CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203975Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 203975
Submission date: 12/18/2012
Drug: umeclidinium bromide and vilanterol triphenylacetate
Sponsor: GlaxoSmithKline
Indication: treatment of airflow obstruction in chronic obstructive pulmonary diseases

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 umeclidinium bromide and vilanterol triphenylacetate and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above. This product is a combination of umeclidinium bromide, which is a new molecular entity long-acting muscarinic antagonist, and vilanterol which is a long acting $beta_2$-agonist that is a component of another previously approved combination product.

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.

The carcinogenicity of umeclidinium 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. No drug-related neoplasms were noted in either study.

Developmental and Reproductive Toxicity
The only apparent effect of vilanterol in developmental and reproductive toxicity studies was an increase in skeletal variations in rabbits at doses that produced exposures much higher than the maximum human exposures. Similarly, the only apparent effect of umeclidinium in developmental and reproductive studies was an increase in skeletal variations in rats at a dose that produced an exposure substantially higher than that expected in humans.

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Conclusions:
I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. No additional nonclinical studies are recommended. I have provided comments on labeling separately.

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/s/

PAUL C BROWN
12/13/2013

Pharmacology and Toxicology Secondary Review for NDA 203-975

TO: NDA 203-975 (ANORO ELLIPTA; Combination of Umeclidinium bromide and Vilanterol triphenylacetate)

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: August 11, 2013

ANORO ELLIPTA is fixed dose combination, dry powder inhaler of umeclidinium and vilanterol for the treatment of airflow obstruction in chronic obstructive pulmonary diseases (COPD) in adults. ANORO ELLIPTA delivers a mixture of 62.5 mcg of umeclidinium bromide and 25 mcg of vilanterol triphenylacetate in each actuation. The formulation includes magnesium stearate and lactose as excipients.

Dr. Sohn's review focused on the nonclinical safety assessment of umeclidinium alone and umeclidinium in combination with vilanterol. In addition, the daily dose of the excipient, magnesium stearate, was evaluated. The nonclinical program for vilanterol was evaluated under NDA 204-275.

I concur with the recommendations of Dr. Jane Sohn's review dated June 25, 2013 that the nonclinical pharmacology and toxicology of the drug product, a combination of umeclidinium and vilanterol, have been adequately studied and ANORO ELLIPTA should be approved from the nonclinical perspective.

Pharmacology: Umeclidinium is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. Vilanterol is a LABA.

Secondary Pharmacology: Umeclidinium at 1 µM was screened against a panel of receptors, ion channels, and transporters using radioligand binding. The Ki values for the five targets, which were not muscarinic M_1-M_4 receptors, were Kappa opioid receptor (69 nM), Sigma (non-selective) receptor (220 nM), Ca^{2+} channel [L, verapamil site] (330 nM), Na^+ channel [site 2] (170 nM), and Dopamine transporter (780 nM).

Safety Pharmacology: Umeclidinium bromide was assessed for effects on CNS, cardiovascular, and respiratory functions in safety pharmacology studies with rats and dogs following inhalation exposure. Moderately dilated pupil was observed for rats at inhaled doses of 322 and 1994 mcg/kg, which was an expected pharmacological effect, but no other changes in CNS were observed. An inhaled dose of 10 µg/kg administered to dogs increased heart rate up to 49 bpm from a predose average of 65 bpm, which

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recovered 30 min after infusion. In agreement with increased heart rate, RR interval decreased, but returned to predose values by 30 min postdose. Changes in heart rate and RR interval were associated with the tachycardia seen following dosing with 10 mcg/kg. Second degree atrioventricular (AV) block was observed in 3 of 4 animals treated with 10 mcg/kg. Increased heart rate, decreased RR interval, and AV block can be attributed to the antimuscarinic effects of umeclidinium. Single inhaled doses of umeclidinium at 215 and 2260 mcg/kg administered to rats produced increases in respiratory rate (18 to 45%) and decreases in tidal volume (3 to 17%).

ADME: The absorption, distribution, metabolism, and excretion of umeclidinium were examined in mice, rats, dogs, and humans. The half-lives of umeclidinium in rats and dogs following intravenous administration were 1.42 and 11.6 hr, respectively. The half-life of umeclidinium at an inhaled dose of 62.5 µg in human subjects was 11 to 15 hr. Plasma protein binding in mouse, rat, rabbit, dog, and human ranged from 74.7 to 88.8% and was concentration-independent. Studies in rats and dogs suggested extensive distribution into tissues and rapid clearance suggestive of metabolic clearance. Umeclidinium was a substrate for p-glycoprotein. Metabolism was examined *in vitro* using hepatocytes from mouse, rat, rabbit, dog and human. Studies with human hepatocytes and microsomes found that the main routes of metabolism of umeclidinium were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The main routes of metabolism in humans were also present in rat and dog. Metabolism of umeclidinium in human liver microsomes was mediated primarily by CYP2D6, with some contribution from CYP3A4; umeclidinium was an inhibitor of both enzymes.

Inhalation Toxicology: Pivotal general toxicology studies to support the use of umeclidinium were 26 and 39 week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. Relevant target organs were the lung and tracheal bifurcation in the rat and the heart, lung, larynx, and nasal turbinates in the dog. A 13-week toxicology study with the combination of umeclidinium and vilanterol in dogs found that observed toxicity was consistent with the monoproducts. There was no evidence of additive or synergistic toxicity with the combination. See Dr. Sohn's review for further details.

Genotoxicity: Umeclidinium was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation assay*, in vitro* mouse lymphoma assay, and *in vivo* rat micronucleus test.

Carcinogenicity: The sponsor determined the carcinogenic potential of umeclidinium in 2-year nose-only inhalation bioassays conducted with mice and rats. Both bioassays were negative for test-article related tumors. See Dr. Sohn's review dated May 23, 2013.

Reproductive and developmental toxicology: Reproductive and developmental studies showed that umeclidinium had no effects on fertility or reproductive performance in rats and was not teratogenic in rats or rabbits. Umeclidinium caused a skeletal variation in

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rats in a dose-dependent manner. Umeclidinium did not have any effects on pre- or post-natal development in rats.

Excipients: The drug product includes lactose and magnesium stearate as excipients. There are no safety concerns for either compound. Lactose is used as an excipient in a number of currently marketed inhalation drug products. The proposed exposure to magnesium stearate from ANORO ELLIPTA is 200 µg/day. To support the safety of magnesium stearate as an excipient, the sponsor submitted a 26-week inhalation toxicology study with magnesium stearate in rats. No treatment-related effects were identified.

Minor errata in Dr. Sohn's Review: A few minor errata in Dr. Sohn's review are corrected here. For the table on Page 83, the last row of the table as well as the footnote both refers to the viability of F_2 pups on Day 4. On Page 90 in reference to the embryofetal development study with rabbits, body weight gains for rabbits in the mid and high doses from gestation days 7 to 18 (i.e., period of dosing) were reduced by 14% relative to the concurrent control. On Page 94, Dr. Pei's review was dated June 7, 2000.

Established Pharmacological Class (EPC): ANORO ELLIPTA is a combination anticholinergic/long-acting beta2-adrenergic agonist (Anticholinergic/LABA). The EPC for umeclidinium as an anticholinergic was discussed with Dr. Paul Brown.

Labeling: Dr. Sohn's review recommends changes to product labeling in Section 1 (Indications and Usage), Section 8.1 (Pregnancy), Section 8.3 (Nursing Mothers), Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility). I concur with Dr. Sohn's recommendations for changes to the product label. See Dr. Sohn's review for additional details of changes to the product labeling.

Control of potential genotoxic impurities: Genotoxic impurities in the drug substance were controlled to the Threshold of Toxicological Concern (TTC) of less than 1.5 µg/day. Descriptions of genotoxic impurities were provided below; however, there were no safety concern given that all were less than the TTC.

(b) (4)

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A number of other structurally related genotoxic impurities are potential impurities of [redacted] (b) (4) The sponsor determined that [redacted] (b) (4) (Study #2010N110596) and [redacted] (b) (4) (Study #2011N111625) were Ames-positive mutagens. These 5 impurities were controlled by strict limits in the specification for the starting material [redacted] (b) (4) There are no tests for these potential impurities in umeclidinium bromide drug substance.

Other impurities that were considered as part of the genotoxic risk assessment for the synthesis of umeclidinium bromide were as follows:

[redacted] (b) (4)

Level for all three impurities were [redacted] (b) (4) in clinical batches of [redacted] (b) (4) Levels of these three impurities were confirmed to be [redacted] (b) (4)

[redacted] (b) (4) There is no test for [redacted] (b) (4) in the drug substance specification.

[redacted] (b) (4)

(b) (4)

There is no test for (b) (4) in the specifications for any intermediates or drug substance.

Recommendation: From the nonclinical perspective, approval of the application is recommended.

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

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/s/

TIMOTHY W ROBISON
08/11/2013

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 203975
Supporting document/s:	SD-1 (New NDA), SD-18 (Non-clinical/Response to Information Request), SD-22 (Labeling/Package Insert Draft), SD-23 (Non-clinical/Response to Information Request)
Applicant's letter date:	12/18/12, 5/16/13, 6/7/13, 6/11/13
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Product:	Umeclidinium-Vilanterol
Indication:	Treatment of airflow obstruction in COPD
Applicant:	GlaxoSmithKline
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Jane J. Sohn, Ph.D.
Supervisor/Team Leader:	Timothy W. Robison, Ph.D., DABT
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.
Project Manager:	Leila P. Hann

Template Version: September 1, 2010

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Any information or data necessary for approval of NDA 203975 that GlaxoSmithKline does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203975.

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1 Executive Summary

1.1 Introduction

GlaxoSmithKline (GSK) has submitted this NDA for registration of ANORO ELLIPTA for treatment of airflow obstruction in chronic obstructive pulmonary diseases (COPD). ANORO ELLIPTA is a dry powder inhaler that delivers a dry powder mixture of umeclidinium bromide salt (umeclidinium) and vilanterol triphenylacetate salt (vilanterol) with excipients, magnesium stearate and lactose monohydrate. The sponsor proposes that each actuation deliver 62.5/25 mcg of umeclidinium/vilanterol, respectively, with one actuation proposed per day.

The application included nonclinical studies with umeclidinium alone, vilanterol alone, and the two active pharmaceutical ingredients (APIs) in combination. Vilanterol is approved for use in combination with fluticasone furoate for the maintenance treatment of airflow obstruction in patients with COPD. Studies for vilanterol were submitted previously to and reviewed under NDA 204275 (fluticasone furoate and vilanterol). Some studies for umeclidinium were previously submitted under INDs 104479 and 106616 for supporting umeclidinium alone and the combination of umeclidinium and vilanterol, respectively.

Regarding umeclidinium, most nonclinical studies to support its use utilized GSK573719A (umeclidinium bromide salt, proposed commercial form) with excipients, magnesium stearate and lactose monohydrate. Pharmacokinetic studies were conducted using ^{14}C-GSK573719 (as the bromide salt, GSK573719C, or as the trifluoroacetate salt, GSK573719G) and a pharmacology study was conducted using ^{3}H-GSK573719. Micronized GSK573719A was used for nonclinical inhalation studies, and both non-micronized and micronized forms of GSK573719A were used for other routes of administration. Clinical studies by the inhalation route used micronized GSK573719A (umeclidinium bromide salt), with excipients magnesium stearate and lactose monohydrate. Early nonclinical and clinical studies used lactose and (b) (4)) as excipients.

Regarding vilanterol, nonclinical studies to support its use were performed for the most part with GW642444M (vilanterol triphenylacetate salt, commercial form), and GW642444H (the α-phenylcinnamate salt). A few studies (not used for safety assessment) used GW642444A (the acetate salt). The justification for the use of these test articles is summarized in the nonclinical review for NDA 204275.

1.2 Brief Discussion of Nonclinical Findings

1.2.1 Umeclidinium

Umeclidinium is a long-acting muscarinic antagonist (LAMA), which is being recommended to labeled as an anticholinergic to be consistent with the established pharmacological classification for similar approved drugs in its class. A complete and

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extensive nonclinical program has been conducted to support the safety of umeclidinium from the nonclinical perspective.

Pivotal general toxicology studies to support the use of umeclidinium were 26 and 39 week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. Relevant target organs were the lung and tracheal bifurcation in the rat and the heart, lung, larynx, and nasal turbinates in the dog. Safety margins were 30 and 19 times the maximum recommended human dose (MRHD) on an AUC basis for rats and dogs, respectively.

A 13-week toxicology study with the combination of umeclidinium and vilanterol in dogs found that observed toxicity was consistent with the monoproducts. There was no evidence of additive or synergistic toxicity with the combination.

Two-year carcinogenicity studies were conducted in rats and mice. Both bioassays were negative for test-article related tumors. Safety margins were 26 and 22 times the MRHD on an AUC basis for male and female mice, respectively. The safety margin in rats was 22 times the MRHD on an AUC basis.

Reproductive and developmental studies showed that umeclidinium did not affect fertility in rats, and was not teratogenic in rats or rabbits. Umeclidinium caused a skeletal variation in rats in a dose-dependent manner, but it did not have any pre- or postnatal effects in rats. With regard to fertility, safety margins were 100 and 108 times the MRHD on an AUC basis for male and female rats, respectively. For teratogenicity, safety margins were 287 and 108 times the MRHD on an AUC basis for rabbits and rats, respectively. The peri- and postnatal study in rats provided a safety margin of 80 times the MRHD on an AUC basis.

1.2.2 Vilanterol

The following is based on the review for NDA 204275 by Dr. Luqi Pei, dated 3/12/13. The dose multiples are changed to reflect the higher exposure to VI (AUC 0.615 ng•hr/mL) in combination with UMEC for NDA 203975, versus VI (AUC 0.266 ng•hr/mL) in combination with fluticasone furoate under NDA 204275. To adjust for the higher exposure to VI (in combination with UMEC), dose multiples for VI have been decreased by a factor of 2.31.

The toxicological profile of vilanterol has been characterized previously under NDA 204275. Briefly, vilanterol is a long acting beta$_2$-agonist (LABA). Pivotal general toxicology studies to support the use of vilanterol were 26 and 39 week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. Target organs identified were the upper airways, lung, heart, liver and testes. Findings were consistent with other beta$_2$-agonists.

Two-year carcinogenicity studies were conducted in rats and mice. Rats showed a dose-dependent shortening of latency for pituitary neoplasms in both sexes, and increased incidence of mesovarian leiomyomas in females (at greater than or equal to

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20 times the MRHD on an AUC basis). In mice, there were increases in tubulostromal carcinomas of the ovaries in females (at greater than or equal to 3800 times the MRHD on an AUC basis). No tumors were observed at approximately 1 time the MRHD on an AUC basis. Findings were typical of $beta_2$-agonists.

Reproductive and developmental studies showed that vilanterol caused dose-dependent skeletal variations in both rats and rabbits. There were no teratogenic effects in rats and rabbits at approximately 13,000 and 70 times, respectively, the MRHD in adults (on a mcg/m^2 basis at a maternal inhaled dose of 33,700 mcg/kg/day in rats and on an AUC basis at a maternal inhaled dose of 591 mcg/kg/day in rabbits). However, fetal skeletal variations were observed in rabbits at approximately 500 times the MRHD 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 MRHD in adults (on a mcg/m^2 basis at a maternal dose of 10,000 mcg/kg/day). Vilanterol did not have an affect on fertility in either males or females at approximately 12,000 and 14,000 times the MRHD in adults (on a mcg/m^2 basis at inhaled doses of 31,500 and 37,100 mcg/kg/day in males and females, respectively.

1.3 Recommendations

1.3.1 Approvability

The applicant has provided complete nonclinical pharmacology and toxicology programs for umeclidinium and vilanterol, which support the safety of the proposed clinical dose of 62.5/25 mcg/day of umeclidinium/vilanterol, respectively, for the treatment of airflow obstruction in COPD. From the Pharm/Tox perspective, the application is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

See Labeling Recommendations.

1.3.3 Recommended Labeling Edits

Recommended line editing of the proposed text for the nonclinical sections of the ANORO ELLIPTA label is provided below. Changes are presented as strikethroughs for deletions or in red font for additions.

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1 INDICATIONS AND USAGE

ANORO ELLIPTA is a combination anticholinergic/long-acting beta$_2$-adrenergic agonist (Anticholinergic/LABA) indicated for the long-term, once-daily, maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. (1)

[redacted] (b) (4)

~~Important Limitations of Use: ANORO ELLIPTA is NOT indicated for the relief of acute bronchospasm or for the treatment of asthma.~~

8.1 Pregnancy

Teratogenic Effects: [redacted] (b) (4) Pregnancy Category C. There are no adequate and well-controlled trials [redacted] (b) (4) of ANORO ELLIPTA or its individual components, umeclidinium and vilanterol, in pregnant women. [redacted] (b) (4) Because animal reproduction studies are not always predictive of human response, ANORO 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 physicians if they become pregnant while taking ANORO ELLIPTA.

Umeclidinium: There was no evidence of teratogenic effects in rats and rabbits at approximately [redacted] (b) (4) respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 278 mcg/kg/day in rats and at maternal subcutaneous doses up to 180 mcg/kg/day in rabbits). [redacted] (b) (4)

Vilanterol: (b) (4) There were no teratogenic effects in rats and rabbits at approximately 13,000 and (b) (4) 70 times, respectively, the MRHD (b) (4) in adults (on a mcg/m^2 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 (b) (4) 450 times the MRHD (b) (4) 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. (b) (4)

(b) (4)

Nonteratogenic Effects:

Umeclidinium: There were no effects on peri-natal and post-natal developments in rats at approximately 80 times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 180 mcg/kg/day).

Vilanterol: There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHD in adults (on a mcg/m^2 basis at a maternal oral doses up to 10,000 mcg/kg/day.)

8.3 ~~8.3~~ Nursing Mothers

ANORO ELLIPTA: It is not known whether ANORO ELLIPTA is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when ANORO ELLIPTA is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of ANORO ELLIPTA by nursing mothers, based on the data for the individual components, a decision should be made whether to discontinue nursing or to discontinue ANORO ELLIPTA, taking into account the importance of ANORO ELLIPTA to the mother.

Umeclidinium: It is not known whether umeclidinium is excreted in human breast milk. However, administration to lactating rats at approximately 25 times the MRHD in adults resulted in measurable umeclidinium in the milk (on an AUC basis at a maternal subcutaneous dose of 60 mcg/kg/day).

Vilaterol: It is not known whether vilanterol is excreted in human breast milk. However, other beta$_2$-agonists have been detected in human milk.

(b) (4)

(b) (4)

12.1 Mechanism of Action

ANORO ELLIPTA: (b) (4) ANORO ELLIPTA contains both umeclidinium and vilanterol;, the mechanisms of action described below for the individual components apply to ANORO ELLIPTA. These drugs represent 2 different classes of medications (an anticholinergic (b) (4) and a LABA) that have different effects on clinical and physiological indices- (b) (4)

Umeclidinium: Umeclidinium is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methacholine and acetylcholine-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of umeclidinium is predominantly a site-specific effect. (b) (4)

(b) (4)

Vilanterol: Vilanterol is a LABA. (b) (4) *In vitro* tests have shown the functional selectivity of vilanterol was similar to salmeterol. The clinical relevance of this (b) (4) *in vitro* finding is unknown.

Although beta$_2$-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta$_1$-receptors are the predominant receptors in the heart, there are also beta$_2$-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$_2$-agonists may have cardiac effects.

———The pharmacologic effects of beta$_2$-adrenergic agonist drugs, including vilanterol, are at least in part attributable to stimulation of intracellular adenyl 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

ANORO ELLIPTA: No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with ANORO ELLIPTA; however, studies are available for individual components, umeclidinium and vilanterol, as described below.

