	1	0	0
	F	0	0	0	0
Papillary muscle fibrosis	M	0	0	1	0
	F	0	0	0	0
Thymic involution	M	1 (1)	1 (1)	2 (1)	1 (3)
	F	1 (1)	1 (1)	2 (2.5)	3 (2)

The severity was in a scale of 1-5 from minimum to severe degree

The liver findings, based on the PAS staining, are not considered toxicologically significant. The heart findings of myocardial mineralization or fibrosis are typical for beta agonist. Thymic atrophy might be treatment related and NOALE can be defined as the LD of 10 mcg/kg.

Toxicokinetics: Due to lack of bioanalytical quality of control for GW642444, no valid plasma concentrations or associated toxicokinetic parameters were determined.

Following inhalation of GW642444M for 4 weeks to male and female dogs, GI179710 <sup>(b)(4)</sup> was mainly quantifiable in the MD and HD groups. In general, systemic exposure to GI179710 (as measured by  $AUC_{(0-t)}$  and  $C_{max}$ ) increased approximately proportionally with increasing dose. There was no gender related findings in systemic exposures. The table below presents the TK parameters of GI179710 observed.

		AUC <sub>0-t</sub> , ng.h/mL			Cmax, ng/mL		
		Day 1	Day 16	Week 4	Day 1	Day 16	Week 4
LD	M	NC	NC	NC	NQ	NQ	NQ
	F	NC	NC	NC	NQ	NQ	NQ
MD	M	NC	NC	NC	23.4	22.6	14.5
	F	NC	31.2	NC	21.7	24.2	20.3
HD	M	352	151	143	202	169	168
	F	836	194	187	486	222	176

NC: not calculable; NQ: not quantifiable (below limit of quantification 5.0 ng/mL)

The corresponding pulmonary doses of GW642444 (25% of delivered doses) for these days are the following:

Group	LD, mcg/kg	MD, mcg/kg	HD, mcg/kg
Day 1	2.5	31	502
Day 16	2.5	16	143
Week 4	2.5	16	143

**Study title:** Toxicity study by Inhalation administration to beagle dogs for 13 weeks  
Followed by a 4 week recovery period

**Key study findings:** Dogs given GW642444 (delivered doses of 9.3, 66, 120/501 mcg/kg) exhibited systemic toxicities of liver findings (hepatocyte rarefaction, glycogen deposition, increased in periportal region and decreased in centrilobular region) and heart findings of left ventricle papillary muscle mineralization and/or fibrosis. Plasma troponin I assessed in Week 4 of the 13-w study showed increased troponin I in animals at the MD and HD. The troponin I levels in LD animals were similar to that of the controls. In addition to the systemic toxicities, nasal epithelial lymphoid infiltration was seen in the HD animals. The NOAEL was concluded to be 9.3 mcg/kg (AUC of 7 ng.h/mL) based on a minimal concern for the single incidence (1/4 male) of liver finding.

**Study no.:** (b) (4) Study No. BVR/783; GSK reference No. D26082

**Volume #, and page #:** Vol 23, page 1; report No. WD2006/01711/00

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 2/21/2006

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:** GW642444M, Batch No. R204768, Purity not provided, expiry dated 10/31/2006; Lactose, GSK lot No 051089255, no purity information was provided

## Methods

Doses: Delivered doses of 0, 10, 65 and 120 /500 mcg/kg (given 120 mcg/kg for 3 days and followed by 500 mcg/kg for additional 13 weeks)

Species/strain: Beagle dog

Number/sex/group or time point (main study): n=4/sex/dose

Route, formulation, volume, and infusion rate: micronized GW642444 was blended in lactose at a nominal concentration of 7% w/w. Dogs were administered with the test compound via oronasal inhalation mask, for 30 minutes per day.

Satellite groups used for toxicokinetics or recovery: Recovery study used 2/sex for the control and the HD groups

Age: 12-15 month old

Weight: males, 8.3-15 kg; females, 7.0-11.4 kg

Sampling times: see "Observation and Times" below

Unique study design or methodology (if any): The HD groups were designed to use an escalating dose regimen, and the animals were given GW642444 at 130 mcg/kg for 3 days (Phase I) and followed by 500 mcg/kg for additional 13 weeks (Phase II).

## Observation and Times:

**Clinical signs:** Twice a day (near the start and end of each working day)

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: once prior to initiation of dosing and during Week 13 of treatment

Femoral pulse rate: On Days 1, 2, and 3 of Phase 1 (HD group) and Days 1, 7, 28 and 91 of Phase 2 (all groups). On these occasions, pulse rates were recorded pre-dose, immediately after dosing, 0.5, 1, 2, 4, 8 and 23 hours after completion of dosing. In addition, pulse rate was recorded at pre-dose and immediately after dosing on Days 2, 3, 4, 5 and 6 of Phase 2.

EKG: Once prior to initiation of dosing and during week 13 of treatment (pre-dosing and immediately after dosing).

Clinical pathology (Hematology and clinical chemistry): Blood samples were collected once prior to initiation of dosing, and before dosing during Weeks 4 and 13 and during Week 4 of the recovery period.

Troponin I assay: During Day 1 (Phase 1 and 2) and Week 4 of Phase 2, blood samples were collected immediately after and 4, 8 and 23 hours after the completion of dosing. A single sample was also collected on one occasion from each recovery animals during Week 4 of the recovery period.

Urinalysis: Overnight (approximately 16 hours) urine samples were collected prior to the initiation of dosing and during Week 4 and 13.

Gross pathology: At necropsy

Organ weights: Adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, testes and thymus. All paired organs were weighted together.

Histopathology: A complete battery of tissues from all dogs was examined (see histopathology inventory). HE staining was used for most of tissue slides. Bone marrow smears were air-dried and subsequently stained using a Romanowski procedure. The additional samples of liver taken for glycogen analysis were processed to wax block and stained with PASH (Periodic Acid-Schiff with heamatoxylin)

The histopathology tissue battery is adequate. A peer review of selected microscopic tissue sections and pathology data interpretation was completed. A further peer-review by the sponsor's pathologist was conducted.

## Results:

Pulmonary deposited doses: The pulmonary deposited doses are 25% of the delivered doses based on the measured MMAD of 1.5-2.1 mcm. Therefore, averaged Day 1-28 doses are 2.32, 16.5 and 125.3 mcg/kg (see the table of drug exposure under methodology part)

Group	Delivered dose, mcg/kg	MMAD, mcm	Deposition factor	Lung deposited dose, mcg/kg
Vehicle	0	-	-	-
LD	9.31	1.6	0.25	2.32
MD	66.0	1.7	0.25	16.5
HD phase 1	120	1.95	0.25	30.0
HD phase 2	501	2.15	0.25	125.3

The sponsor assumed 100% deposition in the respiratory tract and concluded the delivered doses as achieved doses.

Mortality: None

Clinical signs: No treatment related findings seen during phase 1 of the study. During Phase 2, vasodilation of the gums was seen in a proportion of animals before the start of exposures on the following day. The highest incidence of this sign was seen at 0.5-2 hour pose dose check. There was remarkable finding in the 4-week recovery animals.

Body weights: An increased body weight gain was seen in the HD (501 mcg/kg) animals (1.1 kg versus 0.3-0.4 kg in controls)

Food consumption: A slight increase in food consumption over the 13-week period was noted for females at the MD and HD (1.11-1.16 X control)

Ophthalmoscopy: No treatment related finding

EKG: Decreased heart rates were observed for all groups at pre-dosing in Week 13 (controls, 0.91-0.88X pretreatment; treated groups, 0.82-0.62X pretreatment and 0.83-0.68X control), with the degree of effect being greatest in the treated groups.

Increased heart rates were seen for all groups given the test article immediately after dosing during Week 13 (1.24-1.86 X predose) and was dose-related in both sexes. The duration of the heart rate increase was not recorded

During Week 4 of recovery, dogs previously given the HD showed similar heart rate to that of pre-treatment.

Hematology: By week 13, WBC counts were increased (1.48X pretreatment) for three females at the HD, predominantly due to an increase in neutrophils, monocytes and large unstained cells (1.62, 2.24 and 4.25X pretreatment, respectively). The 4-week recovery animals showed these changes were completely reversible.

Clinical chemistry: No treatment related findings, except changes in Troponin I levels (see below)

Troponin I: Noteworthy (>0.15 mcg/L) increases in serum cardiac troponin (cTnI) were as the following:

On Day 1 of Phase 1, increased in cTnI were observed 4-23 hour pose dose in 4/6 females given 120 mcg/kg (up to 5.66 mcg/L at 8 hours post dose).

On Day 1 of Phase 2, increases in cTnI was observed 4-23 hours pose dose in 2/4 females and in 1/4 males given 66.0 mcg/kg (up to 2.97 mcg/L at 8 hours pose dose) and 0-23 hours post dose in 2/6 females and 4/6 males given 501 mcg/kg (up to 3.89 mcg/L at 4 hours pose dose). Increase in cTnI was also seen in a 1/6 control females on this occasion (0.59 mcg/L, 4 hours post dose).

During Week 4, increases in cTnI were observed in 1/4 female given 9.31 mcg/kg (0.32 mcg/L immediately post dose) and in 1/6 male given 501 mcg/kg (0.16 mcg/L at 4 hours post dose). A similar increase in cTnI was also observed in 1/6 male (0.17 mcg/L at 23 hours post dose).

In conclusion, treatment related increases of Troponin I were seen in the dogs given the HD and MD. The changes in dogs given the LD were similar to that of the controls.

Urinalysis: No treatment related finding

Gross pathology: No treatment related finding

Organ weights: Reduced thymus weights were observed in treatment groups (all treated male groups, 15-32%; HD females, 11%)

Histopathology:

See the table below

Incidence (severity) of histopathological findings in the dog 13-W IH study (4/sex/dose)

		vehicle	LD	MD	HD
<b>Liver</b>					
Increased periportal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction	M	0	1 (2)	2 (2)	3 (1.7)
	F	0	0	1 (2)	2 (2)
<b>PAS staining</b>					
Decreased centrilobular glycogen	M	0	0	3 (1.3)	4 (1.2)
	F	0	1 (1)	3 (2)	4 (2.2)
Decreased periportal glycogen	M	2	0	0	0
	F	0	1 (1)	0	0
Generalized glycogen	M	2	4	1	0
	F	4	2	1	0
<b>Heart</b>					
Left ventricle papillary muscle fibrosis	M	0	0	1	2 (1.5)
	F	0	0	0	0
<b>Thymic</b> involution	M	4 (1.3)	4 (1.7)	3 (1.3)	3 (2.3)
	F	1 (1)	3 (1)	4 (1.7)	4 (1.5)
<b>Nasal turbinates</b>					
Olfactory epi-lymphoid infiltration in lamina propria	M	0	0	0	4 (1)
	F	0	0	0	2 (1)
Respiratory epi-lymphoid infiltration in lamina propria	M	0	0	0	4 (1)
	F	1 (1)	0	1 (1)	2 (1)

Most of the above findings were not observed in the recovery animals except that one of two females previously given the HD (501 mcg/kg) showed myocardial fibrosis in the left ventricle papillary muscle.

Toxicokinetics:

Systemic exposure to GW642444 (AUC<sub>0-t</sub>, C<sub>max</sub>) increased in approximately proportion with increasing dose. There was no gender related finding in the toxicokinetic parameters. Plasma drug accumulation was not evident except in females given the HD (Week 13 AUC was 3- fold of Week 4 value).

The metabolite GW630200 was only quantifiable in the plasma samples from the HD group. The ratio of the metabolite GW630200 to GW642444 (parent) based on AUC<sub>0-t</sub> was approximately 0.008 across the study. The metabolite GSK932009 was only quantifiable in the plasma samples from the HD and MD groups. The ratio of the metabolite GSK932009 to GW642444 based AUC<sub>0-t</sub> was approximately 0.06 across the study. (b)(4) GI179710 was only quantifiable in the plasma samples from the HD and MD groups. The ratio of GI179710 to GW642444 based on ACU<sub>0-t</sub> was approximately 0.46 across the study.

A summary of the toxicokinetic parameters for GW642444, corrected for the overall estimated achieved doses, in male and female dogs are presented below:

Parameter	Week	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )					
		Male			Female		
		9.31	66.0	501	9.31	66.0	501
$\text{AUC}_{0-1^{\text{a}}}$ (ng.h/mL)	Day 1	3.88	44.4	235	2.79	30.7	345
	4	4.54	51.3	357	5.80	39.3	331
	13	9.96	58.7	400	7.88	55.6	1117
$\text{C}_{\text{max}}^{\text{a}}$ (ng/mL)	Day 1	2.09	16.2	111	1.70	14.1	155
	4	2.24	17.6	139	2.78	16.0	126
	13	3.72	22.4	178	3.51	24.5	314

A summary of the toxicokinetic parameters for the metabolite GW630200, corrected for the overall estimated achieved doses, in male and female dogs are presented below:

Parameter	Week	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )					
		Male			Female		
		9.31	66.0	501	9.31	66.0	501
$\text{AUC}_{0-1^{\text{a}}}$ (ng.h/mL)	4	NC	NC	4.51	NC	NC	2.71
	13	NC	NC	3.26	NC	NC	3.01
$\text{C}_{\text{max}}^{\text{a}}$ (ng/mL)	4	NQ	NQ	1.35	NQ	NQ	0.902
	13	NQ	NQ	0.852	NQ	NQ	0.752

NQ Not quantifiable

NC Not calculated

A summary of the toxicokinetic parameters for the metabolite GSK932009, corrected for the overall estimated achieved doses, in male and female dogs are presented below:

Parameter	Week	Estimated Achieved Dose ( $\mu\text{g}/\text{kg}/\text{day}$ )					
		Male			Female		
		9.31	66.0	501	9.31	66.0	501
$\text{AUC}_{0-1^{\text{a}}}$ (ng.h/mL)	4	NC	2.84	32.1	NC	2.24	21.5
	13	NC	2.24	28.1	NC	2.90	24.0
$\text{C}_{\text{max}}^{\text{a}}$ (ng/mL)	4	NQ	0.57	4.7	NQ	0.620	3.41
	13	NQ	0.44	4.0	NQ	0.700	3.56

NQ Not quantifiable

NC Not calculated



Lymph nodes mandibular	X	X		
Lymph nodes, mesenteric	X	X		
Mammary Gland	X	X		
Nasal cavity	X	X		
Optic nerves	X	X		
Ovaries	X	X		
Pancreas	X	X		
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary	X	X		
Prostate	X	X		
Rectum	X	X		
Salivary gland	X	X		
Sciatic nerve	X	X		
Seminal vesicles				
Skeletal muscle	X	X		
Skin	X	X		
Spinal cord	X	X		
Spleen	X	X		
Sternum	X	X		
Stomach	X	X		
Testes	X	X		
Thymus	X	X		
Thyroid	X	X		
Tongue	X	X		
Trachea	X	X		
Urinary bladder	X	X		
Uterus	X	X		
Vagina	X	X		
Zymbal gland				

X, histopathology performed

\*, organ weight obtained

#### 2.6.6.4 Genetic toxicology

. GW642444 was negative in an Ames test, SHE cell assay, in vivo micronucleus assay and was weak positive in a mouse lymphoma assay. In light of the negative effects in all other genotoxicity studies, this drug was concluded not significant genotoxic. Reviewed previously (review for submission dated July 18, 2007)

#### 2.6.6.5 Carcinogenicity

No study report submitted

#### 2.6.6.6 Reproductive and developmental toxicology

Deferred to a later time

#### 2.6.6.7 Local tolerance

Not done

### 2.6.6.8 Special toxicology studies

No study report submitted

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

### Summary:

GW642444 is a novel long acting beta 2 agonist. Several in vitro and in vivo studies demonstrated that GW642444 acts through beta 2 receptors and effectively relaxes airway smooth muscle.

Safety pharmacology studies demonstrated that GW642444 moderately reduced body temperature and locomotor activity, inhibited hERG channel (IC<sub>50</sub>= 4.8 μM), inhibited electric activities in isolated Purkinje fibers and increased heart rate and decreased blood pressure in dogs. Pulmonary function is not affected by this drug.

Toxicities and human safety for the proposed clinical study have been evaluated previously (see preliminary safety evaluation for this submission), the following represents these evaluations:

Toxicology studies were conducted in rats and dogs for up to 13 weeks by inhalation administration. The review for the rat studies can be found in the previous reviews for the submissions dated 7/18/2007.

Rats received GW642444M by inhalation for 13 weeks (delivered doses of 56, 658, 10400 and 38845 mcg/kg; pulmonary deposited doses of 5.6, 65.8, 1040 and 3884.5 mcg/kg) showed toxicities in nasal cavities, nasopharynx, trachea, tracheal bifurcation and bronchi, as inflammation, epithelial degeneration/regeneration, epithelial ulceration, squamous metaplasia, and olfactory nerve atrophy. The NOAELs were defined as 56 mcg/kg for local toxicities (tracheal epithelial degeneration/regeneration was seen at 658 mcg/kg) and 38800 mcg/kg (AUC<sub>0-t</sub>=1485 ng·h/mL) for systemic toxicities.

Dog inhalation toxicology reports include a 4-week and a 13-week studies. The doses used in these two studies are the following:

- 4-Week study: delivered doses of 10, 97, 135-2010 mcg/kg; pulmonary deposited doses of 2.53, 24 and 33.75-503 mcg/kg. Of note, the HD group received delivered dose of 132 mcg/kg for the three days namely phase I and 2010, 135, 1220 and 571 mcg/kg for Days 1-2, Days 3-4, Days 5-15 and Days 16-28 of phase II, respectively. The average dose for the phase 2 was 891 mcg/kg. The MD group received delivered doses of 123 mcg/kg for the first 15 days and 64.2 mcg/kg for additional 13 days. The average dose for the MD was 97 mcg/kg.
- 13-week study: delivered doses of 9.3, 66, 120/501 mcg/kg; pulmonary deposited doses of 2.32, 16.5, 30/125.3 mcg/kg. Of note, the HD group was given 120 mcg/kg for 3 days and followed by 501 mcg/kg for additional 13 weeks.

Systemic toxicities in these two studies are similar and include liver findings (hepatocyte rarefaction, glycogen deposition, increased in periportal region and decreased in centrilobular region) and heart findings of left ventricle papillary muscle mineralization and/or fibrosis. Plasma troponin I assessed in Week 4 of the 13-w study showed increased troponin I in animals at the MD and HD. The troponin I levels in LD animals were similar to that of the controls. In addition to the systemic toxicities, nasal epithelial lymphoid infiltration was seen in the HD animals in the 13-week study. The 4-week recovery animals in the 13-week study showed only finding of myocardial fibrosis. Therefore, the liver and nasal findings are reversible. The table below presents the incidence and severity histopathological findings in the 4-week and 13-week studies.

Incidence (severity) of histopathological findings in the dog studies

4-W dog IH study (3/sex/dose)					
Delivered dose, mcg/kg		vehicle	10	97	135-2010
<b>Liver</b>					
Increased periportal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction	M	0	0	1 (1)	2 (1.5)
	F	0	0	3 (1)	3 (1.7)
<b>Heart</b>					
Papillary muscle mineralization	M	0	1	0	0
	F	0	0	0	0
Papillary muscle fibrosis	M	0	0	1	0
	F	0	0	0	0
13-W dog IH study (4/sex/dose)					
Delivered dose, mcg/kg		vehicle	9.31	66	120/501
<b>Liver</b>					
Increased periportal hepatocyte rarefaction/decreased centrilobular hepatocyte rarefaction	M	0	1 (2)	2 (2)	3 (1.7)
	F	0	0	1 (2)	2 (2)
<b>Heart</b>					
Left ventricle papillary muscle fibrosis	M	0	0	1	2 (1.5)
	F	0	0	0	0
<b>Nasal turbinates</b>					
Olfactory epi-lymphoid infiltration in lamina propria	M	0	0	0	4 (1)
	F	0	0	0	2 (1)
Respiratory epi-lymphoid infiltration in lamina propria	M	0	0	0	4 (1)
	F	1 (1)	0	1 (1)	2 (1)

Number in parenthesis indicate the severity at a scale of 1-5 as 1=minimal, 2=slight, 3=moderate, 4= marked, 5=severe

The nasal findings in the 13-week dog study are considered clinically irrelevant as there would not be significant nasal exposure in humans by oral inhalation. The findings in the heart and liver are considered treatment related. The Division considers heart rate as an acceptable biomarker for cardiac effects of beta agonists (preIND 74,696 meeting minutes). Therefore, clinical doses are not restricted by the heart findings in dogs. The

toxicological significance of liver changes in glycogen deposition is unclear. As compared to the 4-week study, the severity and incidence in the 13-week study is slightly increased. The recovery data indicates that after 13 weeks of treatment, the liver findings are reversible in 4 weeks. The sponsor explained that this finding represent a reversible alteration in glycogen storage, as beta 2 adrenoreceptor agonists can increase hepatic glycogenolysis (Smith, 1984; Phillips, 1980). This reviewer considers this explanation possible. A follow-up in chronic dog study would be helpful to clarify the toxicity significance of the liver finding.

The NOAEL for 4-week study is considered to be 10 mcg/kg (AUC of 5 ng.h/mL) as the heart lesion was not reproduced in the 13-week study. The NOAEL for the 13-week study is defined as 9.3 mcg/kg based on a minimal concern for the single incidence (1/4 male) of liver finding. The 13-week NOAEL is associated with an AUC of 7 ng.h/mL. Based on the NOAELs defined in the rat and dog studies, there are adequate safety margins for the proposed human dose of 50 mcg (table below).

	NOAEL, pulmonary deposited dose, mcg/kg		Safety margin	
	Local	Systemic	Local	Systemic
Rat 13-w IH	5.6	3885	13.3x	4496x
Dog 4-w IH	33.8	2.5	43x	15x
Dog 13-w IH	125.3	2.3	163x	21x

Note: safety margins for local and systemic toxicities were based on lung burden and AUC values, respectively.

GW642444 was negative in the Ames assay, rat bone marrow micronucleus assay, in vitro UDS assay and SHE cell assay but produced a weak positive/equivocal response in the mouse lymphoma assay in the presence of S9-mix that was not reproduced in another similar assay. Therefore, this drug is concluded to not represent a significant genotoxic risk.

Based on a cursory review of the teratogenicity study in rabbits, GW642444 was found to be teratogenic (open eyelid, cleft palate etc). No NOAEL was defined as findings occurred at the lowest (inhalation dose of 63 mcg/kg (AUC=3.76 ng.h/mL). The sponsor reported a NOAEL of 30 mcg/kg (AUC=22.4 ng.h/mL) by subcutaneous administration in a dose ranging study in rabbits. The sponsor plans to conduct a definitive subcutaneous rabbit study.

Lastly, the clinical formulation contains magnesium stearate (125 mcg/blister) which gives a maximum human exposure of 125 mcg/day. Mg stearate has been used extensively as an excipient for orally administered drugs. Studies in rats up to 6 months of treatment were conducted and used to support the approval of NDA 21-592 (IND (b)(4)) that allowed for Mg stearate exposures (b)(4). The current IND proposal results in (b)(4) daily Mg stearate exposures (125 mcg/day). In December

2005, GSK submitted questions regarding the development of Mg stearate as an inhalation excipient. At that time, they stated that they had right of reference to a 6 month repeat dose IH study in rats that was conducted (b) (4). The Division responded that, pending review of the 6-month IH study, GSK's proposed maximum daily exposure to Mg stearate (b) (4) appears to be supported. GSK has provided right of reference to the (b) (4) data and submit the study report for the 6-month study in rats in the submission dated 6/27/2008 (see the review for this submission).

Internal comments: none at this time

External comments (to sponsor): None at this time

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

Linked Applications

Sponsor Name

Drug Name

-----  
IND 74696

-----  
GLAXOSMITHKLINE

-----  
GW642444 INHALATION POWDER

-----  
**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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HUIQING HAO

10/29/2008

LUQI PEI

10/29/2008

I concur.

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**IND number:** 74,696

**Review number:** 1

**Sequence number/date/type of submission:** 008 and 009 on July 18, 2007, SX

**Information to sponsor:** Yes (X) No ( )

**Sponsor and/or agent:** GlaxoSmithKline

**Manufacturer for drug substance:** Not provided

**Reviewer name:** Huiqing Hao

**Division name:** Pulmonary and Allergy Products

**Review completion date:** 5/29/08

#### Drug:

Trade name: Not available

Generic name: Not available

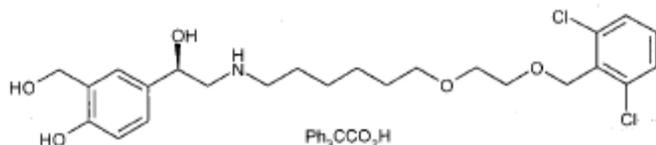
Code name: GW642444

Chemical name: Triphenylacetic acid-4-{(1R)-2[(6{2-[2,6-dichloro]oxy]ethoxy)amino]-1-hydroxyethyl}—(hydroxymethyl)phenol (1:1)

CAS registry number: Not available

Molecular formula/molecular weight:  $C_{24}H_{33}Cl_2NO_5 \cdot C_{20}H_{16}O_2$  (as the triphenylacetate salt), MW=774.78 (as the triphenylacetate salt)

Structure:



**Relevant INDs/NDAs/DMFs:** None

**Drug class:** Long acting beta agonist

**Intended clinical population:** COPD and Asthma patients

**Clinical formulation:** Dry powder blend of GW642444M in magnesium stearate and lactose

**Route of administration:** Oral inhalation

**Proposed clinical protocol:** None at this time

**Previous clinical experience:** A total of 7 studies have been conducted outside of US. The study with longest treatment duration was a 15 day study which employed doses up to 400 mcg/day of GW642444H.

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

**Studies reviewed within the submissions:**

GSK Document No.	Study Title (Study No., GSK Ref. No.)	Submission Serial No.
WD2006/01713/00	GW64244M: Preliminary Toxicity study by Inhalation administration to CD-1 mice for 13 weeks (Study No. BVR 0864/062050, GSK Ref. No. M26583)	009
WD2006/01716/00	GW64244M: A 13-week Inhalation Toxicity Study of Powder Aerosol Formulation in the rat (Study No. 79041, GSK Ref. No. R26581)	008
WD2003/01017/00	GW64244H: Bacterial Reverse Mutation Test (Study No. BVR 344/032827, GSK Ref. No. M26583)	009
WD2003/01411/00	GW64244H: Intravenous Micronucleus Assay in Rats (Study No. BVR 342/032953, GSK Ref. No. R24331)	009
WD2003/01463/00	GW64244H: In Vitro Mutation Assay Using L5178Y Mouse Lymphoma Cells (Study No. BVR 343/032869, GSK Ref. No. V24328)	009
WD2004/00169/00	In Vitro Transformation of Syrian Hamster Cells (SHE) by 7-day exposure (Study No. 7274-59, GSK Ref. No. V24843)	009
WD2003/01713/00	In Vivo DNA Repair (UDS) Test Using Rat Hepatocytes (Study No. BVR429/033425, GSK Ref. No. R24908)	009
WD2005/00325/00	GI179710X: High Throughput Fluctuation Test (GSK Study No. HTFT-295)	009
WD2005/00277/00	GI179710X: L5178Y Mammalian Cell Mutation Screen (GSK Study No. MLA-369)	009

**Studies not reviewed within this submission:**

None

**Background:**

GW642444 is being developed as the LABA component of a once-daily inhaled corticosteroid (ICS)/long-acting beta agonist (LABA) combination product for treatment of asthma in patients (b)(4). This combination product and GW642444 monotherapy are also planned for treatment of COPD.

The sponsor plans to use doses up to 50 mcg/day for future clinical trials and the anticipated Maximum Recommended Human Dose (MRHD) is 6.25 mcg/day. The AUC associated with 6.25 mcg/kg is assumed to be 56 pg.h/mL based on a linear extrapolation from AUC of 896 pg.h/mL at 100 mcg/day.

The sponsor's initial development of GW642444 (b)(4) was subsequently switched to triphenyl acetate salt (GE642444M). There is no toxicity concern with this switch as the salts are expected to be dissociated from the base (per discussion with CMC reviewer, Dr. Parasad Peri) in the body.

The formulation tested in nonclinical studies contains no magnesium stearate which is planned to be included in commercial formulation. This discrepancy has been evaluated in the preIND phase and no bridging studies are recommended since magnesium stearate is an excipient in an approved inhalation drug product (see 1/31/07 meeting minutes).

Based on the in vitro cultures with cryopreserved hepatocytes, all major metabolic routes of GW642444 (O-dealkylation and oxidation) seen in man were also seen in the rat and the mouse. Plasma protein binding was 92, 94 and 97% in the rat, mouse and human, respectively (summary information in the current submission).

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### 2.6.6.1 Overall toxicology summary

#### General toxicology:

The mouse 13-week inhalation study used GW642444M nominal doses of 58.6, 1020, 6490, 63600/38200 mcg/kg/day. Due to severe clinical signs and mortalities, the HD group was given 63600 mcg/kg for the first 8 days and then switched to 38200 mcg/kg for the remaining duration of the 13 weeks. The modified HD 382000 mcg/kg is considered MTD based on clinical signs. The treatment-related histopathological findings were of minimal to slight degree including epithelial degeneration/regeneration, epithelial metaplasia and increased eosinophilic inclusions in nasal turbinate epithelial cells, squamous metaplasia in larynx, decreased hepatocyte cytoplasm rarefaction and uterus myometrial hypertrophy. For local toxicities, no NOAEL was defined due to the increased eosinophilic inclusions. For systemic toxicities, the NOAEL is the low dose of 58.6 mcg/kg based on the observed uterine findings.

The rat 13-week inhalation study used GW642444M nominal doses of 56, 658, 10392 and 38845 mcg/kg. Drug-related effects were minimal to slight changes in hematology and blood chemistry parameters, and histopathological findings in upper airways including epithelial inflammation, ulceration, degeneration/regeneration and squamous metaplasia in part or all upper airways (nasal cavities, nasopharynx, larynx, trachea, tracheal bifurcation and bronchi). There was no systemic histopathological finding. The MTD is 658 mcg/kg based on the findings of epithelial ulceration. For local toxicities, the LD of 56 mcg/kg is the NOAEL based on the findings of epithelial degeneration and squamous metaplasia. For systemic toxicities, the HD of 38845 mcg/kg is the NOAEL.

#### Genetic toxicology:

GW642444H exhibited negative effects in an Ames test, a micronucleus test in rats (I.V. dose for 2 days), a SHE cell transformation assay (7-day exposure protocol), and an unscheduled DNA synthesis test in rat hepatocytes. In a mouse lymphoma assay, GW642444H was negative in the absence of S9, and weakly positive/equivocal in the presence of S9 mix.

### 2.6.6.3 Repeat-dose toxicity

**Study title:** GW642444M: Preliminary Toxicity study by Inhalation administration to CD-1 mice for 13 weeks

#### **Key study findings:**

1. An initial high nominal dose of 63600 mcg/kg was given for the first 8 days of dosing. However, due to the severity of clinical signs (underactive behavior, irregular and/or labored breathing and half closed eyelid) and deaths (10/60 died including 4 killed for humane reasons and 6 found dead in the restraint tube) at this dose level, the dose was reduced from Day 9 of the study to 38200 mcg/kg.
2. At 38200 mcg/kg underactive behavior, irregular and/or labored breathing and half closed eyelid were evident, however all signs had resolved in Week 4. Minor

- microscopic changes were seen at this dose including olfactory and respiratory epithelial degeneration/regeneration, and metaplasia, decreased incidence of hepatocyte cytoplasm rarefaction and uterus myometrial hypertrophy.
3. The MTD in this study is the modified high dose of 382000 mcg/kg based on no dose-limiting toxicity observed up to 38200 mcg/kg.
  4. A NOAEL for local effects could not be defined based on the findings of eosinophilic inclusions in respiratory and olfactory epithelial cells at all doses. The low nominal dose of 58.6 mcg/kg was the NOAEL for systemic effects based on the observed uterine findings.

**Study no.:** WD2006/01713/00

**Volume #, and page #:** volume 1, page 55

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 1/12/2006

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW642444M, batch No. R204768, Expiry date 10/31/2006, purity, 98.5%; Lactose, batch B143232, expiry date 9/9/2006

### Methods

Doses: Nominal doses of 0 (lactose only), 58, 1020, 6490, 63600/38200 mcg/kg/day; pulmonary deposited doses of 5.8, 102, 649, 6360, 3820 mcg/kg/day based on pulmonary deposition factor of 0.1

Species/strain: CD-1 mice

Number/sex/group or time point (main study): 12/sex/dose

Route, formulation, volume, and infusion rate: Snout only inhalation for 60 min/day, in lactose formulation

Satellite groups used for toxicokinetics or recovery: 18/sex/dose for TK study

Age: 6-7 weeks old

Weight: males, 31.1-38.6 g; female, 22.7-29.7 g

Sampling times: see the section of Observation and Times

Unique study design or methodology (if any): Aerosol was generated (b) (4)

Samples were collected daily from each dose group for gravimetric analysis. Samples were collected for chemical analysis daily during Week 1, four times per week during Week 2, three times per week during Week 3 and 4 and twice weekly for week 5 to 14. Particle size distribution was measured (b) (4)

Samples were collected during week 1, 5, 9 and 13. (b)(4)

. The dose calculation was based on the following equation:  $D = (RMV \times T \times C) / (BW)$  where D=dose (mcg/kg);  $RMV = 0.499 \times bw$  (kg)<sup>0.809</sup> (Bide et al, 2000); T=duration of exposure/day (minutes); C=GW642444×aerosol concentration (mcg/L); BW=group average body weight for study (kg)

**Observation and Times:**

Clinical signs: Daily, immediately before and after dosing, as well as between 1/2 to 2 hours after completion of dosing

Body weights: 2X during week -1, on the day of treatment commence, weekly during treatment and on the day of necropsy

Food consumption: During the week before treatment, and weekly throughout the treatment period

Ophthalmoscopy: once prior to initiation of dosing, and during week 13

EKG: Not done

Hematology: Before dosing during Week 13 and prior to necropsy during Week 14

Clinical chemistry: See hematology part

Urinalysis: Not done

Gross pathology: At necropsy

Organ weights: Brain, heart, kidney, liver with gall bladder, lungs including bronchi, ovaries (paired), spleen, testes (paired) and thymus.