———Umeclidinium: Umeclidinium produced no treatment-related increases in the incidence of tumors in 2-year inhalation studies in rats and mice at inhaled doses up to 137 mg/kg/day and 295/200 mcg/kg/day (male/female), respectively (approximately 20 and 25/20 times the MRHD in adults on an AUC basis, respectively).

(b) (4)

———Umeclidinium tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* mouse lymphoma assay, and *in vivo* rat bone marrow micronucleus assay.

(b) (4)

———No evidence of impairment of fertility was observed in in male and female rats at subcutaneous doses up to 180 mcg/kg/day and inhaled doses up to 294 mcg/kg/day, respectively (approximately 100 and 110 times, respectively, the MRHD in adults on an AUC basis).

(b) (4)

———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 (b) (4) times the MRHD (b) (4) in adults on an AUC basis). No increase in tumors was seen at an inhalation dose of 615 mcg/kg/day (approximately (b) (4) times the MRHD~~ID~~ 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 (b) (4) 20 times the MRHD~~ID~~ in adults on an AUC basis). No tumors were seen at an inhalation dose of 10.5 mcg/kg/day (approximately (b) (4) 1 times the MRHD (b) (4) 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* (b) (4) (b) (4) Ames assay, *in vivo* (b) (4) rat bone marrow micronucleus assay, *in vivo* (b) (4) rat unscheduled DNA synthesis (UDS) assay, and *in vitro* (b) (4) Syrian hamster embryo (SHE) cell assay. Vilanterol tested equivocal in the *in vitro* (b) (4) mouse lymphoma assay.

———

No evidence of impairment of fertility was observed in reproductive studies conducted in male and female rats at inhaled vilanterol doses up to 31,500 and 37,100 mcg/kg/day, respectively (approximately 12,000 and 14,500 times, respectively, the MRHD (b) (4) in adults on a mg/m^2 basis).

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 869113-09-7 (umeclidinium bromide salt), 503068-34-6 (vilanterol)

Generic Name:
1) Umeclidinium bromide salt (UMEC)
2) Vilanterol triphenylacetate salt (VI)

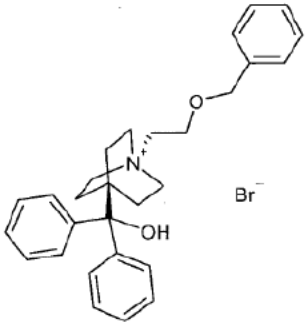
Code Name:
1) GSK573719A (umeclidinium bromide salt)
2) GW642444M (vilanterol triphenylacetate salt)

Chemical Name:
1) 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide
2) Triphenylacetic acid-4-{(1R)-2-[(6-{2-[2,6-dicholorobenzyl)oxy]ethoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol (1:1)

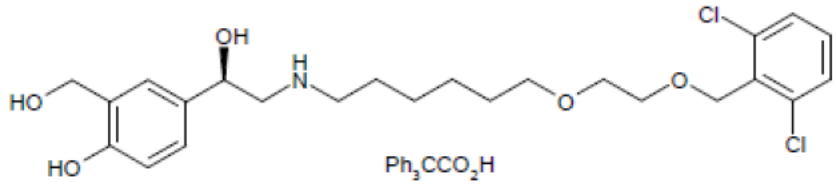
Molecular Formula/Molecular Weight:
1) $C_{29}H_{34}NO_2 \cdot Br$
508.5 (GSK573719A as quaternary ammonium bromide compound)
428.6 (as free cation, GSK573719);
2) $C_{24}H_{33}Cl_2NO_5.C_{20}H_{16}O_2$
774.78 (as the triphenylacetate salt)
486.43 (as the free base)

Reference ID: 3330354

Structure:



Umeclidinium bromide salt



Vilanterol triphenylacetate salt

Pharmacologic Class:
1) anticholinergic
2) long-acting β2-adrenergic agonist (LABA)

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 204,275 (GSK, BREO ELLIPTA (Fluticasone furoate/vilanterol trifenatate) Dry Powder Inhaler)
IND 106,616 (GSK, GSK573719 (umeclidinium)/GW642444 (vilanterol)
IND [redacted] (b) (4)
IND [redacted] (b) (4)
IND 104,479 (GSK, GSK573719)
IND 74,696 (GSK, GW642444)
IND 77,855 (GSK, GW642444 + Fluticasone furoate)
IND [redacted] (b) (4)
DMF 026,339 (GSK, GSK573719)
DMF 025,906 (GSK, GW642444)

2.3 Drug Formulation

The drug product is a plastic inhaler that contains two separate blister strips. Each strip contains blisters, each containing either umeclidinium or vilanterol. The umeclidinium/vilanterol inhalation powder 62.5/25 mcg contains 62.5 mcg of umeclidinium (UMEC: as bromide) and 25 mcg of vilanterol (VI; as trifenatate) per inhalation. There is a manufacturing overage of up to [redacted] (b) (4) of umeclidinium and vilanterol [redacted] (b) (4), respectively.

Blister strip	Ingredient	Quantity/blister (mcg)*	Function

UMEC 62.5 mcg	Umeclidinium bromide	74.2	Active
	Magnesium Stearate	75	(b) (4)
	Lactose Monohydrate	to 12.5	(b) (4)
VI 25 mcg	Vilanterol trifenatate	40	Active
	Magnesium Stearate	125	(b) (4)
	Lactose Monohydrate	to 12.5	(b) (4)

* (b) (4) of UMEC and VI, respectively
Note: Each actuation (daily dose) releases 62.5 mcg umeclidinium bromide, 25 mcg vilanterol trifenatate, 200 mcg magnesium sulfate, and 25 mcg lactose monohydrate, for a total of 375 mcg of drug product.

2.4 Comments on Novel Excipients

The product uses magnesium stearate and lactose as inactive ingredients. Each actuation (daily dose) releases 200 mcg of magnesium sulfate, and 25 mcg of lactose monohydrate. The proposed level of lactose is supported by its use in other approved inhalation products. The expected exposure to magnesium stearate is higher than levels in approved products. The applicant submitted additional nonclinical data (up to 6 months in rats) to support the proposed use of magnesium stearate. The NOAEL in the 6 month toxicity study provided adequate safety margins for the proposed use of the excipients.

Lactose: Lactose is a common excipient used in formulations for dry powder inhalers. The expected daily exposure to lactose from the proposed product is 25 mg/day, which is present in equal or greater amounts in other approved products.

Magnesium stearate: Magnesium stearate is present as an excipient in approved products, including BREO ELLIPTA. The exposure to magnesium stearate from BREO ELLIPTA is 125 mcg/day. The proposed exposure to magnesium stearate from ANORO ELLIPTA is 200 mcg/day. Due to the higher exposure of patients to magnesium stearate, safety relies upon nonclinical data. The data includes inhalation toxicity studies with magnesium stearate of up to 6 months duration in rats (see Pharm/Tox review by Dr. Luqi Pei, dated June 7, 2000). Briefly, there were no safety concerns identified in the rodent study. Sprague-Dawley rats were exposed via nose-only inhalation of up to 180 mcg/kg/day of magnesium stearate (pulmonary deposited dose) for 26 weeks (GSK Report WD2006/03154; (b) (4) 724353). The expected exposure to magnesium stearate from ANORO ELLIPTA is 3.3 mcg/kg/day (for a 60 kg individual). Thus, these data provide a safety margin of 54 for the proposed clinical doses, which is considered adequate.

2.5 Comments on Impurities/Degradants of Concern

There are no concerns for impurities or degradants from the nonclinical perspective.

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2.6 Proposed Clinical Population and Dosing Regimen

Adults with COPD, including chronic bronchitis and emphysema, are recommended to use one oral inhalation (one actuation) of ANORO ELLIPTA daily. Each actuation delivers 62.5 mcg of UMEC, and 25 mcg of VI.

2.7 Regulatory Background

The NDA (203975) relies upon NDA 204275 for nonclinical data supporting VI, and INDs 104479 and 106616 for nonclinical data supporting UMEC alone and the combination of UMEC and VI, respectively. NDA 204275 (combination of VI and fluticasone furoate) received approval for marketing on May 10, 2013, with no outstanding issues from the nonclinical perspective. In general, studies for VI to support NDA 204275 were submitted to IND 74696, and combination of VI and fluticasone furoate studies were submitted to IND 77855. Some data for VI only were submitted and discussed in both INDs 74696 and 77855. The previous review of VI by the Agency led to the current review for NDA 203975 to focus on UMEC.

DPARP and GSK held numerous meetings and exchanged written communications to discuss the development of UMEC alone and in combination with VI under INDs 104479 and 106616, respectively. Key interactions are summarized in the table below.

Application	API	Key regulatory events	Dates
NDA 203975	UMEC/VI	Filing of NDA	12/18/2012
NDA 204275	VI/FF	Approved	5/10/2013
IND 104479	UMEC	Pre-IND meeting	5/26/2009
		Carcinogenicity protocol assessment review	6/3/2009
		IND filing	7/14/2009
		Carcinogenicity protocol amendment review	2/18/2011
IND 106616	UMEC/VI	IND filing	11/16/2009
		EOP2 meeting	10/29/2010
		pre-NDA meeting	1/18/2012
		pre-NDA CMC comments	6/5/2009
IND 74696	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
IND 77855	VI/FF	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

UMEC = umeclidinium
VI = vilanterol
FF = fluticasone furoate

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Under IND 104479, GSK developed UMEC alone for the maintenance treatment of bronchospasm in patients with COPD including chronic bronchitis and emphysema. A pre-IND meeting was held on 5/26/2009, during which the Agency outlined the duration of nonclinical studies needed to support clinical dosing, the use of magnesium stearate via the inhalation route, findings in 4-week dog studies, and proposed carcinogenicity studies. GSK filed the initial IND submission on 7/14/2009. Carcinogenicity protocols were assessed by the Executive Carcinogenicity Assessment Committee (ECAC) on 6/3/2009, and amendments to the protocols were evaluated by the ECAC on 2/18/11.

Under IND 106616, GSK developed UMEC in combination with VI for the maintenance treatment of bronchospasm in patients with COPD including chronic bronchitis and emphysema. GSK filed the IND on 11/16/09, with 4 week combination (UMEC/VI) toxicology studies in rat and dog to support a 4-week clinical protocol. On 10/29/10, an EOP2 meeting was conducted during which GSK asked if the available nonclinical studies were adequate to support Phase III clinical trials Further, GSK asked if the submitted 4 week combination studies in rats and dogs, and the 13 week combination study in dogs were sufficient to support registration. The Pharm/Tox reviewer stated that the 13 week combination (UMEC/VI) toxicology study in dogs was intended to determine if the combination led to toxic effects that were additive or synergistic, compared to the monoproducts. Further histopathologic assessment of tissue from the 13 week dog study was requested to complete the toxicity assessment. The reviewer noted that a pre-adaptation phase was used for some drug-treated groups in the 13 week combination dog study, which could have potentially induced tolerance to toxic effects. To address the Division's concerns about the use of a pre-adaptation phase in the 13 week study, GSK conducted a 4 week combination study in dogs to determine the effect of pre-adaptation.

To support a 52 week clinical protocol, the sponsor submitted the requested data for the 13 week combination (UMEC/VI) study in dogs, and additional studies with UMEC only were reviewed (26 week study in rats, 39 week study in dogs). The reviewer determined that there were adequate safety margins for UMEC based on the monoproduct studies, and that there were no additive or synergistic toxicities due to the combination. Therefore, Phase III studies were determined to be safe to proceed from the nonclinical perspective. The outstanding 4-week combination study dogs to determine the effect of pre-adaption is reviewed in this NDA review.

On 1/18/12, a pre-NDA meeting under IND 106616 was held. It was agreed that no further nonclinical studies were required to support registration of the combination product.

See the review for NDA 204275 for a written summary of key regulatory events under INDs 74696 and 77855 to support the development of VI.

Reference ID: 3330354

3 Studies Submitted

3.1 Studies Reviewed

Study no.	Test article	Title
Toxicology		
Dog - Inhalation - Short		
2010N109790	GW642444M, GSK573719A	A 4-Week Combination Inhalation Toxicity Study in Dogs
Reproductive and Developmental		
Fertility and early embryonic development		
CD2010/00187	GSK573719A	Subcutaneous Male Fertility Study in Rats
EFD		
CD2010/00253	GSK573719A	Subcutaneous Embryofetal Development Study in Rabbits
PPND		
2011N118595	GSK573719A	Subcutaneous Pre-and Postnatal Developmental Study in Rats

3.2 Studies Not Reviewed

Study no.	Test article	Title
Pharmacology		
Primary		
2012N 138876	GSK573719	Pharmacological characterisation of the binding kinetics of [3H]GSK573719 and [3H]Tiotropium at the muscarinic acetylcholine receptor subtypes M2 and M3
Pharmacokinetics		
Analytical Method		
WD2008/00583	GSK573719, GW642444	The Abbreviated Validation of a Method for the Determination of GSK573719 and GW642444 (ranges 0.1 to 100 ng/mL) in Rat Plasma using HPLC-MS/MS
WD2008/00560	GSK573719, GW642444	The Validation of a Method for the Determination of GSK573719 and GW642444 (range 0.1 to 100 ng/ml) in Dog Plasma using HPLC-MS/MS
WD2009/01379	GSK573719, GW642444	The Abbreviated Validation of a Method for the Determination of GSK573719 (range 20 to 20000pg/ml) and GW642444 (range 30 to 30000 pg/mL) in Rabbit Plasma using HPLC-MS/MS
WD2006/03558	GSK573719	The Abbreviated Validation of a Method for the Determination of GSK573719 in Mouse Plasma (range 0.1 to 100ng/ml) using HPLC-MS/MS
2011N117769	GSK573719	The Abbreviated Validation of a Method for the Determination of GSK573719 (Range 20 to 20000 pg/ml) in Mouse Plasma using HPLC-MS/MS
WD2004/01614	GSK573719	Method for the Determination of GSK573719 (range 0.10to 10ng/ml) in Rot Plasma using HPLC-MS/MS
WD2005/00426	GSK573719	The abbreviated validation of a method for the determination of GSK573719 in rat plasma (range 0.10 to 100 ng/ml) using HPLC-MS/MS

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WD2006/003559	GSK573719	The Abbreviated Validation of a Method for the Determination of GSK573719 in Rabbit Plasma (range 0.1 to 50ng/ml) using HPLC-MS/MS
WD2005/00427	GSK573719	The abbreviated validation of a method for the determination of GSK573719 (range 0.10to 100 ng/ml) in dog plasma using HPLC-MS/MS
2011 N114275	GSK573719	The Validation of a Method for the Determination of GSK573719 (range 20 to 20000 pg/ml) in Rat Plasma using HPLC-MS/MS
2011N128044	GSK573719	Supplemental validation data to "The validation of a method for the determination of GSK573719 in (range 20 to 20000pg/mL) in Rat Plasma using HPLC-MS/MS
CD2003/00205	GW642444X	The Validation of Method for the determination of GW642444X in rat plasma (range 0.1·100 ng/ml) using HPLC-MS/MS
CD2003/00206	GW642444X	The Abbreviated Validation of a Method for the determination of GW642444X in dog plasma (range 0.1 to 100 ng/ml) using HPLC-MS/MS
CD2003100640	GW642444X	The Abbreviated Validation of a Method for the determination of GW642444X in mouse plasma (range 0.1 • 100 ng/ ML) using HPLC-MS/MS
WD2005/00112	GW642444	The Validation of a Method for the Determination of GW642444 in Rabbit Plasma (range 0.1 to 100ng/ml) using HPLC-MS/MS
WD2006/01702	GW642444, GW630200, GSK932009	The Validation of a Method for the Determination of GW642444, GW630200 and GSK932009 (range 0.1 to 50 ng/ml) in Dog Plasma using HPLC-MS/MS
WD2006/01892	GW642444, GW630200, GSK932009	The Abbreviated Validation of a Method for the Determination of GW642444. GW630200 and GSK932009 (range 0.1 to 50 ng/mL) in Mouse Plasma using HPLC-MS/MS
WD2006/02001	GW642444, GW630200, GSK932009	The Abbreviated Validation of a Method for the Determination of GW642444. GW630200 and GSK932009 (range 0.1 to 50 ng/mL) in Rat Plasma using HPLC-MS/MS
WD2006/02592	GW642444, GW630200, GSK932009	The Abbreviated Validation of a Method for the Determination of GW642444 and its metabolites GW630200 and GSK932009 (range 0.1 to 50 ng/mL) in Rabbit Plasma using HPLC-MS/MS
WD2007/01855	GSK233705, GW642444	The Abbreviated Validation of a Method for the Determination of GSK233705 (Range 0.05 to 20ng/ml) and GW642444 (range 0.1 to 40 ng/mL) in Rat Plasma using HPLC-MS/MS
WD2007/01373	GSK233705, GW642444	The Validation of a Method for the Determination of GSK233705 (Range 0.05 to 20 ng/ml) and GW642444 (Range 0.1 to 40 ng/mL) in Dog Plasma using HPLC-MS/MS

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WD2008/01085	GSK233705, GW642444	The Validation of a Method for the Determination of GSK233705 (Range 0.05 to 20 ng/ml) and GW642444 (Range 0.1 to 40 ng/mL) in Dog Plasma using HPLC-MS/MS
WD2008/01259	GSK233705, GW642444	The Abbreviated Validation of a Method for the Determination of GSK233705 (Range 0.05 to 20 ng/ml) and GW642444 (Range 0.1 to 40 ng/mL) in Rabbit Plasma using HPLC-MS/MS
WD2005/ 00558	GI179710	Validation of a method for the determination of GI179710 in dog plasma (range 5 to 5000 ng/ml) using HPLC-MS/MS
WD2005/00571	GI179710	The Abbreviated Validation of a Method for the Determination GI179710 in Rat Plasma (range 5 to 5000 ng/ml) using HPLC-MS/MS
WD2005/ 00827	GI179710	The Abbreviated Validation of a Method for the Determination GI179710 in Rabbit Plasma (range 5 to 5000 ng/ml) using HPLC-MS/MS
WD2005/ 01158	GI179710	The Abbreviated Validation of a Method for the Determination of GI179710 in Mouse Plasma (range 5 to 5000ng/ml) using HPLC-MS/MS
FD2006/00311	GW685698, GW642444	The Validation of a Method for the Determination of GW685698 (range 40 to 4000 pg/ml) and GW642444 (range 100-10000 pg/mL) in Rat Plasma using SPE-HPLC MS/MS
FD2006/00312	GW685698, GW642444	An Abbreviated Validation of a Method for the Determination of GW685698 (range 40 • 40000pg/ml) and GW642444 (range 10 to 10000 pg/mL) in Dog Plasma using SPE-HPLC MS/MS
2010N108722	GW685698, GW642444	The Validation of a Method for the Determination of GW685698 and GW642444 (range 100to 10.000 pg/ml) in Dog Plasma HPLC-MS/MS
2011N118541	GW685698, GW642444	Supplemental Validation Data to "The Abbreviated Validation of a Method for the Determination of GW685698 (range 100 to 10000 pg/mL) and GW64244 100 to 10000 pg/mL) to in Dog Plasma using HPLC MS/MS
Distribution		
2012N144582	GSK573719	Plasma protein binding in plasma from renal and hepatically impaired subjects
WD2010/00669	GSK573719	In vitro investigations of the transport GSK573719 via human OCTl. OCT2. OCT3. OCTN1 and OCTN2 expressing cell systems.
Metabolism		
CH2006/00016	GSK573719	A Study to Estimate the In Vitro Intrinsic Clearance of GSK573719 in Rat. Dog. and Human Liver Microsomes
2010N105743	GSK573719	Metabolism of GSK573719 in male mice following a single intravenous administration of [14C]GSK573719 bromide at 1 mg free base/kg.

WD2009/00039	GSK573719	Quantification and characterisation of the major metabolites of GSK573719 following intravenous administration of [14C]GSK573719
Toxicology		
Single-Dose		
Rat · Subcutaneous		
RD2009/01098	GSK573719A	Single -dose subcutaneous toxicokinetic study in male rats
Repeat-Dose		
Rat - Inhalation - Short		
WD2004/01556	GSK573719A	Dose range finding study by inhalation administration to rats for 7 days
WD2005/01063	GSK573719A	A 7-day inhalation dose range finding toxicity study of a powder aerosol formulation in the rat
Dog - Inhalation - Short		
WD2005/ 00370	GSK573719A	A 7-day dose range finding inhalation toxicity study of a nebulised aerosol formulation in the beagle dog
WD2005/ 01061	GSK573719A	A 7-day inhalation dose range finding toxicity study of a powder aerosol formulation in the beagle dog
WD2006/ 03228	GSK573719	A Dose range finding study by inhalation administration to beagle dogs
Mouse · Oral · Short		
FD2009/ 00397	GSK573719	Single oral dose tolerability and 14-day oral dose range toxicity study in CD·1 mice
Rat · Intravenous · Short		
FD2008100339	GSK573719A	Single and 4-day intravenous infusion study in male Sprague Dawley rats
Rat · Subcutaneous · Short		
RD2009/ 01099	GSK573719A	14 day subcutaneous tolerability and toxicokinetic study in rats
20011 N111874	GSK573719A	14 day toxicokinetic and tolerability study in female rats
Reproductive and Developmental		
EFD		
WD2006/03186	GSK573719A	Inhaled dose range and preliminary embryofetal study in female Rabbits
Local Tolerance		
2011N123962	GSK573719A	Local Lymph Node Assay in the Mouse
2011 N 123960	GSK573719A	Determination of Skin Irritation Potential Using the SkinEthic Reconstructed Human Epidermal Model
2011 N 123961	GSK573719A	Determination of Eye Irritation Potential using the SkinEthic Reconstructed Human Corneal Epithelial Model
2012n139906	GSK573719A, GSK233705A	719 vs. 705 non-GLP topical irritancy study
Other Toxicity Studies		
Immunotoxicity		
2010N104308	GSK573719, GW642444	WEIGHT OF EVIDENCE REVIEW OF IMMUNOTOXICITY POTENTION OF LONG ACTING MUSCARINIC ANTAGONIST
Other		
CD2005/00038	GSK573719	In vitro hemolysis testing in rat and dog blood

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2012N147482	GSK573719 ·	Photomicrographs of Foamy Alveolar Macrophage• in the lung in 2-Year Carcinogenicity Study in Rats

*Studies for impurities are not listed, and are addressed in a separate review.

3.3 Previous Reviews Referenced

See NDA 204275 for nonclinical data supporting vilanterol.

For umeclidinium, the following reviews are reference, listed by the corresponding study previously reviewed.

Study no.	Test article	Title	Review
Pharmacology			
Primary PD			
CH2006/00020	GSK573719A	In vitro muscarinic antagonist activity. binding and calcium mobilization at cloned human receptors	IND 104479 (5/25/10)
CH2006/00014	GSK573719A	In vitro muscarinic antagonist on human bronchus in a static organ bath	IND 104479 (5/25/10)
CH2006/00015	GSK573719A	Functional in vitro muscarinic antagonist reversibility profile in human bronchus and guinea pig trachea using superfusion organ batch with carbachol challenge	IND 104479 (5/25/10)
CH2009/00016	GSK1781002 A, GSK339067A	In vitro antagonist potency against cloned human muscarinic ACh receptors	IND 104479 (5/25/10)
CH2006/00018	GSK573719A	In vitro effect on methacholine-induced bronchoconstriction in Balb/C mice	IND 104479 (5/25/10)
CH2005/00954	GSK573719A	Effect of intratracheal GSK573719A on acetylcholine-induced bronchoconstriction in guinea pigs	IND 104479 (5/25/10)
CH2005/ 00953	GSK573719A	Effect of intra-tracheal GSK573719A on acetylcholine-induced bronchoconstriction and bradycardia in guinea pigs	IND 104479 (5/25/10)
Secondary PD			
CH2006/00030	GSK573719A	In vitro receptor and ion channel selectivity screening	IND 104479 (5/25/10)
Safety Pharmacology			
FD2008/00365	GW642444 and GSK573719A	The effect of single intravenous administration on cardiovascular function in the conscious beagle dog	IND106616 (2/10/10)
VD2005/00625	GSK573719A	Acute neurobehavioural effects following inhalation administration in the conscious CD rat	IND 104479 (5/25/10)
CD2005/01385	GSK573719A	Acute effects on respiratory function following inhalation administration in the conscious CD rat	IND 104479 (5/25/10)
FD2005/00167	GSK573719A	The effect of single intravenous administration on a range of doses on cardiovascular function in the conscious beagle dog	IND 104479 (5/25/10)
CH2006/00029	GSK573719A	In vitro hERG channel selectivity screen	IND 104479 (5/25/10)
FD2005100109	GSK573719A	Effect on hERG tail current recorded from stably transfected HEK-293 cells	IND 104479 (5/25/10)

Reference ID: 3330354

Pharmacokinetics			
Absorption			
WD2008/00001	GSK573719	A study to determine the oral absorption and disposition of [14C]GSK573719 following a single administration of [14C]GSK573719 (trifluoroacetate) at a target dose level of 40 mcg free base/kg in the FVBn and mdrfa/1b mouse	IND 104479 (5/25/10)
WD2006/00073	GSK573719	A study to investigate the pharmacokinetics for GSK573719 in mole CD/SD rats following intravenous infusion at target dose level of 0.5 mg/kg	IND 104479 (5/25/10)
CH2006/00012	GSK573719A	Pharmacokinetics of GSK573719A following intravenous infusion, portal infusion and oral administration in male Sprague-Dawley rats	IND 104479 (5/25/10)
CH2006/00001	GSK573719A	Pharmacokinetics of GSK573719A in the male beagle dog	IND 104479 (5/25/10)
WD2006/00075	GSK573719	A study to investigate the pharmacokinetics of GSK573719 in male beagle dogs following intravenous infusion at target dose levels of 0.5 mg free base/kg	IND 104479 (5/25/10)
CH2006/00004	GSK573719A	Pharmacokinetics of GSK573719A in the male Sprague·Dawley rat	IND 104479 (5/25/10)
Distribution			
WD2008/00503	GSK573719	In vitro plasma protein binding of GSK573719 and blood cell association of (14C)GSK573719 in mouse, rat, rabbit, dog and human	IND 104479 (6/3/09)
WD2006/02657	GSK573719	An in vitro investigation of both the transport via heterologously expressed human P-glycoprotein and the passive membrane permeability of [14C]GSK573719 in MDCKII-MDR1 cells	IND 104479 (5/25/10)
WD2006/02596	GSK573719A	An in vitro investigation of the inhibition by GSK573719A of xenobiotic transport via human P-glycoprotein, heterologously expressed in MDCKII cells	IND 104479 (5/25/10)
CH2006/00002	GSKS73719A	A study to estimate the lung retention and systemic exposure of GSKS73719A following intranasal administration in the male BALB/c mouse	IND 104479 (5/25/10)
FD2005/ 00236	GSK573719	Quantitative whole-body autoradiography following a single oral or a single intravenous administration of (14C)GSK573719 bromide to male Lister Hooded pigmented rats at a target dose level of 1 mg free base/kg	IND 104479 (5/25/10)
CH2006/00013	GSK573719A	Investigation of the brain to plasma ratio of GSK573719A in the conscious male Sprague Dawley rat	IND 104479 (5/25/10)
Metabolism			
WD2009/00030	GSK573719	Preliminary Characterization of Metabolites of GSK573719 in Human Plasma and Urine (submitted to IND 104479, not found under Nonclinical Section of NDA 203975)	IND 104479 (6/3/09)
WD2006/00147	GSK573719	An in vitro investigation of the metabolism of [14C]GSK573719 in human, rat and dog	IND 104479 (6/3/09)

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CH2006/00011	GSK573719A	A preliminary study to estimate the intrinsic clearance of GSK573719A in rat. dog and human hepatocytes	IND 104479 (6/3/09)
WD2007/01370	GSK573719	An in vitro investigation of the metabolism of (14C)GSK573719 in human, female rabbit and mouse hepatocytes	IND 104479 (6/3/09)
WD2006/03367	GSK573719	A preliminary in vitro investigation into the human oxidative enzymology of GSK573719	IND 104479 (5/25/10)
WD2005/01195	GSK573719	A study to investigate the biotransformation of [14C]GSK573719 in the isolated perfused rat liver model	IND 104479 (5/25/10)
WD2006/00172	GSK573719	Quantification and identification of the metabolites of GSK573719 in the male Sprague Dawley rat following a single oral intravenous administration	IND 104479 (6/3/09)
WD2006/00250	GSK573719	Quantification and identification of the metabolites of GSK573719 in the male beagle dog following a single oral or single intravenous administration	IND 104479 (5/25/10):
CH2005/00950	GSK573719A	A preliminarily screen of the in vitro concentration dependent inhibition of human cytochrome P450 enzymes	IND 104479 (5/25/10):
WD2005/01627	GSK573719A	The effect of GSK573719A on the mRNA levels of cytochrome P450 genes in rat livers obtained following inhaled administration	IND 104479 (5/25/10):
Excretion			
FD2005/00208	GSK573719	Elimination of drug-related material following a single oral or a single intravenous administration of [14C]GSK573719 bromide to rats	IND 104479 (6/3/09)
FD2005/00164	GSK573719	Elimination drug-related material following a single oral or a single intravenous administration of [14C]GSK573719 bromide to dogs	IND 104479 (5/25/10):
WD2007/01907	GSK573719	Elimination of drug-related material following intravenous administration of [14C]GSK573719 trifluoroacetate o male bile duct-cannulated dogs	IND 104479 (5/25/10):
Toxicology			
Repeat-Dose			
Rat - Inhalation - Short			
FD2009/00392	GW642444M, GSK573719A	Toxicity Study by Inhalation Administration to Rats for 4 Weeks	IND 104479 (8/31/09)
WD2006/03225	GSK573719A	A 14 day inhalation toxicity study of a powder aerosol formulation in the rat (magnesium stearate bridging study)	IND 104479 (5/25/10)
WD2005/01422	GSK573719A	A 28 day inhalation study of a powder aerosol formulation in the rat	IND 104479 (6/3/09)
Dog - Inhalation - Short			
FD2009/00391	GW642444M, GSK573719A	A 4-Week Combination Inhalation Toxicity Study in Dogs (Note: Includes 3-day pretreatment w/GSK573719 and GW642444, or GW642444 alone)	IND 104479 (8/31/09)
WD2006/03669	GSK573719A	A 14 day study to examine the influence of magnesium stearate on the toxicity and toxicokinetics of GSK573719A by inhalation administration in beagle dogs	IND 104479 (5/25/10)

WD2008/01504	GSK573719A	Dose range finding by inhalation administration to beagle dogs for 4 weeks	IND 104479 (5/25/10)
WD2005/01423	GSK573719A	A 28 day inhalation toxicity study of a powder aerosol formulation in the beagle dog	IND 104479 (5/25/10)
WD2006/03294	GSK573719A	A 28 day inhalation investigative toxicity study of a powder aerosol formulation in the beagle dog (Note: Issue with feed regimen.)	IND 104479 (5/25/10)
Dog - Inhalation - Medium			
WD2010/00677	GSK573719A, GW642444M	A 13-Week Combination Inhalation Toxicity Study in Dogs	IND 106616/104 479 (2/14/11)
Mouse - Inhalation - Short			
WD2007/00074	GSK573719A	Preliminary Toxicity study by inhalation administration to CD-1 mice for 2 weeks	IND 104479 (6/3/09)
Mouse - Inhalation - Long			
WD2007/01600	GSK573719	13 week inhaled dose range finding study in the mouse	IND 104479 (6/3/09)
Mouse · Oral · Medium			
WD2010/00349	GSK573719A	Preliminary Toxicity Study By Oral Gavage Administration To CD-1 Mice for 13 Weeks	IND 104479 (5/4/10)
WD2010/00556	GSK573719A	Preliminary Toxicity Study by Oral Gavage Administration to CD·1 Mice for 13 Weeks	IND 104479 (5/4/10)
Rat • Inhalation • Long			
WD2007/02012	GSK573719A	A 13 week inhalation toxicity study of a powder aerosol formulation in the rat with a 4 week recovery period	IND 104479 (6/3/09)
FD2009/00467	GSK573719	A 26 Week Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat Followed by a 6 week Recovery	IND 106616/104 479 (2/14/11), correction in (9/6/12)
Dog · Inhalation · Long			
WD2007/01512	GSK573719A	Toxicity Study by Inhalation administration to beagle dogs for 13 weeks followed by a 4-week recovery period	IND 104479 (5/25/10)
FD2009/00466	GSK573719A	Toxicity Study by Inhalation administration to Beagle Dogs for 39 weeks with a 6 Week Recovery Period	IND 106616/104 479 (2/14/11)
Genotoxicity			
In vitro			
WD2005/00750	GSK573719A	Bacterial mutation assay (Ames test) with Salmonella typhimurium and Escherichia coli	IND 104479 (6/3/09)
WD2005!00751	GSK573719A	In vitro mutation assay with L5178Y mouse lymphoma cells at the TK locus	IND 104479 (6/3/09)
In vivo			
WD2005/01079	GSK573719A	Intravenous bone marrow micronucleus assay in rats	IND 104479 (6/3/09)
Carcinogenicity			

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2012N131619	GSK573719A	Carcinogenicity Study by Inhalation Administration to Crl: CD(SD) Rats for 104 Weeks	NDA 203975 (4/25/13)
2012N131664	GSK573719A	Carcinogenicity Study by Inhalation Administration to CD-1 Mice for 104 Weeks	NDA 203975 (4/25/13)
Reproductive and Developmental			
Fertility and early embryonic development			
WD2007/00763	GSK573719A	Inhaled Female Fertility and Early Embryonic Development in Rats	IND 104479 (5/25/10)
EFD			
CD2009/00970	GSK573719A, GW642444M	Subcutaneous Dose Range Study in Female Rabbits	IND 106616 (5/10/10a)
WD2007/00764	GSK573719A	Inhaled Embryofetal Development Study in Rats	IND 104479 (5/25/10)
WD2007/00762	GSK573719A	Inhaled Embryofetal Development Study in Rabbits	IND 104479 (5/25/10)
Other Toxicity Studies			
Other			
WD2008/01499	GSK573719	In Vitro Haemolytic potential in Rat. Dog and Human Peripheral Blood	IND 104479 (5/4/10)

4 Pharmacology

4.1 Primary Pharmacology

See IND 104479 Pharmacology/Toxicology review dated 5/25/10.

4.2 Secondary Pharmacology

See IND 104479 Pharmacology/Toxicology review dated 5/25/10.

4.3 Safety Pharmacology

See IND 104479 Pharmacology/Toxicology review dated 5/25/10.
See IND 106616 Pharmacology/Toxicology review dated 2/10/10.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

See IND 104479 Pharmacology/Toxicology reviews dated 5/25/10, 6/3/09.

5.2 Toxicokinetics

See General Toxicology.

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6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies were not reviewed (see Studies Not Reviewed).

6.2 Repeat-Dose Toxicity

Study title: GSK573719A AND GW642444M: A 4-WEEK COMBINATION INHALATION TOXICTY STUDY IN DOGS

Study no.:	2010N109790
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	25 June 2010
GLP compliance:	Yes, signed.
QA statement:	Yes, signed.
Drug, lot #, and % purity:	GSK573719A, batch R360975, 83.8% purity GW642444M, batch R336684, 62.3 % purity

Key Study Findings

- The Sponsor conducted a study to assess effects of pretreatment with lower doses of GSK573719/GW642444 for 3 days followed by treatment with higher doses for 28 days. The Division had expressed concerns about the use of pretreatment in the 13-week toxicology study with GSK573719/GW642444 in dogs (study WD2010/00677/01) in terms of offering a protective effect against subsequent treatment with higher doses.
- Pretreatment (Phase 1) was for 3 consecutive days. Main study treatment (Phase 2) was for 4 weeks. Groups received the following target doses: (Estimated achieved doses, and pulmonary deposited doses are listed under Methods, Study Design).
    - Group 1: 0 (vehicle)
    - Group 2 (low dose pretreatment, high dose treatment): 3-day pretreatment with 40/40 mcg/kg/day of GSK573719/ GW642444, 4 week treatment with 160/160 mcg/kg/day GSK573719/ GW642444
    - Group 3 (high dose treatment): 4 week treatment with 160/160 mcg/kg/day GSK573719/ GW642444
    - Group 4 (high dose pretreatment only): 3-day pretreatment with 160/160 mcg/kg/day GSK573719/ GW642444
- There were test article related increases in arrhythmias, which decreased due to pretreatment in Group 2, and tolerance to the test-article with repeated dosing

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during Phase 2 for both Groups 2 and 3. The highest number of ventricular premature couplets (VPCs) was observed in one Group 3 male (#3016m) on day 1, which developed single, pairs and runs of VPCs in 8/8, 8/8 and 5/8 time points, respectively.

- There were test article related increases in heart rate, which decreased due to pretreatment in Group 2, and tolerance to the test-article with repeated dosing during Phase 2 for both Groups 2 and 3. There was an overall trend for lower heart rate in Group 2 versus Group 3, based on Day 1 (213% vs. 256%), Day 2 (70% vs. 120%), Day 14 (123% vs. 137%), and Week 4 (71% vs. 118%) values, compared to baseline.
- Femoral pulse showed test article related increases, with tolerance induced as a result of pretreatment in Group 2, and repeat dosing during Phase 2 in Groups 2 and 3. In Phase 1, Group 2 males developed increased percent change in pulse immediately after dosing (IAD), which was higher in males (101%) than females (75%) on Day 1. In Phase 2, Group 3 males had higher increased pulse (128%) on Day 1, compared to Group 2 males (92%). Both Groups 2 and 3 showed a general trend towards lower levels of pulse with repeat dosing, resulting in partial recovery by Week 4.
- There were test-article related increases in cardiac troponin I, which decreased due to pretreatment in Group 2, and tolerance to the test-article with repeated dosing during Phase 2 for both Groups 2 and 3. During Phase 2, there was an overall trend for lower heart rate in Group 2 versus Group 3, based on SD 1 (Group 2 males 13278%, females 750% ; Group 3 males 47022%, females 14437%, and SD 27 (Group 2 males 117%, females 174%; Group 3 males 107%. females 260%). Dosed animals did not all recover by 23 hrs and 50 min post dose.
- There were consistent elevations in potassium (Group 2 males 13%, females 14%; Group 3 males 9%, females 9%), and phosphorus (Group 2 males 13%, females 14%; Group 3 males 7%, females 13%). Elevations in phosphorus are common to $beta_2$-agonists.
- Decreased heart weight (Group 2 males -21%, females -18%; Group 3 males -16%, females -10%) was observed, when normalized to body weight (%BW), compared to controls (Group 1).
- There were no dose-limiting microscopic findings in the heart.
- There were test article related decreases in rate of tear production, which persisted throughout the study, without recovery. Decreased tear production is an expected pharmacological effect of muscarinic antagonists.