Histopathology: Adequate Battery: yes (X), no ( )—explain

Peer review: yes (X), no ( )

A complete battery of tissues as listed in the histopathology inventory was examined in all animals of control and the high dose groups, as well the majority of premature deaths in all groups. For terminal toxicology animals of the LD, Low MD and UMD, the nasal turbinates, larynx, liver and uterus were also examined microscopically.

The sponsor reported a peer review of selected microscopic tissue sections and pathology data interpretation without providing details.

Toxicokinetics: Blood samples were collected from TK animals (18/sex/group) in Week 13 of treatment.

**Results:**

The achieved delivered doses, pulmonary deposited doses and measured particle sizes are shown below:

Aerosol conc., mcg/L	Delivered dose, mcg/kg	MMAD, mcm	Pulmonary deposition factor	Pulmonary dose, mcg/kg
1.01	58.6	1.7	0.1	5.9
17.9	1020	2.0	0.1	102

114	6490	1.9	0.1	649
1090*	63600	3.3	0.1	6360
671**	38200	2.3	0.1	3820

\* and \*\* : the HD animals were given 1090 mcg/L from Day 1-8 and 671 mcg/L from Day 9-93. This HD dose adjustment was applied to both the main study and the TK study.

Of note, the high dose of 63600 mcg was the maximum practicable dose (50000-70000 mcg/kg) for a 1 hour dosing period based on a total particulate concentration of 2 mg/L using a 40% w/w blend.

The sponsor considered the delivered doses as achieved doses based on an assumption of 100% deposition factor.

Mortality: At the initial high dose of 63600 mcg/kg, during the first week of exposure there was a high incidence of animals showing signs including irregular and/or labored breathing, underactive behavior and half closed eyelid which lead to 10 of 60 animals dying or being killed (3 males and 7 females) for humane reasons (4 were killed for humane reasons, and 6 were found dead in the restraint tube). The mortality details are presented in the table below.

#### Animal mortality in the first 9 days of treatment

Dose, mcg/kg	Group	Day of death	Cause of death
63600	Tox female	1	Humane kill (animal gasping)
63600	Tox female	2	Found dead in restraint tube
63600	Tox female	4	Found dead in restraint tube
63600	Tox female	5	Found dead in restraint tube
63600	TK female	2	Found dead in restraint tube
63600	Tox male	7	Humane kill (animal gasping, & labored breathing)
63600	TK male	4	Humane kill (animal gasping, salivating & with half closed eyelids)
63600	TK female	4	Found dead in restraint tube
63600	TK female	6	Found dead in restraint tube
63600	TK male	6	Humane kill (animal gasping)

Five toxicology animals among the 10 animals were examined microscopically and findings in the larynx and nasal turbinate olfactory epithelium (minimal epithelial degeneration/necrosis and slightly squamous metaplasia) were observed. However, no histopathological finding attributable to death was observed. The dead animals were replaced with spare animals where available. A reduced dose, 38200 mcg/kg, was used from Day 9 onwards for the HD.

There were 10 further deaths (5 in control groups, 1 at the LD, 4 at the HD) during the course of the study and these deaths were considered to be a result of the animal turning in the restraint tubes and/or other reasons unrelated to treatment with GW642444M. The table below presents details of these 10 deaths.

Dose, mcg/kg	Group	Day of death	Cause of death
58.6	Tox female	7	Found dead in restraint tube
0	Tox female	21	Found dead in restraint tube
0	TK female	25	Found dead in restraint tube
6490	TK female	25	Found dead in restraint tube
0	Tox male	32	Humane kill (excessive weight loss)
6490	TK female	29	Found dead in restraint tube
6490	TK female	31	Found dead in restraint tube
0	TK male	32	Humane kill (animal lost use of hindlimbs)
6490	Tox female	53	Found dead in restraint tube
0	TK male	66	Accidental death (animal died following trauma after unloading)

Clinical signs: Irregular/labored breathing and half closed eyelids were seen in the HD group within the first 4 weeks of treatment, but were resolved thereafter (see the table below).

Incidence of clinical signs (number of days sign present during study)

Day 1-8 (63600 ug/kg for HD)						
Dose, mcg/kg		0	58.6	1020	6490	63600/38200
Underactive behavior	Male	0	0	0	0	11(23)
	Female	0	0	0	0	7(2)
Irregular breathing	Male	0	0	0	0	11(2)
	Female	0	0	0	0	8(2)
Labored breathing	Male	0	0	0	0	10(1)
	Female	0	0	0	0	11(1)
Eyelid, left closed	Male	0	0	0	0	1(1)
	Female	0	0	0	0	0
Eyelid, left partially closed	Male	0	0	0	0	9(1)
	Female	0	0	0	1(1)	0
Eyelid, right partially closed	Male	0	0	0	0	9(1)
	Female	0	0	0	1(1)	0
Eyelids, both half closed	Male	0	0	0	1(1)	12(4)
	Female	0	0	0	5(1)	12(4)
Day 9-28 (38200 mcg/kg for HD)						
Irregular breathing	Male	0	0	0	0	3(1)
	Female	0	0	0	0	1(1)
Labored breathing	Male	0	0	0	0	1(1)
	Female	0	0	0	0	12(4)
Eye lids, both half closed	Male	0	0	0	0	12(4)
	Female	0	0	0	0	12(2)

Body weights: Body weight gains were increased in males (1.37-1.46 fold of control) and females (1.21-1.58 fold of control) exposed to GW642444M, without a dose-relationship.

Food consumption: No treatment-related findings

Ophthalmoscopy: No treatment-related findings

Hematology: Treatment related decrease of platelet count (males, 0.93-0.83 fold; females, 0.90-0.86 fold) and increase of WBC counts (males, 1.28-1.5 fold; females, 1.24-1.46 fold) were observed. The increased WBC was due to elevated levels of monocyte (males, 1.18-1.27 fold; females, 1.14-1.57 fold), neutrophil (males, 1.4-1.76 fold; females, 1.18-1.53 fold), and eosinophil (males, 1.21-2.07 fold; females, 1.14-1.64 fold) counts. However, there was no clear dose-relationship observed in any of these changes and the toxicological significance of these findings is not clear.

Clinical chemistry: Slightly increased total protein (1.05-1.07 fold) was observed in both HD male and female groups and the UMD male group. A slight increase of albumin level (1.06 fold) was also observed in HD groups. However, such small changes are not considered toxicologically significant.

Gross pathology:

Stomach:

Roughening of the forestomach epithelium was seen in 2/12 and 4/12 male mice given the UMD and HD, respectively. However, there was no associated microscopic finding and, therefore, this stomach change is not considered toxicologically significant.

Uterus:

An increased incidence of thickening of uterus was seen in treated females (0/11, 5/11, 9/12, 8/11, 9/12 for control, 58.6, 1020, 6490 and 38200 mcg/kg groups, respectively). The associated microscopic finding of myometrial hypertrophy was also reported (see histopathology section).

Organ weights:

Body weight adjusted liver weights (7%-15%) were decreased in all male treatment groups and females at the UMD and the HD.

Body weight adjusted lung and bronchi weight were higher in groups at the UMD and the HD (9-15%).

Absolute ovary weights were increased (29-104%) in most female treatment groups except for that of the LD group which showed no difference from the control.

Body weight adjusted brain weights were decreased (4-8%) in male treatment groups. However, due to the lack of associated microscopic findings, this finding is not considered significant.

**Histopathology:** The treatment-related findings were mostly mild including minimal epithelial degeneration/regeneration, minimal to slight epithelial metaplasia and eosinophilic inclusions in nasal turbinates, squamous metaplasia in larynx, minimal to slight decreased hepatocyte cytoplasm rarefaction in liver and minimal to slight uterus myometrial hypertrophy. Most of the findings were dose related. Based on the increased eosinophilic inclusions in respiratory and olfactory epithelial cells, no NOAEL could be defined. The following table presents the incidence and severity of these findings.

Incidence (severity) of histopathological findings

Dose, mcg/kg		0	58.6	1020	6490	38200
N	M	11	12	12	12	12
	F	11	11	12	11	12
<b>Nasal turbinates</b>						
Respiratory epi. degen./regen.	M	0	0	0	0	3 (1)
	F	0	0	0	0	1 (1)
Respiratory epi. eosinophilic inclusion	M	0	2(1.5)	1(1)	1(1)	3(1)
	F	2(1.5)	8(1)	6(1.5)	2(1.5)	4(1.7)
Respiratory epi. cystic gland ±luminal debris	M	0	0	0	1(1)	2(1)
	F	0	0	0	0	0
Olfactory epi. degen/regen	M	0	0	0	0	9(1.7)
	F	0	0	0	0	10(1.3)
Olfactory epi. metaplasia	M	0	0	0	0	1(1)
	F	0	0	0	0	0
Olfactory epi. eosinophilic inclusion	M	2(1.5)	4(1.3)	4(1)	5(1.2)	6(1.3)
	F	3(1.3)	6(1.3)	5(1.6)	5(1.2)	7(1.6)
Olfactory epi. cystic gland ±luminal debris	M	0	0	0	0	1(1)
	F	0	0	0	0	0
<b>Larynx</b>						
Larynx ventral epi. squamous metaplasia	M	0	0	7(1.1)	12(1.3)	12(1.6)
	F	0	0	4(1)	10(1.7)	12(1.4)
<b>Liver</b>						
Decreased generalized hepatocyte cytoplasm rarefaction	M	0	1	0	4(1.5)	5(1.6)
	F	0	0	0	0	0
<b>Uterus</b>						
Uterus myometrial hypertrophy	F	0	0	6(1.5)	10(1.4)	12(1.3)

Severity scale was not provided. The average severities presented in this table are converted from the data provided as 1=minimum, 2=slight, 3=moderate

**Toxicokinetics:**

Systemic exposure to GW642444, its metabolites GW630200 and GW932009, and to <sup>(b)(4)</sup> GI17970 (as measured by dose normalized AUC and Cmax) increased with increasing dose, in a less than dose-proportional manner. There was no gender related

difference in TK parameters. The metabolite/parent ratios based on AUC for GW630200 and GSK932009 were approximately 0.004 and 0.08, respectively.

Mouse TK data in Week 13 of inhalation study with GW642444M

	Dose, mcg/kg		58.6	1020	6490	38200
GW642444	AUC0-t, ng.h/mL	M	6.33	98.9	301	611
		F	10.7	67.2	288	565
	Cmax, ng/mL	M	3.69	46.6	175	202
		F	4.30	35.4	156	244
GW630200	AUC0-t, ng.h/mL	M	ND	ND	0.591	2.25
		F	ND	ND	1.56	3.03
	Cmax, ng/mL	M	ND	ND	0.361	0.825
		F	ND	ND	0.552	0.795
GW932009	AUC0-t, ng.h/mL	M	ND	7.21	16.0	68.4
		F	ND	4.36	22.7	72.2
	Cmax, ng/mL	M	ND	3.41	5.11	20.2
		F	ND	1.91	7.98	24.4
GI179710	AUC0-t, ng.h/mL	M	ND	908	4310	16100
		F	ND	1590	7140	15500
	Cmax, ng/mL	M	ND	366	714	1890
		F	ND	300	1170	2780

GW630200 and GW6932009 are metabolites of GW642444. GI79710 is the (b)(4)

**Study title:** GW64244M: A 13-week Inhalation Toxicity Study of Powder Aerosol Formulation in the rat

**Key study findings:**

1. Rats received GW64244M by inhalation at delivered doses of 56, 658, 10392 and 38845 mcg/kg. Drug-related effects included increased body weight gain and food consumption, minimal to slight changes in hematology and blood chemistry parameters, and histopathological findings in upper airways only; there was no systemic histopathological finding. Epithelial inflammation, ulceration, degeneration/regeneration and squamous metaplasia were observed in part or all upper airways (nasal cavities, nasopharynx, larynx, trachea, tracheal bifurcation and bronchi). All of these findings were dose-related.
2. The MTD is 658 mcg/kg based on the findings of epithelial ulceration at the doses of 10390 mcg/kg and higher.
3. The NOAEL for local findings in this study is 56 mcg/kg based on the findings of epithelial degeneration and squamous metaplasia at the doses of 658 mcg/kg and higher.

**Study no.:** 79041, GSK Ref. No. R26581, GSK Report No. WD2006/01716/00

**Volume #, and page #:** Vol. 1, page 48

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 2/1/2006

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW64244M, batch No. R204768, Expiry date 10/31/2006, 98.2-98.3% as free base; lactose, batch B143232, expiry date 9/9/2006

**Methods**

Doses (delivered): nominal inhaled doses: 0 (lactose only), 56, 658, 10392, 38845 mcg/kg/day; pulmonary deposited doses: 5.6, 65.8, 1039.3, 3884.5 mcg/kg/day

Species/strain: Crl:CD (SD) rats

Number/sex/group or time point (main study): 12/sex/dose

Route, formulation, volume, and infusion rate: nose only inhalation for 60 min/day, in lactose formulation, NA, NA

Satellite groups used for toxicokinetics or recovery: 6/sex/dose for TK study

Age: 6-7 weeks old

Weight: males, 163-210 g; female, 134-169 g

Sampling times: see the section of Observation and Times

Unique study design or methodology (if any): Aerosols were generated [REDACTED] (b) (4)

[REDACTED] Samples were analyzed on 2 occasions prestudy, daily during the first week of treatment, and 3

times weekly from Weeks 2 to 4 then 2 times weekly thereafter. (b) (4)

The dose calculation was based on the following equation:  $D=(RMV \times T \times C)/(BW)$  where  $D$ =dose (mcg/kg);  $RMV=0.499 \times bw$  (kg)<sup>0.809</sup> (Bide et al, 2000);  $T$ =duration of exposure/day (minutes);  $C=GW642444 \times$  aerosol concentration (mcg/L);  $BW$ =group average body weight for study (kg)

### **Observation and Times:**

Clinical signs: Daily throughout the pre-treatment and treatment period. Detailed clinical examinations were performed on Days -4, 28, 90, 92 and or 93

Body weights: 2X during the pre-treatment, On Day 1 and weekly thereafter.

Food consumption: During the week before treatment, and weekly throughout the treatment period

Ophthalmoscopy: Once prior to initiation of dosing, and during week 13

EKG: Not done

Hematology: On Days 25 and 87 or 88 of the treatment period.

Clinical chemistry: See hematology part

Urinalysis: Overnight urine samples were collected on Days 24 and 87 of treatment period.

Gross pathology: At necropsy

Organ weights: Adrenals, brain, heart, kidney, liver, lungs, ovaries, prostate, spleen, testes, and thymus were weighed.

Histopathology: Adequate Battery: yes ( X ), no ( )—explain

Peer review: yes ( X ), no ( )

A complete battery of tissues as listed in the histopath inventory was examined in all animals of control and the high dose groups, all unscheduled deaths, and tissues with gross lesions of all animals. Tissues with treatment-related findings in the high dose group were examined in lower dose groups (including bronchi, larynx, lung, nasal cavities, nasopharynx, trachea and tracheal bifurcation).

Performance of a peer review of selected microscopic tissue sections and pathology data interpretation was reported without details.

Toxicokinetics: Blood samples for TK analysis were collected in Week 4 and Week 13 of treatment from TK animals (6/sex/group).

### **Results:**

The achieved delivered doses, the pulmonary deposited doses and measured particle sizes are shown below:

Aerosol conc., mcg/L	Delivered dose, mcg/kg	MMAD, mcm	Pulmonary deposition factor	Pulmonary dose, mcg/kg
1.5	56.2	4.2	0.1	5.6
17.7	657.9	5.0	0.1	65.8
280.6	10392.6	3.0	0.1	1039.3
1044.5	38845.1	3.6	0.1	3884.5

The sponsor considered the delivered doses as achieved doses based on an assumption of 100% deposition factor.

Mortality: Four drug treated and one control rats died (2 at UMD and 1 at the HD died on Days 24 or 25, 1 at the HD died on Day 67, 1 control rat died on Day 25). Other than the one animal that died on Day 67, all deaths occurred immediately after jugular venipuncture. This time frame and necropsy finding of hemorrhage in the cervical region suggests the deaths are likely procedure related. Pathology examinations in the treated animals were no different from that seen in the terminal sacrificed animals (findings were in nasal cavities, nasopharynx, larynx, trachea, tracheal bifurcation and bronchi). The cause of death for the one HD male that died on Day 67 could not be determined from pathology results and the relationship to the test article is unknown.

Clinical signs: No treatment related findings.

Body weights: By the end of the 13 weeks, a dose-related increase of body weight gain was seen in females (up to 19% at the HD). There was no similar finding in males.

Food consumption: A dose-related increase of food consumption was observed throughout the study in both sexes (up to 19-22%) except the HD male group in which only 8% increase was observed.

Ophthalmoscopy: No treatment-related findings

Hematology: At Week-13, a dose-related increase of neutrophil counts (male HD, 35%; female HD, 213%) and decrease of platelet counts (male HD, 30%; female HD, 21%) were observed in treated groups. The platelet finding, in a lower degree, was also seen at Week-4. The table below presents the neutrophil and platelet counts at Week 13.

#### Hematology at Week-13

Delivered Dose, mcg/kg		0	56	658	10393	38845
Neutrophil count, 10 <sup>9</sup> /L	M	1.57	1.29	1.48	1.44	2.13
	F	0.49	0.44	0.67	0.91	1.55
Platelet count, 10 <sup>9</sup> /L	M	1201	1008	955	884	841
	F	1084	945	941	898	860

#### Clinical chemistry:

Decreased triglyceride (up to 65%) and glucose concentrations (up to 36%), increased serum alanine aminotransferase activities (up to 45%) and increased urea levels (up to 26%) were observed in treated groups in Week -4 and Week-13. In Week-13, increased

serum alkaline phosphatase activities (up to 40%) and bilirubin concentrations (up to 39%) were also noted in treated groups. Females at Week 13 also showed a slight increase of potassium concentration (up to 19%), lower total protein (up to 7%) and albumin (up to 11%). The toxicological significances of these changes are not clear. Changes noted in Week-4 but not Week-13 included slightly increased creatinine (up to 18% increase) and albumin concentrations (up to 7% increase) and slightly decreased cholesterol levels (up to 20% decrease). The table below presents the details of the Week -13 findings.

#### Blood chemistry at Week-13

Delivered Dose, mcg/kg		0	56	658	10393	38845
Triglyceride, mmol/L	M	0.808	0.908	0.592	0.428	0.332
	F	0.515	0.471	0.448	0.379	0.363
Total protein, g/L	M	71.1	73.7	71.7	71.9	74.0
	F	74.9	74.8	73.2	69.6	71.2
Albumin, g/L	M	41.3	42.3	42.3	42.0	41.1
	F	47.7	47.5	45.8	43.4	42.4
Glucose, mM	M	6.03	4.37	4.02	3.88	3.86
	F	5.63	4.97	4.62	4.36	4.71
ALT, U/L	M	41.0	44.8	44.4	48.9	53.3
	F	40.1	41.6	39.0	43.1	50.0
Urea, mM	M	5.66	5.71	5.88	6.82	6.86
	F	5.62	5.53	6.38	5.45	5.75
Total bilirubin, mcmol/L	M	2.3	2.5	3.2	2.9	2.9
	F	2.5	3.0	3.3	2.6	2.5
Alkaline phosphatase, U/L	M	90.7	94.6	104.6	107.2	111.5
	F	56.0	60.0	64.3	78.6	70.3
Potassium, mmol/L	M	5.706	5.795	5.895	5.563	5.960
	F	5.061	5.695	6.011	5.192	5.502

#### Gross pathology:

No remarkable finding.

#### Organ weights:

Slightly decreased ovary weights (19%) were noted in females at the HD.

Histopathology: The treatment-related findings were restricted to the lung and included minimal to marked epithelial degeneration/regeneration, slight to marked metaplasia, minimal to severe ulceration, exudate, and inflammation in the nasal cavities, nasopharynx, larynx, trachea, tracheal bifurcation and bronchi. Most of the findings were observed at the UMD and the HD. The low MD (658 mcg/kg) showed a minimal degree of epithelial degeneration/regeneration in nasal cavity, nasopharynx and trachea, and larynx squamous metaplasia. The following table presents the incidence and severity of these findings.

## Rat histopathological findings following 13 weeks of GW642444M inhalation

Delivered Dose, mcg/kg		0	56	658	10393	38845
<b>Nasal cavities/sinuses</b>						
Squamous metaplasia	M	0	0	0	4 (2.8)	12 (2.8)
	F	0	0	0	7 (2.7)	12 (4)
Olfactory ulceration	M	0	0	0	3 (1.7)	11 (2.6)
	F	0	0	0	4 (2)	12 (3.6)
Respiratory epithelial ulceration	M	0	0	0	0	9 (2.2)
	F	0	0	0	0	7 (3.1)
Respiratory/olfactory epi. metaplasia	M	0	0	0	3 (1.3)	11 (3)
	F	0	0	0	1 (2)	11 (3.6)
Respiratory epi. degeneration/regeneration	M	0	0	0	2 (1)	12 (3.1)
	F	0	0	0	4 (2)	12 (3)
Olfactory epi. degeneration/regeneration	M	0	0	0	6 (2.2)	12 (3.3)
	F	0	0	0	10 (2.6)	12 (4)
Transitional epi. degeneration/regeneration	M	0	0	7 (1)	7 (1.3)	11 (2.4)
	F	0	0	3 (1)	5 (1.8)	12 (2.4)
Bowman's gland ectasia/metaplasia	M	0	0	0	2 (2)	12 (3.3)
	F	0	0	0	5 (1.6)	12 (3.5)
Olfactory nerve atrophy	M	0	0	0	2 (1.5)	12 (3.4)
	F	0	0	0	2 (1)	12 (3.4)
Inflammation	M	0	0	0	4 (1)	12 (2.7)
	F	0	0	0	1 (1)	12 (3.2)
Exudate	M	0	0	0	1 (2)	11 (2.2)
	F	0	0	0	3 (1.3)	12 (2.7)
<b>Nasopharynx</b>						
Squamous metaplasia	M	0	0	0	1 (1)	11 (2.6)
	F	0	0	0	0	10 (3.6)
Respiratory epithelial ulceration	M	0	0	0	0	4 (1)
	F	0	0	0	0	1 (1)
Respiratory epi. degeneration/regeneration	M	0	0	1 (1)	2 (2)	12 (3)
	F	0	0	0	2 (2.5)	11 (2.6)
Exudate	M	0	0	0	0	7 (2)
	F	0	0	0	0	3 (2.3)
Inflammation	M	0	0	0	0	12 (2.2)
	F	0	0	0	1 (2)	10 (2.2)
Goblet cell hyperplasia	M	0	2 (1)	0	1 (1)	5 (1.4)
	F	0	0	0	1 (1)	3 (1.3)

Note: numbers in ( ) indicate severities in a scale of 5 as 1=minimum, 2=slight 3=moderate, 4=marked, 5=severe; numbers of animals examined were 12/sex/group including pre-terminal deaths.

## Rat histopathological findings following 13 weeks of GW642444M inhalation (cont'd)

Delivered Dose, mcg/kg		0	56	658	10393	38845
<b>Larynx</b>						
Squamous metaplasia	M	0	0	2 (1.5)	2 (1.5)	12 (1.7)
	F	0	0	2 (1)	0	12 (2.9)
Epithelial hyperplasia	M	0	0	1 (1)	0	7 (1.1)
	F	0	0	0	0	7 (1.7)
Exudate	M	0	0	0	1 (1)	5 (1)
	F	0	0	0	0	1 (1)
<b>Trachea</b>						
Squamous metaplasia	M	0	0	0	0	1 (1)
	F	0	0	0	0	0
Respiratory epi. degeneration/regeneration	M	0	0	3 (1)	3 (1)	5 (1.4)
	F	0	0	0	2 (1)	6 (1)
<b>Tracheal bifurcation</b>						
Squamous metaplasia	M	0	0	0	3 (1.3)	7 (1.7)
	F	0	0	0	5 (1)	9 (1.3)
<b>Bronchi</b>						
Squamous metaplasia	M	0	0	0	0	2 (1)
	F	0	0	0	0	0
Epithelial hyperplasia	M	0	0	0	0	1 (2)
	F	0	0	0	0	1 (1)

Note: numbers in ( ) indicate severities in a scale of 5 as 1=minimum, 2=slight 3=moderate, 4=marked, 5=severe; numbers of animals examined were 12/sex/group including pre-terminal deaths.

**Toxicokinetics:** In general, systemic exposures to GW642444 and (b)(4) (GI179710, (b)(4)) were approximately dose-proportional. Exposure to the parent compound was no detected at the lowest dose with lower limit of quantification of 0.1 ng/mL. There was no drug accumulation observed after comparing Week 4 and Week 13 data. Systemic exposures to the metabolites of GW642444 were much lower than to GW642444. The metabolite/parent ratios based on AUC for GW630200 and GW932009 were approximately 0.004 and 0.03 respectively, across the study.

## AUC (ng.h/mL) levels in rat 13-week inhalation study with GW642444M

Delivered Dose, mcg/kg			56.2	658	10392	38845
GW642444	Week 4	M	NC	63.2	492	1210
		F	NC	31.5	447	816
	Week 13	M	NC	33.5	451	2450
		F	NC	28.4	425	1460
GW630200	Week 13	M	NC	NC	2.12	6.45
		F	NC	NC	1.51	3.57
GW932009	Week 13	M	NC	0.397	11.8	52.8
		F	NC	0.376	17.8	46.2
GI179710	Week 4	M	NC	92.8	1840	5670
		F	NC	60.5	2190	6180
	Week 13	M	NC	77.0	2040	10600
		F	NC	167	3950	18700

## Cmax (ng/mL) levels in rat 13-week inhalation study with GW642444M

Delivered Dose, mcg/kg			56.2	658	10392	38845
GW642444	Week 4	M	3.09	19.1	206	435
		F	2.23	12.3	195	263
	Week 13	M	5.11	13.0	238	555
		F	4.24	7.89	130	282
GW630200	Week 13	M	NC	NC	1.12	2.56
		F	NC	NC	0.790	0.660
GW932009	Week 13	M	NC	0.267	3.88	12.7
		F	NC	0.293	6.17	8.27
GI179710	Week 4	M	19.6	44.5	392	1410
		F	9.33	26.3	763	1340
	Week 13	M	NC	42.0	633	1650
		F	NC	36.1	2050	4080

**Histopathology inventory**

Study	M2658 3	R2658 1		
Species	mouse	rat		
Adrenals	X	X		
Aorta	X	X		
Bone Marrow smear	X	X		
Bone (femur)	X	X		
Brain	X	X		
Cecum	X	X		
Cervix	X	X		
Colon	X	X		
Duodenum	X	X		
Epididymis	X	X		
Esophagus	X	X		
Eye	X	X		
Fallopian tube				
Gall bladder	X			
Gross lesions	X	X		
Harderian gland	X			
Heart	X	X		
Ileum	X	X		
Injection site				
Jejunum	X	X		
Kidneys	X	X		
Lachrymal gland				
Larynx	X	X		
Liver	X	X		
Lungs	X	X		
Lymph nodes, cervical	X			
Lymph nodes, tracheo- bronchial	X	X		
Lymph nodes mandibular		X		
Lymph nodes, mesenteric	X	X		
Mammary Gland	X	X		
Nasal cavity	X	X		
Optic nerves	X	X		
Ovaries	X	X		
Pancreas	X	X		
Parathyroid	X	X		
Peripheral nerve				
Pharynx				
Pituitary	X	X		
Prostate	X	X		
Rectum	X			
Salivary gland	X	X		
Sciatic nerve	X	X		

Seminal vesicles	X	X		
Skeletal muscle	X	X		
Skin	X	X		
Spinal cord	X			
Spleen	X	X		
Sternum	X	X		
Stomach	X	X		
Testes	X	X		
Thymus	X	X		
Thyroid	X	X		
Tongue	X	X		
Trachea	X	X		
Urinary bladder	X	X		
Uterus	X	X		
Vagina	X	X		
Zymbal gland				

X, histopathology performed  
 \*, organ weight obtained

**2.6.6.4 Genetic toxicology**

**Study title:** GW642444 H: Bacterial Reverse Mutation test

**Key findings:** GW642444H was not mutagenic when tested in the absence or presence of S9-mix with any of the *Salmonella Typhimurium* or *Escherichia Coli* strains used in this study, at concentrations up to 5000 mcg (base)/plate.

**Study no.:** Protocol No. BVR344/032827; GSK reference No. V24329; GSK report No. WD2003/01017/00

**Volume #, and page #:** Vol. 2 of 3, page 242

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 4/7/2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW642444H, Lot SS101948-196A1, purity 66.3% of free base (1.508 g of GW642444H=1.00 g of GW642444X)

**Methods**

**Strains/species/cell line:** *Salmonella Typhimurium* TA98, TA100, TA1525, TA1537 and *Escherichia Coli* WP2uvrA (pKM101)

**Doses used in definitive study:** 50, 150, 500, 1500 and 5000 mcg/plate in both the absence and presence of S9-mix. All doses and concentrations of GW642444H are expressed in term of the free base.

Basis of dose selection: The maximum recommended dose by the current guidelines (ICH S2A, 1996)

Negative controls: DMSO

Positive controls:

Strain	-S9		+S9	
	(+) control	mcg/plate	(+) control	mcg/plate
TA1535	NaN3	0.5	2-aminoanthracene	2
TA1537	9-aminoacridine	50	Benso[a]pyrene	5
TA98	2-nitrofluorene	1	Benso[a]pyrene	5
TA100	NaN3	0.5	Benso[a]pyrene	5
WP2 uvrA	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	0.05	2-aminoanthracene	10

Incubation and sampling times: plate incorporation tests were conducted with 3 days of incubation at 37°C before scoring for revertant colonies.

## Results

Study validity: This study was deemed valid based on the following: 1). Doses up to 5000 mcg/plate were used; 2). Two main tests (with the same dose selections) were performed, each test included 6 plates/group for vehicle control, and 3 plates/group for the treated groups and positive controls; 3). Colonies were counted using a Domino image analysis system. 4). Positive criteria: at least a two-fold increase in revertants for TA98, TA100 and WP2uvrA (pKM101), and at least a three fold increase for TA1537 over the vehicle control; the effect should be reproducible in an independent assay; and ideally there should be evidence of dose-relationship. 5). Results of both positive and negative control groups are expected.

Study outcome:

GW642444H showed bactericidal activity at 5000 mcg/plate in all strains in the presence of S9-mix (thin background lawn and reduced revertants). There was no evident toxicity seen at lower concentrations including 1500, 500, 150 and 50 mcg/plate. No precipitation was observed with GW642444H.

No increased number of revertants was observed in the absence or presence of S9-mix at concentrations up to 5000 mcg/plate with any bacterial strains tested.

In conclusion, GW642444H was negative in the bacterial reverse mutation test under the test condition.

**Study title:** GW642444 H: Intravenous Micronucleus Assay in Rats

**Key findings:** Rats given doses up to 12.5 mg/kg for 2 days showed negative findings of micronucleus under the conditions tested.

**Study no.:** Protocol No. BVR342/032953; GSK reference No. R24331; GSK report No. WD2003/01411/00

**Volume #, and page #:** Vol. 3 of 3, page 20

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** 4/9/2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW642444, Lot SS101948-196A1, purity 66.3% of free base (1.508 g of GW642444H=1.00 g of GW642444X)

## Methods

**Strains/species/cell line:** Sprague-Dawley (CrI:CD) rats, males only

**Doses used in definitive study:** 7.8 and 12.5 mg/kg/day. All doses are expressed in terms of free base, GW642444X.

**Basis of dose selection:**

In the dose ranging study, based on the maximum solubility of GW642444H in the vehicle, rats (3/sex/dose) were given GW642444H at 12.5 (twice in two days) or 25 mg/kg (once only due to severe clinical signs). No vehicle control group was included in this study. The observations appear to be clinical signs only. The animals at 25 mg/kg were observed with underactivity, abnormal gait, and irregular breathing, but no deaths occurred. Responses in males and females were similar. Based on the observed clinical signs, the high dose of 12.5 mg/kg was considered the MTD and was chosen for the definitive study.

**Negative controls:** 20/80 PEG400/8% 2-hydroxypropyl beta cyclodextrin

**Positive controls:** Cyclophosphamide

**Incubation and sampling times:**

All animals in the vehicle control and the test material groups were dosed intravenously twice separated by 24 hours. Animals in the positive control group were dosed orally once on Day 2 only. All animals in all groups were dosed at a volume of 10 mL/kg. Bone marrow samples were collected at 24 hours after the final dose. The study design was the following:

Group	Treatment	Conc., mg/mL	Dose, mg/kg/day	N of rats
1	Vehicle	-	-	7
2	GW642444	0.78	7.8	7
3	GW642444	1.25	12.5	7+3*
4	Cyclophosphamide	2	20	5

\*satellite animals for TK analysis, dosed once only. TK sample taken 15 minutes following dosing

## Results

### Study validity:

This study is deemed valid based on the following: 1). 7 rats/group were used; 2). Use of male rats only is acceptable as no substantial difference in toxicities (clinical signs) between the sexes was observed in the dose ranging study; 3). The HD of 12.5 mg/kg for two days is considered the MTD based on the clinical signs at this dose, including underactive behavior, flattened posture, abnormal gait, fast and irregular breathing, prominent eyes and partially closed eyelids; 4). Bone marrow slides were counted manually under a microscope, for proportion of PCE in 1000 total erythrocytes, micronuclei in a total of at least 100 NCE, and the number of micronucleated polychromatic erythrocytes (MPCE) from at least 2000 PCE; 5). Criteria for a positive response are statistically significant dose-related increase in the incidence of micronucleated immature erythrocytes over the concurrent vehicle control ( $p < 0.01$ ); individual and/or group mean values exceed the laboratory historical vehicle control range.

**APPENDIX 2 Micronucleus test - Clinical signs and mortalities**

Treatment / dosage Group / sex	GW642444H (7.8 mg/kg/day)										GW642444H (12.5 mg/kg/day)									
	2M					3M					2M					3M				
Time after first dosing (hr:min)	0:09	0:59	3:33	5:20	7:00	21:41	24:20	25:08	29:03	45:42	0:09	0:44	3:18	5:05	21:24	24:21	25:32	28:47	45:28	
No abnormalities detected	0	0	0	0	7	0	0	0	0	5	0	0	0	0	0	0	0	0	0	
Behaviour - Underactive	7	7	7	7	0	7	7	7	4	0	7	7	7	7	3	7	7	5	4	
Posture - Flattened	7	7	7	7	0	7	7	7	7	1	7	7	7	7	0	7	7	4	0	
Abnormal gait	7	7	7	7	0	7	7	7	7	0	7	7	7	7	0	7	7	6	0	
Respiration - Fast	0	0	0	0	0	7	7	7	4	0	7	7	0	0	3	7	7	5	0	
Respiration - Irregular	7	7	7	7	0	7	7	7	7	2	0	0	7	7	4	7	7	6	7	
Eyes - Prominent	0	0	0	0	0	4	4	4	7	0	7	7	0	0	0	7	0	4	0	
Eyelids - Partially closed	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
Mortalities	0/7										0/7									

Clinical signs of fast respiration were noted in all animals for the vehicle control group approximately 24 hours and 32 minutes after first the dose. Further clinical signs of fast respiration were also noted in three animals 25 hours and 23 minutes after the first dose.

NB No adverse clinical signs were noted for the positive control group throughout the experiment. The number of animals displaying the clinical sign is indicated within each box.

Study outcome:

Based on the effect on erythroblast proliferation, there was no bone marrow toxicity observed at any dose tested.

No statistically significant increases in MPCE were observed at any of the doses tested. The results of positive and negative control groups are expected.

Analysis of plasma from satellite animals, obtained 15 minutes after i.v. dosing at 12.5 mg/kg showed the mean concentration of GW642444 of 967.5 ng/mL.

**Study title:** GW642444 H: In vitro mutation assay using L5178Y mouse lymphoma cells

**Key findings:** GW642444H demonstrated weak positive/equivocal response in the presence of S9 mix, in this mammalian cell mutation assay, under the experimental conditions.

**Study no.:** Protocol No. BVR343/032869; GSK reference No. V24328; GSK report No. WD2003/01463/00

**Volume #, and page #:** Vol. 3 of 3, page 57

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 4/7/2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW642444, Lot SS101948-196A1, purity 66.3% of free base (1.508 g of GW642444H=1.00 g of GW642444X)

## Methods

Strains/species/cell line: Mouse lymphoma L5178Y (TK<sup>+/-</sup>) 3.7.2c cells

Doses used in definitive study: Nine serial dilutions ranged 0.5-3 mcg/mL were used in the first main study (without S9, incubation for 3 hours and 24 hours) and 9 serial dilutions ranged 1.25-35 mcg/mL were used in two tests with a 3-hour incubation in the presence of S9. All doses are expressed in terms of the free base, GW642444X.

Basis of dose selection: Cytotoxicity was observed in the preliminary study. The concentrations reached 1-2% relative suspension growth (as compared to 100% in the control) were 32.3 mcg/mL for the 3- hour incubation with and without S9 mix, and was 8.1 mcg/mL for 24-hour incubation, without S9 mix.

Negative controls: DMSO

Positive controls: Methyl methanesulphonate (MMS) and 3-methylcholanthrene (3MC) were used as positive control in the absence and presence of S9 mix, respectively.

Incubation and sampling times:

In the absence of S9 mix, cultures were treated for 3 hours and 24 hours. In the presence of S9 mix, cultures were treated for 3 hours only.

## Results

Study validity:

Method:

Microwell method was used for this study. After exposure, the cells were washed once, adjusted density (to  $2 \times 10^5$  cells/mL) and planted for 48 hour culture of phenotype expression which include a cell density readjustment (to  $2 \times 10^5$  cells/mL) at 24 hour. Suspension growth was assessed with the 24 and 48 hour cell counts. After the 48-hour expression phase, cells were assessed for cloning efficiency ( $\geq 7$  days' culture) and mutant frequency (10-14 days' culture) by plating in 96 well plates.

Preliminary cytotoxicity assay:

Precipitation was observed at concentrations of 1035-4140 mcg/mL. There was a decrease in osmolality associated with exposure to precipitating concentrations. The maximum change of osmolality was 353 mOsm/kg observed at exposure to 4140 mcg/mL, compared to 441 mOsm/kg in the vehicle.

Toxicity was observed after exposure to GW642444H at concentrations of 2 mcg/mL and greater in the absence of S9 mix, with a 3-hour and a 24-hour exposure; and 16.2 mcg/mL in the presence of S9 mix, with a 3-hour exposure.

Study validity:

This study meets the acceptance criteria except for mutant frequency of vehicle controls that exceed the acceptable range based on CFSAN red-book ( $208 \times 10^{-6}$  vs  $175 \times 10^{-6}$ ). Considering the totality of the study quality, this reviewer concluded this study acceptable. The detailed evaluation of the study validity is presented below.

- 1). Three main tests comprising four independent mutation assays were conducted (-S9, 3- and 24-hour exposures; +S9, 3-hour exposure). Duplicate cultures were employed for each concentration of the test substance in all these tests. Vehicle controls were tested in quadruplicate.
- 2). Dose selection was appropriate: the high dose in each test reached RTG values between 10-20% and at least four analyzable doses were used for each test.
- 3). Results of vehicle controls meet acceptance criteria of CE of (65-120%), and SG of 8-32. MF, however, exceeds the acceptable range of  $(50-170) \times 10^{-6}$  in two tests where MF were  $208 \times 10^{-6}$  (-S9, 24 hour culture) and  $204 \times 10^{-6}$  (+S9, 3 hour culture). The sponsor considered these data acceptable based on the historical control value of  $(188 \pm 72) \times 10^{-6}$  in the conducting laboratory (historical control data are attached at the end of this study review).

Vehicle control	-S9		+S9	
Treatment time	3 hrs	24 hrs	3 hrs, test 1	3 hrs, test 2
CE	87%	100%	114%	75%
MF, $\times 10^{-6}$	179	208	110	204
SG	15.0	41.4	15.04	17.7

The above data are means of quadruplicate of vehicle control.

4). The results of positive controls are acceptable based on the RTG higher than 10%, IMF higher than  $300 \times 10^{-6}$  and small colonies comprised more than 40% of mutant colonies. The results of positive controls demonstrate the ability of the system to detect indirect-acting genotoxic compounds.

Positive control	MMS, 10 mcg/mL, -S9		3-MC, 2.5 mcg/mL, +S9	
Treatment time	3 hrs	24 hrs	3 hrs, test 1	3 hrs, test 2
RTG	73%	38%	39%	53%
IMF, $\times 10^{-6}$	840	2172	921	1877
Small colony%	74%	75%	61%	73%

Study outcome:

Note: All mutant frequency data were based on the counts containing adequate numbers of small colonies (3 h, -S9, 57-69%; 24 h, -S9, 68-88%; 1<sup>st</sup> test of 3 h, +S9, 43-56%; 2<sup>nd</sup> test, 3 h, +S9, 68-75%).

1. GW642444H produced no mutation effects in the absence of S9-mix.

Mutant frequency in the absence of S9.

Test Article <sup>a</sup>	Dose Level µg (active moiety)/mL	3 hr Treatment -S9		24 hr Treatment -S9	
		Mean RTG (%)	Pooled Mutant Freq. ( $\times 10^{-6}$ )	Mean RTG (%)	Pooled Mutant Freq. ( $\times 10^{-6}$ )
DMSO	0	100	179	100	208
GW642444X	0.5			97	202
GW642444X	1			115	160
GW642444X	1.25	103	221		
GW642444X	2			105	171
GW642444X	2.5	104	179	92	189
GW642444X	3			82	175
GW642444X	3.5			77	191
GW642444X	4			75	188
GW642444X	5	99	213		
GW642444X	6			31	267
GW642444X	8			16	262
GW642444X	10	70	146		
GW642444X	15	55	188		
GW642444X	20	40	245		
GW642444X	25	28	161		
GW642444X	30	15	213		
MMS	10 <sup>1</sup>	73	1019 <sup>1</sup>		
MMS	5 <sup>1</sup>			38	2380 <sup>1</sup>

1. Statistical Analysis: p<0.01

<sup>a</sup> Test article as active moiety, and positive control in µg/mL

2. GW642444H produced mutation effects in the presence of S9-mix.

Based on FDA guideline in Redbook (version 10-04-05, IV.C.1.c. Mouse lymphoma Thymidine Kinase Gene Mutation Assay), applying the global evaluation factor of  $126 \times 10^{-6}$  to the MF of vehicle controls, positive results are considered for those cultures that showed MF higher than  $236 \times 10^{-6}$  ( $110 \times 10^{-6} + 126 \times 10^{-6}$ ) in the Test 1 and  $330 \times 10^{-6}$  ( $204 \times 10^{-6} + 126 \times 10^{-6}$ ) in the Test 2. Therefore, GW642444H was found negative at all concentrations in the Test 1 but positive in the Test 2 at concentrations ranging from 22.5-35 mcg/mL, though the responses are not necessarily dose-dependent. Increases in

mutation frequency were 22% or less. In light of the negative findings in Test 1 and the weakly positive findings at the high concentrations of GW642444H, this drug is considered weak positive/equivocal in the mouse lymphoma assay. The sponsor concluded in the study report that GW642444H was positive for genotoxic potential in the presence of S9-mix. In the Overview of Dose Rational for carcinogenicity study designs, the sponsor claimed equivocal results of this mouse lymphoma assay.

Additionally, as per discussion with Dr. Martha Moore, the historical control presented by the conducting laboratory is higher than most other laboratories.

Mutant frequency in the presence of S9.

Test Article	Dose Level µg (active moiety)/mL	Test 1		Test 2	
		Mean RTG (%)	Pooled Mutant Freq. (x10 <sup>4</sup> )	Mean RTG (%)	Pooled Mutant Freq. (x10 <sup>4</sup> )
DMSO	0	100	110	100	204
GW642444X	1.25	93	122		
GW642444X	2.5	119	90		
GW642444X	5	101	105		
GW642444X	10	92	90	86	183
GW642444X	15	74	120	91	195
GW642444X	20	58	144	43	324 <sup>1</sup>
GW642444X	22.5			22	358 <sup>1</sup>
GW642444X	25	18	230	17	449 <sup>1</sup>
GW642444X	27.5			15	343 <sup>1</sup>
GW642444X	30	13	212	15	348 <sup>1</sup>
GW642444X	32.5			12	420 <sup>1</sup>
GW642444X	35			10	412 <sup>1</sup>
3MC	2.5	39	1031 <sup>1</sup>	53	2081 <sup>1</sup>

<sup>1</sup> Statistical Analysis: p<0.01

\* Test article as active moiety, and positive control in µg/mL.

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**Table 19: Historical Control Data**

i) in the absence of S9 mix - 3 hours exposure

		Mean Day <sub>0</sub> RS	Mean suspension growth	Mean Day <sub>2</sub> CE	Mean mutant frequency
solvent controls	mean	88.96	7.79	108.63	0.000188
	maximum	122.60	12.42	169.80	0.000302
	sd	16.81	2.42	29.53	0.000072
positive controls	mean	92.78	7.21	77.48	0.000950
MMS 10 µg/ml	minimum	41.36	3.79	42.15	0.000373
	sd	18.39	1.82	33.89	0.000460

95% confidence limit for solvent controls 0.000290

sd = standard deviation

Number of tests 38

Data collection period 08-May-01 14-Apr-03

ii) in the presence of S9 mix - 3 hour exposure

		Mean Day <sub>0</sub> RS	Mean suspension growth	Mean Day <sub>2</sub> CE	Mean mutant frequency
solvent controls	mean	84.24	8.41	103.16	0.000179
	maximum	129.97	14.72	188.17	0.000337
	sd	16.90	2.64	23.77	0.000065
positive controls	mean	63.39	6.95	89.02	0.000791
3MC 2.5 µg/ml	minimum	26.31	3.02	49.35	0.000346
	sd	19.86	4.02	25.44	0.000290

95% confidence limit for solvent controls 0.000312

sd = standard deviation

Number of tests 61

Data collection period 08-May-01 23-Jun-03

iii) in the absence of S9 mix - 24 hour exposure

		Mean Day <sub>0</sub> RS	Mean suspension growth	Mean Day <sub>2</sub> CE	Mean mutant frequency
solvent controls	mean	90.36	7.91	104.80	0.000178
	maximum	109.12	13.72	160.16	0.000322
	sd	18.17	2.52	26.93	0.000079
positive controls	mean	59.20	7.11	59.23	0.001771
MMS 5 µg/ml	minimum	34.78	3.93	29.90	0.000610
	sd	21.03	1.92	21.23	0.000711

95% confidence limit for solvent controls 0.000291

sd = standard deviation

Number of tests 25

Data collection period 08-May-01 28-Apr-03

**Study title:** In vitro Transformation of Syrian Hamster Embryo (SHE) Cells by 7-Day Exposure

**Key findings:** GW642444H was negative in the SHE cell transformation assay using the standard 7-day continuous exposure protocol.

**Study no.:** GSK reference No. V24843; GSK report No. WD2004/00169/00

**Volume #, and page #:** Vol. 3 of 3, page 104

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 9/11/2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW642444H, Batch No. SS101948-196A1, Lot No. E03L116; purity 66.3% of free base (1.508 g of GW642444H=1.00 g of GW642444X)

## Methods

Strains/species/cell line: Embryo cells derived from timed pregnant Syrian Golden Hamster at 13-13.5 days of gestation

Doses used in definitive study: 0 (DMSO), 22.5, 25.0, 27.5, 30.0, 32.5 mcg/mL

Basis of dose selection: Decrease in plating efficiency at least 50%. GW642444 was cytotoxic as evidenced by the dose-related reduction of relative plating efficiency ranged 101-35% for concentrations of 22.5-32.5 mcg/mL (see the study outcome table )

Negative controls: DMSO

Positive controls: Benzo[a]pyrene, 5.00 mcg/mL

Incubation and sampling times: 7 days of exposure was used.

## Results

Study validity:

This study is considered valid based on the following: 1). 45 replicate cultures for each treatment, vehicle and positive control were employed. 2). Five analyzable concentrations of treatment were tested. 3). Concentrations of test article were appropriate; 4). Morphological transformation of the cells was analyzed blindly and one sided Fisher's exact test was used for the result analysis; 5). Positive criteria were a statistically significant increase in morphological transformation frequency at two or more concentrations compared to concurrent vehicle control, or a statistically significant increased transformation frequency at one concentration plus a statistically significant

positive dose-relationship; 6). Results of both vehicle control and positive control were in expected range.

Study outcome:

None of the five concentrations (22.5, 25.0, 27.5, 30.0, 32.5 mcg/mL) of GW642444H induced a statistically significant increase in the frequency of morphological transformed SHE cell colonies compared to the concurrent vehicle control, according to the Fisher's exact test (see the table below).

**TABLE 4 : SUMMARY OF THE TRANSFORMATION ASSAY (TRIAL B2)**

Test Article: GW642444H  
 Genetic Toxicology Assay No.: 25523-0-485R  
 Treatment Date: 07 January 2004

Treatment Group	MT		Total		Average		No.of Target	
	Frequency <sup>2</sup> (%)	Total MT Colonies <sup>3</sup>	Colonies <sup>3</sup> Scored <sup>4</sup>	Colonies per Dish <sup>5</sup>	Colonies per Dish	Cells seeded per Dish	Average PE <sup>6</sup> ± SD <sup>7</sup>	RPE <sup>8</sup> (%)
Vehicle Control <sup>1</sup>	0.491	6	1222	27.2	130	20.9 ± 4.4	100	
B[a]P 5.00 µg/mL	2.581*	28	1085	24.1	130	18.5 ± 3.1	89	
Test Article:								
22.5 µg/mL	0.568	7	1233	27.4	130	21.1 ± 4.2	101	
25.0 µg/mL	1.178	13	1104	24.5	130	18.9 ± 3.5	90	
27.5 µg/mL <sup>9</sup>	0.587	9	1533	34.1	186	18.3 ± 2.9	88	
30.0 µg/mL <sup>9</sup>	0.499	7	1404	31.2	217	14.4 ± 3.0	69	
32.5 µg/mL <sup>9</sup>	0.247	3	1215	27.0	371	7.3 ± 2.0	35	

<sup>1</sup> Vehicle Control = 0.2% DMSO  
<sup>2</sup> MT Frequency = (Total MT colonies/Total colonies scored) x 100%  
<sup>3</sup> Total MT Colonies = Total number of morphologically transformed colonies  
<sup>4</sup> Total number of colonies from all dishes  
<sup>5</sup> Total colonies scored / total number of dishes  
<sup>6</sup> PE = Plating efficiency = (Number of colonies per dish/number of target cells seeded per dish) x 100%  
<sup>7</sup> SD = Standard deviation  
<sup>8</sup> RPE = Relative plating efficiency = (Average PE of treatment group / vehicle control average PE) x 100%  
<sup>9</sup> The number of cells seeded per dish was increased to adjust for expected toxicity  
 \* p ≤ 0.05 (Fisher's Exact Test)

**Study title:** In vivo DNA repair (UDS) test using rat hepatocytes

**Key findings:** Intravenous administration of GW642444H at doses up to MTD (25 mg/kg) did not induce unscheduled DNA synthesis in the hepatocytes of male SD rats.

**Study no.:** GSK reference No. R24908; GSK report No. WD2003/01713/00

**Volume #, and page #:** Vol. 3 of 3, page 147

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** 8/6/2003

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** GW642444H, Batch No. SS101948-196A1, Lot No. E03L116; purity 66.3% of free base (1.508 g of GW642444H=1.00 g of GW642444X)

### **Methods**

Note: there is a discrepancy on the total doses given to treatment groups in the study report (Section 3.1. page 10 states that the frequency of the formulation is once daily at doses of 3.75 and 12.5 mg/kg/day. However, section 3.1, page 11, stated that the animals in the vehicle control and the test substance groups were dosed intravenously at 16 hours and 2 hours prior to harvesting hepatocytes. Section 3.3, page 12, below the study design table, noted "positive control dosed once daily only 16 hours prior to hepatocyte harvest" which implies that the vehicle and treatment groups were dosed differently). Upon a request, the sponsor provided a clarification: rats were given 2 consecutive intravenous doses of either vehicle control or test article (3.75 or 12.5 mg/kg/day), 14 hours apart, over a two-day period. The animals were killed 2 hours after the second dose, so UDS could be measured following 16 hours and 2 hours exposure to GW642444H. Therefore, rats received total doses of 7.5 or 25 mg/kg, respectively (8/21/07 fax from GSK).

**Strains/species/cell line:** Male rat, Sprague-Dawley (CRI:CD@BR)

**Doses used in definitive study:** Two i.v. injections separated by 14 hour, each injection was at 3.75 or 12.5 mg/kg (total dose was 7.5 mg/kg for the LD and 25 mg/kg for the HD)

**Basis of dose selection:** MTD based on clinical signs (irregular/labored breathing, flattened posture, abnormal gait, etc) observed in a micronucleus test in Sprague Dawley rats ([REDACTED] (b) (4) Study No. BVR/342) at dose of 12.5 mg/kg.

**Negative controls:** 20/80 PEG 400/8% 2-hydroxypropyl beta cyclodextrin

**Positive controls:** 2-acetylaminofluorene (2-AAF), 50 mcg/kg administered orally, once at 16 hours prior to hepatocyte harvest.

Incubation and sampling times: 2 hours after the final dose.

### Results

Study validity: The study is considered acceptable based on the following: 1) adequate doses were used in the study based on clinical signs including underactivity, flattened posture, abnormal, fast respiration, irregular respiration, prominent eyes and partially closed eyelids. 2). A total of 150 hepatocytes were scored for all animals with either the vehicle or the test substance, except for one HD animal where only 106 scoreable cells were found from three slides. Only 75 hepatocytes per animal were scored from slides for animals treated with 2-AAF, as a result of the strong response. 3). the data for the vehicle and positive control were within the historical range of the laboratory. 4).Positive criteria: dose-related statistically significant increase in the NNG count which is accompanied by a substantial increase in the gross nuclear grain count over concurrent vehicle control values.

### Study outcome:

The vehicle and positive control data for this study were within the ranges of historical data from this laboratory. The positive control showed a significant increase in the net nuclear grain (NNG) count which was accompanied by a substantial increase in the gross nuclear grain count.

Animals treated with GW642444H did not show any significant increase in the gross or net nuclear grain count at either dose level (see the table below).

Treatment	Dose, mg/kg	Animal n	Gross NG count	Cytoplasmic grain count	NNG count*
Vehicle	0	4	8.4	14.8	-6.4
GW642444	7.5	4	9.8	17.2	-7.4
GW642444	25	4	9.2	15.5	-6.3
2-AAF	50	2	26.2	14.2	12.0

\*Net grain count=Gross minus cytoplasmic count

**Non-GLP Reports: early development screen for genotoxicity of GI179710X**

## 1. GI179710X: High Throughput Fluctuation Test (GSK Study No. HTFT-295)

The test compound, GI179710X is (b) (4)

This is a high throughput Ames test with Salmonella typhimurium TA98 and TA100. With this method, positive wells, in which reversion mutation has occurred, appear yellow due to acid fermentation and negative wells remain purple. GI179710X was not mutagenic in the absence and presence of S9-mix in this test at concentrations up to 1000 mcg/mL. The table below presents the study results.

**Tests in the Absence and Presence of S9-mix**

Concentration of $\mu\text{g}(\text{base})$ per mL	Number of positive wells			
	TA98		TA100	
	-S9	+S9	-S9	+S9
0 <sup>a</sup>	1	1	12.9	9.7
7.81	1	1	17	15
15.63	1	2	16	11
31.25	0	1	18	14
62.5	1	0	14	13
125	0	0	13	11
250	0	1	11	16
500	0	2	12	17
1000	1	1	15	11
Positive Control	44	25	40	41

Positive control, in the absence of S9-mix : TA100 Sodium Azide (0.5 $\mu\text{g}/\text{mL}$ ), TA98 2-Nitrofluorene (2 $\mu\text{g}/\text{mL}$ ), and in the presence of S9-mix : TA100 and TA 98 2-Aminoanthracene (0.2 $\mu\text{g}/\text{mL}$ ).

<sup>a</sup> Solvent control = DMSO

At least a three fold increase in positive wells for TA98 and at least a two fold increase for TA100 are indicated by a **bold box**

## 2. GI179710X: L5178Y Mammalian Cell Mutation Screen (GSK Study No. MLA-369)

The test compound, GI179710X is (b) (4)

Mouse lymphoma cells, L5178Y were used to detect forward mutation the tk locus. Incubation time was 3 hours in the presence and absence of S9-mix. The maximum test concentration examined was 120.0 and 156.25 mcg (base)/mL in the absence and presence of S9-mix respectively. The sponsor stated this dose selection was due to the cytotoxic effects of the compound. However, the relative total growth (RTG) reached only 19% at the highest concentration in both absence and presence of S9-mix (standard practice requires drug concentrations reaching RTG of 10% for cytotoxic compounds).

Three concentrations (80, 100, 120 mcg/mL) were tested in the absence of S9-mix (at least 4 analyzable concentrations should be tested, based on the standard practice); four concentrations (19.53, 39.06, 78.13 and 156.25 mcg/mL) were tested in the presence of S9-mix. Based on the inadequate concentrations and number of concentrations tested, this study is invalid to conclude the mutagenicity potential of GI179710X. The table below presents the study results.

#### Tests in the Absence and Presence of S9-mix

Concentration of GI179710X $\mu\text{g}(\text{base}/\text{Ml})$	(-)S9		(+)S9	
	RTG (%)	Mutant Freq. ( $\times 10^{-6}$ )	RTG (%)	Mutant Freq. ( $\times 10^{-6}$ )
0 <sup>1</sup>	100	63.69	100	80.01
19.53	-	-	100	97.07
39.06	-	-	107	82.36
78.13	-	-	85	131.24
80.00	64	69.30	-	-
100.00	31	92.25	-	-
120.00	19	90.84	-	-
156.25	-	-	19	61.71
DMBA <sup>2</sup>	-	-	23	1228.83

1. Dimethyl sulphoxide

2. Positive controls fulfilled required criteria.

- Denotes 'Not Tested' or 'Not Plated' for mutant frequency.

DMBA Dimethylbenzanthracene at  $1\mu\text{g}/\text{mL}$  (in the presence of S9-mix).

RTG Relative Total Growth

#### 2.6.6.9 Discussion and Conclusions

The 13-week inhalation studies with GW642444M provided toxicity profiles in mice and rats. The dose limiting toxicities were severe clinical signs and mortalities in mice and upper airway epithelial ulceration in rats. The MTD is nominal doses of 38200 mcg/kg in mice and 658 mcg/kg in rats. Histopathological findings were mostly local (mice, epithelial degeneration/regeneration, epithelial metaplasia and eosinophilic inclusions in nasal turbinates, squamous metaplasia in larynx; rats, epithelial inflammation, ulceration, degeneration/regeneration and squamous metaplasia in part or all upper airways- nasal cavities, nasopharynx, larynx, trachea, tracheal bifurcation and bronchi) and systemic toxicities were seen in mouse study only (decreased hepatocyte cytoplasm rarefaction and uterus myometrial hypertrophy). NOAEL for local toxicities was not defined in mice due to increased eosinophilic inclusions in respiratory and olfactory epithelial cells, and is 56 mcg/kg for rats due to epithelial degeneration and squamous metaplasia in the upper airway. Systemic toxicities were seen in the mouse study only as decreased hepatocyte rarefaction and myometrial hypertrophy in uterus. NOAEL for systemic toxicities is 58.6 mcg/kg for mice based on the observed uterine findings and is 38845 mcg/kg (HD) for rats.

GW642444H exhibited negative mutagenic effects in an Ames test, a micronucleus test in rats (I.V. dose for 2 days), a SHE cell transformation assay (7-day exposure protocol), and an unscheduled DNA synthesis test in rat hepatocytes. In a mouse lymphoma assay, GW642444H was negative in the absence of S9, and weakly positive/equivocal in the presence of S9 mix.

Based on the totality of the genotoxicity profile, this review concludes that this drug does not impose a genotoxic risk to humans.

Additionally, two non-GLP mutagenicity studies for [REDACTED] (b)(4) [REDACTED] (GI179710X) were reported. These studies included a high throughput Ames test and a mouse lymphoma assay. The Ames test used only two bacterial strains (Salmonella typhimurium TA98 and TA100) and the mouse lymphoma assay tested inadequate concentrations and number of concentrations. Therefore, these tests are not valid to make any conclusions.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

### Summary:

GW642444 is being developed as the long-acting beta agonist (LABA) component of a once-daily inhaled corticosteroid (ICS)/(LABA) combination product for treatment of asthma in patients [REDACTED] (b)(4). This combination product and GW642444 monotherapy are also planned for treatment of COPD. The sponsor plans to use doses up to 50 mcg/day for future clinical trials and the anticipated Maximum Recommended Human Dose (MRHS) for marketing is 6.25 mcg/day.

The sponsor's initial development of GW642444 [REDACTED] (b)(4) [REDACTED] was subsequently switched to triphenyl acetate salt (GE642444M). There is no toxicity concern with this switch as the salts are expected to be dissociated from the base in body. Non-GLP, genotoxicity screening studies (Ames test and mouse lymphoma assay) [REDACTED] (b)(4) were conducted to examine genotoxicity potential of the salt, however these studies provided limited value for toxicity evaluation.

The formulation tested in nonclinical studies contains no magnesium stearate which is planned to be included in commercial formulation. This discrepancy has been evaluated in the pre-IND phase and no bridging studies are recommended since magnesium stearate is an excipient in an approved inhalation drug product (1/31/07 meeting minutes).

Based on the cultures with cryopreserved hepatocytes, all major metabolic routes of GW642444 (O-dealkylation and oxidation) seen in the man were also seen in the rat and the mouse. Plasma protein binding was similar across species- 92, 94 and 97% in the rat, mouse and human, respectively.

GW642444H tested negatively in the Ames assay, rat bone marrow micronucleus assay, in vitro UDS assay and SHE cell assay; but produced a weak positive/equivocal response in the mouse lymphoma assay in the presence of S9-mix.

Mouse Carcinogenicity Study Protocol and Dose Selection:

The sponsor proposed a standard 2-year carcinogenicity study in CD-1 mice (60/sex/dose) at GW642444M doses (b) (4)

(b) (4) by snout-only inhalation for 104 weeks with a standard battery of observations/examinations. Histopathology evaluation will be conducted in all main study animals (tissues from animals dying or killed were collected into three phase category: 0-78 weeks, Weeks 78-91, week 91-the terminal sacrifice). 66 mice/sex/group will be included for TK assessment. When survival approaches 25 animals in any treatment group, early termination will be considered. Males and females will be considered separately. The sponsor's dose selection was based an MTD criterion. (b) (4)

In a 13-week dose ranging study, a dose of 63,600 mcg/kg resulted in irregular and/or labored breathing and deaths during the first 9 days of dosing. This dose was concluded to have exceeded the MTD. The dose was reduced to 38,200 mcg/kg and resulted in increased body weight gain and microscopic lesions in the nasal turbinates (epithelial degeneration/regeneration, epithelial metaplasia, eosinophilic inclusions), larynx (epithelial squamous metaplasia), liver (decreased hepatocyte cytoplasm rarefaction) and uterus (myometrial hypertrophy) that were not considered dose limiting.

Rat Carcinogenicity Study Protocol and Dose Selection:

The sponsor proposed a standard 2-year carcinogenicity study in SD rats (60/sex/dose) at GW642444M doses (b)(4) by nose-only inhalation with a standard battery of observations/examinations. Histopathology evaluation will be conducted in all main study animals ((tissues from animals dying or killed were collected into three phase category: 0-78 weeks, Weeks 78-91, week 91-the terminal sacrifice). 6 rats/sex/group will be included for TK assessment. When survival approaches 25 animals in any treatment group, early termination will be considered. Males and females will be considered separately. The sponsor's dose selection rational was based on an MTD criterion (b)(4)

The sponsor considers the proposed (b) (4)

In a 13-week dose ranging study, the MTD was considered to be 658 mcg/kg. Doses of 10,392 mcg/kg and greater resulted in significant respiratory tract lesions including moderate to marked ulceration of the olfactory and/or respiratory epithelium of the nasal

cavity, slight to marked epithelial degeneration/regeneration, olfactory nerve atrophy, and moderate to marked squamous metaplasia. At the lower-mid dose of 658 mcg/kg, lesions were limited to minimal epithelial degeneration/regeneration and minimal to slight laryngeal metaplasia and hyperplasia.

#### Executive CAC Recommendations and Conclusions:

##### Mouse:

- The Committee did not concur with the doses proposed by the sponsor [REDACTED] (b) (4).
- The Committee recommended doses of 0, 3000, 10,000, and 30,000 mcg/kg/day by snout-only inhalation, based on an MTD criterion (mortality and clinical signs at 63,600 mcg/kg). The recommended high dose is approximately one-half of the identified lethal dose. The sponsor may add additional lower doses at their discretion.
- The sponsor should contact the agency prior to terminating any groups or changing any doses.

##### Rat:

- The Committee did not concur with the doses proposed by the sponsor [REDACTED] (b) (4).
- The Committee recommended doses of 0, 80, 220, and 650 mcg/kg/day by nose-only inhalation, based on an MTD criterion (severe respiratory tract irritancy and ulceration of the upper airways at doses of 10,392 mcg/kg and higher). The sponsor may add additional lower doses at their discretion.
- The sponsor should contact the agency prior to terminating any groups or changing any doses.

The above recommendations from CAC have been conveyed to the sponsor on 8/22/2007. Of note, the sponsor followed up with a counter-proposal to the Executive CAC recommendations (submission 012, dated September 7, 2007). The review of the counter-proposal (review #2) was completed on September 17, 2007 and comments were faxed to the sponsor on September 20, 2007. The agreed doses are 0, 10, 80, 220 and 650 mcg/kg for the rat study and 0, 6, 60, 600, 6,000 and 30,000 mcg/kg for the mouse study.

##### Internal comments:

None at this time.

External comments (to sponsor): None at this time.

##### Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

Linked Applications

Sponsor Name

Drug Name

-----  
IND 74696

-----  
GLAXOSMITHKLINE

-----  
GW642444 INHALATION POWDER

-----  
**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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HUIQING HAO

06/03/2008

TIMOTHY J MCGOVERN

06/03/2008

**REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA**  
**Division Of Pulmonary and Allergy Drug Products (HFD-570)**

**REVIEW INFORMATION:**

**Review No.:** 1  
**Date of Completion:** June 7, 2000  
**Reviewer Name:** Luqi Pei, Ph.D.  
**Key Words:** (b) (4)  
**Information to be Conveyed to Sponsor:** Yes  No

**APPLICATION INFORMATION:**

**IND Application No:** IND (b) (4)  
**Serial No., Content and Date of Submission:** 000, Original submission, April 27, 2000 (b) (4)  
**Sponsor:** (b) (4)  
**Drug Name:** *Generic Name:* (b) (4)  
*Code Name:* (b) (4)  
*Brand Name:* (b) (4)  
*Chemical Name:* (b) (4)  
  
*Molecular Weight and Formula:* (b) (4)  
*CAS No.:* (b) (4)  
**Class:** (b) (4)

**Review Summary:**

(b) (4)

**ADDITIONAL INFORMATION:**

3 Page(s) has been Withheld in Full as B4 (CCI/TS) immediately following this page

1. **Six-month Inhalation Toxicity Study with Magnesium Stearate in Rats.** (b)(4) **Projects No, 724353. Vol. 4, page 8-260.**

*Testing Laboratory:* (b)(4)

*Study Number:* (b)(4) Project No. 724353

*GLP Statement* Yes

*Study Date:* 4/21/99-11/3/99; Final Report date: 3/10/2000

*Lot Number:* 96114/2 and S9D050 (expiration date: 3/31/2000)

*End Point:* Toxicity of inhaled Mg stearate on respiratory system

## Method

Wistar rats (10 rats/sex/dose, 7 – 11 weeks of age) were exposed by nose-only inhalation to magnesium stearate or magnesium stearate in lactose (1%, w/w) for 26 weeks. Magnesium stearate dose levels were 0, 0.9, 20, 60 and 180 µg/kg/day (pulmonary deposition). The 0.9 µg/kg/day group was also exposed to 90 µg/kg/day of lactose monohydrate. The control group of rats received room air only. The duration of exposure was 1 hour/day for all groups. Table 1 presents the estimates of magnesium stearate dose levels in the study.

Table 1. Estimates Of Magnesium Stearate Dose Levels in Rats.