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Methods

Doses:	Group 1: 0 (vehicle) Group 2: 3-day pretreatment with 40/40 mcg/kg/day of GSK573719/ GW642444, 4 week treatment with 160/160 mcg/kg/day GSK573719/ GW642444 Group 3: 4 week treatment with 160/160 mcg/kg/day GSK573719/ GW642444 Group 4: 3-day pretreatment with 160/160 mcg/kg/day GSK573719/ GW642444 **Note: Target doses are listed. Estimated achieved doses and pulmonary deposited doses are shown in the table below. Pulmonary deposited doses are used in the review tables below.**
Frequency of dosing:	Daily
Route of administration:	Inhalation (oropharyngeal tube)
Dose volume:	10 minutes
Formulation/Vehicle:	1% (w/w) magnesium stearate in lactose
Species/Strain:	Dog/Beagle
Number/Sex/Group:	Groups 1-3, 3/sex/grp, Group 4, 1/sex/grp
Age:	14-15 months
Weight:	9.0 to 10.2 kg (males), 6.9 to 8.6 kg (females)
Satellite groups:	None
Unique study design:	Animals were dosed with a 3-day pretreatment (Phase 1) or without a 3-day pretreatment (Phase 2) to determine the effect of pretreatment on drug-induced tachycardia.

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Study Design:

			Phase 1: 3-day pretreatment			**Phase 2**: 4-week dosing		
			mcg/kg/day			mcg/kg/day		
Group	Blend	N/sex/ group	Target dose	Achieved dose	Pulmonary deposited dose	Target dose	Achieved dose	Pulmonary deposited dose
1	Vehicle (1% magnesium stearate in lactose)	3	-	-	-	0	0	0
2	GSK573719/ GW642444	3	40/40	42/47	10.5/11.8	160/160	189/201	47.3/50.3
3	GSK573719/ GW642444	3	-	-	-	160/160	188/199	47.0/49.8
4	GSK573719/ GW642444	1	160/160	220/234	55.0/58.5	-	-	-

NR indicates not measured or reported by sponsor
"-" indicates no dosing

The particle size distribution shows that the test articles tested were within range for pulmonary deposition:

Group	Blend	MMAD	GSD
1	Vehicle (1% magnesium stearate in lactose)	(b) (4) [redacted]	
2	GSK573719/ GW642444		
3	GSK573719/ GW642444		
4	GSK573719/ GW642444		

Observations and Results

Mortality

A viability check was performed near the start and end of each working day. There were no mortalities.

Clinical Signs

During the dosing period, all animals were examined at least twice daily, including during and immediately after dosing, 30 minutes to 2 hours after completion of dosing, and late in the work day. Detailed clinical examinations, in which animals were removed from cages, were performed during the pre-treatment period (Week -4) and weekly until necropsy. Examinations included observations of general condition, skin and fur, eyes, nose, abdomen and external genitalia as well as an evaluation of respiration and observations for any unusual behavior or clinical signs of toxic or pharmacologic effects. All abnormalities were recorded.

On May 10, 2013, an Information Request was sent regarding inconsistencies in the data for clinical observations:

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"2. In the study report titled "GSK573719A and GW642444M: A 4-Week Combination Inhalation Toxicity Study In Dogs" (Study No. 2010N109790), some statements are made in the text that do not appear to be reflected in the Clinical Observation Summary Tables (Tables 1 and 2). For example, the study report states, "Swelling of the neck was seen....in both animals given 220/234 GW642444/GSK573719 mcg/kg/day (Group 4). The finding was first observed during dosing and was still present immediately post dose in affected animals.

Provide corrected summary tables, or clarify where findings are represented in the summary tables and how they correspond to the text."

On May 16, 2013, GSK provided a corrected summary table as an appendix to SD-18. Observations were made in Groups 2 and 4 during Phase 1, and Groups 1-3 during Phase 2 (Group 1 is the control group).

Due to the lack of data from controls in Phase 1, it was difficult to determine if findings were test article related. No findings, however, were observed at an incidence greater than 1 of 3 animals/sex, therefore none were determined to be test article related based on low incidence.

During Phase 2, excessive salivation was noted in a higher number of dosed animals versus controls (control 2/6, Group 2 5/6, Group 3 6//6) during dosing, and IAD (control 1/6, Group 2 4/6, 6/6). Unilateral neck swelling was observed at a higher rate in dose animals during dosing (control 1/6, Group 2 2/6, Group 3 2/6), and IAD (control 2/6, Group 2 4/6, Group 3 4/6). Bilateral neck swelling was also observed in a higher number of dosed animals during dosing and IAD (control 3/6, Group 2 6/6, Group 3 6/6). These findings were test-article related, but did not correlate with histopathological findings.

Also during Phase 2, panting was observed at a slightly higher incidence in Group 3 (2/6) than controls (1/6) during dosing; no increase was observed for Group 2 (2/6) over controls. IAD, panting was observed only in 1/6 Group 2 animals. Red exudates on tubing were observed only in dosed animals during dosing (Group 2 1/6, Group 3, 1/6) and IAD (Group 2 1/6). Based on the low incidence and inconsistent relationship with dosing, these findings were determined to be incidental.

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Table 1: 4 week combination inhalation dog study: Clinical observations

		Clinical observations (# animals/# incidences)							
		Males				Females			
	Group:	1	2	3	4	1	2	3	4
	N:	3	3	3	3	3	3	3	3
Excessive salivation	Dosing	2/6	3/32	3/43	-	0	2/25	3/40	-
	IAD	0	3/4	3/4	-	1/1	1/2	3/3	-
Neck swelling - Unilateral	Dosing	0	0	2/4	-	1/1	2/4	0	-
	IAD	1/3	2/4	2/4	-	1/2	2/4	2/5	-
Neck swelling - Bilateral	Dosing	1/6	3/13	3/26	-	2/3	3/20	3/34	-
	IAD	1/1	3/26	3/35	-	2/3	3/32	3/51	-
Panting	Dosing	1/3	1/8	2/4	-	0	0	0	-
	IAD	0	1/2	0	-	0	0	0	-
Red exudates on tube	Dosing	0	1/2	1/2	-	0	0	0	-
	IAD	0	2/2	2/2	-	0	0	1/1	-

"-" = not evaluated

Body Weights

Body weights were recorded 4 times during the pre-treatment period (including the day before Phase 1 and 2 dosing periods), weekly during dosing, and just prior to necropsy. There were no adverse changes in body weight gain compared to control.

Table 2: 4 week combination inhalation dog study: Body weight gain, percent change

	GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)					
	Males			Females		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Time point	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8
Pretreatment (g)	8.7	9.7	9.6	7.3	7.6	7.0
Week 4 (g)	8.9	10.4	10.4	7.5	8.3	7.4
Δ BW (g, w.r.t. randomization/ pretreatment)	0.2	0.7	0.8	0.2	0.7	0.4
%Δ BW (w.r.t. pretreatment)	2%	7%	8%	3%	9%	6%

PT indicates pretreatment

Feed Consumption

Feed consumption was measured daily, beginning 1 week prior to dosing. Feed consumption for Group 1 (control) was not determined at the end of Phase 1, after 3 days of pretreatment dosing, therefore percent change in feed consumption for Groups 2 and 4 were not calculated at this time point with respect to controls. Instead, percent

change in feed consumption was calculated for Group 2 and 4 at the end of Phase 1 with respect to their respective weights at randomization. Percent change in feed consumption was calculated at the end of Phase 2, after 4 weeks of dosing, for Groups 2 and 3, compared to Group 1 (control).

There were no consistent test-article related effects on feed consumption. After Phase 1 (3-day pretreatment), Group 2 males (-27%) and females (-32%), and Group 4 males (-16%) appeared to have a decrease in feed consumption, when compared to values measured at randomization. These changes, however, did not persist after Phase 2 in Group 2 animals. The change in feed in Group 4 animals was inconsistent between males and females.

Table 3: 4 week combination inhalation dog study: Feed consumption, percent change

	GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)							
	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Time point	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8	55.0/58.5 PT	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8	55.0/58.5 PT
% Δ @Day 3 after PT (g/day) vs. randomization	N/A	-27%	N/A	-16%	N/A	-32%	N/A	0%
% Δ @Week 4 (g/day) vs. control	-	5%	-1%	N/A	-	-3%	-2%	N/A

PT indicates pretreatment

Ophthalmology

Not performed.

ECG, Femoral Pulse, Cardiac Troponin I Assay

Methods:
Dogs were jacketed to provide 24-hour ECG interval and heart rate recordings. With the exception of Group 4, electrocardiograms were recorded from each animal once during Pretest (sham dosing) for all groups, at Phase 1 for Group 2 only, and Phase 2 SD 1, 2, 14 and Week 4 for all groups.

Femoral pulse was measured daily during Phase 1 for Groups 2 and 4. Measurements were made predose, within 10 minutes after completion of dosing and 30 minutes and

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1, 2, 4, 8 and 23 hours 50 minutes after the completion of dosing. Femoral pulse was measured for all animals during Phase 2 on SD 1 and during Week 4 at predose, within 10 minutes after completion of dosing and 30 minutes and 1, 2, 4, 8 and 23 hours 50 minutes after the completion of dosing. Pulses were also measured during Phase 2 SD 2, 3, and 7 at predose and within 10 minutes after dosing.

Concentrations of cardiac troponin I (cTnI) in serum from Groups 1 to 3 were determined using a Bayer Centaur CP Chemiluminescence analyzer. Blood (~1 mL; jugular or cephalic vein) was collected from Group 2 the day before Phase 1, on Phase 1 SD 1 (Day -3 with respect to Phase 2) within 5 minutes after dosing and 30 minutes and 1, 2, 4, 8 and 23 hours and 50 minutes after dosing. Blood from Groups 1 to 3 were collected on Phase 2 SD 1 pre-dose, within 5 minutes after dosing, 30 minutes after dosing, and 1, 2, 4, 8 and 23 hours and 50 minutes after dosing.

Results:

Arrhythmias

There were test article related increases in arrhythmias, which decreased due to pretreatment in Group 2, and tolerance to the test-article with repeated dosing during Phase 2 for both Groups 2 and 3.

During Phase 1 (3-day pretreatment phase), one animal in Group 2 (#2016m) developed single ventricular premature complexes (VPCs) post dose at 5 time points, compared to no VPCs predose.

Arrhythmias during Phase 2 (4-week treatment phase) were highest on Day 1, and lower on Day 2 due to tolerance to the test articles. The highest number of VPCs was observed in one Group 3 male (#3016m) on Day 1, which developed single, pairs and runs of VPCs in 8/8, 8/8 and 5/8 time points, respectively. On Day 2, this animal developed single VPCs in 4/8 time points. Additional animals that had slightly increased (2 or less time points per day, on SD 1 or SD 2) test-article related VPCs during Phase 2 were Group 2 animal (#2016m), and three Group 3 animals (#3015m, #3017m, #3516f).

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Table 4: 4 week combination inhalation dog study: Ventricular premature complexes on Day 1 (Phase 2 Dosing)

			Ventricular premature complexes					
			Predose			Post dose		
	Sex	ID#	Single	Pair	Run	Single	Pair	Run
Group 1 (vehicle)	M	1015m	0	0	0	0	0	0
	M	1016m	0	0	0	0	0	0
	M	1017m	0	0	0	0	0	0
	F	1515f	0	0	0	0	0	0
	F	1516f	1	0	0	1	0	0
	F	1517f	0	0	0	1	0	0
Group 2 (pretreatment)	M	2015m	0	0	0	0	1	0
	M	2016m	1	0	0	2	0	0
	M	2017m	0	0	0	0	0	0
	F	2515f	0	0	0	0	0	0
	F	2516f	0	0	0	0	0	0
	F	2517f	0	0	0	0	0	0
Group 3	M	3015m	0	0	0	2	0	0
	M	**3016m**	**0**	**0**	**0**	**8**	**8**	**5**
	M	3017m	0	0	0	2	0	1
	F	3515f	0	0	0	0	0	0
	F	3516f	0	0	0	0	0	0
	F	3517f	0	0	0	0	0	0

Numbers indicate the number of time points at which VPCs were observed
Bold indicates highest number of VPCs observed in individual animal

Heart rate
There were test article related increases in heart rate, which decreased due to pretreatment in Group 2, and tolerance to the test-article with repeated dosing during Phase 2 for both Groups 2 and 3. During Phase 1 (Group 2 only evaluated), Group 2 animals had up to 152% increase in heart rate (T_{max} at 1 hr post dose), compared to baseline (1 hr pre dose).

During Phase 2 (Groups 1-3 evaluated), both Groups 2 and 3 showed test article related increases in heart rate, with the highest increases noted on Day 1 in Group 3 (256%) compared to Group 2 (213%), relative to baseline values (1 hr predose; baseline values were similar to Group 1 controls). This suggests that tolerance to tachycardia was induced in Group 2 due to pretreatment during Phase 1. The trend for lower maximum percent change in heart rates in Group 2 versus Group 3 continued on Day 2 (70% vs. 120%), Day 14 (123% vs. 137%), and Week 4 (71% vs. 118%). Tolerance to tachycardia was noted in both Group 2 and Group 3, as a result of repeated dosing with the test-articles (shown in Table 7 and Table 8). Test-article related increases in heart rate reversed at approximately 14-18 hrs post dose (see Figures below). In vehicle-only treated animals (Group 1), there was no notable increase in heart rate, as shown in

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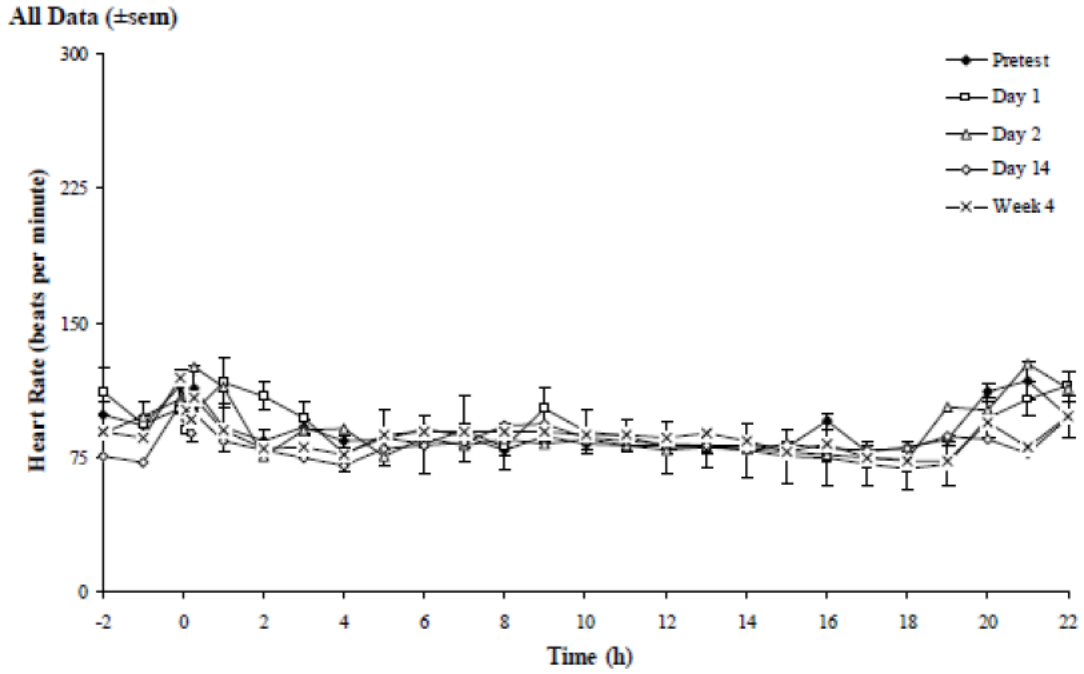
Table 5: 4 week combination inhalation dog study: Heart rate, percent change

		Time post dose (hrs)								
		0.08	0.25	1	2	3	>>>	20	21	22
Phase 1: Day 1	Group 2	33%	98%	**152%**	151%	129%		-20%	-13%	27%
Phase 2: Day 1	Group 1	-4%	6%	**24%**	16%	3%		3%	15%	22%
	Group 2	47%	144%	196%	**213%**	197%		65%	41%	34%
	Group 3	91%	**256%**	**256%**	232%	211%		88%	95%	73%
Phase 2: Day 2	Group 1	0%	**28%**	16%	-23%	-8%		3%	30%	15%
	Group 2	-10%	44%	**70%**	66%	56%		6%	-18%	-10%
	Group 3	34%	114%	**120%**	109%	95%		11%	5%	-7%
Phase 2: Day 14	Group 1	33%	**39%**	17%	10%	4%		18%	7%	35%
	Group 2	29%	100%	**123%**	115%	88%		13%	17%	12%
	Group 3	33%	138%	**137%**	112%	100%		20%	13%	15%
Phase 2: Week 4	Group 1	17%	**26%**	6%	-7%	-6%		9%	-6%	14%
	Group 2	14%	65%	**71%**	43%	26%		-2%	0%	3%
	Group 3	30%	**118%**	116%	93%	75%		29%	13%	6%

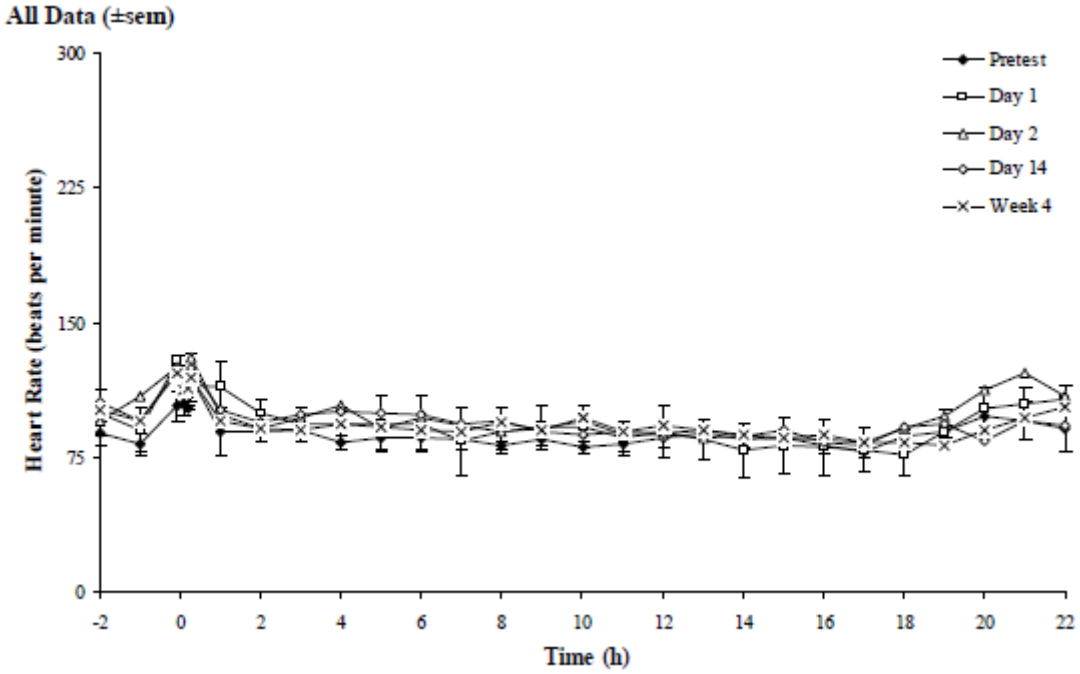
>>> indicates time points not shown for brevity
Bold indicates highest percent change compared to baseline (1 hr predose)

Table 6: 4 week combination inhalation dog study: Heart rate in vehicle treated animals (Group 1)

Males:



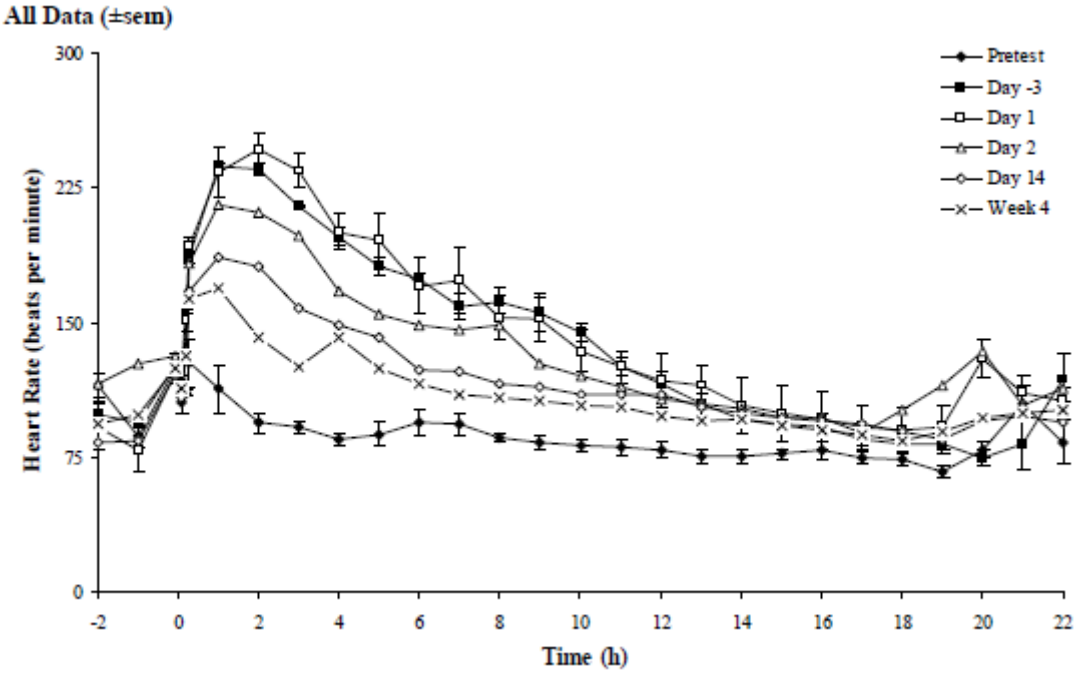
Females:



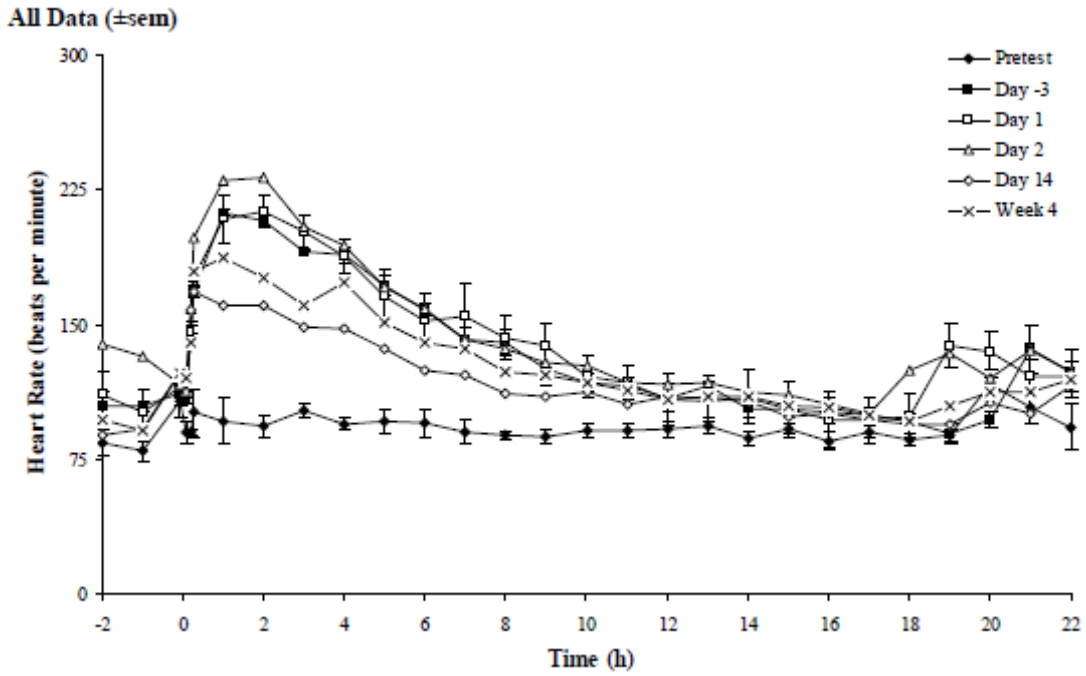
Sponsor's figures

Table 7: 4 week combination dog study: Heart rate in animals with 3-day pretreatment and 4 weeks treatment (Group 2)

Males:



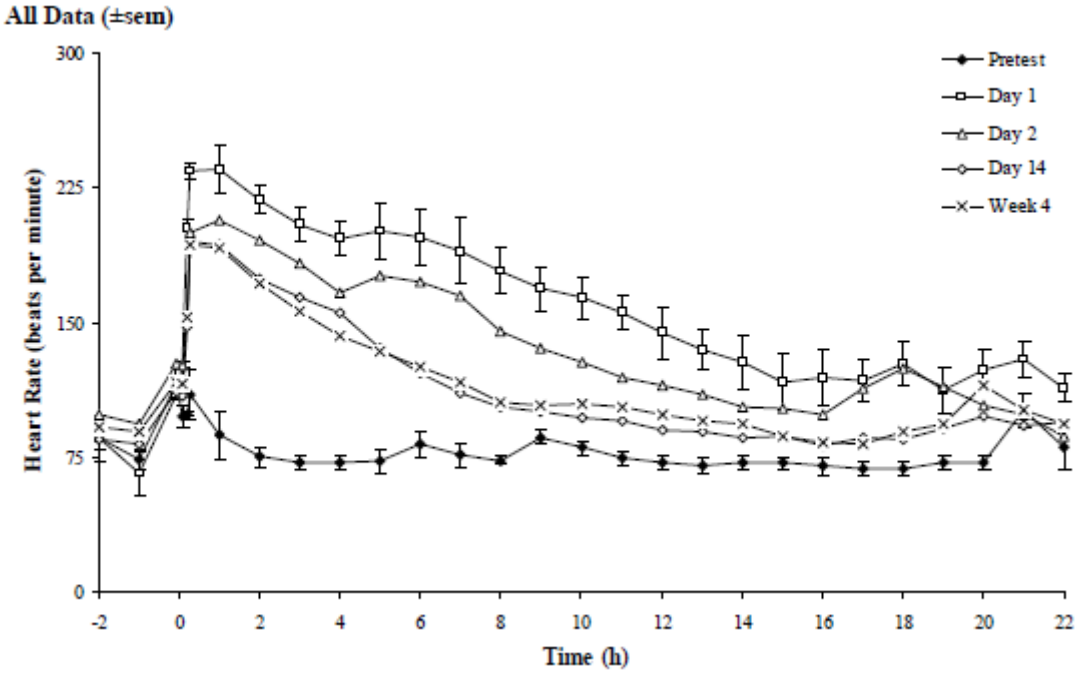
Females:



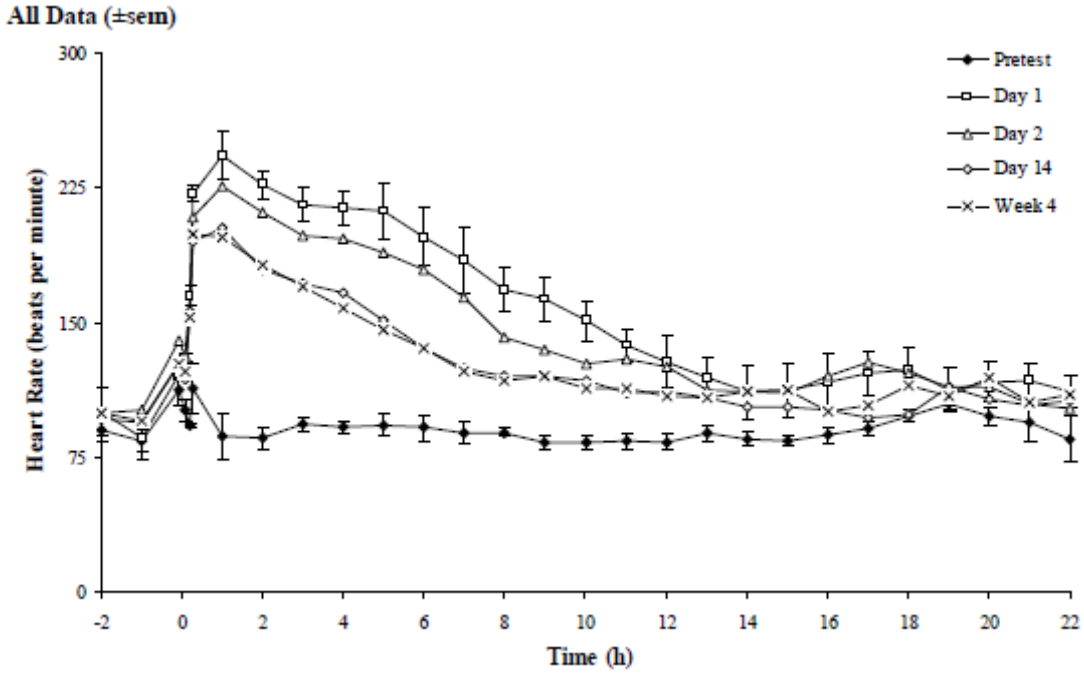
Sponsor's figures

Table 8: 4 week combination inhalation dog study: Heart rate in animals with 4 weeks treatment (Group 3)

Males:



Females:



Sponsor's figures

Femoral pulse

Femoral pulse showed test article related increases, with tolerance induced as a result of pretreatment in Group 2 males, and repeat dosing during Phase 2 in Groups 2 and 3. In Phase 1, Group 2 males developed increased percent change in pulse immediately after dosing (IAD), which was higher in males (101%) than females (75%) on Day 1. This affect decreased with each additional day of dosing, as observed by lower increases on Day 2 (males 42%; females 44%) and Day 3 (males 14%, females 20%), compared to Day 1. Data for Group 4 animals is difficult to interpret as only 1 animal/sex/group was evaluated, and only during Phase 1. In general, Group 4 animals showed a similar trend to dosing as Group 2 animals.

In Phase 2, Group 3 males had higher increased pulse (128%) on Day 1, compared to Group 2 males (92%). Group 3 (84%) females on Day 1 had a similar increase pulse compared to Group 2 (93%). Both Groups 2 and 3 showed a general trend towards lower levels of pulse with repeat dosing, resulting in partial recovery by Week 4. Thus, Phase 1 (pretreatment) appeared to induce partial tolerance during Phase 2 on males that was pronounced on Day 1. (Data for Phase 2 SD 3 and 7 are not shown in the table below for brevity.)

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Table 9: 4 week combination inhalation dog study: Femoral pulse, percent change

Males:

		Time point (hrs)						
Phase 1	Grp 2 (n=3/sex/grp), Grp 4 (n=1/sex/grp)	IAD	0.5	1	2	4	8	23 hr/50 min
Day 1	Group 2	101%	51%	71%	90%	97%	43%	-2%
	Group 4	143%	60%	138%	173%	130%	50%	30%
Day 2	Group 2	42%	42%	40%	42%	40%	41%	14%
	Group 4	23%	19%	19%	15%	0%	0%	4%
Day 3	Group 2	14%	24%	19%	21%	11%	15%	-4%
	Group 4	15%	7%	4%	19%	-11%	-11%	-26%
Phase 2								
Day 1	Group 1	-8%	21%	-9%	-14%	-18%	-9%	-22%
	Group 2	92%	30%	62%	72%	76%	24%	15%
	Group 3	128%	30%	69%	58%	94%	47%	8%
Day 2	Group 1	40%	-	-	-	-	-	-
	Group 2	36%	-	-	-	-	-	-
	Group 3	83%	-	-	-	-	-	-
Day 3	Group 1	-13%	-	-	-	-	-	-
	Group 2	26%	-	-	-	-	-	-
	Group 3	36%	-	-	-	-	-	-
Day 7	Group 1	16%	-	-	-	-	-	-
	Group 2	45%	-	-	-	-	-	-
	Group 3	120%	-	-	-	-	-	-
Week 4	Group 1	-16%	-12%	-16%	-12%	-22%	-6%	-19%
	Group 2	40%	32%	47%	58%	9%	13%	-14%
	Group 3	43%	64%	55%	38%	15%	35%	-7%

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Females:

		Time point (hrs)						
Phase 1	Grp 2 (n=3/sex/grp), Grp 4 (n=1/sex/grp)	IAD	0.5	1	2	4	8	23 hr/50 min
Day 1	Group 2	75%	59%	60%	83%	106%	30%	-4%
	Group 4	30%	42%	80%	98%	40%	24%	8%
Day 2	Group 2	44%	40%	54%	45%	24%	31%	16%
	Group 4	35%	19%	33%	19%	37%	7%	4%
Day 3	Group 2	20%	3%	16%	9%	15%	2%	-9%
	Group 4	7%	14%	14%	14%	11%	7%	-21%
Phase 2	n-3/sex/group							
Day 1	Group 1	19%	24%	4%	10%	24%	14%	15%
	Group 2	84%	45%	35%	76%	96%	16%	7%
	Group 3	93%	21%	55%	59%	90%	28%	8%
Day 2	Group 1	10%	-	-	-	-	-	-
	Group 2	69%	-	-	-	-	-	-
	Group 3	67%	-	-	-	-	-	-
Day 3	Group 1	-2%	-	-	-	-	-	-
	Group 2	7%	-	-	-	-	-	-
	Group 3	28%	-	-	-	-	-	-
Day 7	Group 1	5%	-	-	-	-	-	-
	Group 2	62%	-	-	-	-	-	-
	Group 3	72%	-	-	-	-	-	-
Week 4	Group 1	-19%	-14%	-23%	-16%	-16%	-19%	-22%
	Group 2	26%	55%	63%	75%	26%	22%	-7%
	Group 3	39%	60%	55%	30%	2%	28%	-11%

IAD indicates immediately after dosing
"-" indicates not evaluated

Cardiac Troponin I Assay

There were test-article related increases in cardiac troponin I (cTnI), which decreased due to pretreatment in Group 2, and tolerance to the test-article with repeated dosing during Phase 2 for both Groups 2 and 3. During Phase 1, data from only Group 2 were collected, and thus these were analyzed based on percent change relative to predose values (0.006 mcg/L) in Group 2; this predose value was within the range for control (Group 1) animals during Phase 2 SD 1 and 27, which was from 0.006 to 0.014 mcg/L. On Day 1 of Phase 1 (pretreatment), cTnI was increased up to 14445% in males and 728% in females, with T_{max} at 4 and 8 hrs post dose, respectively. After 23 hrs and 50 min, there was partial reversal in males (4717%), and full reversal in females.

During Phase 2 SD 1, animals in Groups 1-3 were evaluated; therefore percent change was calculated relative to controls (Group 1). Animals in Group 2 had slightly elevated levels of cTnI prior to dosing in Phase 2 (males 78%, females 22%), due to pretreatment with test-article for 3 consecutive days during Phase 1. After dosing on Phase 2 SD 1, Group 2 males (13278%) and females (750%) had lower maximum levels compared to Group 3 males (47022%) and females (14437%), suggesting that

pretreatment induced acclimatization to test article. On Phase 2 SD 27, both Group 2 (males 117%, females 174%) and Group 3 animals (males 107%, females 260%) had notably lower values than previously observed in the study. Dosed animals did not all recover by 23 hrs and 50 min post dose, especially in the case of a female in Group 2 (# 2516) that had a cTnI level of 0.060 mcg/L. Overall, repeated treatment with test article led to tolerance to test article in both Group 2 and 3, as measured by cTnI levels.

Table 10: 4 week combination inhalation dog study: Cardiac Troponin I, percent change

Phase 1: Percent change, relative to baseline (Group2)

		Cardiac Troponin I (mcg/L)	
		Males	Females
Day	Time point	Group 2	Group 2
Phase 1			
1	IAD	0%	0%
1	0.5	0%	0%
1	1	450%	0%
1	2	4812%	95%
1	4	**14445%**	728%
1	8	13128%	**742%**
2	23 hr/50 min	4717%	0%

Phase 2: Percent change, relative to controls (Group1)

		Cardiac Troponin I (mcg/L)			
		Males		Females	
Day	Time point	Group 2	Group 3	Group 2	Group 3
Phase 2					
1	PreDse	78%	0%	22%	0%
1	Immed	72%	0%	-38%	-35%
1	30 Min	50%	372%	0%	117%
1	1 hr	147%	947%	0%	1533%
1	2 hrs	2955%	9145%	172%	9605%
1	4 hrs	11060%	23077%	**750%**	**27833%**
1	8 hrs	**13278%**	47022%	407%	14437%
2	23 hrs/50 min	2100%	11228%	45%	5067%
27	PreDse	71%	39%	-50%	41%
27	Immed	-14%	-7%	**174%**	**260%**
27	30 Min	61%	107%	-33%	71%
27	1 hr	-8%	22%	-25%	198%
27	2 hrs	95%	72%	-19%	186%
27	4 hrs	**117%**	**112%**	-32%	108%
27	8 hrs	78%	88%	-17%	77%
28	23 hrs/50 min	24%	19%	100%	-31%

"PreDse" indicates before dosing
"Immed" indicates immediately after dosing
Bold indicates maximum value for Day

Hematology

Following overnight fasting, blood samples were obtained via jugular or cephalic venipuncture from all dogs once pre-treatment (Week -2) and during Week 4. Approximately 0.5 mL of blood was assayed for Hematocrit, Platelet count, Hemoglobin, Red blood cell distribution width, Total red blood cell count, Reticulocytes (absolute count), Mean cell volume, Blood smear for cell morphology, Mean cell hemoglobin, Total leukocyte count, Mean cell hemoglobin concentration, Leukocyte differential count (absolute), and abnormalities of blood morphology (manually). Coagulation parameters, prothrombin time and activated partial thromboplastin time, were also assayed.

At Phase 2 Week 4, there were no notable and consistent changes in any hematology parameters. There was a minimal increase in lymphocytes in both Group 2 and 3, for both males and females (19%-38%). Other parameters showed changes ≥20%, compared to control (Group 1), but they were not consistent in both Groups 2 and 3, and were generally minimal.

Table 11: 4 week combination inhalation dog study: Hematology and coagulation parameters, percent change

	GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)			
	Group 2		Group 3	
	10.5/11.8 PT, 47.3/50.3		47.0/49.8	
	Males	Females	Males	Females
Reticulocytes	-59%	-4%	-63%	-24%
Platelets	-1%	46%	6%	43%
Lymphocytes	19%	22%	38%	28%
Monocytes	14%	40%	10%	26%
Eosinophils	54%	47%	44%	7%
Basophils	-33%	20%	-33%	0%
Large unstained cells	0%	-29%	-20%	-29%

Shading indicates <20% change, compared to controls (Group 1)

Clinical Chemistry

Following overnight fasting, blood samples were obtained via jugular or cephalic venipuncture from all dogs once pre-treatment (Week -2) and during Week 4. Approximately 1.0 mL of blood was centrifuged for serum, which was assayed for Alkaline phosphatase, Triglycerides, Alanine aminotransferase, Total protein, Aspartate aminotransferase, Albumin, Total bilirubin, Sodium, Blood urea nitrogen, Potassium, Creatinine, Calcium, Glucose, Chloride, Cholesterol, Phosphorus, and Glutamate dehydrogenase.

There were consistent elevations in potassium (Group 2 males 13%, females 14%; Group 3 males 9%, females 9%), and phosphorus (Group 2 males 13%, females 14%; Group 3 males 7%, females 13%). There were inconsistent changes in glutamate dehydrogenase, total bilirubin, and creatinine.