Group	Aerosol Particles		Mg stearate (µg/L air)	Estimated exposure (µg/kg/day)	
	MMAD	GSD		Total body	Pulmonary
1	-	-	0	0	0
2	1.52	2.49	0.293	9.3	0.9
3	0.92	2.50	6.31	200	20
4	0.89	2.73	18.94	600	60
5	0.86	2.87	56.81	1800	180

The report gave the following as the rationale for the dose selection. The low dose (9.3 µg/kg/day, total inhaled dose) was a human equivalent dose<sup>2</sup> and the high dose gave a dose ratio of 180 between rats and humans. According to the current division practice that only pulmonary dose should be used as the actual exposure in rats, the low dose is only one-tenth of the expected clinical dose. Such a low dose is meaningless for the purpose of safety evaluation. Nonetheless, the selection of the high dose is reasonable.

2. The sponsor calculated their human dose as the following: (b)(4)

(b)(4)

(b)(4)

(b)(4)

The following parameters were monitored during the study:

<i>Clinical Signs:</i>	Daily
<i>Clinical exams:</i>	Weekly
<i>Body Weight:</i>	Weekly
<i>Food Consumption:</i>	Weekly
<i>Ophthalmology:</i>	Pre-exposure and Week 26
<i>Clinical Pathology:</i>	Hematology, blood chemistry, and urinalysis examinations in weeks 6 and 26 of the exposure
<i>Pathology:</i>	Time of sacrifice
<i>Organ Weights:</i>	Adrenals, brain, heart, kidneys, liver, lungs, spleen, testes/ovaries, thymus, thyroids
<i>Necropsy:</i>	All animals
<i>Histopathology:</i>	Respiratory tract of animals at all doses: nasal cavity, nasopharynx, larynx, lungs, lymph-nodes, trachea and bronchi. All organs/tissues from animals that died spontaneously. All organs/tissues with gross lesions.

## Results

*Clinical signs:* No treatment-related clinical signs were observed. One mid-dose male (rat No. 34) died of purulent prostatitis on day 179. This death is not considered treatment-related.

*Body Weights:* No treatment-related effects were observed.

*Food Consumption:* No treatment-related effects were observed.

*Ophthalmology:* No treatment-related effects were observed.

*Hematology:* No treatment-related effects were observed.

*Clinical Chemistry:* No treatment-related effects were observed.

*Organ Weight:* No treatment-related effects were observed.

*Histopathology:* No treatment-related effects were observed.

*Key Study Observations:* Administration of magnesium stearate to Wistar rats at inhalation doses of up to 180  $\mu\text{g}/\text{kg}/\text{day}$  did not result in any treatment related findings. The no-observed-adverse-effect-level (NOAEL) for magnesium stearate can be identified as 180  $\mu\text{g}/\text{kg}/\text{day}$  in rats.

## Summary of Toxicity Studies

The effect of magnesium stearate on the respiratory system was evaluated in two inhalation studies in rats. Their durations of exposure were (b)(4) 6-month, respectively. The doses (pulmonary deposition) were (b)(4) 1, 20, 60 and 180 for the 6-month study. Neither study revealed any treatment-related abnormalities in the respiratory system. The 6-month NOAEL value for magnesium stearate in rats was 180  $\mu\text{g}/\text{kg}/\text{day}$  with the inhalation route of exposure.

### 3. Use of Magnesium Stearate Excipient in Lactose Formulations, (b)(4) project No. 708401, page 8-15.

This is a literature review conducted by (b)(4) in 1998. (b)(4) is the contract laboratory that performed the above two inhalation-toxicity studies of magnesium stearate in rats. The (b)(4) review contains no significant information for the safety evaluation of inhalation magnesium stearate, but the following is note-worthy:

The American Conference of Governmental Industrial Hygienists (ACGIH) has a TLV-TWA of 10 mg/m<sup>3</sup> for magnesium stearate. The effect of magnesium stearate on the respiratory system after long-term inhalation exposure is unknown. The laboratory has limited experience with the mixture of magnesium stearate and lactose in repeat-dose inhalation toxicity studies, but it cannot provide this formation to the sponsor due to confidentiality. Overall, the review states “Short-term bridging inhalation toxicity studies in rodents, generally required by regulatory authorities, are warranted....”

#### OVERALL SUMMARY AND EVALUATION

(b)(4)

## Lactose

Lactose is an excipient used both in the new and old formulations. The Division has determined that the safety of lactose as an excipient in inhalation drug products is established by available clinical and preclinical data.

## Magnesium Stearate

Magnesium stearate is (b)(4) added in the formulation. Magnesium stearate is a well-known excipient for oral drug products. The CDER Inactive Ingredient Guide (1996) indicates that over 2,000 drug products contain magnesium stearate. However, magnesium stearate has not been used as an excipient for inhalation drug products. Thus, inhalation use of magnesium stearate becomes a safety concern in the safety evaluation of the DPI. This concern was conveyed to the sponsor in a pre-IND meeting held on June 30, 1999. (b)(4) was asked to conduct inhalation toxicity studies with treatment duration of up to 6 months to evaluate the effect of magnesium stearate on the respiratory tract in animals.

(b)(4) has conducted and submitted two inhalation toxicity studies with exposure durations of (b)(4) six-months in rats to evaluate the toxic effect of magnesium stearate on the respiratory system. These studies showed that magnesium stearate treatment by inhalation for 6 months at doses of up to 180 µg/kg/day, the highest dose tested, did not cause any treatment-related abnormalities in the respiratory system in rats. This dose, considered a 6-month NOAEL value in rats, is approximately 16-fold greater than the expected exposure in humans (b)(4). It is concluded that there is sufficient margin of safety to support the safety of magnesium stearate in the proposed protocol.

## Recommendation

From the preclinical viewpoint, this protocol is safe to proceed.

## Comment to Sponsor:

Justify your selection of the rat as the most appropriate species for the 6-month inhalation toxicity study. Generally, species selection is based on short-term studies conducted in rodent and non-rodent species.

---

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

---

Robin Huff, Ph.D.  
Pharm/Tox Team Leader

Ori: IND (b)(4)/HFD-570/Division File  
HFD-570/Drs. Pei /Huff /Anthracite /Bertha/ Jani

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LUQI PEI  
03/12/2013

MARCIE L WOOD  
03/12/2013

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

**PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION**

Application number: NDA 204,275  
Supporting document/s: Sequences 0000  
Applicant's letter date: July 12, 2012  
CDER stamp date: July 12, 2012  
Product: Breo Ellipta (Fluticasone furoate /Vilanterol  
trifenatate) Dry Powder Inhaler  
Indication: COPD  
Applicant: GSK  
Review Division: Pulmonary, Allergy and Rheumatology  
Reviewer: Luqi Pei, Ph.D.  
Team Leader: Marcie Wood, Ph.D.  
Division Director: Badrul Chowdhury, M.D., Ph.D.,  
Project Manager: Angela Ramsey

*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

This review evaluates the carcinogenicity potential of vilanterol, a long acting beta<sub>2</sub> agonist (LABA) being developed for a chronic obstructive pulmonary disease (COPD) indication.

The carcinogenicity potential of vilanterol was studied in two traditional 2-year inhalation bioassays in rats and mice (one each). The Executive Carcinogenicity Assessment Committee (ECAC) concurred with the dose selection and some major protocol deviations in both studies. The deviations included dose-adjustments and early terminations in the rat study and animal relocations in the mouse study. The Committee gave its concurrence on major protocol deviations in the rat study. The Committee did not give concurrence to the animal relocation (i.e., from scheduled TK section to the main study due to excessive mortalities in the main study), but this deviation did not adversely affect the outcome of the study results. Detailed discussions about these deviations can be found in the regulatory history and study evaluation sections.

The ECAC evaluated the final reports of the vilanterol carcinogenicity studies on November 27, 2012. The Committee concluded that vilanterol tested positive for carcinogenicity in mice and rats as described in Section 1.2 Summary of Nonclinical Findings.

## 1.2 Summary of Nonclinical Findings

Vilanterol caused dose-dependent increases in tumor incidences in the female reproductive organs in rats and mice and pituitary tumors in both sexes in rats. The tumors in the female reproductive organs included the tubulostromal adenomas in the ovaries in mice ( $p < 0.05$ ) and leiomyomas in mesovarian ligaments in rats ( $P < 0.01$ ). Decreases in the latency period of pituitary adenomas were observed in rats. Evidence of tumorigenicity was observed at  $\geq 84/28.8$  and  $\geq 62 \mu\text{g}/\text{kg}/\text{day}$  in rats and mice, respectively. These doses were approximately 25 and 10 times the maximum recommended human daily inhalation dose (MRHDID) on a  $\text{mg}/\text{m}^2$  basis. No increases in tumor incidences were observed at  $10.5/3.5$  and  $6 \mu\text{g}/\text{kg}/\text{day}$  in rats and mice, respectively. These doses were approximately 3 times and equal to the MRHDID on a  $\text{mg}/\text{m}^2$  basis, respectively.

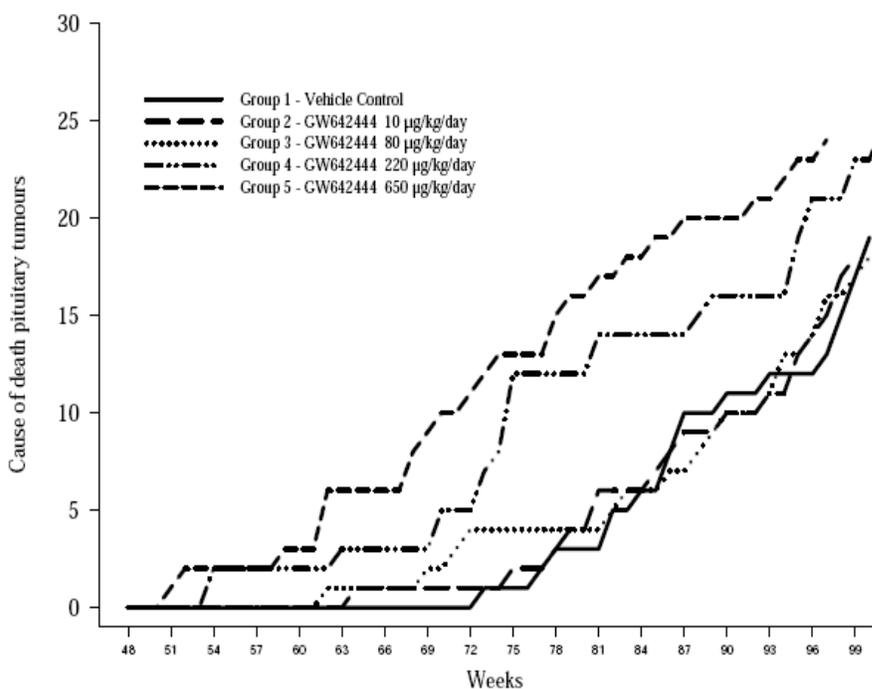
Sprague-Dawley rats (60/sex/dose) were exposed by nose-only inhalation to vehicle (C), which was lactose powder, low-dose (LD), mid dose (MD), mid-high dose (HD-1), and high dose (HD-2) of vilanterol for up to 104 weeks. Specifically, males were treated with 0, 10.5, 84.4, 223, 657- $\mu\text{g}/\text{kg}/\text{day}$  vilanterol (achieved doses) for 101 weeks. Females were exposed to the same doses for 85 weeks and dose adjustments were made subsequently due to excessive mortalities in the vilanterol-treated groups. The dose adjustments consisted of the following: dosing was discontinued in the HD-1 and HD-2 groups and vilanterol doses in the LD and MD groups were reduced to 3.5 and 28.2  $\mu\text{g}/\text{kg}/\text{day}$ , respectively. The three top-dose

groups were terminated during weeks 95 – 96 when the number of survivors reached 15/group.

**Table 1: Mesovarian Leiomyoma Incidences in Rats**

Sex	Incidence (p-value)				
	0	10.5/3.5	84.4/28.2	223	657
F	0/60	0/60	5/60 (0.007)	4/60 (0.020)	4/60 (0.020)

Both male and female rats had dose-related mortality ( $P < 0.01$ ) and shortened latency to pituitary neoplasms, which were considered to be the cause of death, although the increases in overall tumor incidence did not reach the statistically significant level of 0.01 for the common tumor. Control incidences were 70% for males and 90% for females. The three highest dose groups of females also had increased incidences of mesovarian leiomyomas. Table 1 (above) presents the leiomyoma incidences in the mesovarian ligament in rats. Figure 1 presents the time-course of pituitary adenoma-related deaths in males as an example.



**Figure 1: Pituitary tumor-related deaths in male rats.**

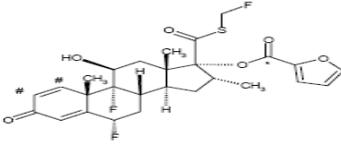
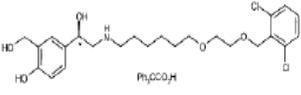
Mice (84/sex/dose, CD-1) were treated by nose-only inhalation with 0 (C), 6 (LD-1), 62 (LD-2), 615 (MD), 6,150 (HD-1), or 29,500 (HD-2)-µg/kg/day vilanterol (achieved doses) for 101 – 104 weeks. The HD-2 female group showed a statistically significant increase in ovarian tubulostromal adenoma (Table 2).

**Table 2: Incidences of Tubulostromal Adenomas in Ovaries in Female Mice**

	Vilanterol (µg/kg/day)					
	0	6	62	615	6150	29,500
Incidence (overall)	0/84	0/83	1/84	0/84	2/84	6/83
P-value (vs. vehicle)	-	-	0.500	-	0.249	0.0137

## 2 Drug Information

### 2.1 Drug

CAS Registry Number:	90566-53-3	503070-58-4
Generic Name:	Fluticasone Furoate	Vilanterol trifenate
Code Name:	GW685698	GW642444M
Chemical Name:	Androsta-1,4-diene-17-carbothioic acid, 6,9-difluoro-11,17-dihydroxy-16-methyl-3-oxo-, S-(fluoromethyl) ester, (6α,11β,16α,17α)-	4-((1R)-2-((6-(2-(2,6-dichlorophenyl)methoxy)ethoxy)hexyl)amino)-1-hydroxyethyl)-2-(hydroxymethyl)phenol
Molecular Formula/weight:	C <sub>27</sub> H <sub>29</sub> F <sub>3</sub> O <sub>6</sub> S/538.6	C <sub>24</sub> H <sub>33</sub> C <sub>12</sub> NO <sub>5</sub> .C <sub>20</sub> H <sub>16</sub> O <sub>2</sub> /774.8
Structure:		
Pharmacologic Class:	Corticosteroid	Long acting beta-2 agonist

### 2.3 Drug Formulation

Fluticasone and vilanterol are stored in two separate blister strips. Contents of the blisters are released and mixed when the device is actuated. A mixture of dry powder is released from the mouth piece of the device. Table 3 shows the contents of each blister.

**Table 3: Breo Ellipta Composition**

Blister strip	Ingredient	Quantity/ blister	Function
FF strip	Fluticasone furoate	100 µg	Active
	Lactose	(b)(4)	(b)(4)
VI strip	Vilanterol	25 µg	Active
	Magnesium stearate	0.125 mg	(b)(4)
	Lactose	(b)(4)	(b)(4)

### 2.6 Proposed Clinical Population and Dosing Regimen

Adults with COPD will receive one oral inhalation (one actuation) of Breo Ellipta daily. Each actuation of Breo Ellipta delivers 92-µg FF and 22-µg VI from its mouth piece, although the dosage strength of the drug is 100-µg FF and 25-µg VI.

## 2.7 Regulatory Background

GSK submitted to IND 74,696 two draft protocols of 2-year inhalation toxicity studies of vilanterol in rats and mice (one each) on July 18, 2007. The respective proposed doses were [REDACTED] (b)(4) mg/kg/day in rats and [REDACTED] (b)(4) mg/kg/day in mice. Animals would be treated at the above doses for 104 weeks.

The Agency's Executive Carcinogenicity Committee (ECAC) evaluated the above dose selection on August 21, 2007. The Committee recommended revising the doses to 0, 0.08, 0.2 and 0.65 mg/kg/day in rats and 0, 0.3, 10 and 30 mg/kg/day in mice. DPARP informed GSK of the Agency's recommendations on August 22, 2007.

On September 7, 2007, GSK accepted the Agency's recommendation for the rat protocol and proposed to revise the dose selection for vilanterol in the mouse study protocol to 0.06, 0.6, 6, and 30 mg/kg/day. This proposal broadened the range of vilanterol doses.

The ECAC concurred with GSK's new proposal on September 19, 2007.

GSK started the studies with the recommended dose selections in November 2007. GSK subsequently proposed amendments to each study protocol during the in-life period of the studies. The amendments included proposals for dose modifications and early terminations of some groups. The ECAC accepted some proposal and rejected others as discussed below.

**Rat Study:** On Sept 9, 2009 (SDN # 69), GSK proposed to stop dosing the two top-dose groups immediately and to reduce vilanterol doses in the two low-dose groups by two thirds because of excessive mortality in the study. The Agency agreed with the proposals on September 30, 2009. See Memo completed by Dr. Lawrence Sancilio on Sept 17, 2009 for the Agency's evaluation of the GSK proposals. The Agency also recommended terminating the HD groups if the number of survival reached 15/sex. Consequently, dosing in top dose groups were stopped in week 85 and survivals were sacrificed in week 95. Vilanterol doses in the two low dose groups were reduced to 0.003 and 0.029 mg/kg/day, respectively, in week 85. All survival rats were sacrificed in weeks 101 - 104.

**Mouse Study:** There was no major change in the dosing schedule of the mouse study, but a significant protocol deviation occurred because the Agency denied GSK's request [REDACTED] (b)(4) GSK proposed [REDACTED] (b)(4) due to excessive mortality in mice on October 31, 2008 (treatment week 46). The Agency denied the request on November 20, 2008. The decision was based on the finding [REDACTED] (b)(4). See a pharmacology review completed by Dr. Molly Shea on November 18, 2008 for the Agency's evaluation of the proposal. Briefly, GSK noted that excessive mortality occurred across groups in the first 35 weeks of treatment. GSK investigated the cause of death [REDACTED] (b)(4) but GSK requested [REDACTED] (b)(4) anyway in the 31-OCT-2008 submission. The Agency denied the request [REDACTED] (b)(4).

To ensure that sufficient mice survived the 2-year treatment period for meaningful statistical analysis, GSK relocated mice (24/sex/dose) from the toxicokinetic section to the main study section. The relocated mice did not undergo any process of blood sampling. The ECAC did not evaluate this protocol deviation, but the deviation did not adversely affect the evaluation and interpretation of the study results as discussed in the review.

The ECAC evaluated the final study reports of both studies on November 27, 2012. The Committee found both studies adequate for evaluating the carcinogenic potential of vilanterol in rodents.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

Report No.	Description	Location
2011N119325	2-yr inhalation carcinogenicity of vilanterol (VI) in mice	4.2.3.4.1
2010N109253	2-yr inhalation carcinogenicity of VI in rats	4.2.3.4.1

#### 3.3 Previous Reviews Referenced

This review references a number of nonclinical reviews completed previously by DPARP staff in IND 74,696. Table 4 below lists these reviews.

**Table 4: Previous Reviews Referenced**

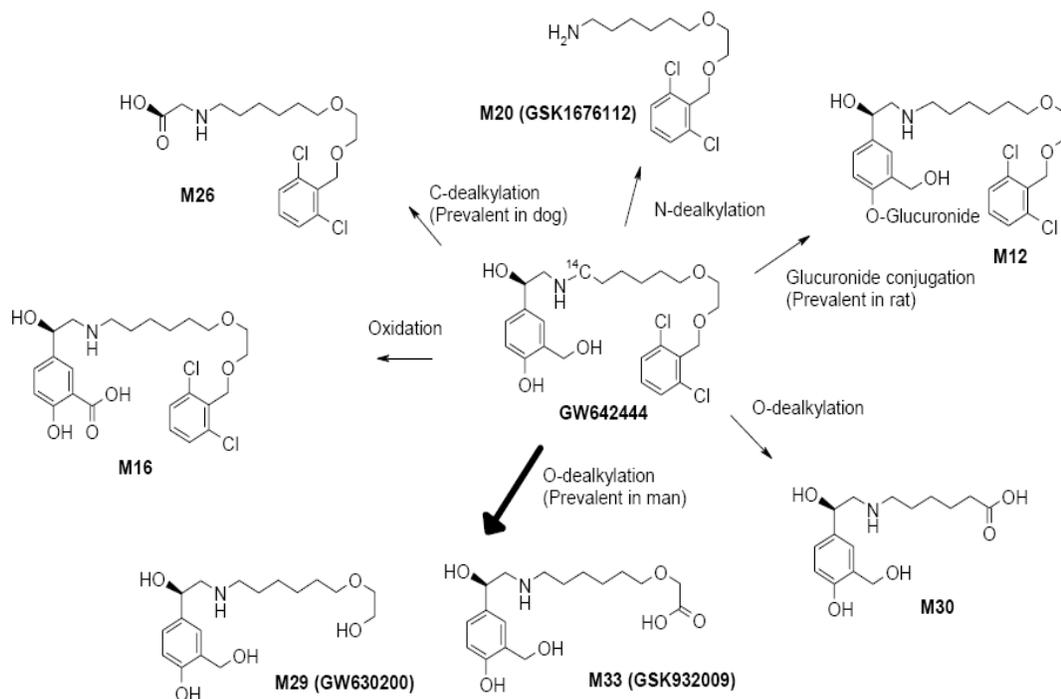
Author	Review Content	Date of Completion
Huiqing Hao	ECAC meeting minutes	8/21/2007
T. McGovern	Carcinogenicity study protocols review	9/17/2007
Huiqing Hao	Genetic toxicity data & 13-wk rat IH study	6/3/2008
Huiqing Hao	Original IND review	10/29/2008
Molly Shea	Mouse carcinogenicity study - interim mortality data	11/18/2008
Larry Sancilio	Rat carcinogenicity study – dose modification proposal	9/17/2009
Larry Sancilio	Rat carc. study – mortality and GI disturbance data	9/23/2009
Larry Sancilio	Chronic toxicity studies in rats and dogs	3/25/2012
Steve Thompson	Statistical review of carcinogenicity data	4/20/2012

### 4 Pharmacology

Vilanterol is a LABA. Similar to formoterol, a currently marketed LABA, it binds to beta-2 adrenoceptors and results in subsequent increases in intracellular cAMP levels, relaxation of smooth muscles located in the airways, and decreases in airway resistance. The potency of vilanterol is comparable to that of formoterol in pEC50s (~9.4). In vivo studies showed that vilanterol relaxed guinea pig airway muscle contractility induced by electric stimulation. Similarly, vilanterol inhibited the contractile response in human bronchus preparation induced by PGF-2 alpha. It also relaxed histamine-induced bronchoconstriction in conscious guinea pig.

## 5 Pharmacokinetics and Toxicokinetics

Vilanterol by the inhalation route of administration was well-absorbed in rat, dog and human, but the oral bioavailability of the drug was variable: 1.1%, <2%, 30% in rats, humans and dogs, respectively. Plasma drug concentrations were generally proportional to the inhalation dose in rats and dogs. Peak plasma drug levels were found at the end of inhalation exposure that lasted 60 minutes. Vilanterol is moderately, highly bound to plasma proteins (92-99%) in animals and humans. Vilanterol is metabolized by the CYP 3A4 enzyme. The main route of metabolism was by O-dealkylation to a range of metabolites with significantly reduced  $\beta$ 1- and  $\beta$ 2-agonist activity. Figure 2 presents the major metabolic pathways in animals and humans.



**Figure 2: Important metabolic pathways of vilanterol in animals and humans. The bold arrow indicates the major metabolic pathways in man.**

## 6 General Toxicology

Inhalation toxicity studies of up to 6 and 9 months in treatment duration were completed in rats and dogs, respectively. Drs. Huiqing Hao and Larry Sancilio completed reviews of these studies on October 29, 2008 and March 25, 2012, respectively. The following is a brief summary of findings in the chronic toxicity studies.

Sprague-Dawley rats (12/sex/dose) were dosed with 0 (C, lactose), 5.8 (LD), 53.7 (MLD), 267 (MHD), or 1,025 (HD)  $\mu\text{g}/\text{kg}/\text{day}$  of vilanterol (pulmonary deposits) for 6 months. The target organs of vilanterol toxicity included the lung, nose, and ovaries. All treatment related findings were limited to the HD group in the males and  $\geq 53.7$

µg/kg/day in the females. Findings included degeneration and squamous metaplasia of olfactory epithelium in the nose and increases in incidences of alveolar macrophages in the lung and hemorrhage in the tracheobronchial lymph node, and increases in the incidence of follicular cysts in the ovaries. The respective NOAELs in males and females were 267 and 5.77 µg/kg/day on a body-weight basis and 334 and 3.7 ng.h/mL in AUC<sub>0-t</sub>.

Beagle dogs (4/sex/dose) were dosed with 0 (lactose), 2.4, 15.6, or 127 µg/kg/day of vilanterol (pulmonary deposits) for 9 months. The target organs of toxicity included the heart (myocardial fibrosis), lung (alveolar hemorrhage), nose (degeneration of olfactory epithelium), liver (increased glycogen deposition), and kidney (fibrosis and tubular atrophy in the cortex). Most of the findings occurred in the females. The NOAEL was 15.6 µg/kg/day, corresponding to AUCs<sub>0-t</sub> of 34.8 and 39.0 µg/kg/day in males and females, respectively.

## 7 Genetic Toxicology

Vilanterol tested negative in the Ames assay, rat bone marrow micronucleus assay, in vitro UDS assay, and SHE cell assay; and equivocal in the mouse lymphoma assay according to the nonclinical review completed by Dr. Huiqing Hao on October 29, 2008.

## 8 Carcinogenicity

### 8.1 Mouse Carcinogenicity

#### Study title: GW642444M: Inhalation carcinogenicity study in mice

<i>Study no.:</i>	G07259; GSK document#: 2010N209253_00; GSK reference #: G7260
<i>Study report location:</i>	eCTD 4.2.3.4
<i>Conducting laboratory and location:</i>	 (b)(4)
<i>Date of study initiation:</i>	Nov 20, 2007
<i>GLP compliance:</i>	Yes, with a signed GLP statement
<i>QA statement:</i>	Yes, with a signed GLP statement
<i>Drug, lot #, and % purity:</i>	GW642444M, Batch# K064845 (99.4%) and K064213 (99.4%)
<i>CAC concurrence:</i>	Yes, see minutes of 21-AUG-2007 ECAC meeting.

#### Key Study Findings

- Vilanterol treatment for 2 years increased tumor incidences in the reproductive system in female mice. The male mice did not show any evidence of tumorigenicity.
- Mice (84/sex/dose, CD-1) were treated by nose-only inhalation with 0, 6, 62, 615, 6150, or 29,500-µg/kg/day vilanterol (achieved doses) for 101 – 104 weeks.

- The 29,500- $\mu\text{g}/\text{kg}/\text{day}$  group females showed a statistically significant increase in tubulostromal adenoma ( $p = 0.014$ ) in ovaries while the mid-high dose (MDH) group showed a numerical increase in the total incidence of tubulostromal adenoma.
- The  $\geq 6,150$ - $\mu\text{g}/\text{kg}/\text{day}$  groups showed statistically non-significant increase in leiomyosarcomas in the uterus ( $p = 0.014 - 0.06$ ).
- The  $\geq 62$ - $\mu\text{g}/\text{kg}/\text{day}$  groups showed numerical increases, but statistically non-significant increases, in total incidences of leiomyomas and leiomyosarcoma in the uterus.
- The 6- $\mu\text{g}/\text{kg}/\text{day}$  showed no evidence of tumorigenicity at any organs.

### Adequacy of Carcinogenicity Study:

The study was adequate for assessing the carcinogenicity potential of vilanterol in mice. The ECAC concurred with the vilanterol dose selections on August 21, 2007. The exposure duration was adequate; the total exposure duration was 101 and 104 weeks in males and females, respectively. The termination of groups met the Agency's criteria for early termination. Dose-related effects typical of beta agonists were observed. These effects included body weight changes in both sexes and increases in the incidence of tubulostromal adenomas in the ovaries in females. The ECAC concurred with the above conclusion in a meeting held on November 27, 2012.

The study had a major protocol violation. The violation, however, did not adversely impact the evaluation and interpretation of the study results. The violation was the relocation of additional mice (24/sex/dose) from the toxicokinetic section to the main study section. The relocation of mice was prompted by excessively high mortalities (27-40%) that occurred across groups in the early phase of the study (i.e., the first 33 weeks). The action was taken to ensure that there were sufficient numbers of mice surviving the 2-year treatment period.

The study was initiated with 60 and 42 mice/sex/dose in the main study section and 42 for the toxicokinetic section, respectively. Excessively high mortalities occurred across groups in the early phase of the study as alluded to above. The cause of mortality was (b)(4) discussed in the memo completed by Dr. Molly Shea on November 18, 2008. Modifications (b)(4) resulted in stabilization of mortality by week 35. To compensate for the potential small sample sizes of at the end of study, 24 mice (per sex/group) were relocated from the

(b)(4)

toxicokinetics section to the main study. The relocated mice did not go through blood sampling. The animal relocation was reasonable and acceptable.

There were sufficient animals at the end of the study for a sound scientific evaluation of the drug effect although high mortality occurred across groups throughout the study. The overall survival rate at the end of the study ranged between 24% to 35% in males and 19% - 33% in females, respectively. However, there were sufficient numbers of mice (20 – 25 and 16 – 28 mice/group in males and females, respectively). These numbers of survival met the Agency's criteria set in the memo completed by Dr. Larry Sancilio on September 17, 2009.

### ***Appropriateness of Test Models:***

The model was appropriate. The ECAC evaluated the model in a meeting held on August 21, 2007 and accepted the model. See minutes of the meeting for details.

### ***Evaluation of Tumor Findings:***

Female mice treated with vilanterol for 2 years showed dose-related increases in the incidences of tumors in organs of the reproductive system. The male mice did not show any evidence of tumorigenicity. Females receiving  $\geq 62$ - $\mu\text{g}/\text{kg}/\text{day}$  vilanterol showed statistically significant increases in the tubulostromal adenomas in the ovaries, and statistically non-significant, but numerical increases ( $P = 0.014 - 0.06$ ) in the incidence of leiomyomas and leiomyosarcomas, alone or in combination in the uterus. There was no evidence of tumorigenicity at the 6- $\mu\text{g}/\text{kg}/\text{day}$  dose.

CD-1 mice (84/sex/dose) were exposed via nose-only inhalation to GW642444 at the achieved doses of 0 (C, lactose), 6 (LD), 62 (MLD), 615 (MD), 6,150 (MHD) or 29,500 (HD)  $\mu\text{g}/\text{kg}/\text{day}$  for up to 104 weeks. The females showed dose-related increases in the incidences of tumors in the ovaries and uterus (Table 3, next page). The incidence of tubulostromal adenomas in the ovaries was 0, 0, 1, 0, 2 and 6 ( $p = 0.014$ ) in the C, LD, MDL, MD, MHL and HD groups, respectively.<sup>2</sup> The respective incidence of uterine tumors in the C, LD, MDL, MD, MHD and HD groups (among 84 mice/group) was 2, 2, 5, 5, 1, and 2 in leiomyomas; 0, 1, 2, 4 ( $p = 0.06$ ), 6 ( $p = 0.014$ ), and 4 ( $p = 0.06$ ) in leiomyosarcomas; 2, 3, 7, 9, 7 and 6 in the sum of uterine leiomyomas and leiomyosarcomas. All the tumors were observed in terminally killed mice or those that survived for most of the treatment period as indicated below.

The analysis of the study results based on the overall tumor incidence rates (above), however, could be misleading because of excessive mortality (21 – 28/group) that occurred in the first 34 weeks of the study. The reason is that tumorigenicity is a time dependent process and the mice that died early may not have sufficient time to develop tumors. If this hypothesis is true, the analysis of the overall tumor incidence rate would undermine the tumorigenicity effect of vilanterol. Analyses of the tumor rates among subgroups of mice based on the duration of exposure may overcome the potential bias from the early mortality.

To overcome the bias of early mortality, the review analyzed the tumor rates by subgroups: those surviving for at least 34 weeks and those terminally sacrificed. Not surprisingly, all the

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<sup>2</sup> P values cited in the reviews were pairwise comparison against the vehicle group. These values were taken from the statistical reviews of these studies completed by Dr. Steven Thompson on April 10 (DARRTS Reference IN# 3117343).

tumors were found in the mice survived for at least 34 weeks (n = 56 – 61/dose). The early death subgroups (n = 28 – 21) had none. The following discussion is based on the tumor rate (in %) because of the difference in denominators among groups. Dose-response relationships were observed in both ovary and uterine tumors in each analysis. Table 5 summarizes the results of the overall and subgroup analysis of the uterine and ovary tumors.

#### Tubulostromal adenomas in ovaries:

The ovaries showed vilanterol dose-dependent increase in the incidence of tubulostromal adenoma. The respective prevalence of the tumor in the C, LD, MDL, MD, MHD and HD groups was 0%, 0%, 1.2%, 0%, 2.4% and 7.1% in the overall analysis; 0%, 0%, 1.7% 0%, 3.4%, and 10.7% in mice survived for at least 34 weeks; and 0%, 0%, 5.6%, 0%, 0% and 11.1% in the terminally-sacrificed mice. The tumor prevalence rate of the MHD and HD groups in mice that survived for at least 34 weeks (3.4% - 10.7%) was significant higher than the historic value of 0.6% (5/821) among mice the laboratory during the period of 2001 – 2009.