Table 12: 4 week combination inhalation dog study: Clinical chemistry parameters, percent change

	GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)			
	Group 2		Group 3	
	10.5/11.8 PT, 47.3/50.3		47.0/49.8	
	Males	Females	Males	Females
Potassium	13%	14%	9%	9%
Phosphorus	13%	24%	7%	13%
Glutamate dehydrogenase	-32%	-34%	-28%	5%
Total bilirubin	-8%	-30%	-8%	0%
Creatinine	17%	20%	0%	0%

Shading indicates inconsistency with other values in other dosed group, or sex within same group.

Urinalysis

Urine samples were collected overnight (over approximately 16 hours) from once pre-treatment (Week -2) and during Week 4. Food and water were removed during urine collection. The following parameters were measured: Volume, Protein, pH, Creatinine, Specific gravity, Glucose total output, Appearance, Creatinine Total Output, Glucose, and Protein Total Output. There were no test article related urinalysis findings.

Gross Pathology

After 4 weeks of treatment, Group 1- 3 animals were fasted overnight, and euthanized by sedation with acepromazine followed by intravenous administration of sodium pentobarbital and then exsanguination.

There were no clear test-article related gross findings, based on a lack of incidence above 1/3 animals/sex/group. Discoloration of the lymph nodes was noted in males of both Group 2 and 3. All gross findings at or greater than 1/sex/group in Group 2 or 3 are shown in the table below. Correlating microscopic findings were observed in the lungs, lymph node, and pituitary that could possibly be test article related, but none were dose-limiting.

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Table 13: 4 week combination inhalation dog study: Gross findings

	GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)					
	Males (n=3/sex/grp)			Females (n=3/sex/grp)		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Organ/Finding	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8
Lungs Discolored	0	0	1	0	0	0
Lymph Node other Discolored	0	1	1	0	0	0
Pituitary Cyst(s)	0	0	0	0	0	1
Popliteal LN Enlarged	0	0	0	0	0	1
Spleen Small	0	0	0	0	0	1

PT indicates pretreatment during Phase 1

Organ Weights

The following organs were weighed: Adrenals (paired), Liver, Testes (paired), Brain, Lung, Thymus, Heart, Ovaries (paired), Kidneys (paired), Prostate.

Decreased heart weight (Group 2 males -21%, females -18%; Group 3 males -16%, females -10%) was observed, when normalized to body weight (%BW), based on percent change compared to controls (Group 1). The prostate consistently showed increases in weight in both Group 2 (23% %BrW) and Group 3 (70% %BrW), when normalized to brain weight (%BrW) and %BW. Ovaries consistently showed decreases in weight in both Group 2 (-29% %BW) and Group 3 (-24% %BW), when normalized to %BW and %BrW. The testes showed increases in absolute weight in Group 2 (6%) and Group 3 (14%), but these changes were not consistent when normalized to %BW or %BrW. There were no test-article related macroscopic changes in the heart. No gross changes were noted in the prostate, ovaries and testes; therefore they were not examined microscopically.

Table 14: 4 week combination inhalation dog study: Organ weight, percent change

		GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)			
		Group 2		Group 3	
		10.5/11.8 PT, 47.3/50.3		47.0/49.8	
		Males	Females	Males	Females
Heart	Absolute	-7%	-11%	-2%	-12%
	%BW	-21%	-18%	-16%	-10%
	%BrW	-11%	-5%	5%	-11%
Prostate/Ovaries	Absolute	26%	-24%	57%	-26%
	%BW	12%	-29%	42%	-24%
	%BrW	23%	-18%	70%	-25%
Testes	Absolute	6%	-	14%	-
	%BW	-11%	-	-4%	-
	%BrW	2%	-	21%	-

"-" indicates not relevant
Shading indicates ≥20% change, compared to control (Group 1)

Histopathology

Adequate Battery: Yes. Only the heart and gross abnormalities were examined for Groups 1-3. Sections of the heart were also stained with Masson's Trichrome stain.

Peer Review: Yes. The sponsor stated "The Peer Review Statement is not presented in the report but is maintained in the raw data."

Histological Findings

There were no clear test article related findings, based on the low incidence of 1/sex/group for all findings. All heart findings are reported below; perivascular neutrophilic infiltrate was observed in 1/3 males in Group 2, but the severity was only Grade 1. Mononuclear cell infiltrate was observed in both controls and Group 3 animals, therefore it was not considered to be test article related. Other findings in the lungs, lymph node (other), pituitary, and popliteal lymph were low in incidence (1/sex/group), and were not dose-limiting.

Table 15: 4 week combination inhalation dog study: Histopathological findings*

	GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)					
	Males			Females		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Organ (number examined)/Finding	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8	Vehicle	10.5/11.8 PT, 47.3/50.3	47.0/49.8
Heart	3	3	3	3	3	3
PAPILLARY MUSCLE: PERIVASCULAR NEUTROPHILIC INFILTRATE						
Grade 1	0	1	0	0	0	0
MYOCARDIUM: MONONUCLEAR INFLAMMATORY CELL INFILTRATE						
Grade 1	0	0	1	1	0	0
Lungs	0	0	1	0	0	0
BRONCHIOLAR LUMEN:FOAMY MACROPHAGES						
Grade 2	0	0	1	0	0	0
Lymph Node other	0	1	1	0	0	0
SINUSES: FREE ERYTHROCYTES						
Grade 4	0	1	0	0	0	0
Grade 5	0	0	1	0	0	0
Pituitary	0	0	0	0	0	1
CYSTS						
Grade 1	0	0	0	0	0	1
Popliteal LN	0	0	0	0	0	1
SINUSES: FREE ERYTHROCYTES/ERYTHROPHAGOCYTOSIS						
Grade 1	0	0	0	0	0	1

Shading indicates that finding was also observed in control at same incidence
*Determined not be test article related based on low incidence

Special Evaluation: Schirmer Tear Test

The tear test was performed on Phase 2 Study Day (SD) 2 and 24, after which artificial tears were administered. The rate of tear production was measured by placing the wick of a sterile test strip in the lower conjunctival sac for 1 minute and recording the distance travelled by tear secretion in both eyes of all animals. Measurements were made predose and within 10 minutes after completion of dosing. Percent change in distance travelled by tear secretion in each eye was analyzed by calculating percent change in Group 2 and Group 3, compared to Group 1 (controls).

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There were test article related decreases in rate of tear production, based on distance travelled by tear secretion. Before dosing (Week -1), baseline values show a range of -32% to 17 % change. At Week 1 (Phase 2) post dose, both Group 2 (-83% to -48%) and Group 3 (-96% to -78%) showed a marked decrease in both sexes. These decreases persisted at Week 4 (Phase 2): Group 2 (-72% to -50%) and Group 3 (-100% to -71%) had decreased test scores predose, and these values were consistent post dose (Group 2: -82% to -53%; Group 3: -90% to -77%).

Table 16: 4 week combination inhalation dog study: Rate of tear production, percent change

			GSK573719/ GW642444 Pulmonary Deposited Dose (mcg/kg/day)			
			Males		Females	
			Group 2	Group 3	Group 2	Group 3
		Side	10.5/11.8 PT, 47.3/50.3	47.0/49.8	10.5/11.8 PT, 47.3/50.3	47.0/49.8
Week -1	Pretest	L	17%	-6%	-11%	-6%
		R	-14%	-23%	-32%	-11%
Week 1 (Phase 2)	Predose	L	-18%	-45%	-28%	-67%
		R	-6%	-28%	-45%	-70%
	Post dose	L	-74%	-78%	-48%	-83%
		R	-83%	-78%	-68%	-95%
Week 4 (Phase 2)	Predose	L	-67%	-71%	-72%	-89%
		R	-50%	-82%	-58%	-100%
	Post dose	L	-73%	-77%	-55%	-90%
		R	-82%	-77%	-53%	-84%

L and R indicate left and right, respectively

Toxicokinetics

Blood samples were obtained only during the main 4-week dosing phase from Groups 1-3. TK was not evaluated for Group 4, which was dosed only during the 3-day pretreatment period. For Groups 1-3, blood (~1 mL) was collected from the each unanesthetized animal via jugular, cephalic or saphenous venipuncture, on Day 1 and during Week 4 immediately after completion of dosing (within 5 minutes), and 0.5, 1, 2, 4, 8 and 23 hours 50 minutes after the completion of dosing. Additionally, naïve blood samples (approximately 2.0 mL) were collected from 12 animals. Concentrations of GSK573719 and GW642444 in plasma from all animals (including controls) were determined by HPLC-MS/MS.

There was large individual animal variation in exposure to GSK573719 and GW642444. For GSK573719, Day 1 exposures were less Week 4 exposures for both sexes, based on AUC_{0-t}. This suggests an accumulation of GSK573719 to achieve steady state levels.
There was no notable affect of 3-day pretreatment (Group 2; Phase 1) on exposure, based on AUC_{0-t} after 4 weeks of consecutive treatment (Phase 2). At week 4, there

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were also no notable sex differences in GSK573719 exposure based on AUC_{0-t}, based on the overlap of ranges between males and females.

For GW642444, Day 1 and Week 4 exposures were relatively comparable. There was no consistent effect of 3-day pretreatment (Group 2; Phase 1) on exposure, based on AUC_{0-t} after 4 weeks of consecutive treatment (Phase 2). In males, 3-day pretreatment increased AUC_{0-t} after 4 weeks of treatment, but the trend was reversed in females. At week 4, there were also no consistent sex-related differences in GW642444 exposure based on AUC_{0-t}, but it is noted that females in Group 2 (with Phase 1 pretreatment) had a lower mean AUC_{0-t}, compared to females in Group 3 and males in both Group 2 and 3.

Table 17: 4 week combination inhalation dog study: Toxicokinetic Parameters with and without pretreatment

Male dogs:

		Group 2 (w/pretreatment)		Group 3	
		Pulmonary deposited dose (mcg/kg/day)			
		GSK573719	GW642444	GSK573719	GW642444
		47.3	50.3	47	49.8
Day 1	AUC_{0-t} (ng.h/mL)	6.91 [3.23– 13.8]	239 [88.8– 325]	2.57 [1.09 – 3.59]	95.9 [52.8 – 173]
	C_{max} (ng/mL)	9.11 [2.62 – 19.5]	61.1 [31.5 – 85.2]	4.32 [2.07 – 6.98]	34.8 [19.6 – 51.6]
	T_{max} (h)	0.17	0.17	0.17	0.17
Week 4	AUC_{0-t} (ng.h/mL)	17.3 [11.8 – 22.7]	264 [227 – 333]	18.6 [15.1 – 21.8]	130 [86.7 – 193]
	C_{max} (ng/mL)	8.58 [3.00 – 12.9]	98.9 [61.9 – 134]	10.5 [8.11 – 13.3]	70.8 [58.4 – 87.5]
	T_{max} (h)	0.17	0.17	0.17	0.17

Female dogs:

		Group 2 (w/pretreatment)		Group 3	
		Pulmonary deposited dose (mcg/kg/day)			
		GSK573719	GW642444	GSK573719	GW642444
		47.3	50.3	47	49.8
Day 1	AUC_{0-t} (ng.h/mL)	2.34 [1.62– 3.02]	60.6 [33.4 – 96.8]	7.23 [2.24 – 11.0]	166 [145 – 200]
	C_{max} (ng/mL)	2.35 [0.760 – 4.68]	26.8 [13.8 – 35.9]	11.1 [4.16 – 18.1]	75.9 [59.6 – 95.4]
	T_{max} (h)	0.17	0.17	0.17	0.17
Week 4	AUC_{0-t} (ng.h/mL)	9.26 [5.33 – 15.5]	84.4 [29.8 – 164]	13.9 [9.61 – 21.7]	187 [142 – 255]
	C_{max} (ng/mL)	4.8 [1.24 – 6.74]	43.8 [10.7 – 64.7]	7.9 [4.21 – 15.3]	114 [81.6 – 171]
	T_{max} (h)	0.17	0.17	0.17	0.17

Average of males and females:

		Group 2 (w/pretreatment)		Group 3	
		Pulmonary deposited dose (mcg/kg/day)			
		GSK573719	GW642444	GSK573719	GW642444
		47.3	50.3	47	49.8
Day 1	AUC_{0-t} (ng.h/mL)	4.625	149.8	4.9	130.95
	C_{max} (ng/mL)	5.73	43.95	7.71	55.35
	T_{max} (h)	0.17	0.17	0.17	0.17
Week 4	AUC_{0-t} (ng.h/mL)	13.28	174.2	16.25	158.5
	C_{max} (ng/mL)	6.69	71.35	9.2	92.4
	T_{max} (h)	0.17	0.17	0.17	0.17

Dosing Solution Analysis

A minimum of 2 samples per group were collected on each day of exposure. A measured volume of air was drawn from exposure chambers at a rate of approximately 2 Liters/minute through a Pall type A/E glass fiber filter, fitted to an open-faced filter holder attached to a spare animal breathing port. The first sample was analyzed for GSK573719 and/or GW642444, as appropriate. GSK573719 and for GW642444 content were measured using a single High Performance Liquid Chromatography (HPLC) method with Ultra Violet (UV) detection and a quantification range of 1.0 to 16 mcg/mL.

The estimate achieved doses for both test articles were notably higher (4-46%) than the target dose, based on percent change. This did not affect the validity of the study.

Table 18: 4 week combination inhalation dog study: Target and estimated achieved doses

	GSK573719			
Group	**Target concentration (mg/kg)**	**% change from target concentration**	**Estimated achieved dose (mcg/kg)**	
			Males	**Females**
1	0	0	0.00	0.00
			Average = 0.00	
2	**Phase 1**			
	40	4%	40.7	42.7
			Average = 41.7	
	Phase 2			
	160	18%	185	193
			Average = 189	
3	**Phase 2**			
	160	18%	182	194
			Average = 188	
4	**Phase 2**			
	160	38%	214	225
			Average = 220	

	GW642444			
Group	**Target concentration (mg/kg)**	**% change from target concentration**	**Estimated achieved dose (mcg/kg)**	
			Males	**Females**
1	0	0	0.00	0.00
			Average = 0.00	
2	**Phase 1**			
	40	18%	46.2	48.4
			Average = 47.3	
	Phase 2			
	160	26%	196	205
			Average = 201	
3	**Phase 2**			
	160	24%	192	205
			Average = 199	
4	**Phase 2**			
	160	46%	228	240
			Average = 234	

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

See IND 104479 Pharmacology/Toxicology review dated 6/3/09.

7.2 *In Vitro* Assays in Mammalian Cells

See IND 104479 Pharmacology/Toxicology review dated 6/3/09.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

See IND 104479 Pharmacology/Toxicology review dated 6/3/09.

7.4 Other Genetic Toxicity Studies

None.

9 Reproductive and Developmental Toxicology

9.1 Male Fertility

Study title: GSK573719A: Subcutaneous Male Fertility Study in Rats

Study no.:	Study #G09187 (GSK Report #CD2010/00187)
Study report location:	SD-1, eCTD 4.2.3.5.1
Conducting laboratory and location:	GlaxoSmithKline Safety Assessment 709 Swedeland Rd. King of Prussia, PA, USA
Date of study initiation:	18-Dec-2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK573719A, batch #R360972, 99.8%

Key Study Findings

- Male rats were dosed subcutaneously with up to 180 mcg/kg/day umeclidinium for 2 weeks before mating with untreated females and through the mating period for a total of 50 to 54 days. There were no test-article related affects on male fertility.
- Dosed males overall showed a decrease in body weight gain compared to controls (LD 85%, MD 87%, HD 92%), but the effect was not dose-dependent. Based upon the overall decrease in body weight gain, the dose selection was adequate.

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- There were no notable test article related effects in the mating index for males. Treatment of males with the umeclidinium up to 180 mcg/kg/day had no effects related to female fertility or offspring malformations.

Methods

Doses:	0 (vehicle), 30, 60, or 180 mcg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/Kg.
Route of administration:	Subcutaneous
Formulation/Vehicle:	0.9% aqueous sodium chloride
Species/Strain:	Crl:CD(SD) Rat
Number/Sex/Group:	25/sex/group (Males treated, females untreated)
Satellite groups:	None.
Study design:	Males were treated for 14 days, and cohabited 1:1 with untreated females for up to 7 days. Females were checked daily for the presence of sperm in vaginal lavage to confirm mating. Mated females were separated from males on Day 0 postcoitum (pc). After 7 days, unmated males were paired with a second female for up to 7 days. Treatment of males was continued through cohabitation until termination on Day 50 to 54. Mated females and their litters were euthanized on Day 20 pc.
Deviation from study protocol:	On one occasion, one male in the HD group (R09M-10452) received 11 to 14% higher dose (200 to 252 mcg/kg/day) and on another occasion, two males (R09M-10371 and R09M-10407) in the HD group received a dose of 184 and 154 mcg/kg/day, respectively. The sponsor reported no adverse findings as a result of these errors. This does not affect the validity of this study.

Evaluation

The study design, the duration of treatment, and dose selection were acceptable. Dose selection was based on minimal toxicity expected with the HD of 180 mcg/kg/day, based on a 2 week subcutaneous study with doses of 60, 180, and 1600 mcg/kg/day (study RD2009/01099/00). In the 2 week study dose-ranging study, the sponsor reported that 180 mcg/kg/day resulted in a mild decrease of body weight gain (5.81%) when compared to body weight gain in controls (16.4%). The pretreatment duration of 2 weeks is acceptable based upon a lack of findings in male reproductive organs by histopathological evaluation in a 26-Week Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat followed by a 6-Week Recovery Period (reviewed under IND 104479/106616 on 2/14/11/). This is allowed by an addendum to the ICH Guideline on

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Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility S5 (R2).

In the reviewed male fertility study, dosed animals overall showed a decrease in body weight gain compared to controls (LD 85%, MD 87%, HD 92%), but the effect was not dose-dependent.

Observations and Results

Mortality

During the Dosing period, viability checks were done once before dosing, and once near the end of each working day.

There were no test article related deaths. One untreated female was euthanized due to a mass in the axillary region.

Clinical Signs

During the Dosing period, clinical observations were done once before dosing, and once immediately after dosing. Detailed clinical observations were performed on males on treatment Days 1, 15, and 50, and on females on Days 0 and 20 post coitum.

There were no test article related clinical signs.

Body Weight

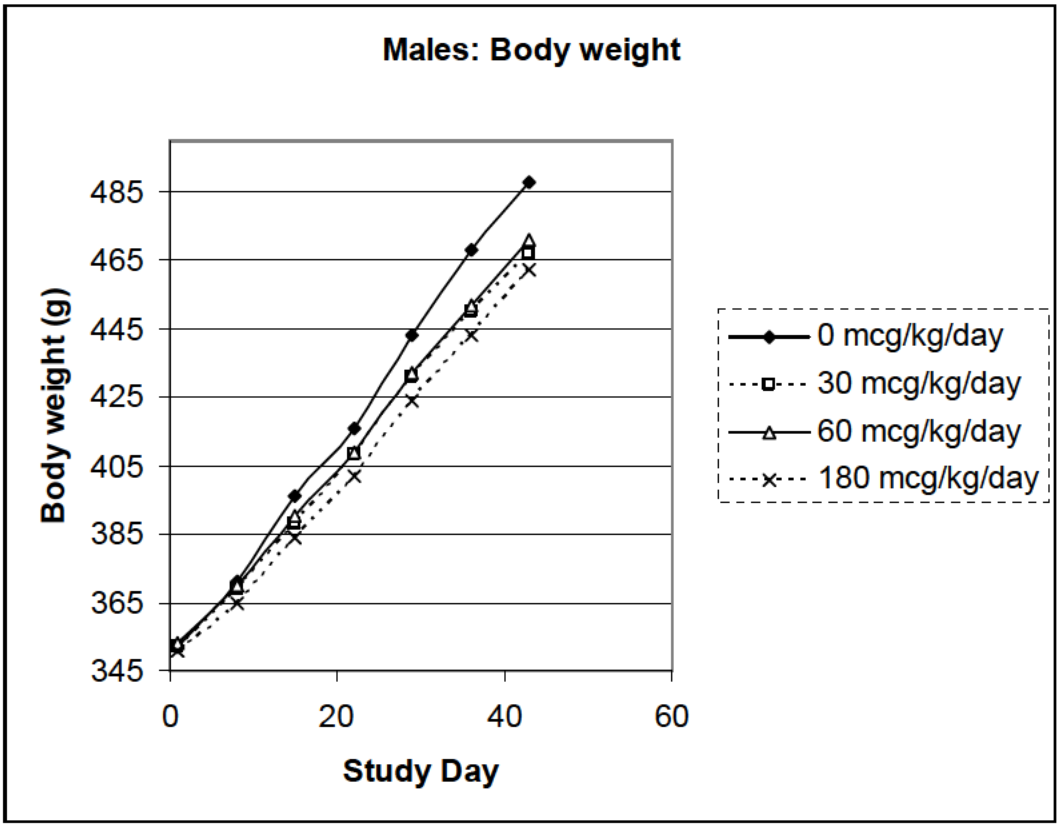
Body weights were measured daily in males from SD 1 to 43, and on Day 0 and Day 20 post coitum in females.