**Table 5: Ovarian and Uterine Tumors in Female Mice**

Organ/ tumor	Parameter	Vilanterol (µg/kg/day)					
		0	6	62	615	6150	29500
Uterus:	N	84	84	84	84	84	84
Overall	Leiomyoma #, (%) <sup>a</sup>	2 (2.4)	2 (2.4)	5 (6.0)	5 (6.0)	1 (1.2)	2 (2.4)
	Leiomyosarcoma (%)	0 (0)	1 (1.2)	2 (2.4)	4 (4.7)	6 (7.1) *	4 (4.7)
	Leiomyoma/sarcoma (%)	2 (2.4)	3 (3.6)	7 (8.3)	9 (10.7)	7 (8.3)	6 (7.1)
> Wk 34: N		61	63	59	59	59	57
	Leiomyoma # (%)	2 (3.3)	2 (3.2)	5 (8.5)	5 (8.5)	1 (1.7)	2 (3.6)
	Leiomyosarcoma # (%)	0 (0)	1 (1.6)	2 (3.4)	4 (6.8)	6 (10.2) *	4 (7.0)
	Leiomyoma/sarcoma #, (%)	2 (3.3)	3 (4.8)	7(11.9)	9(15.3)	7(11.9)	6(10.7)
Terminal: N		26	28	18	24	16	27
	Leiomyoma # (%)	2 (7.7)	2 (7.1)	0 (0)	2 (8.3)	1 (6.3)	2 (7.4)
	Leiomyosarcoma # (%)	0 (0)	1 (3.5)	0 (0)	3(12.5)	1 (6.3)	2 (7.4)
	Leiomyoma/sarcoma # (%)	2 (7.7)	3(10.7)	0 (0)	5(20.8)*	2 (12.5)	4 (14.8)
Ovary:	Overall, incidence (%)	0/84	0/83	1/84	0/84	2/84	6/84 *
Tubulostromal adenoma <sup>b</sup>		(0)	(0)	(1.2)	(0)	(2.4)	(7.1)
	> Wk 34, % (incidence)	0/61	0/63	1/59	0/59	2/59	6/56 *
		(0)	(0)	(1.7)	(0)	(3.3)	(10.7)
	Terminal % (incidence)	0/26	0/27	1/18	0/24	0/16	3/27
		(0)	(0)	(5.6)	(0)	(0)	(11.1)

\*, p = 0.012 – 0.014 by pairwise comparison.

- The historic background of leiomyomas and leiomyosarcomas, alone and in combination, in this laboratory during the period of 2001 – 2009 was 2.3% (19/828) and 2.7% (22/821), 5.0% (41/828), respectively.
- The historic value of tubulostromal adenoma in the laboratory during the period of 2001 – 2009 was 0.6% (5/821).

The MHD and HD groups also showed increases in the incidence of tubulostromal hyperplasia. The incidence of hyperplasia was 1, 1, 3, 0, 6 and 7 in the C, LD, MDL, MD, MHD and HD groups, respectively. The total incidence of tubulostromal hyperplasia and

adenoma was 1, 1, 4, 0, 7 and 12 in the C, LD, MDL, MD, MHD and HD groups, respectively. The tubulostromal hyperplasia was mostly detected in the terminally sacrificed mice.

The review considers the tubulostromal adenoma of the ovaries in MHD and HD groups a treatment-related finding. The tumor prevalence rate of the MHD and HD groups (3.4% - 10.7%) was significantly higher than the historic value of 0.6% (5/821) among mice the laboratory during the period of 2001 – 2009. These two groups also showed increases in the incidence of tubulostromal hyperplasia. In contrast, the study report does not consider the MHD finding a treatment related effect. The report argues that “the incidence ... was comparable to historic control data and was not statistically significant from the concurrent control either by pairwise comparison or trend evaluation” (p 66).

Tubulostromal adenoma is a benign ovarian epithelial tumor observed in mice and rats, but rarely seen in other animal species. Morphologically it is characterized by the presence of tubular structures and interstitial stroma.

It is noted that the  $\geq 62$ - $\mu\text{g}/\text{kg}/\text{day}$  groups also showed an incidence of sex cord associated tumors (2 – 4/84, total) that was slightly higher than the historic background of 1.0%. The review does not consider the finding a treatment-related effect because of the lack of the statistical significance and the lack of dose-response relationship.

#### Uterine leiomyomas and leiomyosarcomas:

The uterus showed a vilanterol dose-dependent increase in the incidence of leiomyosarcomas; there was, however, no dose-response relationship for leiomyomas, alone or in combination with leiomyosarcomas. The respective tumor incidence in the C, LD, MDL, MD, MHL and HD groups was 0, 1, 2, 4 ( $p = 0.06$ ), 6 ( $p = 0.014$ ), and 4 ( $p = 0.06$ ) in leiomyosarcomas; 2, 2, 5, 5, 1, and 2 in leiomyomas; 2, 3, 7, 9, 7 and 6 in the sum of uterine leiomyomas and leiomyosarcomas. The discussion is again based on the tumor prevalence (i.e., rate) among groups because this rate normalizes the sample sizes.

The prevalence rate of leiomyosarcomas in the C, LD, MLD, MD, MHD, and HD groups was 0%, 1.2%, 2.4%, 4.7%, 7.1%, and 4.7% in overall rate ( $n = 84$  each) and 0%, 1.6%, 3.4%, 6.8%, 10.2% and 7.0% in mice survived for at 34 weeks; and 0%, 3.5%, 0%, 12.5%, 6.3% and 7.4% in the terminally sacrificed mice. The prevalence rate of leiomyomas in the C, LD, MLD, MD, MHD, and HD groups was 2.4%, 2.4%, 6.0%, 6.0%, 1.2%, and 2.4% in overall rate ( $n = 84$  each) and 3.3%, 3.1%, 8.5%, 8.5%, 1.7% and 3.6% in those survived for at 34 weeks; and 7.7%, 7.1%, 0%, 8.3%, 6.3% and 7.4% in the terminally sacrificed mice. The prevalence rate of leiomyomas and leiomyosarcomas in combination in the C, LD, MLD, MD, MHD, and HD groups was 2.4%, 3.6%, 8.3%, 10.7%, 8.3% and 7.1% in overall rate ( $n = 84$  each) and 3.3%, 4.8%, 11.9%, 15.3%, 11.9% and 10.7% in those survived for at least 34 weeks; and 7.7%, 10.7%, 0%, 20.8%, 12.5% and 14.8% in the terminally sacrificed mice. The historic background of leiomyomas and leiomyosarcomas, alone and in combination, in this laboratory during the period of 2001 – 2009 was 2.3% (19/828) and 2.7% (22/821), and 5.0% (41/828), respectively.

The uterus also showed dose-dependent increases in the incidence of myometrial hypertrophy and hyperplasia. The total incidence of hypertrophy or hyperplasia (mostly minimal to slight in severity) was 0, 0, 9, 40, 46 and 41 in C, LD, MLD, MD, MHD, and HD groups ( $n = 84$ ), respectively.

The study report concluded that vilanterol at doses  $\geq 62$   $\mu\text{g}/\text{kg}/\text{day}$  increase incidences of tumors in the ovaries and uterus in female mice. This conclusion was based on the dose-related, statistically significant increases in the incidences of tumors in the ovary (tubulostromal adenoma) at 6,150  $\mu\text{g}/\text{kg}/\text{day}$ , and numerical increases in the incidence of leiomyosarcomas, alone or in combination with leiomyomas in the uterus. Furthermore, the incidence of these tumors were above the historic background levels. Finally, other beta agonists have been known to increase the incidence of tumors in the female reproductive organs in rodents. For example, increases in leiomyomas and leiomyosarcomas have been reported in mice exposed to formoterol.<sup>3</sup> The conclusion that vilanterol treatment was associated with the increases in the incidence of uterine leiomyosarcomas was in agreement with the applicant, but the ECAC concluded that the uterine tumors were not a treatment-related effect because none of the increases in tumor incidences reached the statistical significant level of  $p < 0.01$  for common tumors.

## Methods

- Doses:* Achieved dose: 0, 6, 62, 615, 6,150 or 29,500 mg/kg/day of vilanterol trifenate in lactose (vehicle). These dose groups were referenced as Groups 1, 2, 3, 4, 5 and 6 in the Study Report; or C, LD, MLD, LD, MHD, and HD groups in the review, respectively.
- Frequency of dosing:* 60 min/episode/day
- Dose volume:* Not applicable
- Route of administration:* Nose-only inhalation exposure
- Formulation/Vehicle:* Vilanterol (w/w) powder in lactose; GW642444M concentration (w/w), 0, 0.4%, and 20% for Groups 1, 2 – 3, and 4 – 6, respectively.
- Basis of dose selection:* A 3-month inhalation dose-finding study
- Species/Strain:* CD-1 mice
- Number/Sex/Group:* 84/sex/group [See Deviation from Study Protocol section (below) for this unusual number.
- Age:* 7 weeks old on day 1 of treatment

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<sup>3</sup> The formoterol (NDA 20-831) label approved on 9/27/2012 states: “The incidence of uterine leiomyomas and leiomyosarcomas was increased at [formoterol] doses of 2 mg/kg and above ... Increases in leiomyomas of the rodent female genital tract have been similarly demonstrated with other beta-agonist drugs.”

- Weight at start of exposure* Male: 23.1 – 36.5 g; females: 17.7 – 29.6 g
- Animal housing:* 5 per cage
- Paradigm for dietary restriction:* Certified Rodent Diet No. 2016C pellets at lib except during the inhalation exposure period
- Dual control employed:* No
- Chamber aerosol drug concentration:* Gravimetric (daily) and chemical analysis (daily for the first 7 weeks, and 3 times/week thereafter)
- Aerosol particle distribution:* Monthly except for the week 1 and 3 (once/week)
- Satellite groups:* 42/sex/group used for TK analysis; see TK section
- Toxicokinetics:* 3/sex/time point on weeks 4 and 26 (0.08, 0.5, 1, 2, 4, 8 and 23 hours after the end of exposure)
- Deviation from study protocol:*
- Major deviations:* The number of mice in the main study section was increased to 84 (per sex/dose) from 60). These 24 mice were relocated from the toxicokinetic section to the main study section in week 46. The relocation was to compensate for the unexpected mortality (b)(4) in the first 35 weeks of treatment. See Regulatory history section for additional information.
- Minor deviations:*
- 1) Males were sacrificed in week 101 when the number of surviving mice in the control group reached 20.
  - 2) Exposure deviations: Several groups of animals were not exposed for specified time on one or more occasions (b)(4). The variations are listed below:  
Group 1 animal did not receive exposure on day 71/72, groups 5 and 6 animal exposure on day 178/179 was 57 and 30 min, respectively, group 3 exposure was for 35 min on day 584, group 4 animals were exposed to 47 min on day 660. Also, Group 3 used 0.4%-GW642444M formulation on days 1 and 2. These minor deviations do not affect the outcome of the study.

### Study Design:

CD-1 mice (84/sex/dose) were exposed via nose-only inhalation to GW642444 at the achieved doses of 0 (lactose), 6, 62, 615, 6150 or 29,500 µg/kg/day for up to 104 weeks. Table 6 summarizes the exposure conditions and dosimetry. The achieved dose was estimated based on the exposure duration of 60 minutes/day. The animal number of 84 per sex per dose was a major unique design. See the Protocol Deviation and History of Regulatory Submission sections for the reasons for the unique design.

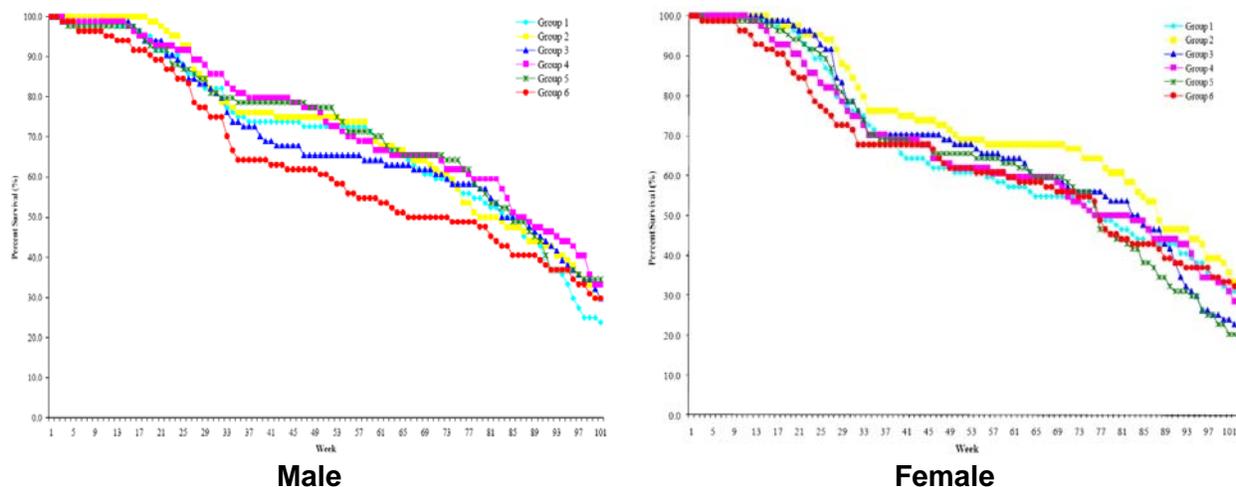
**Table 6: Study Design of the Mouse Study**

	Group					
	1	2	3	4	5	6
Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ ), target <sup>a</sup>	0	6	60	600	6,000	30,000
Achieved ( $\mu\text{g}/\text{kg}/\text{day}$ )	0	6.2	62	615	6,150	29,500
Vilanterol chamber concentration						
Target ( $\mu\text{g}/\text{L}$ )	0	0.1	1	10	100	500
Achieved ( $\mu\text{g}/\text{L}$ ) <sup>b</sup>	0	0.11	1.06	10.7	107	509
MMAD ( $\mu\text{m}$ ) <sup>c</sup>	7.6	2.0	2.8	2.2	2.9	4.0
GSD	2.88	2.15	2.34	1.96	1.96	2.12
Number of mice/sex <sup>d</sup>	84	84	84	84	84	84
# mice for TK/sex	42	42	42	42	42	42

- a. The reported numbers were from Study Design Table on page 49 of the study report. The mean body weight for calculating the achieved dose ranged from 0.0355 – 0.0382 kg in males and 0.0267 – 0.0284 kg in females, respectively (p801).
- b. Extracted from Group Particle Size Distribution Table (page 803) of the report.
- c. 24 mice (per sex/dose) were relocated to the main study group (from the toxicokinetic section) in week 45 because of excessive mortality (b) (4)

## Observations and Results

**Mortality:** Animals were observed for mortality twice a day, necropsy was performed on animals that died or killed due to moribund conditions. There was no evidence of a treatment-related effect on mortality in either sex. Figure 3 presents the survival (%) – time course of the study, although there appeared to be a dose-response relationship in males. High mortality (75 – 81%) occurred across groups in both sexes, especially during the period of week 9 and 33. The high mortality during the early phase of the treatment (prior to week 35), however, was unrelated to vilanterol treatment. Rather, high mortality occurred as (b) (4) discussed later. Overall, there were no differences in mortality at the time of terminal sacrifice.

**Figure 3: Survival curve in mice**

The Agency had evaluated the early mortality data and the cause of deaths. Dr. Molly Shea evaluated the causal effect of (b) (4) mortality in November 2008. Dr. Larry

Sancilio completed an evaluation of the causal effect of GI distension and mortality in September 23, 2009. Both evaluations were conducted in consultation with the ECAC members. These evaluations concluded that there was no evidence of vilanterol treatment effect on the mortality. See reviews completed by Dr. Molly Shea on November 18, 2008 and Dr. Larry Sancilio on September 23, 2009 for complete details. Table 7 summarizes the mortality during specific periods of the study in males and females.

**Table 7: Unscheduled deaths in the Mouse Study**

Time <sup>a</sup> (week)	Male						Female					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
1 – 8	0	0	1	1	2	3	0	0	0	0	1	1
9 – 25	10	6	9	6	9	10	9	4	6	14	7	18
26 – 34	10	13	12	8	6	15	14	16	19	11	17	8
35 – 53	3	2	7	8	4	7	10	6	2	7	4	5
54 – 73	11	12	5	9	9	7	5	2	10	7	8	6
73 - 101	30	26	25	24	25	17						
73 - 104							20	28	29	21	31	19
Total (1 – 104)	64	59	59	56	55	59	58	56	66	60	68	57
Terminal kill <sup>b</sup>	20	25	25	28	29	25	26	28	18	24	16 <sup>b</sup>	27
Survival (%)	24	30	30	33	35	30	31	33	21	29	19 <sup>b</sup>	32

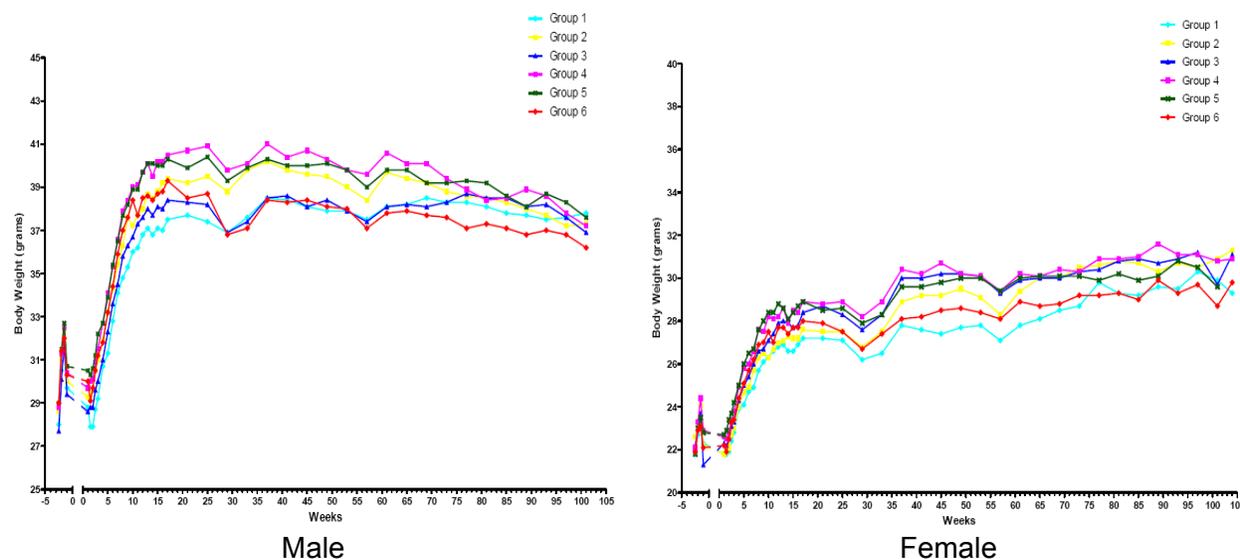
a.

(b) (4)

b. The time for terminal kill was weeks 101 and 105 in males and females, except for G5 females that was killed in week 104.

**Clinical Signs:** Clinical signs were observed daily. Physical examinations and palpation for tissue masses was done weekly. No treatment-related effects were observed.

**Body Weight:** Body weights were recorded twice (weeks 1 and 2 only) or once per week (weeks 3 to 16), or monthly thereafter. The final body weight was recorded on the day of necropsy. A bell-shaped curve of body weight and dose response relationship was apparent. Such a phenomenon is typical of beta agonist effects in rodents. Particularly, Group 4 (mid dose) in both sexes mostly had the highest increases in body weights. The HD had decreases or no significant changes in body weights for the majority of the treatment period. Figure 4 presents the body weight – time curve of the study in males and females.



**Figure 4: Body weight-time course curve in mice. Vilanterol doses were 0, 6, 62, 615, 6150, and 29,500 µg/kg/day in Groups 1, 2, 3, 4, 5, and 6, respectively.**

Table 8 summarizes the body weights and weight gains at several milestones of the study. Statistically significant increases in body weights and weight gains were observed in treatment groups and the highest increases generally occurred in the mid dose groups.

**Table 8: Body Weight Changes in the Mouse Study**

Estimated Achieved Dose <sup>a</sup> (µg/kg/day)	Male						Female						
	0	6.4	62	615	6150	29500	0	6.4	62	615	6150	29500	
<b>Body Weight (g)<sup>c</sup></b>													
Week 1	28.8	29.3	28.6	29.7*	30.5***	30.0***	21.8	21.8	22.3	22.6*	22.7*	22.2*	
Week 13	37.1	38.7**	38.0	40.1***	40.1***	38.6**	26.9	27.1	28.0***	28.6***	28.6***	27.7*	
Week 29	36.9	38.8*	36.9	39.8***	39.3**	36.8	26.2	26.8	27.6**	28.2***	27.9***	26.7	
Week 53	37.9	39.0	37.9	39.8	39.8*	38.0	27.8	29.1*	30.1***	30.1***	30.0***	28.4	
Week 101 (males) or Week 104 (females except Group 5 – Week 101)	37.8	37.3	36.9	37.2	37.6	36.2	29.3	31.3	31.1	30.9	29.6	29.8	
<b>Body Weight gain (g)<sup>c</sup></b>													
Week 1-13	7.4	8.8**	8.6***	9.6***	9.4***	8.1**	4.7	4.9	6.7***	5.8***	5.8***	5.6***	
Week 1-29	7.2	8.8	7.6	9.3**	8.6	6.4	3.9	4.7	6.2***	5.4**	5.0*	4.7	
Week 1-53	8.1	9.2	8.8	9.6*	9.0	7.6	5.2	7.0***	8.7***	7.2***	7.1***	6.2	
Week 1-101/104	8.0	7.6	7.6	7.1	7.1	5.6	7.0	9.0	10.0	8.4	7.1	7.9	

**Food Consumption:** Changes in food consumption were generally reflective of body weight changes, but to a lesser degree. Table 9 summarizes the food consumption during the study.

**Ophthalmology:** Ophthalmic examinations using slit lamp bio-microscopy were done at pre-dosing, and weeks 13, 52, 78 and 100. No treatment-related effects were observed.

**Table 9: Food Consumption in the Mouse Study**

Estimated Achieved Dose <sup>a</sup> (µg/kg/day)	Male						Female						
	0	6.4	62	615	6150	29500	0	6.4	62	615	6150	29500	
<b>Food Consumption (g/animal/day)<sup>c</sup></b>													
Week 1-2	2.8	3.1*	2.9*	3.3***	3.3***	3.2***	2.8	2.9	2.8	2.9	3.0	3.0	
Week 12-13	4.0	4.4**	4.3	4.6***	4.6***	4.4*	3.2	3.2	3.3	3.4	3.5**	3.4**	
Week 28-29	3.8	4.2	3.9	4.5***	4.5***	4.1	3.1	3.0	2.9	3.2	3.4*	3.2*	
Week 52-53	3.8	3.9	3.8	4.2	4.2	3.9	3.0	3.1	3.2*	3.4***	3.4***	3.2***	
Week 100-101 (males) or Week 104 (females except Group 5 – Week 100-101)	4.5	4.4	3.9	4.5	4.5	4.3	4.1	4.2	4.0	4.3	4.9*	4.6	

**Toxicokinetics:** Plasma levels of vilanterol and 3 metabolites were determined in 3 mice/sex/time point on weeks 4 and 26 (0.08, 0.5, 1, 2, 4, 8 and 23 hours after the end of exposure. The metabolites included GSK932009, GW630200 and GI179710. Dose-related increases in plasma drug exposures of vilanterol and its metabolites were observed. Table 10 summarizes the results.

**Table 10: Plasma Vilanterol and Metabolite Levels in the Mouse Study**

Estimated Achieved Dose <sup>a</sup> (µg/kg/day)	Male						Female					
	0	6.4	62	615	6150	29500	0	6.4	62	615	6150	29500
	<b>GW642444M</b>											
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 4<sup>b</sup></b>	NQ	14.1	46.1	143	880	2428	NQ	13.1	52.3	139	750	2230
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 26<sup>b</sup></b>	NQ	3.20	27.3	125	1052	4012	NQ	1.31	13.8	132	996	5694
C <sub>max</sub> (ng/mL): <b>Week 4<sup>b</sup></b>	NQ	2.60	19.2	34.1	305	729	NQ	2.67	12.2	34.7	429	605
C <sub>max</sub> (ng/mL): <b>Week 26<sup>b</sup></b>	NQ	0.520	9.05	37.5	418	1127	NQ	0.736	5.39	76.9	502	1227
	<b>GSK932009</b>											
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 4<sup>b</sup></b>	NQ	NC	NC	3.74	41.8	131	NQ	NC	NC	3.66	35.0	138
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 26<sup>b</sup></b>	NQ	NC	NC	1.88	16.4	89.1	NQ	NC	NC	2.65	24.4	161
C <sub>max</sub> (ng/mL): <b>Week 4<sup>b</sup></b>	NQ	NC	0.212	2.03	18.7	40.4	NQ	NC	0.181	1.69	24.5	53.7
C <sub>max</sub> (ng/mL): <b>Week 26<sup>b</sup></b>	NQ	NC	0.125	0.873	5.38	17.0	NQ	NC	0.178	1.43	12.4	38.4
	<b>GW630200</b>											
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 4<sup>b</sup></b>	NQ	NC	NC	NC	1.61	8.67	NQ	NC	NC	NC	1.62	5.72
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 26<sup>b</sup></b>	NQ	NC	NC	NC	1.06	4.54	NQ	NC	NC	NC	1.33	5.31
C <sub>max</sub> (ng/mL): <b>Week 4<sup>b</sup></b>	NQ	NC	NC	0.162	0.886	1.64	NQ	NC	NC	NC	0.775	1.61
C <sub>max</sub> (ng/mL): <b>Week 26<sup>b</sup></b>	NQ	NC	NC	NC	0.550	1.16	NQ	NC	NC	0.145	0.769	1.20
	<b>GI179710</b>											
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 4<sup>b</sup></b>	NQ	NC	NC	536	9410	23217	NQ	NC	NC	824	11439	45135
AUC <sub>(0-t)</sub> (ng.h/mL): <b>Week 26<sup>b</sup></b>	NQ	NC	NC	266	3721	23895	NQ	NC	NC	578	5197	52805
C <sub>max</sub> (ng/mL): <b>Week 4<sup>b</sup></b>	NQ	NC	15.9	183	3881	3157	NQ	NC	18.7	267	1943	4130
C <sub>max</sub> (ng/mL): <b>Week 26<sup>b</sup></b>	NQ	NC	8.49	88.6	549	2980	NQ	NC	9.05	112	793	7346

Vilanterol and GI179710 accounted for nearly all drug related material in the plasma. Little or no GSK932009 and GW630200 (<0.4%) was present. Table 11 summarizes the plasma AUCs and percentage of vilanterol and metabolites in plasma in week 26. Vilanterol and GSK932009 accounted for approximately 10 – 22% and 77% - 90% of the total material. Only the two high dose groups were calculated because data in the low dose groups were incomplete.

**Table 11: Proportions of Vilanterol and Metabolites in the Plasma in Mice (week 26)**

Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Plasma AUC ( $\mu\text{g}\cdot\text{h}/\text{mL}$ ) <sup>a</sup>				Percent (%) of Total AUC <sup>b</sup>			
	Male		Female		Male		Female	
	6150	29500	6150	29500	6150	29500	6150	29500
GW642444	1052	4012	996	5694	21.96	14.33	16.02	9.71
GSK932009	16.4	89.1	24.2	161	0.34	0.32	0.39	0.27
GW6320200	1.06	4.54	1.33	5.31	0.02	0.02	0.02	0.01
GI179710	3721	23895	5197	52805	77.67	85.34	83.57	90.01
Total	4790.5	28000.6	6218.5	58665.3	100	100	100	100

a. Taken from Table 8. Only the two high dose groups were calculated because data in the low dose groups were incomplete.

b. Calculated using reported plasma AUC values

**Necropsy:** Survival mice were killed in weeks 101 (males) or weeks 104 – 105 (females). Necropsy was conducted on all mice (terminal and pre-terminal killing). A complete panel of organs/tissues was collected for each mouse.

**Neoplastic changes:** The ovaries and uterus in the females showed dose-related increases in tumor incidences. Specifically, the MHD and HD groups showed increases in the incidence of tubulostromal adenomas and the  $\geq$ MD groups showed increases in the incidence of leiomyosarcomas. There was no treatment-related effect in tumor incidences in males. Table 12 summarizes the tumor incidences in uterus and ovaries. Appendices A and B (pages 39 - 43) present the overall tumor incidences in different tissues and organs in males and females, respectively.

Of the tumors in the uterus and ovaries, the respective tumor incidence in the C, LD, MDL, MD, MHD and HD groups (among 84 mice/group) was 0, 1, 2, 4 ( $p = 0.06$ ), 6 ( $p = 0.014$ ), and 4 ( $p = 0.06$ ) in uterine leiomyosarcomas; 2, 3, 7, 9, 7 and 6 in the sum of uterine leiomyomas and leiomyosarcomas; and 0, 0, 1, 0, 2 and 6 ( $p = 0.014$ ) in ovary tubulostromal adenomas.

The  $\geq 62$ - $\mu\text{g}/\text{kg}/\text{day}$  groups also showed the incidence of sex cord associated tumors (2 – 4, total) slightly higher than the historic background of 1.0%. The review does not consider the finding a treatment related effect because of the lack of the statistical significance and the lack of dose-response relationship.

The analysis of the study results based on the overall tumor incidence rates (above) could be misleading because of excessive mortality (27-40%) that occurred in the early phase of the study (i.e., the first 34 weeks). The reason is that tumorigenicity is a time-dependent process and the mice that died early may not have had sufficient time to develop tumors. If this hypothesis is true, the analysis of the overall tumor incidence rate would undermine the tumorigenicity effect of vilanterol. To overcome the potential bias from the early mortality, this review conducted an analysis of the tumor rates among subgroups of mice which survived for at least 34 weeks. See the Evaluation of Tumor Findings Section (p 11 – 14) for these analyses

Table 12: Tumor Incidences in Ovaries and Uterus in Female Mice

Organ/ Lesion	Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )					
	0	6	62	615	6150	29500
Ovaries, # examined	84	83	84	84	84	84
Sex cord: Stromal adenoma, benign	0	1	2	1	0	2
Luteoma [B]	0	0	1	1	0	0
Granulosa cell tumor, benign [B]	0	0	1	1	2	0
Granulosa cell tumor [M]	0	0	0	0	1	0
Total sex cord associated tumors <sup>a</sup>	0	1	4	3	3	2
Tubulostromal adenoma, [B]	0	0	1	0	2	6*
Cyst adenoma, [B]	0	1	3	2	1	0
Malignant hemangiosarcoma	0	0	0	0	0	1
Total ovarian tumors [B] and [M]	0	2	8	5	6	9
Uterus: # examined	84	84	84	84	84	84
Leiomyoma, [B]	2	2	5	5	1	2
Leiomyosarcoma [M] <sup>b</sup>	0	1	2	4	6*	4
Leiomyoma and leiomyosarcoma, total	2	3	7	9	7	6
Hemangioma [B]	1	2	2	0	0	0
Hemangiosarcoma [B]	0	1	0	0	2	0
Endometrial stromal sarcoma [M]	0	1	1	1	0	2
Endometrial adenocarcinoma [M]	1	1	1	1	1	1
Endometrial stromal polyp [B]	2	2	5	2	5	3
Total uterine tumors [B] + [M]	5	10	16*	12	15	12

\*, =  $p < 0.05$  (vs vehicle control) by pair-wise test.

a. Incidence of uterine leiomyosarcoma in historical control data is 2.7% (22/828; 7 studies).

b. Incidence of "sex cord-stromal tumor" in historical control data is 2.0% (16=821; 7 studies)

**Non-neoplastic changes:** Non-neoplastic changes were observed in the nasal cavity in both sexes and the ovaries and uterus in females. Table 13 summarizes the findings in these organs. Lesions in the nasal cavity included generation/regeneration, inflammation, and erosion/ulceration of the nasal mucosal epithelium. Tubulostromal hyperplasia and smooth muscle metaplasia were observed in the ovaries. Myometrial hypertrophy and hyperplasia and glandular metaplasia were observed in the uterus. The non-neoplastic findings in the ovaries and uterus generally corresponded well with the neoplastic findings in these organs. There were, however, no correlations between non-neoplastic and neoplastic finding in the nasal cavity in either sexes.

**Table 13: Non-Neoplastic Findings in Mice**

Sex Group	Male						Female					
	1	2	3	4	5	6	1	2	3	4	5	6
<b>Nasal mucosa</b>												
Generation/ regeneration	5	8	11	11	53	70	7	9	21	11	46	69
Inflammation, olfactory mucosa	1	2	0	2	9	36	3	3	0	3	8	31
Ulcer/erosion, olfactory epithelium	1	0	0	0	8	17	1	0	0	1	5	11
<b>Ovaries</b>												
Hypertrophy/ hyperplasia /sex cord stroma	-	-	-	-	-	-	1	3	6	3	6	3
Tubulostromal hyperplasia	-	-	-	-	-	-	1	1	3	0	6	7
Smooth muscle metaplasia	-	-	-	-	-	-	0	0	0	0	0	4
<b>Uterus</b>												
Glandular squamous metaplasia	-	-	-	-	-	-	0	0	0	0	2	8
Myometrial hypertro./hyperplasia	-	-	-	-	-	-	0	0	9	40	46	41
Vagina, anestrus appearance							10	20	20	19	26	21

## 8.2 Rat Carcinogenicity

### Study title: GW642444M: Inhalation carcinogenicity study in Rats

Study no.: 79088 (b)(4) GSK document#: 2010N209253\_00; GSK reference #: G7260

Study report location: eCTD 4.2.3.4

Conducting laboratory and location:

(b)(4)  
GlaxoSmithKline at 709 Swedeland Rd, King of Prussia, PA 19406-0939 for hormone analysis, and WW DMPK: Park Road, Ware, Hertfordshire, UK SG 12 0DP for immunochemistry

Date of study initiation: January 15, 2008; report issue date: Aug 19, 2011

GLP compliance: Yes, with a signed GLP statement

QA statement: Yes, with a signed GLP statement

Drug, lot #, and % purity: GW642444M, Batch# R29605 (99.4%)

CAC concurrence: Yes, see minutes of 21-AUG-2007 ECAC meeting

### Key Study Findings

- Rats exposed to vilanterol by nose-only inhalation in a traditional 2-year bioassay showed dose-related increases in the incidence of tumors (pars distalis) in the pituitary gland and in mortality caused by these tumors in both sexes. The females also showed increases in the incidence of mesovarian leiomyomas.
- Statistically significant increases in tumorigenicity and mortality were observed in males receiving  $\geq 223$   $\mu\text{g}/\text{kg}/\text{day}$  and females receiving  $\geq 84.2/28.2$   $\mu\text{g}/\text{kg}/\text{day}$  of

vilanterol. Pituitary adenomas were the primary cause of mortality in all of the above but the 10.5/3.5 µg/kg/day group females.

- No evidence of tumorigenicity was observed at vilanterol doses ≤ 84 µg/kg/day in males or 10.5 µg/kg/day in females, respectively.

**Adequacy of Carcinogenicity Study:** The study was adequate. The ECAC concurred with the conclusion on November 27, 2012. See the 27-NOV-2012 ECAC meeting minutes.

Dose-selection of the study was appropriate. The ECAC concurred with the selections and adjustments of vilanterol doses during the study. See minutes of the 17-AUG-2007 and the 30-SEP-2009 General Advise letter. Earlier terminations of some groups were in compliance with the Agency's recommendations in the 30-SEP-2009 General Advise letter. The exposure duration was adequate: the total exposure duration was up to 101 and 104 weeks in males and females, respectively. Dose-related effects typical of beta agonists were observed. These effects included body weight changes in both sexes and mesovarian leiomyomas/leiomyosarcomas in females. Vilanterol also causes dose-related increases in the incidence of pituitary adenomas and shorter latency of these tumors in both sexes.

Major protocol deviations occurred during the study. The deviations included adjustments of vilanterol dose in females and earlier terminations of several groups in both sexes. The deviations, however, do not affect of the interpretation of the study results. The dose adjustments resulted in different vilanterol dosing schedules between males and females. Males were dosed with 0, 10.5, 84.4, 223 and 657-µg/kg/day vilanterol for 100 weeks. The males were terminated in week 101 because the number of survivors in the control group went down to 18. Due to excessive mortality or toxicity females were dosed with the same doses for 85 weeks followed by dose modifications in week 86: the MHD and HD received no further treatment; the two lower-dose groups received reductions (i.e., to 3.5 and 28.2 µg/kg/day). Further, the MLD, MHD and HD groups were terminated during the period of weeks 95 to 96 because the number of surviving rats went down to 15/group. The C and LD female groups were sacrificed in week 105.

The Agency concurred with the dose adjustments in females. Terminations of the groups met the criteria outlined in the pharmacology reviews completed by Dr. Larry Sancilio on September 17 and 23, 2009. Mortality data indicated that the MTD has been exceeded. The memo completed by Dr. Larry Sancilio on September 17, 2009 indicated that the respective survival rate in the C, LD, MDL, MDH and HD groups in week 83 was 77%, 80%, 78%, 58% and 58% in males and 85%, 62%, 45% 47% and 42% in females. Dose-related increases in the incidence of tumors in the pituitary gland and mesovarian ligament were also observed. The respective number of decedent rats that showed pituitary tumors in the same week in the C, LD, MDL, MHD and HD groups was 6, 5, 5, 16 and 17 in males and 8, 16, 29, 28 and 25 in females. Overall, the protocol deviations of the rat study had no negative impact on the evaluation and interpretation of the study results. This study is considered adequate in evaluating the carcinogenicity effects of vilanterol in rats.

**Appropriateness of Test Models:** The model was appropriate. The ECAC accepted the model in a meeting held on August 21, 2007. See meeting minutes of the ECAC meeting for details.

**Evaluation of Tumor Findings:** Vilanterol is an epigenetic carcinogen in rats. Rats exposed to vilanterol by nose-only inhalation in this traditional 2-yr bioassay showed dose-related increases in the incidences of deaths, pituitary tumors, and pituitary tumor-related deaths in both sexes; and mesovarian leiomyomas in females. Significant effects were observed at vilanterol doses  $\geq 223 \mu\text{g}/\text{kg}/\text{day}$  in males and  $\geq 84/28.2 \mu\text{g}/\text{kg}/\text{day}$  in females, respectively. No increases in tumor incidences were observed at lower doses ( $\leq 84 \mu\text{g}/\text{kg}/\text{day}$  in males and  $10.5 \mu\text{g}/\text{kg}/\text{day}$  in females, respectively).

The study has shown positive evidence of tumorigenicity. Both males and females showed dose-related increases in the incidences of pituitary tumors, decreases in the latency of these tumors, and increases in the deaths caused by these tumors (Table 14). The females also showed dose-related increases in the incidence of mesovarian leiomyomas. Both the tumors in both the pituitary gland and mesovarian ligaments appear to be effects of beta-2 agonists in rodents.

**Table 14: Pituitary Tumors in the Rat Study**

Section	Tumor/time	Sex	Prevalence (incidence) <sup>a</sup>				
			0	10.5/3.5	84.4/28.2	223	657
Overall	Adenoma	M	70% (42)	62% (37)	60% (42)	65% (39)	75% (45)
		F	75% (45)	73% (44)	78%** (47)	80%** (48)	88%** (53)
	Adenoma + carcinoma	M	75% (45)	68% (41)	72% (42)	67% (40)	77% (46)
		F	90% (54)	92% (55)	90%** (54)	95%** (57)	100%** (60)
Dead rats (adenoma + carcinoma)	Pre-terminal	M	45% (19/42)	50% (18/36)	59% (19/32)	60% (25/42)	65% (26/42)
		F	88% (23/26)	59% (20/34)	78% (35/42)	91% (41/45)	82% (37/45)
	Week 78	M	20% (2/10)	25% (2/8)	40% (5/12)	60% (12/20)	71% (15/21)
		F	100% (7/7)	56% (10/18)	79% (19/24)	91% (24/25)	82% (23/27)

\*\* Statistically significant ( $p < 0.01$ ) for pair-wise comparison using Peto's one side trend test.

a. The denominator = 60 unless specified.

An analysis of overall tumor incidence data alone may understate the significance of the finding because of the high background incidence of the tumors, especially in females. Table 14 presents the summary data of pituitary tumors. In week 78, the incidence of deaths in the C, LD, MLD, MHD, and HD groups (of 60 rats/sex/group) was 10, 8, 12, 20 and 21 in males and 7, 18, 24, 25 and 27 in females. Among them, the death rate due to pituitary tumors was 20%, 25%, 40%, 60%, and 71% in the control, LD, MLD, MHD, and HD groups in males, respectively. The females; however, showed no dose-response relationship (56% - 100%) for death rate due to pituitary tumors. Despite the clear dose-response relationship in death rate in males during the study (e.g., week 78), there were no significant difference in the overall incidence of pituitary tumors (adenomas and carcinomas), which ranged from 68% - 77% in males and 90% - 100% in females. Further, the overall pituitary tumor incidence was not much different from the historic background values which ranged between 80 - 100%.<sup>4</sup>

<sup>4</sup> The historic background data in the laboratory during the period of 2002 - 2007: the respectively rates of pituitary adenoma and carcinoma (pars distalis) was 58% (556/955, ranged 33% - 80%) and 1.8%

The above discussions indicate that interpretation of the study results based on the overall tumor data was misleading because of the high spontaneous rate of pituitary tumors in aged rats. Analyses of time-course of the tumors were more indicative of any drug effects. Also, there were no clear separations in dose-response curves of the 3 top doses in females. The lack of separation was probably due to the adjustments of vilanterol doses and excessive mortalities in these groups as alluded to earlier. These adjustments were appropriate as discussed in the review completed by Dr. Larry Sancilio on September 17, 2009. The number of survival rats went down to 15 in each of the groups (i.e., 84/28.2 – 657 µg/kg/day groups). In addition, these groups showed increases in incidences of pituitary and ovary tumors and in the pituitary tumor-related mortalities.

The pituitary tumors are not the only positive findings of the study. Female rats treated with ≥ 84/25-µg/kg/day vilanterol also showed statistically significant increases in mesovarian leiomyomas (incidence: 0, 0, 5, 4, 4 in the C, LD, MLD, MHD and HD groups, respectively). Mesovarian leiomyomas are beta-agonist-associated tumors that are rodent specific and irrelevant to humans.

The tumorigenic findings correlated well with the necropsy and microscopic finding in the brain, ovaries and mesovarian ligaments. Both necropsy and microscopic examinations reveals dose-related increases in the incidence and degree of brain tissue compression and ovarian cysts. The females also showed hypertrophy and hyperplasia of the smooth muscles in the mesovarian ligaments.

Vilanterol tested negative in a number of in vivo and in vitro assays of genetic toxicity testing. Vilanterol caused dose-related increases in pituitary tumors and mortality from these tumors in male and female rats. The drug also caused mesovarian leiomyomas in female rats. Mesovarian leiomyomas are typical effects of beta agonists in rodents. Vilanterol is a beta 2 receptor agonist. The evidence demonstrated that vilanterol is an epigenetic carcinogen in rats.

### Interpretation of tumor findings in rats to humans

Reference Report 2010N209253 showed that vilanterol treatment caused dose-related increases in pituitary tumors in both sexes and mesovarian tumors in females in rats. The applicant argued that neither is relevant to humans. The argument is generally correct based on the available evidence; however, the review will use the statement that relevance of the rat tumor findings to humans are unknown to be consistent with the labeling of the currently marketed LABAs.

**Pituitary tumors:** The applicant argued that dose-related increases in pituitary tumors were class effects of beta agonists in rats. GSK referenced salmeterol, a currently marketed long acting beta 2 agonist to support its argument. GSK resubmitted a report of 2-year carcinogenicity study salmeterol in rats (GSK Reference Report #PWT/89/026). GSK also submitted GSK Reference Report #2011N115341 that summarizes salmeterol data and discussed the relevance of these findings rat findings to humans.

GSK Reference Report #PWT/89/026 (Glaxo Study #R11169, GXO 209/89601) was previously submitted to NDA 20-236 and IND (b)(4). Dr. Larry Sancilio completed a review

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(17/955, ranged 0% - 8.3%) in males, 78% (748/955, ranged 58% - 88%) and 7.1% (68/955, ranged 1.7 – 15%).

of the study on May 28, 1991 in IND (b)(4) and summarized the findings in a review completed on July 9, 1992 in NDA 20-236. Briefly, rats (60/sex/dose, CD) were dosed with salmeterol by both oral and inhalation routes of administration. The respective salmeterol dose in the C1 (air control), C2 (vehicle control), LD, MD and HD groups was 0, 0, 0.06, 0.18 and 0.58-mg/kg/day in inhaled doses and 0, 0, 0.15, 0.5 and 2.0 mg/kg/day in oral doses. Dose-related increases in the incidence of pituitary tumors were observed in both sexes.

Table 15 presents the overall incidences of pituitary tumors in rats treated with salmeterol and vilanterol. Figure 5 presents the time-course of the number of rats that died of pituitary tumors in each group during the study in male rats treated with salmeterol and vilanterol. The salmeterol data were from taken from GSK Reference Report #2011N115341.

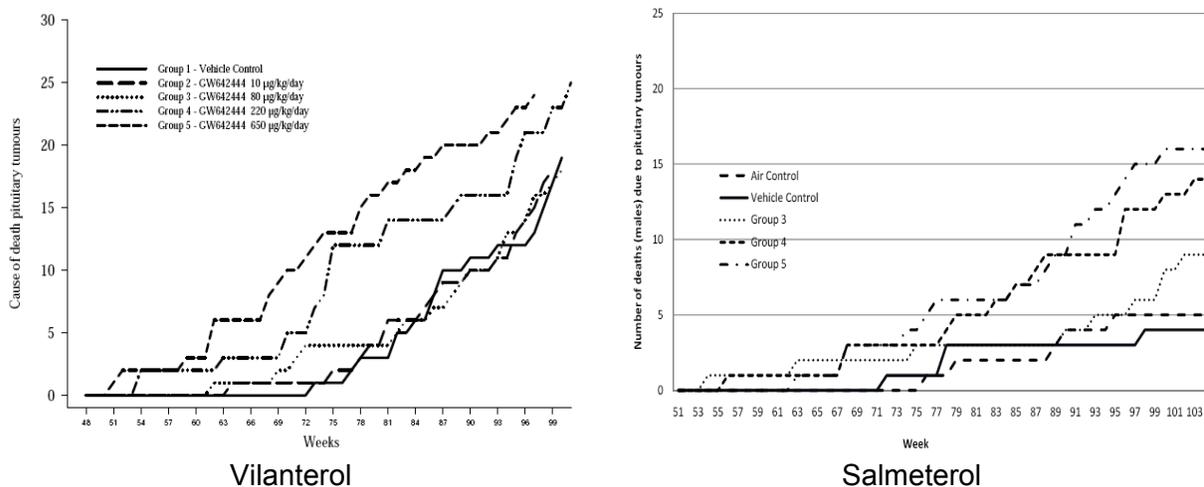
A comparison of data in Table 15 and Figure 6 showed similarities and two differences between the vilanterol and salmeterol studies. Major similarities were dose-related increases in the tumor incidence and shortening of the tumor latency period. The differences were the background tumor incidences and the tumor locations. Specifically, the vilanterol study had higher background tumor incidences in the control groups and higher overall pituitary tumor rate than salmeterol. Regarding the tumor location, salmeterol caused adenomas in pars anterior while vilanterol in pas distalis.

**Table 15: Pituitary tumors in Salmeterol and Vilanterol Studies in Rats**

Group	Vilanterol					Salmeterol				
	1	2	3	4	5	1	2	3	4	5
LABA, IH (mg/kg/day)	0	0.01	0.84	0.22	0.66	0	0	0.06	0.18	0.58
LABA, PO (mg/kg/day)	-	-	-	-	-	0	0	0.15	0.50	2.0
M Pit. Adenoma (A)	42	37	42	39	45	19	18	24	33*	30*
Pit. Carcinoma (B)	3	7	5	9	8	1	1	0	0	1
Total ( A + B)	45	44	47	48	53	20	19	24	33*	31*
F Pit. Adenoma (A)	45	44	47**	48**	53**	28	35	38	34	42*
Pit. Carcinoma (B)	9	11	14	17	60**	6	1	6*	3	4
Total ( A + B)	54	55	54**	57**	60**	34	36	44*	37	46*

\*, p = 0.05; \*\* p < 0.01.

A comparison of data in Table 15 and Figure 6 showed similarities and two differences between the vilanterol and salmeterol studies. Major similarities were dose-related increases in the tumor incidence and shortening of the tumor latency period. The differences were the background tumor incidences and the tumor locations. Specifically, the vilanterol study had higher background tumor incidences in the control groups and higher overall pituitary tumor rate than salmeterol. Regarding the tumor location, salmeterol caused adenomas in pars anterior while vilanterol in pas distalis.



**Figure 5: The number of pituitary tumor-related deaths in salmeterol and vilanterol treated rats (n = 60/group).**

The pituitary tumor finding, however, was not described in the salmeterol label.<sup>5</sup> The exact reason for not including the finding was unclear.<sup>6</sup> Regardless of the reasoning, GSK's argument that the pituitary tumor was a class effect of beta agonists appears reasonable because salmeterol and vilanterol showed similar effects in rats.

**Mesovarian leiomyomas:** Female rats treated with  $\geq 84/25$ - $\mu\text{g}/\text{kg}/\text{day}$  vilanterol also showed statistically significant increases in mesovarian leiomyomas (incidence: 0, 0, 5, 4, 4 in the C, LD, MLD, MHD and HD groups, respectively). Mesovarian leiomyomas is a finding typical of LABAs and its relevance to human use is unknown (Ref. Serevent label, NDA 20-236, See Footnote 6). No additional discussion is necessary.

<sup>5</sup> Serevent (Salmeterol, NDA 20-236) label approved on September 28, 2004 states: "In a 24-month inhalation and oral carcinogenicity study in Sprague Dawley rats, salmeterol caused dose-related increases in the incidence of mesovarian leiomyomas and ovarian cysts at inhalation and oral doses of 0.68 mg/kg/day and above (approximately 55 times the maximum recommended human daily inhalation dose in adults on a mg/m<sup>2</sup> basis). No tumors were seen at 0.21 mg/kg/day (approximately 15 times the maximum recommended human daily inhalation dose in adults on a mg/m<sup>2</sup> basis). These findings in rodents are similar to those reported previously for other beta-adrenergic agonist drugs. The relevance of these findings to human use is unknown."

<sup>6</sup> The review completed by Dr. Larry Sancilio on July 9, 1992 in NDA 20-236 states (p137): "The neoplasm seen in M was pituitary adenomas (LD, HD). This may be compound related although pituitary adenomas are common in these rats at this age. However, as the incidence of adenomas increased with dose, there was a corresponding decrease in the incidence of hyperplasia in the pars anterior. This suggests that salmeterol may have enhanced the progression from hyperplasia. Further, the rats that died revealed a high incidence of ventral depression. This was probably caused by the enlarged pituitary glands found in these animals.... As seen in the M, there was an increase in the incidence of pituitary tumor (HD) [in the females]..." The Nonclinical labeling review completed by Dr. Sancilio on April 26, 1993 in NDA 20-236 has no mentioning of the pituitary findings in rats. There was no documentation about the discrepancies between the two reviews.

## Methods

<i>Doses:</i>	Achieved dose: 0, 10.5/3.47, 84.4/28.2, 223 and 657 µg/kg/day of vilanterol trifenate (GW642444M).
<i>Frequency of dosing:</i>	60 min/episode/day
<i>Dose volume:</i>	Not applicable
<i>Route of administration:</i>	Nose-only inhalation exposure
<i>Formulation/Vehicle:</i>	Vilanterol powder in lactose; vilanterol concentration (w/w): 0, 0.4%, and 20% for Groups 1, 2 - 3, and 4 – 5, respectively.
<i>Basis of dose selection:</i>	A 3-month inhalation dose-finding study in which 1000 µg/kg/day was found to be irritating to the respiratory tract
<i>Species/Strain:</i>	Male and female Crl:CD (SD) rats
<i>Number/Sex/Group:</i>	60/sex/group
<i>Age:</i>	6 weeks old on day 1 of treatment
<i>Weight at start of exposure</i>	Means of 255.2 – 265.8 g and 181.5 - 196.7 g in males and females at the beginning of exposure, respectively
<i>Animal housing:</i>	2 – 3 rats per cage
<i>Paradigm for dietary restriction:</i>	No caloric restriction. Certified Rodent Diet No. 2016C pellets at lib except during the inhalation exposure period
<i>Dual control employed:</i>	No
<i>Chamber aerosol drug concentration:</i>	Gravimetric (daily) and chemical analysis (daily for the first 7 weeks, and 3 times/week thereafter)
<i>Aerosol particle distribution:</i>	Monthly except for week 1 and 3 (once/week)
<i>Satellite groups:</i>	9/sex/group used for TK analysis; see TK section
<i>Toxicokinetics:</i>	3/sex/time point on weeks 4 and 26 (0.08, 0.5, 1, 2, 4, 8 and 23 hours after the end of exposure)
<i>Immunochemistry staining of pituitary masses:</i>	Selected pituitary masses (9 – 13/sex/group) in control and HD groups were examined immunochemically for prolactin activity and growth hormone levels.
<i>Deviation from study protocol:</i>	There were some minor deviations. The deviations do not affect the outcome of the study.

## Study Design:

Rats (60/sex/dose) were exposed via nose-only inhalation to GW642444 at achieved doses of 0 (lactose), 10.5, 84.4, 223 or 657 µg/kg/day for up to 104 weeks. Due to excessive mortality, both the vilanterol treatment duration and dose were adjusted during the study. All males were terminated in week 101 without adjusting vilanterol dose levels because the number of surviving animals in the control group had gone down to 18. More complicated adjustments were made in the females: the vilanterol treatment in the two top-dose groups was stopped in week 85; the vilanterol doses in the two lower-dose groups were reduced to 3.5 and 24.4 µg/kg/day in week 86; and the time of sacrifice was weeks 104, 104, 95, 96 and 96 for C, LD, MLD, MHD, and HD groups, respectively. The 3 top-dose groups were terminated early because the number of survivors had gone down to 15 in each group. Table 16 summarizes the study design.

**Table 16: Study Design of the Rat Study**

	Sex	Time (week)	Group				
			1	2	3	4	5
Number of rats /sex			60	60	60	60	60
# Rats for TK/sex			9	9	9	9	9
Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ ) <sup>a</sup>	M	1 - 101	0	10.5	84.4	223	657
		F	0	10.5	84.4	223	657
		86 - 95	0	3.5	28.2	-	-
		97 - 104	0	3.5	NA <sup>c</sup>	NA	NA
Time of group termination	M		101	101	101	101	101
	F		104	104	95	96	96

a. Achieved dose.

b. -, not treated.

c. NA, not applicable because the group has been terminated.

## Observations and Results

**Dosimetry and aerosol characteristics:** Gravimetric (daily) and chemical analysis (daily for the first 7 weeks, and 3 times/week thereafter) of aerosol drug concentrations were performed. Aerosol particle size characterization was done monthly except for the first month (once in weeks 1 and 3 each). Table 17 summarizes the exposure conditions and vilanterol dosimetry. The achieved vilanterol aerosol concentrations were quite close to the target concentrations. The MMAD was acceptable, although the MMAD for the MHD group was less than optimal. The reason is that overt drug effects were observed in this and other groups. The achieved dose was estimated based on the exposure duration of 60 minutes/day.

**Table 17: Dosimetry of the Rat Study**

	Group				
	1	2	3	4	5
Vilanterol dose ( $\mu\text{g}/\text{kg}/\text{day}$ ), target	0	10/3	80/25	220	650
Achieved <sup>a</sup>	0	10.5/3.5	84.4/24.5	223	657
Vilanterol chamber concentration					
Target ( $\mu\text{g}/\text{L}$ )	0	0.28	2.40	6.16	18.2
Achieved ( $\mu\text{g}/\text{L}$ ) <sup>b</sup>	0	0.30	2.41	6.44	18.9
MMAD ( $\mu\text{m}$ ) <sup>c</sup>		3.7	2.8	4.9	3.9
GSD		2.2	2.3	2.0	2.0
Particles of respirable fraction (%)		$\geq 61$	$\geq 74$	$\geq 49$	$\geq 63$
The duration of exposure (min)	60	60	60	60	60

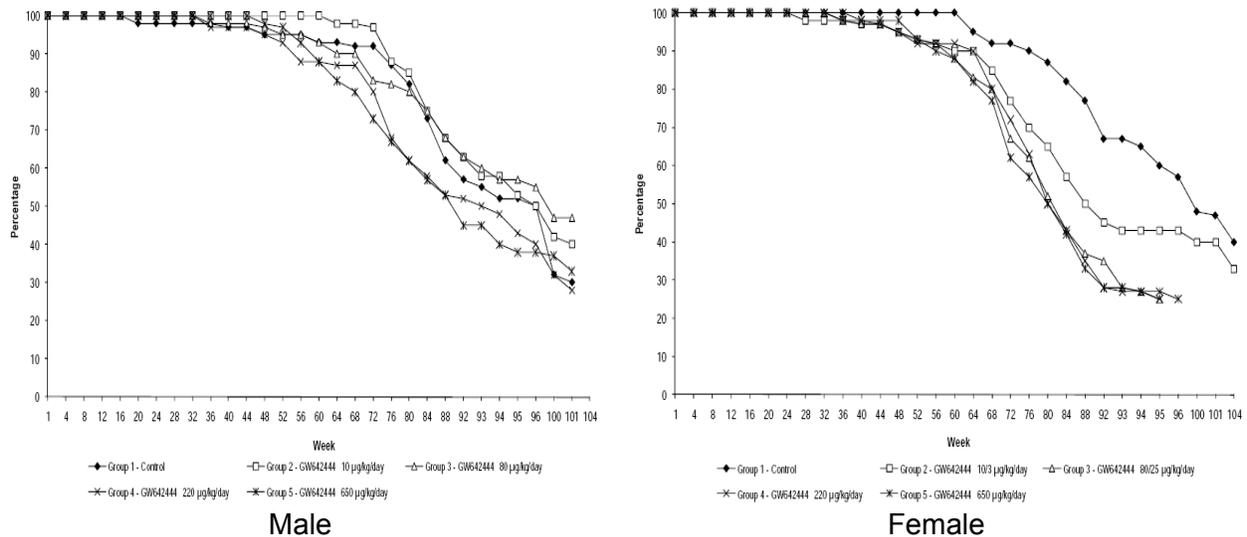
a. Based on chemical analysis results. These numbers were from Study Design Table on page 43 of the study report.

b. Results of chemical analysis.

c. Extracted from Group Particle Size Distribution Table (page 71) of the report.

**Mortality:** Mortality was observed twice a day. Dose-related increases in mortality were observed in both sexes. Figure 6 presents survival curves in males and females. In males, the MHD and HD groups generally showed high mortality during the study (prior to week 96), although the mortality did not differ from the control at the terminal sacrifice. The lack of differences in overall mortality in males was apparently attributed to the sudden increases in mortality in the vehicle group during the period of the last 3 – 4 weeks. In females, the

mortality in Groups 3 – 5 was so high that these groups were terminated during the period of weeks 95 – 96. Only 15 rats remained in each group at the time of termination.



**Figure 6: Survival curves in rats. Groups 4 and 5 females were not treated during the period of week 85 – 95.**

Table 18 summarizes the overall numerical survival data at milestones used for constructing Figure 6. A dose-response relationship in males was apparent in the period of weeks 52 – 96, but not in the period of weeks 97 – 101, because of the sudden increase in mortality in the control group. The reason for the sudden increase in mortality in the male control group is unknown.

**Table 18: Mortality Summary of the Rat Study**

Vilanterol (µg/kg/day)	Male					Female				
	0	10.5	84	223	657	0	10.5 <sup>#</sup>	84 <sup>\$</sup>	223	657
# of Deaths (total)	42	36	32	43	40	37	42	45	45	45
# Survivor at termin.	18	24	28	17	20	23	18	15	15	15
% Survival, terminal <sup>a</sup>	30	40	47	28	33	38	30*	25**	25**	25**
% Survival, wk 52	95	100	95	93	97	100	93	93	92	93
, wk 64	93	98	90	87	83	95	90	83	90	82
, wk 84	73	75	75	58	57	49	57	43	43	42
, wk 95	52	53	57	43	38	60	43	25	25	27
, wk 96	50	50	55	40	38	57	43	- <sup>b</sup>	-	25
, wk 101	30	40	47	28	33	47	40	-	-	-
, wk 104	-	-	-	-	-	40	33	-	-	-

a. Time for the terminal sacrifice was weeks 101 in all male groups; and weeks 95, 96 and 104 in female Groups 3, 4, and 5, respectively. The final survival rates were extracted from the Pathologist report (p1244).

b. -, no data available because the group has been terminated.

\*, p < 0.05; \*\*, p < 0.01.

<sup>#</sup>, the dose was reduced to 3.5 µg/kg/day from week 85 onward.

<sup>\$</sup>, this dose was reduced to 28.8 µg/kg/day from week 85 onward.

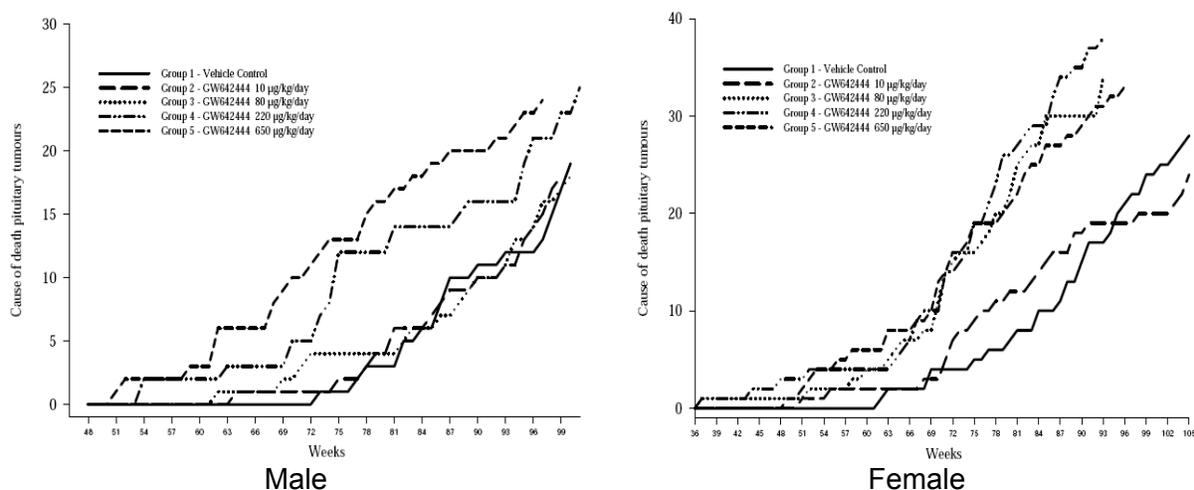
**Cause of Deaths:** Pituitary tumors (adenoma) were the primary cause of deaths. The prevalence of pituitary tumor-related deaths ranged 35 – 60% in males and 55 – 84% in females (Table 19). The males showed a clear dose-response relationship in pituitary

tumor-related deaths: 35%, 50%, 56%, 58% and 60% in C, LD, MLD, MHD, and HD groups, respectively.

**Table 19: Causes of Pre-terminal Deaths in the Rat Study**

Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Male					Female				
	0	10.5	84	223	657	0	10.5	84	223	657
# Deaths	42	36	32	43	40	37	42	45	45	45
#Death due to p. tumors	19	18	18	25	24	28	23	34	38	33
% of pit. tumor deaths	35.2	50	56.3	58.1	60	75.7	54.8	75.6	84.4	73.3
Cause undetermined	5	8	8	5	8	-	-	-	1	2
Mammary neoplasm	-	-	-	-	-	6	13	6	4	4
Other neoplasm	7	4	4	8	5	2	5	1	-	6
Non-neoplastic causes	11	6	2	4	3	1	-	4	2	1

Figure 7 shows a time-course of pituitary tumor-related deaths. Both males and females showed dose-response and time-response in tumor-related mortality, although there was no clear separation among 3 top doses in females. The primary reason for the lack of dose-response in top doses in females appears to be the modification of vilanterol doses during the study. The dose-response in the males was most striking in the middle phase of the study.



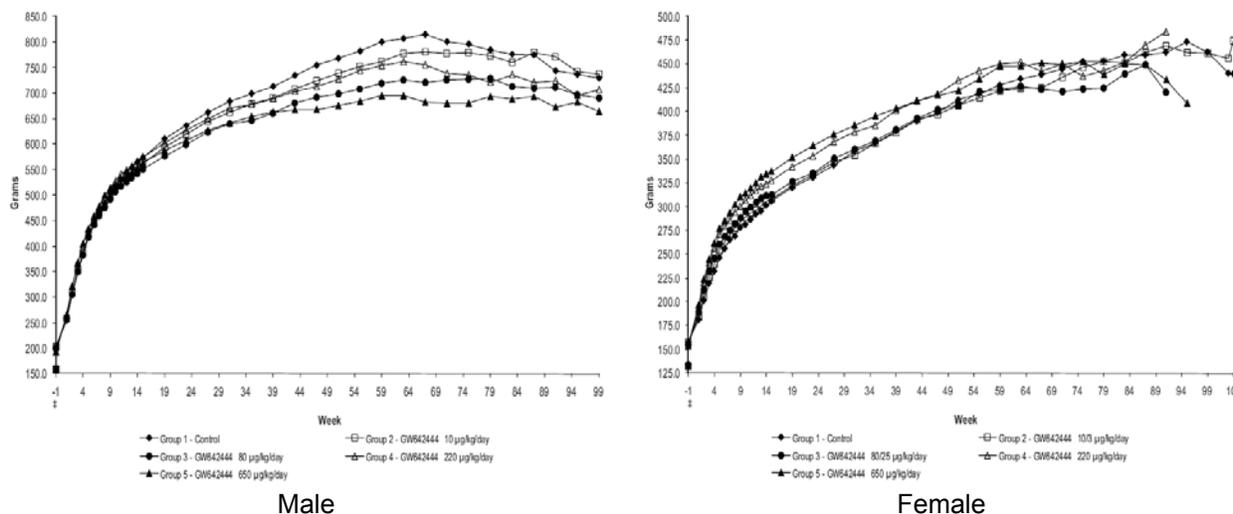
**Figure 7: Pituitary tumor-related deaths in rats. Groups 4 and 5 females were not treated during the period of week 85 – 95.**

Overall, vilanterol treatment increased the incidence of pituitary tumor-related deaths in males at  $\geq 223 \mu\text{g}/\text{kg}/\text{day}$  and in females at  $\geq 84.4/23.3 \mu\text{g}/\text{kg}/\text{day}$ . See the Pituitary Tumor section (later) for additional discussion.

**Clinical Signs:** Clinical signs were observed daily. Physical examinations and palpation for tissue masses were done weekly. No treatment-related effects were observed.

**Body Weight:** Body weights were recorded twice (weeks 1 and 2) or once per week (weeks 3 to 16), or monthly thereafter. The final body weight was recorded on the day of necropsy. A bell-shaped curve of body weight in dose response relationship was apparent. Such a

phenomenon is typical of beta agonist effects in rodents. Particularly, Group 3 (mid dose) in both sexes had the highest increases in body weights. The HD had decreases or no significant changes in body weights for majority of the treatment period. Figure 8 presents the body weight – time curve in males and females over the course of the study.