Dosed males, overall, showed a decrease in body weight gain compared to control males (LD 85%, MD 87%, HD 92%), but the effect was not dose-dependent.

Table 19: Subcutaneous Male fertility study: Body weight gain in dosed males

		Dose (mcg/kg/day)			
	Study Day	0	30	60	180
BW (g)	1	352	352	353	351
	43	488	467	471	462
	ΔBW (g)	136	115	118	111
	BW gain, % initial	39%	33%	33%	32%
	BW gain, % control	100%	85%	87%	82%

Figure 1: Subcutaneous Male fertility study: Body weight



Feed Consumption

Feed consumption was measured approximately weekly for males during the treatment period, except for during cohabitation with females. Feed consumption was not measured in one male in the HD group; the sponsor reported that the male discarded its allotment of feed.

At the last week of the study, dosed males had decreased percent change in food consumption (5-9%), compared to control, but this was not dose-dependent. This correlated roughly with decreased weight gain in dosed animals.

Table 20: Subcutaneous Male fertility study: Food consumption, percent change

	Dose (mcg/kg/day)	30	60	180
Study Days	1-8	-2%	-2%	-9%
	8-15	-2%	1%	-4%
	29-36	-6%	-4%	-8%
	36-43	-7%	-5%	-9%

Toxicokinetics

Not performed.

Dosing Solution Analysis

Formulations of 0.003, 0.006 and 0.018 mg/mL of test article in 0.9% sodium chloride were prepared weekly, and were stated by the sponsor in the reviewed study report to be stable for 15 days when stored refrigerated (2-8ºC), protected from light. An Information Request was sent on May 10, 2013 requesting the study report referenced (study WD2010/00081/00). The sponsor provided the study report on May 16, 2013. The stability data showed that the sponsor tested nominal concentrations of 0.003, 0.03, 0.3, and 3 mg/mL in sodium chloride on Day 0, 8 (ambient temperature and 4º C), and Day 15 (4º C). Results show that readings were within 10% of the nominal concentration, with the exception of 0.003 mg/mL at Day 0 which had a reading of 87.4%. This data is acceptable.

Samples of control and test article formulations prepared on 2 occasions (January 5, 2010 and February 9, 2010) were analyzed for GSK573719 concentration by high performance liquid chromatography with ultraviolet detection (HPLC/UV) within 24 hours of preparation, and concentrations were within 15% of the nominal dose (89.7-100%).

Necropsy & Organ weight

Males were sacrificed and necropsied on Study Day 50-54. Each testis (left intact, right parenchyma), each epididymis (left intact; right caput and corpus together, separate for right cauda), ventral prostate, and 1 seminal vesicle(s) with coagulating gland attached from each male were weighed.

There were no notable test article related changes in organ weights. Fold changes compared to control were no greater than 10%.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.):

Male fertility:

Males were treated for 14 days, and cohabited 1:1 with untreated females for up to 7 days. Females were checked daily for the presence of sperm in vaginal lavage to confirm mating. Mated females were separated from males on Day 0 post coitum (pc). After 7 days, unmated males were paired with a second female for up to 7 days. Treatment of males was continued through cohabitation until termination on Day 50 to 54 by carbon dioxide asphyxiation.

There were no notable differences in mating index. All groups had a mating index of 100%, and successful mating took 2.2-3.3 days. There were no remarkable observations in mated males at sacrifice.

There were no notable differences in fertility index (control 100%, LD/MD/HD 96%).

Female reproductive parameters:

Mated females were euthanized by carbon dioxide asphyxiation on Day 20 post coitum and the cervix and vagina were inspected microscopically. Ovaries were removed and the corpora lutea were counted for pregnant females. Each gravid uterus with at least 1

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live fetus was weighed. Implantation sites (stained with ammonium sulfide if apparently not pregnant), resorptions and live and dead fetuses were counted and their relative positions were recorded. The placentas from live and dead fetuses were microscopically inspected. Each live fetus was weighted individually and euthanized by an overdose of sodium pentobarbital. Pregnancy status was determined for mated females that died prior to scheduled termination. Females that reached the end of the 7 day cohabitation period without mating were continued on the study until 20 days after the end of their cohabitation, or until they delivered a litter. If females did not appear pregnant, the uterus was examined by ammonium sulfide staining, and the number of implantation sites were recorded, and macroscopic inspection of the vagina and cervix were performed.

There were no notable changes in numbers of corpora lutea, implantation sites, live and dead fetuses, sex ratios or gravid uterine weights. Preimplantation loss (%) was higher in dosed groups (control 4.1, LD 9.1, MD 9.1, HD 5.3), but did not increase in a dose-dependent manner. The number of early resorptions was higher in dosed animals versus controls (control 0.3, LD 0.8, MD 0.8, HD 0.6), but did not increase in a dose-dependent manner.

Table 21: Subcutaneous Male fertility study: Reproductive parameters

	Dose (mcg/kg/day)			
Parameter	0	30	60	180
Mating Index % (no. examined)[a]	100 (25)	100 (25)	100 (25)	100 (25)
Fertility Index % (no. examined)[b]	100 (25)	96 (25)	96 (25)	96 (25)
No. of pregnant rats	25	24	24	24
Litter mean no. of corpora lutea	15.3	15.8	15.3	16.0
Litter mean no. of implantation	14.6	14.2	13.9	15.1
Litter mean no. of live fetuses	14.3	13.3	12.9	14.5
Live males %	50.6	47.3	50.3	49.4
No. of dead fetuses	0.0	0.0	0.0	0.0
Preimplantation loss %	4.1	9.1	9.1	5.3
Postimplantation loss %	2.2	5.8	6.5	3.9

Offspring (Malformations, Variations, etc.)

External fetal examinations were performed on live and dead fetuses for malformations. There were no test article related fetal malformations. One litter at the MD had 1 fetus (in 23 litters) that developed gastroschisis, but this was an isolated event.

9.2 Embryonic Fetal Development

Study title: GSK573719A: Subcutaneous Embryo-Fetal Development Study in Rabbits

Reference ID: 3330354

Study no.:	Study #G09239 (GSK Report #CD2010/00253/00)
Study report location:	SD-1, eCTD 4.2.3.5.2
Conducting laboratory and location:	GlaxoSmithKline UK Research & Development PCD-DMPK Park Road Ware, Hertfordshire SG12 0DP United Kingdom
Date of study initiation:	May 24, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK 573719A bromide salt, Batch R360972, 99.8%

Key Study Findings

- Umeclidinium at doses of 0.04, 0.1 and 0.18 mg/kg/day was administered subcutaneously in pregnant rabbits. Animals were treated during gestation days (GD) 7 to 19 and sacrificed on GD 29.
- Rabbits at the MD and HD showed slight decreases in body weight gain (-14%) compared to controls from GD 7 to 18, which correlated to decreases in feed consumption (Study Days 7-19; MD -12%, HD -13%), compared to control. Dose selection appeared adequate.
- Umeclidinium was not teratogenic in rabbits at subcutaneous doses up to 0.18 mg/kg/day. Lack of ossification at the hind limb talus (control 0, LD 0, MD 2.14%, HD 1.86%) was not covered by historical control data, but there is lack of clear dose-response, and this may be an effect of maternal toxicity. Therefore, this finding is not clearly test-article related.

Methods

Doses:	0.04, 0.1 and 0.18 mg/kg/day
Frequency of dosing:	Daily from Day 7 to 19 postcoitum
Dose volume:	2 mL/kg
Route of administration:	subcutaneous
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	New Zealand White Hra:(NZW) SPF rabbits
Number/Sex/Group:	22/females/group
Satellite groups:	None
Study design:	Mated (F0) female rabbits were randomized to 22/group and dosed from Study Day 7 to 19 and sacrificed on gestation day (GD) 29. Evaluations were performed starting on Day 7.
Deviation from study protocol:	None provided.

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Evaluation
Dose selection was based on a dose range finding study (GSK report #CD2009/00970) in which doses of 0.02, 0.1, 0.18 and 1.5 mg/kg/day GSK573719 were given to pregnant New Zealand White rabbits via the subcutaneous route from Day 7 post coitum (PC) to Day 19 PC. At 1.5 mg/kg/day, rabbits had decreased body weight and food consumption, and animals were terminated after 8-10 days of dosing. A dose of 0.18 mg/kg/day produced transient decreases in body weight gain between Day 7 and 20 post coitum. Doses of 0.1 mg/kg/day and lower were well tolerated. Based on this data, a dose of 0.18 mg/kg/day was expected to produce maternal toxicity.

Observations and Results

Mortality

During the non-dosing period, animals were observed once daily for viability. During the dosing period, animals were observed once prior to dosing, immediately after dosing, and once at the end of the working day.

There were no test-article related mortalities. One control female (#H10F-7326) aborted on GD 21.

Clinical Signs

During the non-dosing period, animals were observed once daily. During the dosing period, animals were observed once prior to dosing, immediately after dosing, and once at the end of the working day. A detailed clinical examination was performed on GD 7, 8, 20, 23, 25, and 29.

Fecal aberrations were noted during and after the treatment period. Dosed animals developed elongated feces, with no incidence in controls. HD animals had more incidences of soft feces compared to controls, LD and MD animals. The table below show how many times fecal findings were recorded, with the duration of each incident lasting 1 to several days.

Table 22: Subcutaneous EFD study in rabbits: Clinical signs

	Dose (mg/kg/day)			
	0	0.04	0.1	0.18
Number examined	22	22	22	22
Feces, soft	3	2	1	6
Feces, elongated	0	24	22	22

Body Weight

Body weight was measured daily on GD 7 to 20, 25 and 29.

There was a slight decrease in body weight gain from GD 7 to 18 compared to controls at the MD and HD (MD 86%, HD 86%), but data were difficult to interpret as animals in

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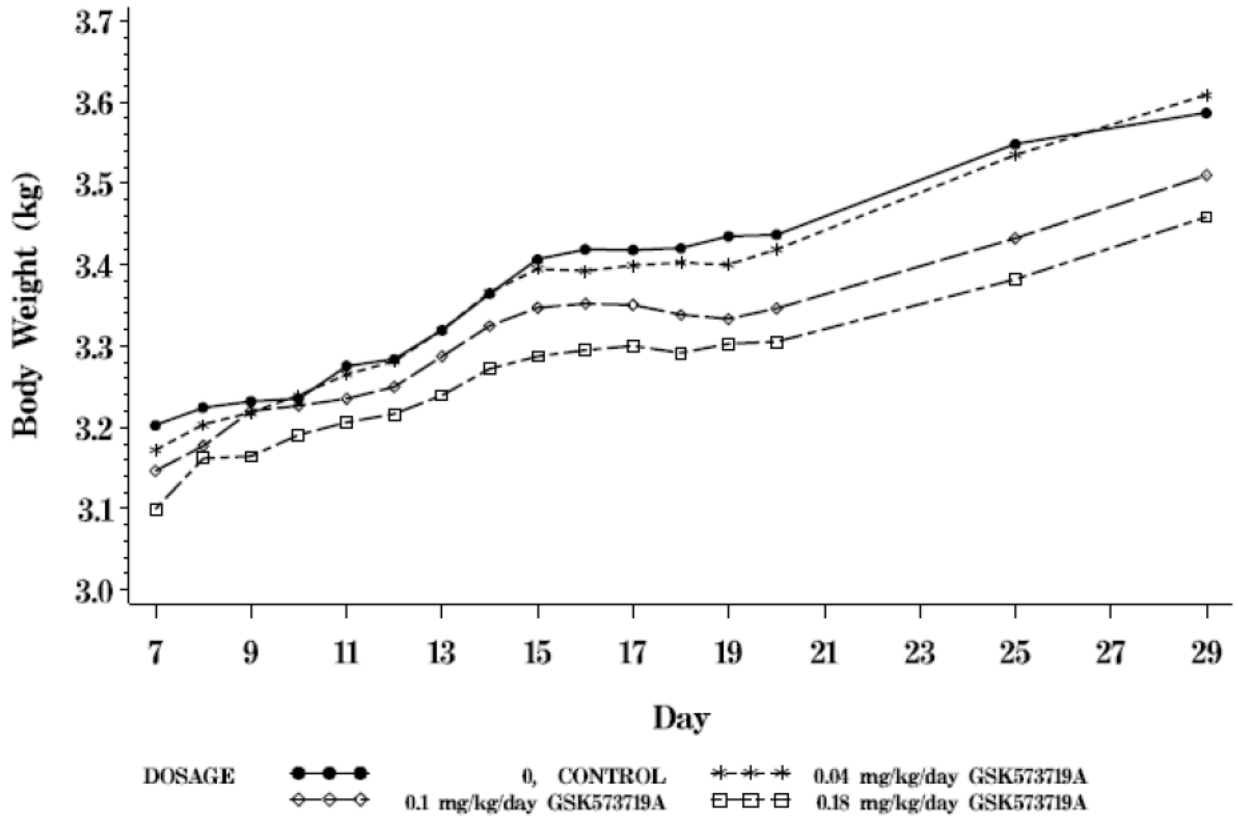
the MD and HD groups started with less weight than those in the control and LD groups. There was as slight recovery in body weight gain after dosing was discontinued, based on percent change in body weight on GD 29 (MD 92%, HD 92%).

Table 23: Subcutaneous EFD study in rabbits: Body weight analysis

	Dose (mg/kg/day)			
Study Day	0	0.04	0.1	0.18
GD 7 (kg)	3.2	3.17	3.15	3.1
GD 18 (kg)	3.42	3.4	3.34	3.29
ΔBW (kg)	0.22	0.23	0.19	0.19
BW gain, % initial	7%	7%	6%	6%
BW gain, % control	100%	105%	86%	86%
GD 29 (kg)	3.59	3.61	3.51	3.46
ΔBW (kg)	0.39	0.44	0.36	0.36
BW gain, % initial	12%	14%	11%	12%
BW gain, % control	100%	113%	92%	92%

Figure 2: Subcutaneous EFD study in rabbits: Body weight



Feed Consumption

Food consumption was measured daily on GD 7 to 29.

There were dose-dependent changes in feed consumption during the dosing period (Study Days 7-19; LD -4%, MD -12%, HD -13%), compared to control. After dosing, feed consumption returned to levels similar to controls (Study Days 19-29).

Table 24: Subcutaneous EFD study in rabbits: Feed consumption, percent change

Dose (mg/kg/day)	0.04	0.1	0.18
Study Days 7-19	-4%	-12%	-13%
Study Days 19-29	6%	-1%	2%

Toxicokinetics

Blood samples (~0.5 mL) were collected from the ear vessel. On Day 5 of dosing (Day 11 pc), 3 subgroups of 3 rabbits (total 9 rabbits) within each group were each bled approximately two or three times: the first subgroup at 0.25, 4 and 8 hours after dosing, the second subgroup at 0.5, 2 and 6 hours after dosing and the third subgroup at 1 and 24 hours after dosing. Plasma samples from non-dosed naïve rabbits that were previously collected were tested with samples from the study. Plasma samples were analyzed for GSK573719 using a validated analytical method based on protein precipitation, followed by HPLC-MS/MS analysis. The lower limit of quantification (LLQ) for GSK573719 was 0.1 ng/mL with a higher limit of quantification (HLQ) of 50 ng/mL.

Systemic exposure to GSK573719 increased in a dose proportional manner based on both AUC_{0-t} and C_{max}. T_{max} was observed at 0.25 hours, which was the first sampling time point after dosing. Control and naïve rabbit plasma samples did not show levels of test article (i.e. below the LLQ).

Table 25: Subcutaneous EFD study in rabbits: TK parameters

Sampling Occasion	Parameter	Dose GSK573719 (mg/kg/day)		
		0.04	0.10	0.18
Day 5 of Dosing (Day 11 post coitum)	AUC_{0-t} (ng.h/mL)	13.1	37.9	61.4
	C_{max} (ng/mL)	14.1	39.1	65.4
	T_{max} (h)	0.25	0.25	0.25

Sponsor's table

Dosing Solution Analysis

Formulations of 0.02, 0.05 and 0.09 mg/mL of test article in 0.9% sodium chloride were prepared weekly, and were stated by the sponsor in the reviewed study report to be stable for 15 days when stored refrigerated (2-8ºC), protected from light. The stability data were determined to be acceptable (study WD2010/00081), and were reviewed

under the study titled "GSK573719A: Subcutaneous Male Fertility Study in Rats" (study #G09187; GSK Report #CD2010/00187") reviewed in this document.

Samples of control and test article formulations prepared on 2 occasions (May 25, 2010 and June 7, 2010) were analyzed for GSK573719 concentration by high performance liquid chromatography with ultraviolet detection (HPLC/UV) within 24 hours of preparation. All were within 10% of the target concentration.

Necropsy

Mated females were euthanized by IV injection of sodium pentobarbital into the ear vein on GD 29 and necropsied. Regarding unscheduled deaths or abortions, treated females that aborted on GD 28 or earlier were euthanized, necropsied, and examined for pregnancy status. All conceptuses GD 15 or older, and any abortuses were euthanized and not examined further.

There were no test article related necropsy findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Corpora lutea were enumerated for pregnant animals. Each gravid uterus with at least 1 live fetus was weighed and the contents examined. Implantation sites, resorptions, and live/dead fetuses were counted and their relative positions were recorded. Each placenta and live/dead fetus was examined microscopically.

There were no test-article related effects. At the LD, there was a statistically significant ($P < 0.05$) decrease in the percent of live males, but this was not a dose-dependent effect.

Table 26: Subcutaneous EFD study in rabbits: Reproductive parameters

	Dose (mg/kg/day)			
Parameter	0	0.04	0.1	0.18
No. of pregnant rabbits	19	20	20	20
No. of pregnant rabbits with no viable fetuses	1	0	0	0
Litter mean no. of corpora lutea	9.1	10.3	10.8	10.1
Litter mean no. of implantation	8.6	9.5	10.1	9.3
Litter mean no. of live fetuses	8.3	9.3	9.2	8.8
Live males %	60.3	41.4*	45.5	54.6
No. of dead fetuses	0.0	0.0	0.0	0.0
Preimplantation loss %	5.9	6.9	5.8	7.8
Postimplantation loss %	9.2	2.3	8.3	5.1

* indicates $P < 0.05$ for pair-wise test compared to control

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Offspring (Malformations, Variations, etc.)

Live fetuses were weighed and euthanized by IP injection of sodium pentobarbital. Live fetuses were examined grossly. Abdominal and thoracic viscera of all fetuses were examined. Approximately one-half of the live (euthanized) fetuses were decapitated and the heads preserved in Bouin's solution then subsequently sectioned and examined. The remaining portion of these fetuses, and all intact live (euthanized) fetuses, were eviscerated, skinned and processed for skeletal examination. All skeletons, except fetal skulls fixed in Bouin's solution, were stained with Alizarin Red S and subsequently examined. Malformations and other fetal observations were noted.