**Figure 8: Body weight-time course curve in rats.**  
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Table 20 summarizes the body weights and weight gains at several milestones of the study. Statistically significant increases in body weights and weight gains were observed in treatment groups and the highest increases generally occurred in the 223 µg/kg/day group

**Table 20: Body Weights and Weight-Gains in Rat Study**

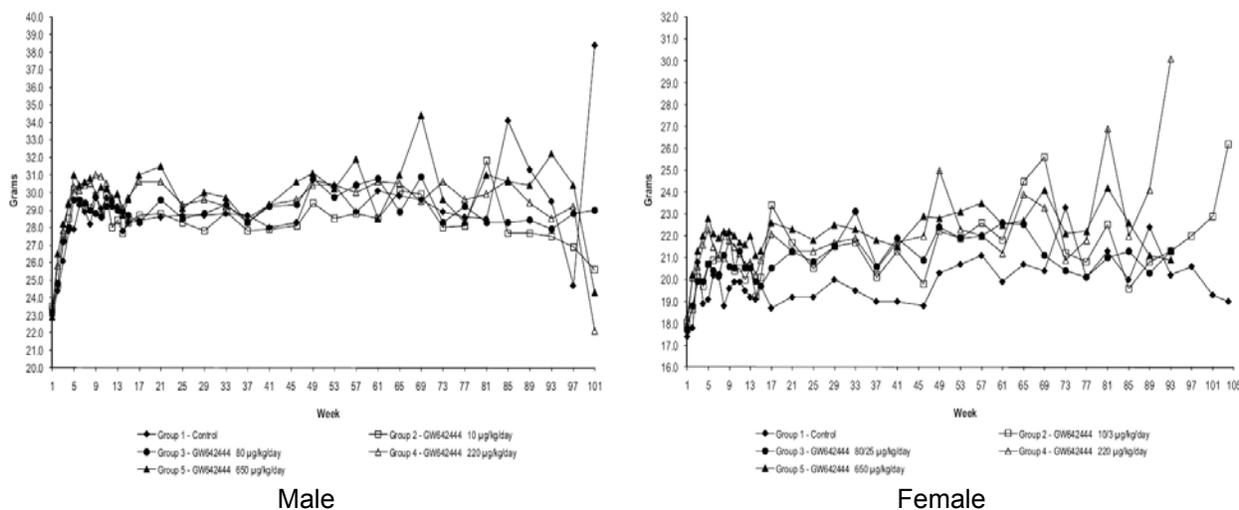
Estimated Achieved Dose <sup>a</sup> (µg/kg/day)	Male					Female				
	0	10.5	84.4	223	657	0	10.5/3.47	84.4/28.2	223	657
<b>Body Weight (X control)<sup>c</sup> g</b>										
Week 4	382.1	1.01	1.00	1.05 C	1.06 C	232.6	1.03	1.06 C	1.10 C	1.13
Week 8	484.3	1.00	0.98	1.03	1.03	269.5	1.03	1.05 A	1.09 C	1.12 C
Week 19	610.2	0.97	0.94 B	0.99	0.96	320.3	1.01	1.02	1.07 B	1.10 C
Week 43	734.6	0.96	0.93 B	0.96	0.91 C	390.6	1.00	1.01	1.05	1.05
Week 83	776.0	0.98	0.92	0.95	0.89 B	459.7	0.98	0.96	0.98	0.98
Week 91	743.6	1.04	0.96	0.97	0.90 A	462.5	1.01	0.91	1.05	0.94
Week 99	729.3	1.02	0.95	0.97	0.91	462.5	1.00	-	-	-
Week 104	-	-	-	-	-	439.7	1.08	-	-	-
<b>Body Weight Gain (X control)<sup>c</sup> g</b>										
Start-Week 1	51.9	1.06 A	1.12 C	1.40 C	1.39 C	23.3	1.09	1.50 C	1.70 C	1.82 C
Start-Week 4	178.8	1.03	1.03	1.17 C	1.19 C	74.4	1.09 A	1.23 C	1.38 C	1.44 C
Start-Week 8	281.0	1.00	0.98	1.09 C	1.09 C	111.3	1.08 A	1.15 C	1.26 C	1.33 C
Start-Week 19	407.0	0.96	0.92 B	1.01	0.97	162.1	1.01	1.06	1.16 C	1.22 C
Start-Week 27	459.1	0.96	0.92 B	0.99	0.95	185.8	1.02	1.06	1.15 C	1.19 C
Start-Week 43	531.3	0.95	0.91 B	0.96	0.90 C	232.4	1.00	1.03	1.11 A	1.10
Start-Week 83	574.8	0.97	0.90	0.95	0.86 A	300.9	0.98	0.95	0.99	0.98
Start-Week 91	542.6	1.05	0.94	0.99	0.89	304.0	1.03	0.87	1.09	0.92
Start-Week 99	529.1	1.01	0.93	0.97	0.89	304.6	1.00	-	-	-
Start-Week 104	-	-	-	-	-	281.9	1.13	-	-	-

**Food Consumption:** Changes in food consumption were generally reflective of body weight changes, but to a lesser degree. Table 21 summarizes the food consumptions during the study. Figure 9 is a graphic presentation of the food consumption data of the study.

**Table 21: Food Consumption in Rat Study**

Estimated Achieved Dose <sup>a</sup> (µg/kg/day)	Male					Female				
	0	10.5	84.4	223	657	0	10.5/3.47	84.4/28.2	223	657
<b>Food Consumption (X control)<sup>c</sup> g/animal</b>										
Start-Week 4	548.7	1.02	1.02	1.06 F	1.07 F	402.0	1.02	1.02	1.07 F	1.10 F
Start-Week 8	1348.3	1.03	1.03	1.06 C	1.07 C	948.3	1.05 A	1.04	1.08 C	1.12 C
Start-Week 21	3156.7	1.01	1.01	1.05 C	1.05 C	2170.1	1.06 C	1.05 A	1.08 C	1.12 C
Start-Week 46	4360.2	1.00	1.01	1.04 B	1.05 B	2977.3	1.07 C	1.06 C	1.09 C	1.13 C
Start-Week 85	6343.5	0.99	1.01	1.04 A	1.04 A	4439.8	1.05 A	1.05	1.08 B	1.11 C
Start-Week 93	6797.6	0.98	1.01	1.03	1.04	4726.1	1.05	1.05	1.08 B	1.10 C
Start-Week 101	7206.7	0.97	0.99	1.01	1.02	4999.5	1.05 G	-	-	-
Start-Week 104	-	-	-	-	-	5045.3	1.08 H	-	-	-
<b>Hormone Analysis (Week 55)</b>										
Dihydrotestosterone (pg/mL)	241	233	261	196	178	-	-	-	-	-
Estradiol (pg/mL)	47	50	44	44	47	73	133 A	98	90	94
Testosterone (ng/mL)	1.27	1.51	1.31	1.10	0.82	0.22	0.20	0.16	0.10	0.06

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**Figure 9: Food consumption-time course curve in rats.**

**Ophthalmology:** Ophthalmic examinations using slit lamp biomicroscopy were done at pre-dosing, weeks 13, 52, 78 and 100. No treatment-related effects were observed.

**Toxicokinetics:** Plasma levels of vilanterol and 3 metabolites were determined in 3 mice/sex/time point on weeks 4 and 26 (0.08, 0.5, 1, 2, 4, 8 and 23 hours after the end of exposure). The metabolites included GSK93209, GW630200 and GI179710. Dose-related increases in plasma levels of vilanterol and its metabolites were observed. Table 22 summarizes the results.

**Table 22: Plasma Levels of Vilanterol and Metabolites in Rat Study**

Parameter+	Week	Male				Female			
		Estimated Achieved Dose				Estimated Achieved Dose			
		10.5 µg/kg/day	84.4 µg/kg/day	223 µg/kg/day	657 µg/kg/day	10.5 µg/kg/day	84.4 µg/kg/day	223 µg/kg/day	657 µg/kg/day
<b>GW642444</b>									
AUC <sub>(0-24)</sub> (ng.h/mL)	Week 4	NC	5.63	14.4	36.0	NC	6.94	18.5	38.4
	Week 26	0.839	11.4	19.3	67.5	0.429	12.5	18.9	72.9
C <sub>max</sub> (ng/mL)	Week 4	0.302	2.84	7.47	12.5	0.255	3.33	6.73	13.3
	Week 26	1.04	4.41	6.56	26.8	0.381	5.79	6.89	32.5
<b>GW630200</b>									
AUC <sub>(0-24)</sub> (ng.h/mL)	Week 4	NC	NC	NC	NC	NC	NC	NC	6.21
	Week 26	NC	NC	NC	NC	NC	NC	NC	NC
C <sub>max</sub> (ng/mL)	Week 4	0.502	0.644	1.21	1.65	0.492	0.225	1.24	2.37
	Week 26	1.90	NC	NC	NC	NC	NC	NC	NC
<b>GSK932009</b>									
AUC <sub>(0-24)</sub> (ng.h/mL)	Week 4	NC	NC	NC	3.03	NC	NC	NC	2.13
	Week 26	NC	NC	0.202	0.614	NC	NC	0.308	1.80
C <sub>max</sub> (ng/mL)	Week 4	NC	NC	0.145	0.436	NC	0.122	0.169	0.521
	Week 26	NC	NC	0.147	0.370	NC	0.0878	0.183	0.670
<b>GI179710</b>									
AUC <sub>(0-24)</sub> (ng.h/mL)	Week 4	NC	NC	NC	95.3	NC	NC	NC	174
	Week 26	NC	NC	18.4	36.0	NC	NC	70.0	240
C <sub>max</sub> (ng/mL)	Week 4	NC	NC	11.2	33.0	NC	7.53	14.9	60.4
	Week 26	NC	5.87	10.2	34.5	NC	14.6	21.6	91.3

+ Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose  
 NC: Not calculated due to insufficient data

Vilanterol and GI179710 accounted for nearly all drug related material in the plasma. Little or no GSK932009 and GW632200 ( $\leq 0.6\%$ ) was present. Table 23 summarizes the plasma AUCs and percentage of vilanterol and metabolites in plasma in week 26. Vilanterol and GSK932009 counted for approximately 10 – 22% and 77% - 90% of the total material. Only the two high dose groups were calculated because data in the low dose groups were incomplete.

**Table 23: Proportion of Vilanterol and Metabolites in the Rat Plasma (week 26)**

Vilanterol (µg/kg/day)	Plasma AUC (µg.h/mL) <sup>a</sup>				Percent (%) of Total AUC			
	Male		Female		Male		Female	
	223	657	223	657	223	657	223	657
GW642444	19.3	67.5	18.9	72.9	50.9	64.8	21.2	23.2
GSK932009	0.20	0.61	0.31	1.8	0.5	0.6	0.3	0.6
GI179710	18.4	36.0	70	240	48.5	34.6	78.5	76.3
Total	37.9	104.1	89.2	314.7	100	100	100	100

a. Taken from Table 9. Only the two high dose groups were calculated because data in the low dose groups were incomplete.

**Necropsy:** Survival rats were killed in weeks 101 (males) or weeks 95 – 104 (females). Necropsy was conducted on all rats (terminal and pre-terminal killing). A complete panel of organs/tissues was collected in each rat. Major organs were examined for masses. The males showed dose-related increases in pituitary masses while the females showed no such a trend. Table 24 presents the summary results of incidences of pituitary masses between pre-terminal killing, terminal killing and overall incidence. The respective overall incidence of pituitary masses in the C, LD, MLD, MHD, and HD groups was 43%, 35%, 40%, 52% and 57% in males; and 75%, 75%, 80%, 85% and 78% in females. Also, males in both the pre-terminal death and terminal killing sections showed dose-related increases in the incidence of pituitary tumors.

**Table 24: Pituitary Masses by Gross Examinations in the Rat Study**

Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Male					Female				
	0	10.5	84	223	657	0	10.5	84	223	657
Overall mass incidence	26	21	24	31	34	45	45	48	51	47
Prevalence (%)	43.3	35	40	51.7	56.7	75	75	80	85	78.3
Pre-terminal: # deaths	42	36	32	43	40	37	42	45	45	45
# Pituitary neoplasm	23	18	18	25	24	28	23	34	38	33
Prevalence (%) of pit. tumors	54.8	50	56.3	58.1	60	75.7	54.8	75.6	84.4	73.3
Terminal: # killed	18	24	28	17	20	23	18	15	15	15
# w/ pituitary neoplasm	3	3	7	5	8	13	14	9	10	12
Prevalence (%) of pit. tumors	16.7	12.5	25	29.4	40	56.5	77.7	60	66.7	80

The females also showed dose-dependent increases in the incidence of ovarian cysts. Table 25 presents the number of ovarian cysts in females upon necropsy. The ovarian cysts were present in 7, 15, 21, 34 and 38 rats in the C, LD, MLD, MHD and HD groups, respectively.

**Table 25: Ovarian Cysts in Rat Study**

Vilanterol dose ( $\mu\text{g}/\text{kg}/\text{day}$ )	Incidences of Ovary Cysts				
	0	10.5/3.5	84.4/24.5	223	657
Decedent	3/37	7/42	14/45	25/45	27/45
Terminal killing	4/23	8/18	7/15	9/15	11/15
Total	7/60	15/60	21/60	34/60	38/60

**Microscopic examinations:** A complete panel of organs/tissues was examined microscopically for non-neoplastic and neoplastic changes in each rat. Treatment-related non-neoplastic and neoplastic findings were observed in both sexes. Non-neoplastic changes were observed in the ovaries in female rats. Neoplastic changes were observed in pituitary gland in both sexes and in the ovaries in females. Appendices A and B (pages 44 - 48) present the overall tumor incidences in males and females.

**Neoplastic changes:** A complete panel of organs/tissues was examined microscopically for non-neoplastic and neoplastic changes. Neoplastic changes were observed in the pituitary gland in both sexes and in ovaries in females. Table 26 presents the incidence of these tumors among groups. There is overwhelming evidence indicating that vilanterol treatment is associated with pituitary tumors, although the overall incidence of pituitary tumors in Table 26 does not appear to be remarkably different among groups. See discussions below for the evidence.

**Table 26: Key Tumor Findings in the Rat Study**

Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Tumor incidence (N/60 rats)									
	Male					Female				
	0	10.5	84	223	657	0	10.5	84	223	657
Pituitary gland: adenoma	42	37	42	39	45	44	47	48**	51**	53**
Mesovarian ligaments: Leiomyoma	-	-	-	-	-	0	0	5*	4*	4*

\*, \*\*, Statistically different at  $p < 0.05$  and  $p < 0.01$  by pairwise comparison from the vehicle control group using Peto's one side test, respectively.

**Pituitary tumors:** Vilanterol treatment groups showed dose-related increases in the incidence of adenoma in Pars distalis of pituitary gland and in pituitary tumor-related deaths in both in males and females. Table 27 presents the number of deaths, the incidence of pituitary tumors, and the number of pituitary tumor-related deaths.

**Table 27: Pituitary Tumors (PT) and PT-related Deaths in the Rat Study<sup>a</sup>**

	Time (week)	Male					Female				
		0	10.5	84	223	657	0	10.5	84	223	657
No. of decedent rats	52 (D)	3	0	4	4	2	0	4	3	4	3
	65 (D)	4	1	6	8	10	3	6	11	6	11
	78 (D)	10	8	12	20	21	7	18	24	25	27
	86 (D)						11	29	39	43	41
	91 (D)	25	22	22	29	30	17	33	39	43	41
	95/96 (D)						26	34	45	45	45
	95/96 (T)								60	60	60
	101/4 (D)	42	36	32	42	40	37	41			
	104 (T)	60	60	60	60	60	60	60			
Adenoma Pars distalis	52 (D)	0	0	1	0	1	0	1	2	4	3
	65 (D)	0	1	3	3	8	3	3	8	5	11
	78 (D)	4	4	6	12	15	4	12	20	21	23
	86 (D)						8	20	31	28	34
	91 (D)	16	13	12	19	24	14	24	32	34	37
	95/96 (D)						20	26	37	38	39
	95/96 (T)								48	51	53
	101/4 (D)	28	22	20	29	32	26	31			
	104 (T)	42	37	42	39	45	44	47			
Carcinoma Pars distalis	52 (D)	0	0	0	0	0	0	0	0	0	0
	65 (D)	0	0	0	0	0	0	0	0	0	0
	78 (D)	0	0	1	0	1	0	0	0	1	0
	86 (D)						3	2	1	2	3
	91 (D)	2	1	1	0	1	3	4	3	4	4
	95/96 (D)						5	4	4	5	6
	95/96 (T)								6	6	7
	101/4 (D)	3	3	1	0	1	9	6			
	104 (T)	3	4	1	1	1	10	8			
Adenoma + carcinoma	Terminal	45	41	43	40	46	54	55	54	57	60
Pituitary tumor as cause of death	52 (D)	0	0	0	0	2	0	1	1	3	2
	65 (D)	0	1	2	3	8	3	3	6	6	10
	78 (D)	2	2	5	12	15	7	10	19	24	23
	86 (D)						12	16	31	32	29
	91 (D)	10	10	11	17	22	17	19	31	32	29
	95/96 (D)						23	20	35	41	37
	101/4 (D)	19	18	19	25	26	30	24			

a. Extracted from pages 64-66 of the report.

Take week 78 as an example. The respective incidence of accumulative deaths in C, LD, MLD, MHD, and HD groups in was 10, 8, 12, 20 and 21 in males; and 7, 18, 24, 25 and 27 in females. Among them, pituitary tumors were identified as the cause of deaths with the following respective incidences in C, LD, MLD, MHD, and HD groups: 2, 2, 5, 12 and 15 in

males; and 7, 10, 19, 24 and 23 in females. The respective percentage of pituitary tumors as the cause of deaths in the C, LD, MLD, MHD, and HD groups were identified in 20%, 25%, 42%, 60% and 71% in males and 100%, 56%, 79%, 96% and 85% in females.

**Ovaries:** Vilanterol treatment at  $\geq 84 \mu\text{g/kg/day}$  showed increases in the incidence of mesovarian leiomyomas. The incidence of the tumor was 0, 0, 5, 4 and 4 in C, LD, MDL, MDH and HD groups, respectively. Mesovarian leiomyomas are a classic effect of beta-agonists in rodents (Kelly *et al.*, *L Amer Col Toxicol*, 1993;12:13-21) and irrelevant to humans.

**Non-neoplastic changes:** Non-neoplastic findings were observed in the brain, ovaries and mesovarian ligaments. Table 28 summarizes the noticeable non-neoplastic findings. Both sexes showed dose-related increases in the degree and incidence of brain tissue compression. The females also showed dose-related increases in the incidence and severity of ovarian cysts and hypertrophy/hyperplasia of the smooth muscles in the mesovarian ligaments.

**Table 28: Non-neoplastic Findings in the Rat Study**

Organ - finding	Sex	Incidence (n = 60/groug)				
		0	10.5	84	223	657
Brain – tissue compression, total	M	20	17	18	27	28
Minimal		4	0	2	1	4
Slight		3	5	9	13	12
Moderate		13	12	7	10	8
Marketed		0	0	0	3	4
Total	F	37	35	46	45	45
Minimal		5	9	12	5	14
Slight		22	10	16	11	17
Moderate		10	14	17	27	11
Marketed		0	2	1	2	3
Ovary, cysts – follicular - total	F	6	17	22	37	39
Minimal		4	9	8	6	15
Slight		1	6	10	10	8
Moderate		1	2	3	19	15
Marketed		0	0	1	2	1
Mesovarian ligaments - smooth muscle hyperplasia /hypertrophy, total	F	0	0	2	7	12
Minimal		0	0	1	6	6
Slight		0	0	1	1	6

**Hormone levels in blood and pituitary masses:** Hormonal levels in the blood and pituitary gland were measured in week 55 in toxicokinetic section of rats. Prolactin levels were measured in some rats that died during the study or were terminally sacrificed. Hormonal levels in the pituitary masses in selected main study animals were measured via immunochemistry after sacrifice.

**Blood hormone levels:** The following hormones in the blood were measured (n = 6 - 9/sex/dose): progesterone, estradiol, follicle-stimulating hormone (FSH), luteinizing hormone, prolactin, testosterone, and dihydrotestosterone. Table 29 presents the hormone

levels at week 55 in males as an example. Results were so variable that no conclusions can be drawn.

**Table 29: Serum Testosterone, Estradiol and Dihydrotestosterone in Male Rats**

GW642444 <sup>1</sup> (µg/kg/day)	Test <sup>2,3</sup> Levels (ng/mL Serum)	E2 <sup>2,3</sup> Levels (pg/mL Serum)	T to E2 <sup>2,3</sup> Ratios	DHT <sup>2,3</sup> Levels (pg/mL Serum)
0	1.27 ± 0.87	73 ± 27	26.8 ± 17.7	241 ± 92
	1.00, 0.22, 3.22	64, 40, 115	22.3, 5.0, 64.4	248, 89, 360
10	1.51 ± 0.68	*133 ± 58	32.0 ± 15.3	233 ± 56
	1.45, 0.68, 2.41	122, 57, 218	32.3, 8.0, 51.9	226, 143, 333
80	1.31 ± 0.56	98 ± 28	30.5 ± 14.9	261 ± 113
	1.44, 0.50, 2.24	99, 60, 138	30.6, 10.9, 59.0	248, 130, 494
220	1.10 ± 0.50	90 ± 32	24.9 ± 10.3	196 ± 54
	1.05, 0.48, 1.92	93, 46, 140	21.8, 10.9, 40.9	192, 118, 284
650	0.82 ± 0.39	94 ± 23	18.3 ± 9.2	178 ± 56
	0.79, 0.33, 1.50	87, 65, 125	17.7, 6.1, 31.9	164, 124, 277

1. Targeted dose for aerosol administration.
2. Test = testosterone, E2 = 17β-estradiol; DHT = dihydrotestosterone.
3. Summary data are means ± standard deviations and medians, minimums, maximums. Rats were about 14.2 of age when blood was collected for hormone analyses during Study Week 55.

Prolactin levels were measured in some scheduled or unscheduled sacrificed rats during the period of week 65 and 105. The treated males, not the females, showed statistically non-significant decreases in serum prolactin levels, but the degree of the decreases were unrelated to vilanterol dose (Table 30).

**Table 30: Serum Prolactin levels on the Day of Termination in Rats**

MALE RATS		FEMALE RATS	
GW642444 <sup>1</sup> (µg/kg/day)	Prolactin <sup>2</sup> Levels (ng/mL Serum)	GW642444 <sup>1</sup> (µg/kg/day)	Prolactin <sup>2</sup> Levels (ng/mL Serum)
0	282 ± 196	0	298 ± 212
	324, 12.9, 676		332, 12.9, 937
10	71.8 ± 104	10 / 3 <sup>&amp;</sup>	280 ± 205
	26.7, 2.22, 519		257, 5.35, 804
80	88 ± 155	80 / 25 <sup>&amp;</sup>	254 ± 156
	30.8, 0.62, 908		259, 12.9, 737
220	101 ± 161	220 <sup>\$</sup>	255 ± 131
	27.4, 3.04, 620		248, 29.0, 676
650	80.1 ± 129	650 <sup>\$</sup>	286 ± 207
	38.9, 3.11, 741		255, 17.1, 790

1. Targeted dose for aerosol administration.
2. Summary data are means ± standard deviations and medians, minimums, maximums.
- <sup>&</sup> Due to increasing mortality, doses for Group 2 and 3 females were reduced to 3 and 25 µg/kg/day, respectively.
- <sup>\$</sup> Dosing was suspended in Week 86 for Group 4 and 5 females. Rats were 17 to 29 months of age when blood was collected for terminal hormone analyses.

**Pituitary hormone levels:** Table 31 showed the summary results of immunochemistry staining in pituitary masses. The HD males showed a low incidence of positive prolactin staining, and the significance of this finding is unknown at the present time because of the limited sample size.

**Table 31: Hormone Levels in Pituitary Masses**

Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )	Male		Female	
	0	657	0	657
Prolactin (# of positive/total)	3/9	0/10	9/13	6/12
% of prolactin positive	33	0	69	50
Growth hormone (# of positive/total)	0/9	0/10	1/13	0/12
% of GH positive	0	0	8	0

## 12 Appendices

Appendix A: Overall tumor incidences in male mice.

Appendix B: Overall tumor incidences in female mice.

Appendix C: Overall tumor incidences in male rats.

Appendix D: Overall tumor incidences in female rats.

Appendix E: Minutes of the 27-NOV-2012 ECAC meeting.

Appendix F: Memorandum completed by Dr. Larry Sancilio on September 17, 2009.

Appendix G: Memorandum completed by Dr. Molly Shea on November 18, 2008.

Appendix H: Review completed by Dr. Timothy McGovern on September 17, 2007.

Appendix J: Minutes of the 27-AUG-2007 ECAC meeting.

**Appendix A:****Tumor Incidence Table in Male Mice**

Organ/ Tumor	Incidence					
	Veh	LowLow	HiLow	LowMed	HiMed	High
ADRENAL GLANDS						
# Evaluated	83	84	84	84	84	84
Cortex: adenoma	1	0	2	1	2	1
subcapsular adenoma	1	2	0	0	0	0
BONE						
# Evaluated	13	9	7	10	9	5
Osteogenic sarcoma	0	0	1	0	0	0
osteoma	2	0	1	0	0	0
Bone						
# Evaluated	84	84	84	84	84	84
Osteoma/osteosarcoma	0	0	1	0	0	0
COLON						
# Evaluated	84	83	84	84	84	83
ADENOMA	1	0	0	0	0	0
DUODENUM						
# Evaluated	81	82	80	78	79	82
Adenocarcinoma	0	0	1	0	0	0
EXTREMITY						
# Evaluated	14	14	14	21	18	15
squamous papilloma	1	1	0	0	0	2
FEMORAL MARROW						
# Evaluated	84	84	84	84	84	84
Hemangioma	0	1	0	0	0	0
hemangiosarcoma	1	0	0	0	0	0
GALLBLADDER						
# Evaluated	82	83	83	82	77	81
Adenocarcinoma	0	1	0	0	0	0
adenoma/papillary adenoma	0	1	0	0	0	0
HARDERIAN GL						
# Evaluated	84	84	84	83	84	84
Adenoma	3	1	4	4	2	1
Carcinoma	0	0	0	0	1	0
HEAD						
# Evaluated	0	1	1	1	0	0
Squamous cell carcinoma	0	0	0	1	0	0
LIVER						
# Evaluated	84	84	84	84	84	84
Adenoma/Carcinoma hepato.	12	13	6	9	7	5
Hemangioma	0	0	0	1	1	0
hemangiosarcoma	0	1	0	0	0	0
hepatocellular adenoma	8	8	4	7	4	1
hepatocellular carcinoma	5	5	2	2	4	4
LUMBAR SC						
# Evaluated	84	84	84	83	84	84
Malignant meningioma	0	0	0	0	1	0
LUNGS						
# Evaluated	84	84	84	84	84	84
Adenoma/Carcinoma bronch. alv.	13	16	12	14	12	6
Bronchiolo/alveolar adenoma	9	9	8	9	7	3
bronchiolo/alveolar carcinoma	4	7	4	5	5	3
Lymph/retic sys						
# evaluated	84	84	84	84	84	84

Granulocytic leukemia	0	0	2	0	0	0
Histiocytic sarcoma	1	1	1	0	1	0
Malignant lymphoma	10	6	2	4	5	9
Mast cell tumor	0	0	1	0	0	1
Pituitary						
# evaluated	84	84	84	83	84	84
Adenoma pars dit./inter.	1	1	1	1	0	0
Pars distalis-adenoma	1	1	1	1	0	0
Pars intermedia: adenoma	1	0	0	0	0	0
Preputial/clit gl						
# evaluated	83	83	84	83	82	80
Adenoma	0	1	0	0	0	0
Prostate						
# evaluated	84	83	84	84	84	82
Adenoma	0	0	1	0	0	0
Skin						
# evaluated	84	84	84	84	84	84
Fibrosarcoma	0	1	0	2	2	0
Fibrous histiocyoma	0	1	0	1	0	0
Hemangioma	0	0	0	1	0	0
Hemangiosarcoma	0	0	0	0	1	0
Keratoacanthoma	2	0	0	0	0	0
Rhabdomyosarcoma	0	1	0	0	0	0
Squamous cell papilloma	1	1	0	0	1	0
Sq. Cell papilloma/keratocanthom	3	1	0	0	1	0
Soft tissue						
# evaluated	2	0	1	1	0	0
Neuroendocrine tumor	1	0	0	0	0	0
Spleen						
# evaluated	84	84	84	84	84	84
Hemangiosarcoma	0	0	0	0	0	1
Stomach						
# evaluated	84	84	84	84	84	84
Forestomach: squamous cell papil	1	0	0	0	0	0
Leiomyosarcoma	0	1	0	0	0	0
Squamous cell carcinoma	0	0	0	1	0	0
Systemic						
# evaluated	84	84	84	84	84	84
Hemangioma	0	1	0	2	1	0
Hemangiosarcoma	1	1	0	1	1	1
hemangioma/-sarcoma	1	2	0	3	2	1
Tail						
# evaluated	13	10	10	8	12	6
Hemangiosarcoma	0	0	0	1	0	0
Testes						
# evaluated	84	84	84	84	84	84
Adenoma/interstitial tumor	0	1	0	0	0	2
Benign interstitial cell tumor	0	0	0	0	0	2
Right testis: adenoma	0	1	0	0	0	0
Thyroid						
# evaluated	84	84	83	84	83	84
C-cell adenoma	0	0	0	1	0	0
Follicular cell adenoma	0	0	0	1	0	0
Vessel						
# evaluated	84	84	84	84	84	84
Hemangioma	0	1	0	2	1	0
Hemangiosarcoma	1	1	0	1	1	1

**Appendix B****Tumor Incidence Table in Female Mice**

Organ/ Tumor	Incidence					
	Veh	LowLow	HiLow	LowMed	HiMed	High
Adipose tissue						
# evaluated	1	1	0	1	2	1
Lipoma	0	1	0	0	0	0
Adrenal glands						
# evaluated	83	84	84	84	84	84
Adenoma	1	1	0	1	0	1
Cortex: adenoma	1	0	0	1	0	1
Subcapsular adenoma	0	1	0	0	0	0
Bone						
# evaluated	5	8	7	9	9	9
Osteogenic sarcoma	0	1	2	0	0	0
Osteoma	0	0	0	0	1	0
Bone						
# evaluated	84	84	84	84	84	84
Osteoma/osteosarcoma	0	2	2	0	0	0
Distal femur						
# evaluated	84	84	84	84	84	84
Chondroma	0	1	0	0	0	0
Extremity						
# evaluated	4	6	5	4	9	5
Squamous papilloma	0	0	1	1	0	1
Femoral marrow						
# evaluated	84	84	84	84	84	84
Hemangioma	0	1	0	0	0	0
Harderian gl						
# evaluated	84	84	84	84	84	84
Adenoma	5	2	3	4	0	2
carcinoma	1	0	1	0	0	0
Ileum						
# evaluated	83	83	81	80	82	81
Adenocarcinoma	0	0	0	0	0	1
Liver						
# evaluated	84	84	84	84	84	84
Hepatocellular adenoma	1	0	3	2	0	0
Lumbar sc						
# evaluated	84	84	84	84	84	84
Benign meningioma	0	0	0	0	1	0
Lungs						
# evaluated	84	84	84	84	84	84
Adenoma/carcinoma bronch. Alv.	8	7	5	8	6	5
Bronchiolo/alveolar adenoma	5	3	3	3	2	4
Bronchiolo/alveolar carcinoma	3	4	2	5	4	1
Lymph/retic sys						
# evaluated	84	84	84	84	84	84
Granulocytic leukemia	1	1	0	0	0	0
Histiocytic sarcoma	7	3	8	4	6	1
Malignant lymphoma	11	7	13	9	5	8
Mast cell tumor	0	1	0	0	0	0
Mammary areas						
# evaluated	83	84	83	84	84	84
Adenocarcinoma	0	2	0	2	0	0
Malignant adenoacanthoma	0	0	1	0	0	0

Mesentery/perito						
# evaluated	20	11	22	17	15	10
Malignant mesothelioma	0	0	1	0	0	0
Ovaries						
# evaluated	84	83	84	84	84	83
Benign granulosa cell tumor	0	0	1	1	2	0
Cystadenoma	0	1	3	2	1	0
Granulosa cell tumor b&m	0	0	1	1	3	0
Hemangiosarcoma	0	0	0	0	0	1
Leiomyosarcoma	0	0	0	0	1	0
Luteoma	0	0	1	1	0	0
Malignant granulosa cell tumor	0	0	0	0	1	0
Sex cord stroma adenoma	0	1	2	1	0	2
Tubulostromal adenoma	0	0	1	0	2	6
Pancreas						
# evaluated	84	83	84	84	84	83
Islet cell adenoma	1	0	0	0	0	1
Pituitary						
# evaluated	83	84	82	82	84	82
Adenoma pars dit./inter.	1	0	1	0	0	0
Pars distalis-adenoma	1	0	1	0	0	0
Skin						
# evaluated	84	84	84	84	84	84
Basosquamous tumor	0	0	0	0	1	0
Carcinoma not otherwise specifie	1	0	0	0	0	0
Fibrosarcoma	0	0	0	0	1	1
Myxosarcoma	0	1	0	0	0	0
Squamous cell carcinoma	1	0	0	0	0	0
Soft tissue						
# evaluated	0	0	0	1	3	2
Fibrosarcoma	0	0	0	0	1	0
Leiomyosarcoma	0	0	0	0	0	1
Malignant schwannoma	0	0	0	0	1	0
Spleen						
# evaluated	83	84	84	84	84	84
Hemangiosarcoma	0	0	1	0	0	0
Stomach						
# evaluated	84	84	84	84	83	83
Forestomach: squamous cell papil	1	1	0	0	0	0
Systemic						
# evaluated	84	84	84	84	84	84
Hemangioma	1	3	2	0	0	0
Hemangiosarcoma	0	1	1	0	2	1
Hemangioma/-sarcoma	1	4	3	0	2	1
Thyroid						
# evaluated	83	84	83	84	84	84
Follicular cell adenoma	0	1	0	0	0	2
Uterus w/ cervix						
# evaluated	84	84	84	84	84	84
Benign granular cell tumor	0	1	0	0	0	0
Deciduoma	0	0	0	0	0	1
Endometrial adenocarcinoma	1	1	1	1	1	1
Endometrial stromal polyp	2	2	5	2	5	3
Endometrial stromal sarcoma	0	1	1	1	0	2
Fibroma	0	1	0	0	0	0
Hemangioma	1	2	2	0	0	0

Hemangiosarcoma	0	1	0	0	2	0
Leiomyoma	2	2	5	5	1	2
Leiomyosarcoma	0	1	2	4	6	4
Leiomyoma/leiomyosarcoma	2	3	7	9	7	6
Stromal polyp/sarcoma	2	3	6	3	5	5
Vessel						
# evaluated	84	84	84	84	84	84
Hemangioma	1	3	2	0	0	0
Hemangiosarcoma	0	1	1	0	2	1

**Appendix C:****Tumor Incidence Table in Male Rats**

Organ/ Tumor	Incidence				
	Veh	Low	LowMid	HiMid	High
<b>ABDOMEN</b>					
# Evaluated	1	2	0	2	3
Fibroma	0	0	0	0	1
Fibroma/Fibrosarcoma	0	0	0	1	1
Fibrosarcoma	0	0	0	1	0
<b>ADRENAL</b>					
# Evaluated	60	60	60	60	60
Adenoma: cortical	0	1	0	1	0
Benign pheochromocytoma	2	3	1	1	4
Malignant pheochromocytoma	0	1	0	0	0
Pheochromocytoma [B]&[M]	2	4	1	1	4
<b>BRAIN</b>					
# Evaluated	60	60	60	60	60
Benign granular cell tumor	1	1	0	0	0
Malignant astrocytoma	1	0	1	1	0
Medulloblastoma	0	0	0	1	0
<b>CAVITY NASAL/SINUS</b>					
# Evaluated	60	60	60	60	60
Papilloma: squamous cell	0	0	0	0	1
Sq. Cell Carcinoma/Papilloma	0	0	0	0	1
<b>CAVITY ORAL</b>					
# Evaluated	0	0	1	0	0
Carcinoma: squamous cell	0	0	1	0	0
Sq. Cell Carcinoma/Papilloma	0	0	1	0	0
<b>HEAD</b>					
# Evaluated	0	0	2	0	0
Squamous cell carcinoma	0	0	1	0	0
<b>HEMOLYM. TISSUE</b>					
# Evaluated	60	60	60	60	60
Histiocytic sarcoma	1	3	0	1	0
Malignant lymphoma	3	2	1	1	4
<b>JEJUNUM</b>					
# Evaluated	60	60	60	60	60
Adenocarcinoma	0	0	0	1	0
Adenoma	1	0	0	0	0
Leiomyoma	0	1	0	0	0
Sarcoma (not otherwise specified)	0	0	0	0	1
<b>KIDNEY</b>					
# Evaluated	60	60	60	60	60
Liposarcoma	2	0	0	0	0
<b>L. NODE MANDIBULAR</b>					
# Evaluated	59	60	58	59	60
Carcinoma: metastasis	0	0	0	0	1
<b>L.NODE MESENTERIC</b>					
# Evaluated	60	59	60	60	60
Hemangioma	2	1	0	1	1
<b>LIVER</b>					
# Evaluated	60	60	60	60	60
Adenoma/Carcinoma hepato.	4	5	3	3	2
Adenoma: hepatocellular	2	0	2	2	0
Carcinoma: hepatocellular	2	5	1	1	2
<b>LUNG</b>					

# Evaluated	60	60	60	60	60
Carcinoma: alveolar/bronchiolar	0	0	1	0	0
Carcinoma: metastasis	0	0	1	0	0
MAMMARY GLAND					
# Evaluated	60	60	60	59	59
Adenoma	0	0	1	1	1
Adenoma/Adenocarcinoma	0	0	1	1	1
Fibroadenoma	2	0	0	1	0
Fibroma/Fibroadenoma	2	0	0	1	0
MUSCLE SKELETAL MI					
# Evaluated	2	2	1	0	2
Lipoma	0	0	0	0	1
PANCREAS					
# Evaluated	60	60	60	60	60
Adenoma/Carcinoma islet cell	10	7	4	9	2
Adenoma: islet cell	6	5	2	9	2
Carcinoma: islet cell	4	2	2	0	0
PARATHYROID GLAND					
# Evaluated	60	60	58	60	60
Adenoma	0	1	0	1	0
PITUITARY					
# Evaluated	60	60	60	60	60
Adenoma/Carcinoma Any	46	41	43	41	47
Adenoma: pars distalis	42	37	42	39	45
Adenoma: pars intermedia	1	0	0	1	1
Carcinoma: pars distalis	3	4	1	1	1
PROSTATE					
# Evaluated	60	60	60	60	60
Adenocarcinoma	0	0	1	0	0
RECTUM					
# Evaluated	60	60	60	60	60
Adenoma	0	1	0	0	0
SALIV.GL. MANDIBUL					
# Evaluated	60	60	59	59	60
Fibroma	0	0	0	1	0
SKIN MISCELLANEOUS					
# Evaluated	15	17	11	14	19
Adenoma/Carcinoma Basal cell	0	2	1	0	1
Adenoma: basal cell	0	0	0	0	1
Adenoma: sebaceous	0	1	0	0	0
Carcinoma: basal cell	0	2	1	0	0
Keratoacanthoma	4	4	0	4	2
Papilloma: squamous cell	0	0	1	1	0
SPINAL CORD LUMBAR					
# Evaluated	60	60	60	60	60
Chordoma	0	1	0	0	0
SPLEEN					
# Evaluated	60	60	60	60	60
Fibrosarcoma	0	1	0	0	0
STOMACH					
# Evaluated	60	60	60	60	60
Benign neuroendocrine cell tumor	1	0	0	0	0
Sarcoma (not otherwise specified)	0	0	0	0	1
SUBCUTANEOUS TISSU					
# Evaluated	13	8	10	13	7
Fibroma	7	2	8	5	7
Fibroma/fibrosarcoma	7	2	8	5	7

Fibrosarcoma	0	0	0	0	1
Hemangiosarcoma	0	0	0	1	0
Lipoma	1	3	1	1	0
Malignant schwannoma	1	0	0	0	0
Sarcoma (not otherwise specified)	0	0	0	1	0
Squamous cell Carcinoma	1	0	0	0	0
TAIL					
# Evaluated	1	1	0	1	2
Fibrosarcoma	0	0	0	1	0
TESTIS					
# Evaluated	60	60	60	60	60
Adenoma: interstitial cell	4	3	2	2	0
THORAX					
# Evaluated	1	0	0	2	0
Hibernoma	0	0	0	1	0
THYROID					
# Evaluated	60	60	60	60	60
Adenoma/Carcinoma C-Cell	6	8	7	10	4
Adenoma/Carcinoma foll. cell	2	1	2	3	1
Adenoma: C-cell	4	7	4	10	3
Adenoma: follicular cell	1	1	2	2	1
Carcinoma: C-cell	2	1	3	0	1
Carcinoma: follicular cell	1	0	0	1	0
TONGUE					
# Evaluated	60	60	60	60	60
Carcinoma: squamous cell	0	1	1	0	0
Papilloma: squamous cell	0	1	0	0	0
Sq. Cell Carcinoma/Papilloma	0	2	1	0	0

**Appendix D:****Tumor Incidence Table in Female Rats**

Organ/ Tumor	Incidence				
	Veh	Low	LowMid	HiMid	High
<b>ADRENAL</b>					
# Evaluated	60	60	60	60	60
Adenoma: cortical	2	0	1	0	1
Benign pheochromocytoma	1	0	0	1	0
<b>BRAIN</b>					
# Evaluated	60	60	60	60	60
Malignant astrocytoma	2	0	0	0	1
Malignant oligodendroglioma	0	0	0	0	1
<b>CAVITY NASAL/SINUS</b>					
# Evaluated	60	60	60	60	60
Adenocarcinoma	0	0	1	0	1
<b>CECUM</b>					
# Evaluated	60	60	60	60	60
Leiomyoma	1	0	0	0	0
<b>HEAD</b>					
# Evaluated	1	0	0	0	0
Squamous cell carcinoma	1	0	0	0	0
<b>HEMOLYM. TISSUE</b>					
# Evaluated	60	60	60	60	60
Histiocytic sarcoma	1	0	0	0	1
Malignant lymphoma	0	1	0	0	0
<b>JEJUNUM</b>					
# Evaluated	60	60	60	60	60
Adenocarcinoma	0	0	1	0	0
Leiomyoma	0	0	1	0	0
<b>KIDNEY</b>					
# Evaluated	60	60	60	60	60
Adenoma: tubular cell	1	0	0	0	1
<b>LIVER</b>					
# Evaluated	60	60	60	60	60
Adenoma: hepatocellular	0	0	1	0	2
<b>LUNG</b>					
# Evaluated	60	60	60	60	60
Adenoma: alveolar/bronchiolar	0	1	0	0	0
<b>MAMMARY GLAND</b>					
# Evaluated	59	60	60	60	60
Adenocarcinoma	9	14	11	11	10
Adenoma	13	7	9	5	10
Adenoma/Adenocarcinoma	19	18	19	13	17
Fibroadenoma	27	27	19	13	17
Fibroma	0	0	0	1	0
Fibroma/Fibroadenoma	27	27	19	14	17
<b>MESOVARIAN LIGAMEN</b>					
# Evaluated	60	60	60	60	60
Leiomyoma	0	0	5	4	4
<b>OVARY</b>					
# Evaluated	60	60	60	60	60
Benign granulosa-theca cell tumor	0	0	0	0	1
<b>PANCREAS</b>					
# Evaluated	60	59	60	60	60
Adenoma/Carcinoma islet cell	0	1	2	0	2

Adenoma: islet cell	0	1	2	0	1
Carcinoma: islet cell	0	0	0	0	1
PARATHYROID GLAND					
# Evaluated	57	59	59	60	59
Adenocarcinoma	0	0	1	0	0
PITUITARY					
# Evaluated	60	60	60	60	60
Adenoma/Carc. pars distalis	54	55	54	57	60
Adenoma: pars distalis	44	47	48	51	53
Carcinoma: pars distalis	10	8	6	6	7
SALIV. GLAND PAROT					
# Evaluated	60	60	59	57	57
Adenoma	0	0	1	0	0
SKIN MISCELLANEOUS					
# Evaluated	5	8	3	4	6
Carcinoma: squamous cell	0	0	0	0	1
Fibrosarcoma	0	1	0	0	0
STOMACH					
# Evaluated	60	60	60	60	60
Adenocarcinoma	0	0	0	1	0
Benign neuroendocrine cell tumor	0	0	0	0	1
SUBCUTANEOUS TISSU					
# Evaluated	2	4	3	3	2
Fibroma	2	1	0	0	0
Fibroma/fibrosarcoma	2	2	1	0	0
Fibrosarcoma	0	1	1	0	0
Lipoma	0	2	0	1	0
Malignant schwannoma	0	0	0	0	1
THORAX					
# Evaluated	0	1	0	0	0
Hibernoma	0	1	0	0	0
THYROID					
# Evaluated	60	60	60	60	60
Adenoma/Carcinoma C-Cell	4	2	3	2	7
Adenoma: C-cell	2	2	2	2	7
Carcinoma: C-cell	2	0	1	0	0
TONGUE					
# Evaluated	60	60	60	60	60
Carcinoma: squamous cell	0	0	0	0	1
UTERUS					
# Evaluated	60	60	60	60	60
Adenocarcinoma: endometrial	0	1	0	0	0
Benign granular cell tumor	2	0	2	0	0
Leiomyoma	0	0	0	1	0
Polyp: endometrial stromal	9	4	6	9	2
Sarcoma: endometrial stromal	0	1	0	0	0
Stromal polyp/sarcoma	9	5	6	9	2
VAGINA					
# Evaluated	60	59	60	60	60
Benign granular cell tumor	0	0	0	0	1

## **Executive CAC**

**Date of Meeting: November 27, 2012**

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair  
Abby Jacobs, Ph.D., OND IO, Member  
Paul Brown, Ph.D., OND IO, Member  
Lynnda Reid, Ph.D., DRUP, Alternate Member  
Luqi Pei, Ph.D., DPARP, Presenting Reviewer

Author of Draft: Luqi Pei, Ph.D.

**The following information reflects a brief summary of the Committee discussion and its recommendations.**

NDA #: NDA 204,275  
Drug Name: Vilanterol (GW642444)  
Sponsor: GSK

### **Background:**

Vilanterol is a long acting beta 2 adrenergic agonist being developed as a component of a therapy (Breo Ellipta) for chronic obstructive pulmonary disease. Breo Ellipta is a dry powder inhaler using fluticasone and vilanterol as the active pharmaceutical ingredients. This meeting evaluated the carcinogenicity potential of vilanterol only because the Committee evaluated the carcinogenicity potential of fluticasone previously during the review of NDA 22-051.

The evaluation of the carcinogenicity potential of vilanterol included a battery of genetic toxicity testing and traditional 2-year bioassays in rats and mice. In the genetic toxicity testing battery, vilanterol tested negative in the following assays: bacterial mutation assay in *S. typhimurium* and *E. coli* (Ames test), rat bone marrow micronucleus assay, in vitro unscheduled DNA synthesis (UDS) assay, and Syrian Hamster embryonic (SHE) cell transformation assay. Vilanterol tested equivocal in the mouse lymphoma assay.

The bioassays were 2-year inhalation carcinogenicity studies of vilanterol in mice and rats. Animals were exposed to various doses of vilanterol daily for up to 104 weeks. Vilanterol was delivered by nose-only inhalation exposure for 60 minutes per day. The Executive CAC concurred with the dose selection for each study and the dose adjustments during the study in rats. Final reports of the studies were submitted in the NDA submission.

### **Rat Carcinogenicity Study**

Sprague-Dawley rats (60/sex/dose) were exposed by nose-only inhalation to vehicle (C), which was lactose powder, low-dose (LD), mid dose (MD), mid-high dose (HD-1), and high dose (HD-2) of vilanterol for up to 104 weeks. Specifically, males were treated with 0, 10.5, 84.4, 223, or 657- $\mu$ g/kg/day vilanterol (achieved doses) for 101 weeks. Females

were exposed to the same doses for 85 weeks and dose adjustments were made subsequently due to excessive mortalities in the vilanterol-treated groups. The dose adjustments consisted of the following: dosing was discontinued in the HD-1 and HD-2 groups and vilanterol doses in the LD and MD groups were reduced to 3.5 and 28.2 µg/kg/day, respectively. The three top-dose groups were terminated during weeks 95 – 96 when the number of survivors reached 15/group.

Both male and female rats had dose-related mortality ( $P < 0.01$ ) and shortened latency to pituitary neoplasms, which were considered to be the cause of death, although the increases in overall tumor incidence did not reach the statistically significant level of 0.01 for the common tumor. Control incidences were 70% for males and 90% for females. The three highest dose groups of females also had increased incidences of mesovarian leiomyomas. Table 1 presents the leiomyoma incidences in the mesovarian ligament in rats. Figure 1 presents the time-course of pituitary adenoma-related deaths in males as an example.

Table 1: Mesovarian Leiomyoma Incidences in Rats

Sex	Incidence (p-value)				
	0	10.5/3.5	84.4/28.2	223	657
F	0/60	0/60	5/60 (0.007)	4/60 (0.020)	4/60 (0.020)

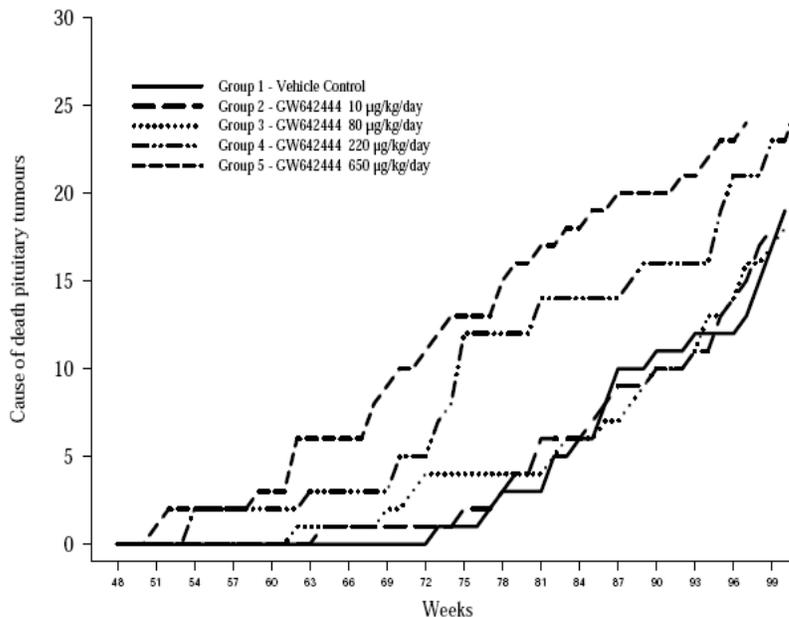


Figure 1: Pituitary tumor-related deaths in male rats.

### Mouse Carcinogenicity Study

Mice (84/sex/dose, CD-1) were treated by nose-only inhalation with 0 (C), 6 (LD-1), 62 (LD-2), 615 (MD), 6,150 (HD-1), or 29,500 (HD-2)-µg/kg/day vilanterol (achieved doses) for 101 – 104 weeks. The HD-2 female group showed a statistically significant increase in ovarian tubulostromal adenoma ( $p = 0.014$ ) (incidence: 0/84, 0/83, 1/84, 0/84, 2/84 and

6/83 in the C, LD-1, LD-2, MD, HD-1 and HD-2, respectively). Although, the four top-dose groups in females showed numerical increases in the incidence of leiomyomas and leiomyosarcomas in the uterus, alone or in combination, none of the increases reached the statistically significant level of  $p < 0.01$ .

Table 2: Incidences of Tubulostromal Adenomas in Ovaries in Female Mice

	Vilanterol ( $\mu\text{g}/\text{kg}/\text{day}$ )					
	0	6	62	615	6150	29,500
Incidence (overall)	0/84	0/83	1/84	0/84	2/84	6/83
P-value (vs. vehicle)	-	-	0.500	-	0.249	0.0137

### Executive CAC Recommendations and Conclusions:

#### *Rat study:*

1. The Committee agreed that the study was acceptable.
2. The Committee considered the following neoplasms to be clearly drug-related:
  - Adenomas of the pituitary gland in males and females (based on dose-related decreases in tumor latency associated with increased lethality)
  - Leiomyomas of mesovarian ligaments in females.

#### *Mouse study:*

- The Committee agreed that the study was acceptable.
- The Committee considered the tubulostromal adenomas in the ovaries to be drug related.

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

cc:\n  
/Division File, DPARP  
/TRobison, DPARP  
/LPei, DPARP  
/ARamsey/PM, DPARP  
/ASeifried, OND IO

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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ADELE S SEIFRIED  
11/30/2012

DAVID JACOBSON KRAM  
11/30/2012

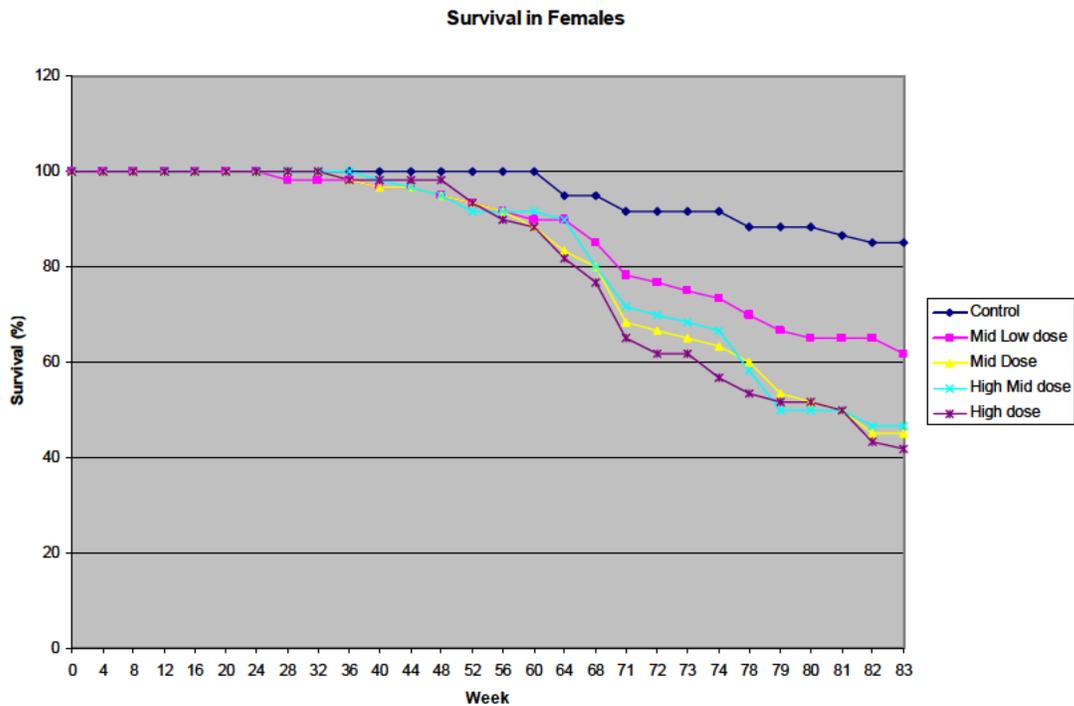
# Memo

To: INDs 77,855, 74,696 (b)(4) Submission date: 9/8/09

Subject: Response to a Request by SmithKlineGlaxo for Adjustment of Doses in the Inhaled Rat Carcinogenicity Assay of GW642444M

Date: September 17, 2009

GW642444 is a beta-2 agonist in development. The Sponsor requested that a dose adjustment be made in female rats in their ongoing rat carcinogenicity assay due to excessive mortality. No adjustment was requested for the male rats. The current inhalation doses are: 10, 80, 220 and 650 mcg/kg. From week 36, there was an increasing number of female deaths as presented in the following survival curve.



The following table presents the incidence of deaths by week 83 in male and females. The number of surviving female animals was: control, 51, low dose, 37, mid dose, 27, mid high dose, 28 and high dose 25.

**Table 1: Incidence of all deaths in rats (main study animals)**

TARGET DOSE	MALES					FEMALES				
	0	10	80	220	650	0	10	80	220	650
ACHIEVED DOSE UP TO WEEK 82	0	10	83	225	634	0	10	83	225	634
NUMBER OF MAIN AT START	60	60	60	60	60	60	60	60	60	60
NUMBER ALIVE AT WEEK 83	46	48	47	35	35	51	37	27	28	25
% SURVIVAL AT WEEK 83	77%	80%	78%	58%	58%	85%	62%	45%	47%	42%

The female decedent animals showed subcutaneous masses the incidence of which was higher than the control group as presented in the following table. In addition, both control and decedent animals showed a similar increased incidence of pituitary masses. The subcutaneous masses may possibly be mammary since in the 6-month inhalation toxicity study in rats, there was an increase in the degree of acinar development and secretory activity in the mammary gland at doses  $\geq 500$  mcg/kg. At this time, the histopathology from the decedent rats was not available for confirmation.

**Table 2: Incidence of selected macroscopic observation from decedents (up to Week 83)**

Target Dose (ug/kg/day)	Males					Females				
	0	10	80	220	650	0	10	80	220	650
Number of animals examined	14	12	13	25	25	9	23	33	32	34
Pituitary mass	6	5	5	16	17	8	16	29	28	25
Pituitary mass (%)	43	42	38	64	68	89	70	88	88	74
Subcutaneous Mass	2	3	-	2	2	2	14	16	13	13
Subcutaneous Mass (%)	14	25	-	8	8	22	61	48	41	38

The sponsor indicated that if the present rates of mortality continue, there unlikely will be a sufficient number of survivors by the end of 2 years. The sponsor proposed to stop dosing the mid high and high dose groups, and reduce the low dose, 10 mcg/kg, and the mid dose, 80 mcg/kg to 3 and 25 mcg/kg, respectively, since the adjustment can easily be made without adjusting the concentration of the inhaled drug: lactose blend.

## EVALUATION

The sponsor requested that adjustment of the doses for females in the inhalation rat carcinogenicity assay be made due to increased mortality. The current inhalation doses were: 10, 80, 220 and 650 mcg/kg. After 36 week of dosing, there was a progressive increase in mortality in all treated groups. These animals manifested an increase incidence of subcutaneous masses which may have contributed to their deaths. These masses may be mammary in origin.

The sponsor proposed to stop dosing the mid high and high dose groups, and reduce the low dose, 10 mcg/kg, and the mid dose, 80 mcg/kg to 3 and 25 mcg/kg, respectively, since the adjustment can easily be made without adjusting the concentration of the inhaled drug: lactose blend. These reductions are reasonable based on the Safety Margins presented in the following table which were based on AUCs and lung burden for the 25 mcg/kg dose and on the AUC for the 3 mcg/kg dose. The AUC for the 3 mcg/kg dose is acceptable since the toxicity seen was predominantly systemic and not local.

Species/Dose mcg/kg/kg	Safety Margin Based on	
	AUC <sup>a</sup>	Lung Burden
at 3	1	3
25	12	25
Human, 25 mcg AUC: 0.23 ng. hr/ml		

<sup>a</sup> From sponsor's submission

The following method was used to determine the lung burden.

Calculation of Lung Burden Safety Margin for 25 mcg (Human dose)

Rat:  $25 \text{ mcg/kg (dose)} \times 10\% \text{ (deposition factor)} = 2.5 \text{ mcg/kg (lung deposition)}$

Lung burden:  $2.5 \text{ mcg/kg} \div 4 \text{ g/kg (rat lung weight)} = 0.625 \text{ mcg/g}$

Human total dose: 25 mcg

Lung burden:  $25 \text{ mcg (total dose)} \div 1000\text{g (human lung weight)} = 0.025 \text{ mcg/g}$

$$\text{Safety Margin} = \frac{\text{Rat Lung burden}}{\text{Human Lung burden}} = \frac{0.625 \text{ mcg/g}}{0.025 \text{ mcg/kg}} = 25$$

In general, dosing of the treated groups should not stop until the number of survivors reach 20 per group and then allowed to complete the study. If the number of survivors is reduced to 15 per group, then the group should be sacrificed and necropsied. Further, if the number of survivors in the control group is reduced to 20, treatment is stopped, and all the animals are necropsied. However, in this particular case, Executive Carcinogenicity Committee (CAC) considered that the two highest doses should be stopped at this stage due to the significant mortality and very high doses applied. The Executive CAC was consulted and concurred the following response to the sponsor.

Letter to the sponsor:

In your request for a change in the inhaled rat carcinogenicity study of GW642444M in females submitted by email on September 8, 2009, we agree with your proposal to stop dosing the mid high and high dose groups and retain them on study until they reach 15 survivors at which time they will be sacrificed. We expect these animals will be necropsied with full histopathology. We also agree that the dose levels of 10 and 80 mcg/kg/day in females be reduced to 3 and 25 mcg/kg/day, respectively.

Pharmacology Reviewer: Lawrence F. Sancilio, Ph.D.

Supervisor (acting): Jean Q. Wu, M.D., Ph.D

cc.

Executive CAC: David Jacobsen-Kram, Ph.D, DABT  
Abigail Jacobs, Ph.D.  
Paul C. Brown. Ph.D.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-77855	ORIG-1	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	GW685698/GW642444 Inhalation Powder

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

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/s/

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LAWRENCE F SANCILIO

09/17/2009

This memo also refers to INDs 74,696 and (b)(4) since it involves the same compound G64244M

JEAN Q WU

09/17/2009

GlaxoSmithKline (GSK) is requesting the Agency's concurrence on a proposal (b)(4) in their on-going mouse carcinogenicity study in IND 74,696.

(b)(4)  
The review finds the available data insufficient to support the sponsor's proposal and recommends that the request be denied.

On October 31, 2008 GSK submitted a General Correspondence regarding their on-going mouse carcinogenicity study. Currently, mice are exposed to dry powder inhalation doses of 6, 60, 600, 6000 and 30,000 mcg/kg/day of GW642444 for 1 hour/day as recommended by the Executive Carcinogenicity Assessment Committee on June 12, 2007. This study has reached dosing Week 46, as of the date of the submission. Through dosing Week 33 an unexpectedly high level of mortality has been observed in all dosing groups with a particular increase in death in the male HD group. The sponsor indicates that this increase in mortality is a result of (b)(4). The sponsor has proposed (b)(4).

Additionally, the sponsor (b)(4) proposed (b)(4).

The following is the sponsor's arguments:

(b)(4)

Graph 1: Survival curve for males (main)



Graph 2: Survival Curves for females (main)



(b)(4)

**Draft mean toxicokinetic data from week 4 & 26 with estimated system overage to clinical doses**

(b)(4)

The above arguments, despite its scientific merits, are insufficient (b)(4)  
(b)(4) based on the following considerations:

(b)(4)

(b)(4)



(b)(4)



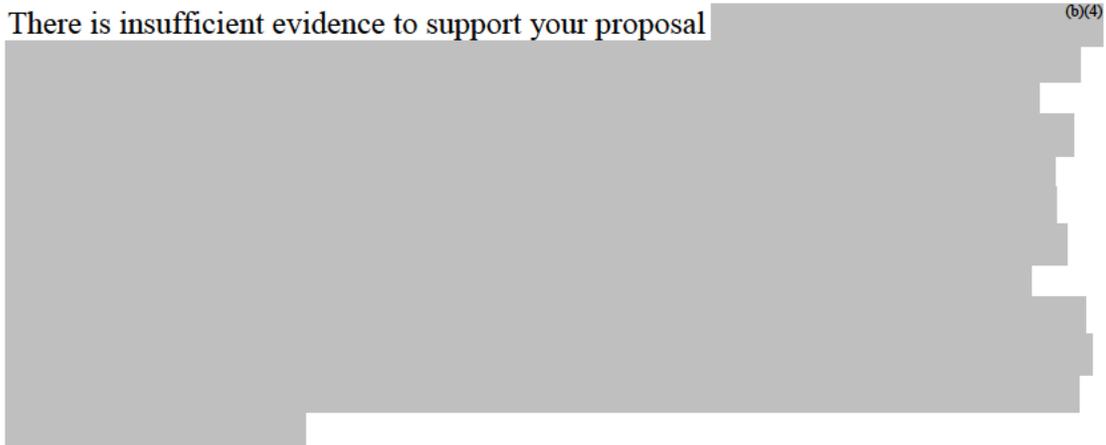
The Executive CAC reviewed the above arguments of the sponsor and the review on November 17, 2008 and concurred with the recommendation of the review.

**Internal Comments:** Inform the sponsor that the proposal (b)(4) for the 2-yr inhalation carcinogenicity study of GW642444 in mice is denied. See the External Comments for addition information.

**External Comments (to the sponsor):**

We have reviewed your recent IND 74,696 submission (SN 0030) dated October 31, 2008 requesting changes to an on-going mouse carcinogenicity study. We have the following comments regarding the request.

There is insufficient evidence to support your proposal (b)(4)



Molly E. Shea, Ph.D.

Linked Applications

Sponsor Name

Drug Name

-----  
IND 74696

-----  
GLAXOSMITHKLINE

-----  
GW642444 INHALATION POWDER

-----  
**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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MOLLY E SHEA

11/18/2008

LUQI PEI

11/18/2008

I occur.

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**IND number:** 74,696

**Review number:** 2

**Sequence number/date/type of submission:** 012/September 7, 2007/IT

**Information to sponsor:** Yes (X) No ( )

**Sponsor and/or agent:** GlaxoSmithKline

**Manufacturer for drug substance:** Not provided

**Reviewer name:** Timothy McGovern, Ph.D.

**Division name:** Pulmonary and Allergy Products

**Review completion date:** September 17, 2007

**Drug:**

Trade name: Not available

Generic name: Not available

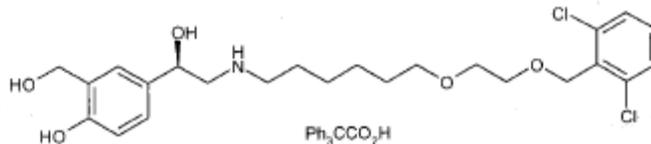
Code name: GW642444

Chemical name: Triphenylacetic acid-4-{(1R)-2[(6{2-[2,6-dichloro]oxy]ethoxy)amino]-1-hydroxyethyl}—(hydroxymethyl)phenol (1:1)

CAS registry number: Not available

Molecular formula/molecular weight:  $C_{24}H_{33}Cl_2NO_5 \cdot C_{20}H_{16}O_2$  (as the triphenylacetate salt), MW=774.78 (as the triphenylacetate salt)

Structure:



**Relevant INDs/NDAs/DMFs:** None

**Drug class:** Long acting beta agonist

**Intended clinical population:** COPD and Asthma patients

**Clinical formulation:** Dry powder blend of GW642444M in magnesium stearate and lactose

**Route of administration:** Oral inhalation

**Proposed clinical protocol:** None at this time

**Previous clinical experience:** None

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

**Studies reviewed within the submissions:** None

**Studies not reviewed within this submission:**  
None

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## OVERALL CONCLUSIONS AND RECOMMENDATIONS

### Summary:

GW642444 is being developed as the long-acting beta agonist (LABA) component of a once-daily inhaled corticosteroid (ICS)/(LABA) combination product for treatment of asthma in patients [REDACTED] (b) (4). This combination product and GW642444 monotherapy are also planned for treatment of COPD. The sponsor plans to use doses up to 50 mcg/day for future clinical trials and the anticipated Maximum Recommended Human Dose (MRHS) for marketing is 6.25 mcg/day.

The sponsor submitted two carcinogenicity protocols in rats and mice for special protocol assessments in July 2007. The protocols were submitted to the Executive CAC on August 21, 2007 and the following comments were forwarded to the sponsor:

### Executive CAC Recommendations and Conclusions:

#### Mouse:

- The Committee did not concur with the doses proposed by the sponsor [REDACTED] (b) (4).
- The Committee recommended doses of 0, 3000, 10,000, and 30,000 mcg/kg/day by snout-only inhalation, based on an MTD criterion (mortality and clinical signs at 63,600 mcg/kg). The recommended high dose is approximately one-half of the identified lethal dose. The sponsor may add additional lower doses at their discretion.
- The sponsor should contact the agency prior to terminating any groups or changing any doses.

#### Rat:

- The Committee did not concur with the doses proposed by the sponsor [REDACTED] (b) (4).
- The Committee recommended doses of 0, 80, 220, and 650 mcg/kg/day by nose-only inhalation, based on an MTD criterion (severe respiratory tract irritancy and ulceration of the upper airways at doses of 10,392 mcg/kg and higher). The sponsor may add additional lower doses at their discretion.
- The sponsor should contact the agency prior to terminating any groups or changing any doses.

In submission 012, the sponsor indicates that they agree with the Executive CAC recommendations for the rat study but will add an additional dose group at a dose of 10 µg/kg/day. Regarding the mouse study, GSK agrees with the recommended high dose of 30,000 µg/kg. However, they would like to gain agreement on adjusting the lower dose

groups to allow them to explore the dose response of anticipated uterine leiomyomas over a wide range of exposures. The CAC recommended mid- and low doses of 10,000 and 3000 mcg/kg. GSK's concern is that if the doses recommended by the CAC are used, it would require 7 dose groups to fully explore the dose response; GSK initially proposed a (b)(4) design.

GSK proposes doses of 30,000, 6,000, 600, 60, 6 and 0 (lactose) mcg/kg. Their rationale for adjusting the recommended mid-dose from 10,000 mcg/kg to 6000 mcg/kg is that if the high dose of 30,000 mcg/kg were to exceed the MTD, 6000 mcg/kg would be within 1/3 the MTD (note: actually more than half) based on the observed sub-proportional increase in systemic exposure. The other proposed lower doses would provide exposure ratios of ~ 1000, 150 and 10-15 fold the anticipated maximum human exposure.

#### Internal comments:

The sponsor's rationale for adjusting the lower doses in the mouse carcinogenicity study is reasonable and the proposed doses still achieve the goals sought by the CAC in recommending the original dose range while allowing the sponsor to investigate the dose response using as limited a number of groups as possible. Members of the Executive CAC (David Jacobson-Kram, Abby Jacobs, and Joe Contrera) were consulted and concurred with the sponsor's approach. Therefore, the sponsor should be informed that the proposal is acceptable.

#### External comments (to sponsor):

We have reviewed your submission dated September 7, 2007 regarding dose selection for your rat and mouse carcinogenicity studies. We agree with your proposal to add an additional dose group of 10 µg/kg/day to the rat study and to administer doses of 30,000, 6000, 600, 60, 6, and 0 µg/kg/day in the mouse study.

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this page is the manifestation of the electronic signature.**  
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/s/

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Timothy McGovern  
9/17/2007 03:11:50 PM  
PHARMACOLOGIST