The sponsor referenced historical control data, but did not submit it with the study report. An Information Request was sent on May 10, 2013 that included the following:

"1. Regarding the study "GSK573719A: Subcutaneous Embryo-Fetal Development Study in Rabbits" (Study No. CD2010/00253/00), please provide the following:

b. Historical control data are referenced in analyzing observed malformations and variations. Provide a copy of the referenced historical control document "GSK Reproductive Toxicology Historical Control Compendium". Ensure that it contains historical control data from animals of similar age in studies of similar duration, from the same testing laboratory, stating the dates of the study(ies) were initiated, the incidence of the relevant findings in each study, and an overall incidence and range from all studies. Separate data based upon the sex of the animals in each study."

On May 16, 2013, the sponsor provided relevant portions of the "GSK Reproductive Toxicology Historical Control Compendium" under Supporting Document 18; data were compiled from New Zealand White Rabbits in EFD studies initiated from August 2004 to June 2006, conducted at GSK Upper Merion, PA, with standard dosing from GD 7-19, with the standard maternal age of 6 months.

There were no test related malformations, based on low incidence (1 litter at the HD), or the incidence falling within historical control ranges. At the HD, 1 fetus had an omphalocele, heart cusp small, malpositioned testis and a short rib, and another fetus had a ventricular septal defect in the heart. The omphalocele (0.50%) was within historical control data (0.08%) and the ventricular septal defect found in 1 fetus was supported by a finding in 2 fetuses, although the % fetal incidence was slightly lower (0.56% versus 0.19%). Historical control data for all malformations, which increased with dose, are shown below. Importantly, malformations were observed in only 1 fetus.

Variations were also analyzed, and those with increased incidence at the HD are shown below. For the most part, findings had a low incidence (1 litter at the HD), or the fell within historical control ranges. The three skeletal variation findings that had the clearest dose response were found at the ribs (#T13 supernumerary), cervical vertebrae (centrum incomplete ossification), and hindpaw (talus not ossified). The supernumerary

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rib was within historical control ranges published by the MARTA Committee[1] (shown as "other" in the table below.) The finding at the cervical vertebrae (centrum incomplete ossification) slightly exceeded the upper end of the historical control range reported by GSK (2.73% versus 2.22%). A relationship to treatment cannot be clearly established because of lack of dose-response (control 0.74%, LD 2.06%, MD 3.84%, HD 2.73%). Lack of ossification at the hind limb talus was not reported in the historical control data from GSK, nor is it reported in the historical control data from the MARTA Committee[1]. While lack of ossification at the hind limb talus (control 0, LD 0, MD 2.14%, HD 1.86%) was not covered by historical control data, there is lack of clear dose-response, and this may be an effect of maternal toxicity. Therefore, this finding is not clearly test-article related. The tables below show external, visceral and skeletal malformations and variations. Variations are marked with a "#".

Table 27: Subcutaneous EFD Study in Rabbits: Fetal External Malformations

	Dose (mg/kg/day)				
Parameter/Finding	0	0.04	0.1	0.18	GSK Historical control (mean %/max %)
No. of fetuses/No. litters examined	141/17	186/20	183/20	176/20	
No. of skulls/No. litters examined	73/17	100/20	97/20	92/20	
External Torso					
Omphalocele (umbilical hernia)	0	0	0	1 (0.50%)	0.08%

Table shows number of fetuses with finding and (% affected fetuses per litter)

Table 28: Subcutaneous EFD Study in Rabbits: Fetal Visceral Malformations/Variations

	Dose (mg/kg/day)				
Parameter/Finding	0	0.04	0.1	0.18	GSK Historical control (mean %/max %)
No. of fetuses/No. litters examined	141/17	186/20	183/20	176/20	
No. of skulls/No. litters examined	73/17	100/20	97/20	92/20	
Heart					
Cusp on Heart Valve Small	0	0	0	1 (0.50%)	none
Ventricular Septal Defect (Membranous)	0	0	0	1 (0.56%)	0.19%
Testis					
Malpositioned	0	0	0	1 (0.50%)	none
Kidney					
#Renal Pelvis Moderately Dilated	0	0	0	1 (0.50%)	none
Thymus					
#Discoloration	0	0	0	1 (0.50%)	none

Table shows number of fetuses with finding and (% affected fetuses per litter)
"#" indicates variations (versus malformations)

Table 29: Subcutaneous EFD Study in Rabbits: Fetal Skeletal

[1] MARTA Committee (1996). Historical Control Data. In Ronald D. Hood (Ed.), Handbook of Developmental Toxicology (pp. 713-733). New York: CRC Press.

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Malformations/Variations

	Dose (mg/kg/day)				
Parameter/Finding	0	0.04	0.1	0.18	GSK Historical control; other* (mean %/max %)
No. of fetuses/No. litters examined	141/17	186/20	183/20	176/20	
No. of skulls/No. litters examined	73/17	100/20	97/20	92/20	
Rib					
Short	0	0	0	1 (0.50%)	none
#Incompletely ossified	0	0	0	1 (0.83%)	none (not ossified)
#T13 Supernumerary	50 (31.65%)	68 (36.51%)	78 (42.24%)	79 (44.67%)	none; 18.55%/76.36%*
Caudal Vertebrae					
#Arch and/or Centrum Incompletely Ossified	0	0	0	2 (0.91%)	none
Cervical Vertebrae					
#Centrum Incompletely Ossified	1 (0.74%)	4 (2.06%)	6 (3.84%)	6 (2.73%)	0.55-2.22%
Hindpaw					
#Talus Not Ossified	0	0	4 (2.14%)	4 (1.86%)	0.55-0.57%
Sternebrae					
#Variation in Shape	0	2 (0.95%)	0	4 (1.86%)	0.39%
Skull					
#Anterior Fonticulus Slightly Enlarged	0	0	0	1 (1.00%)	none
#Extra Suture Between Parietals	0	0	0	1 (0.83%)	1.03-3.19%
#Incompletely Ossified Jugal	0	0	0	1 (1.25%)	none

Table shows number of fetuses with finding and (% affected fetuses per litter)
"#" indicates variations (versus malformations)
* Historical control data from MARTA Committee[1]

<u>Addendum to the Review of the Inhaled Embryo-Fetal Development Study in Rats (see original review dated 5/25/10, under IND 104479</u>
(Study no.: WD2007/00764/00)

The sponsor conducted an inhalation embryo-fetal development study in rat originally reviewed under IND 104479. With respect to histopathological findings in fetuses, a low number of subdural hemorrhages were noted at the time of the review in the mid and high dose groups, and the findings appeared dose-dependent. There was also a discrepancy in the incidence of brain hemorrhages between the study report table from [redacted] (b) (4), and the study report summary from GSK. An Information Request was sent on June 6, 2013, stating the following:

> "1. Regarding the study "GSK573719A: INHALED EMBRYO-FETAL DEVELOPMENT STUDY IN RATS" (Study No. WD2007/00764), we noted a possible dose-dependent effect on brain hemorrhage. Specifically, hemorrhages

of the cerebellum were noted at the MD and HD (4/22 litters MD, 3/19 litters HD), and those within the cerebral hemisphere were only noted at the HD (2/19 litters).

In addition, if all brain hemorrhages are pooled, regardless of location, there remains a potential dose-dependent effect (control 2/22 litters, LD 2/20 litters, MD 4/22 litters, HD 5/19 litters). Our interpretation of the data, based on the provided summary table (p54 of study report) and individual line listings, is provided below:

	Fetuses			
Group	1	2	3	4
Number examined	136	123	135	113
Brain hemorrhages				
between cerebral hemisphere and mid brain	2	3	-	1
cerebellum	-	-	4 (fetus #7, 8, 4, 6)	3 (fetus #6, 10, 1)
within cerebral hemisphere	-	-	-	2 (fetus #3, 9)
3rd ventricle	-	-	1 (fetus #8)	-
all locations	2	3	4	5

	Litters			
Group	1	2	3	4
Number examined	22	20	22	19
Brain hemorrhages				
between cerebral hemisphere and mid brain	2	2	-	1
cerebellum	-	-	4 (dam #47, 52, 56, 64)	3 (dam #78, 84, 85)
within cerebral hemisphere	-	-	-	2 (dam #79, 87)
3rd ventricle	-	-	1 (dam #52)	-
all locations	2	2	4	5

Your analysis of the data in the study report, however, includes the following statement only combining the <u>cerebellum</u>, and <u>between the cerebral hemisphere and mid-brain</u>. It is not clear why only these 2 regions were combined, and why the incidences in your following text are not consistent with the table on p54 of your study report.

Excerpt from p29 of the study report, with the two combined locations underlined:

"The incidence of subdural haemorrhages on the cerebellum and between the cerebral hemisphere and mid-brain was slightly higher in all treated groups, when compared with the concurrent control group. A total of 4 fetuses from 3 litters, 4 fetuses from 4 litters or 4 fetuses from 4 litters showed this abnormality at doses of 0.0317, 0.0969 or 0.278 mg/kg/day, respectively, compared to 2 fetuses from 2 litters in the control group. In the absence of a dose response and in light of the low numbers affected, this is considered unrelated to treatment."

Excerpt from table on p54 of the study report:

Fetal examinations - minor visceral abnormalities - group incidences

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		136	123	135	113	22	20	22	19
Number affected		2	4	8	7	2	3	7	6
Visceral abnormalities									
Brain	dilated 3rd ventricle	-	-	1	-	-	-	1	-
Eye(s)	variation in lens size	-	1	-	-	-	1	-	-
Haemorrhages									
Brain	between cerebral hemisphere and mid brain	2	3	-	1	2	2	-	1
	cerebellum	-	-	4	3	-	-	4	3
	within cerebral hemisphere	-	-	-	2	-	-	-	2
	3rd ventricle	-	-	1	-	-	-	1	-
Eye/surrounding tissue	aqueous humour	-	-	2	-	-	-	2	-
	vitreous humour	-	-	1	1	-	-	1	1

Note: Individual fetuses/litters may occur in more than one category.

a) Provide your assessment of these findings.
b) In your statement from p29, it is not clear why a "total of 4 fetuses from 3 litters" showed hemorrhage (cerebellum and between the cerebral hemisphere and mid-brain) at the dose of 0.0318 mg/kg/day. Your provided table appears to show 3 fetuses from 2 litters with hemorrhage at the reference locations and dose. Please clarify your statement.
c) Provide historical control data for these findings. Ensure that it contains historical control data from animals of similar age in studies of similar duration, from the same testing laboratory, stating the dates of the study(ies) were initiated, the incidence of the relevant findings in each study, and an overall mean incidence and range from all studies."

On June 11, 2013, the sponsor replied with additional justification and a corrected table for the findings. The outline of the justification is stated below. For the purpose of this review, the historical control data is further explored:

"However, the hemorrhage observed in this study is still not considered test article-related for the following reasons (and in more detail below):

1) The incidence is similar to historical control data (see response c) and in fact considered to be an artefact due to the method used during the euthanasia procedure.

2) The rabbit EFD study achieved exposure approximately 4-fold higher than the rat study but did not have similar observations.
3) There is no pharmacological basis for the induction of hemorrhage.
4) Absence of any effects on blood coagulation parameters."

Sponsor's corrected table:

Group	1 (Control)	2 (0.0317 mg/kg/day)	3 (0.0969 mg/kg/day)	4 (0.278 mg/kg/day)
Total Number of Fetuses Examined	136	123	135	113
Total Number of Litters Examined	22	20	22	19
Subdural Hemorrhage Between Cerebral Hemisphere and Mid-Brain	2/2	3/2	0/0	1/1
Subdural Hemorrhage on the Cerebellum	0/0	0/0	4/4	3/3
Hemorrhage within the Cerebral Hemisphere	0/0	0/0	0/0	2/2
3rd Ventricle	0/0	0/0	1/1	0/0
Total Number of Hemorrhages Observed	2/2	3/2	4/4	6/6

Note: Number affected fetuses/Number affected litters (excludes one naturally delivered litter at 0.0317 mg/kg/day).

Based on the sponsor's corrected table, the highest incidence is observed in the HD group with 6/113 (5.3%) affected fetuses, or 6/19 (31.6%) affected litters. Appendices 1 and 2 of the sponsor's response included historical control data from studies in which pups were sacrificed at [redacted] (b) (4) on GD 21 and 20, respectively (i.e. at weaning). The time of sacrifice is comparable to the relevant rat EFD study, in which dams were sacrificed on GD 21 and fetuses were examined. One study (Appendix 1) in which rat fetal heads were examined on GD 21 showed that brain hemorrhages were detected in 3/39 fetuses or (7.7%) or 2/9 litters (22.2%). Eight studies (Appendix 2) in which rats were examined on GD 20 showed that brain hemorrhages were detected in up to 4.7% of fetuses, or 28.6% of litters (see table below). Based on the similar incidence of the upper bound of the historical control data, and the uniformly high incidence of brain hemorrhage across multiple studies, the incidence of brain hemorrhages may be supported by historical control data. Thus, UMEC is determined to not be teratogenic in rats at inhaled doses up to 278 mcg/kg/day.

Table 30: Historical control data for brain hemorrhages in rat EFD studies

	Fetus		Litter		Percent	
Study	Incidence	Total	Incidence	Total	Fetus	Litter
1	3	158	3	22	1.9%	13.6%
2	6	160	4	22	3.8%	18.2%
3	5	170	4	22	2.9%	18.2%
4	6	154	4	21	3.9%	19.0%
5	7	155	6	21	4.5%	28.6%
6	7	149	5	21	4.7%	23.8%
7	5	162	4	22	3.1%	18.2%
8	5	160	5	22	3.1%	22.7%

9.3 Prenatal and Postnatal Development

Study title:

Study no.: Study #20015090 (GSK #2011N118595_00)

Study report location: SD-1, eCTD 4.2.3.5.2

Conducting laboratory and location: (b) (4)

Date of study initiation: 21 June 2011

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: GSK573719A (bromide salt of GSK573719), batch #081168623, 83.8% purity. The sponsor included a correction factor of 1.194 for dose calculations (p. 102 of study report.)

Key Study Findings

- In a pre- and postnatal development study, mated F_0 female rats received subcutaneous injections of the GSK573719A at doses of 0 (vehicle), 0.01, 0.06, 0.18 mg/kg/day from Gestation Day (GD) 6 to Postnatal Day (PND) 20.
- F_0 females that received ≥60 mcg/kg/day exhibited a slight decrease in body weight gain during the gestation period (GD 6-20), with those at the HD weighing 93% compared to controls. During the postnatal period (PND 1-20), HD animals weighted 92% compared to controls. Feed consumption was decreased by 7% at the HD, compared to control, during GD 6-21. From PND 1-14, there was a slight decrease in food consumption at the MD (-4%) and HD (-5%). The sponsor did not measure feed consumption from PND 15-20. Dose selection appears adequate.

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- There were no test article related effects on reproductive parameters for F_0 dams. There were no negative test article related effects on viability of F_1 offspring.
- F_1 generation pups showed dose-dependent decreases in body weight compared to controls at 0.18 mg/kg/day ($P < 0.05$) on PND 7, PND 14, PND 18, and PND 21. After weaning of pups on PND 21 and cessation of dosing (on PND 20), there was a reversal in body weight loss.
- F_1 generation females, which were mated to produce F_2 generation pups, showed an initial decrease in body weight gain (-10%) at the HD between GD 0-7; this effect was reversed by GD 14 and therefore is a transient effect.
- F_1 generation females showed no test-article related changes in reproductive parameters.
- F_1 generation pups showed no clear test article related effects based on evaluation of motor activity, acoustic startle habituation or testing through the modified M-watermaze.
- There were no test article-related effects on F_2 fetuses based on survival, sex, body weight, or malformations (based on external examination).

Methods

Doses:	0 (vehicle), 0.01, 0.06, 0.18 mg/kg/day
Frequency of dosing:	Daily from Gestation Day (GD) 6 to Postnatal Day (PND) 20
Dose volume:	10 mL/kg for control, MD, HD; 2 mL/kg for LD (half of volume was given to each of 2 sites). While the change in dose volume is unusual, the control and HD received the same volume.
Route of administration:	Subcutaneous
Formulation/Vehicle:	0.9% Sodium Chloride
Species/Strain:	Rat: Crl:CD(SD)
Number/Sex/Group:	24 females/group
Satellite groups:	None
Study design:	On PND 21, 2 F_1 generation pups per litter were assigned to 2 subsets: Subset 1 animals were tested for acoustic startle habituation (PND 45 ± 2) and reproductive performance (see Reproduction). Subset 2 animals were tested for motor activity (PND 46 ± 2), acoustic startle habituation (PND 77 ± 3), and modified M-watermaze (PND 70 ± 2).
Deviation from study protocol:	On PND 10, a MD F_0 female (#2457) received two doses of test article, with no adverse clinical observations. Overall there were no deviations that appeared to have affected the validity of the study.

Basis for Dose Selection

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In a 14-day subcutaneous study in female rats (study 2011N111874_00), rats were dosed with 0.03, 0.18 and 1.6 mg/kg/day of test article. At the HD, the sponsor noted dilated pupils, and decreased body weight and food consumption. A high dose of 0.18 mg/kg/day was chosen for the PPND study based on maternal toxicity.

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Observations and Results

F_0 Dams

Survival: Viability checks were performed at least twice daily. There were no test-article related deaths.

Clinical signs: Clinical observations were performed at least once weekly and on GD 0 during the non-dosing period. During the dosing period, clinical observations were performed daily before dosing, shortly after dosing, and at the end of the work day; observations were made on the day of euthanasia. There were no clear test-article related findings. Scabs were observed in 2/24 HD females (compared to 0/24 controls), but due to the low incidence, this is not considered test-article related.

Body weight: Body weights were recorded on GD 0, weekly during the gestation period, on PND 1, and weekly during the post natal phase until PND 21 (euthanasia).

There was a slight decrease in body weight gain during the dosing period (GD 6-20) at HD (93%), based on percent body weight gain versus controls. Body weight gain during the postnatal period was difficult to analyze versus control because MD and HD animals weighed less than controls. There was a decrease in body weight gain from PND 1-20 at the HD (92%). F_0 dams:

Body weight analysis:

	Dose (mg/kg/day)			
Study Day	0	0.01	0.06	0.18
Dosing (Gestation Day)				
GD 6	271.7	271.5	269.7	270
GD 20	386.4	390.8	383.2	376.8
ΔBW (g)	114.7	119.3	113.5	106.8
BW gain, % (GD 6)	42%	44%	42%	40%
BW gain, % control	100%	104%	99%	93%
Postnatal				
PND 1	294.2	296	287	284.2
PND 20	344	344.9	337.1	330.2
ΔBW (g)	49.8	48.9	50.1	46
BW gain, % initial (PND 1)	17%	17%	17%	16%
BW gain, % control	100%	98%	101%	92%

Feed consumption: Feed consumption was measured on the following intervals: GD 0 to 6, 6 to 10, 10 to 14, 14 to 18, 18 to 21 pc, 21 to 25 days post coitum (for the dam that did not deliver a litter), and on PND 1 to 4, 4 to 7, 7 to 10, and 10 to 14.

During the dosing period (GD 6-21), feed consumption was decreased by 7% at the HD, compared to control. From PND 1-

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14, there was a slight decrease in food consumption at the MD (-4%) and HD (-5%). The sponsor did not measure feed consumption from PND 15-20.

F_0 dams: Feed consumption:

	Dose (mg/kg/day)		
Study Day	0.01	0.06	0.18
Gestation			
GD 6-21	-2%	-5%	-7%
Post Natal			
PND 1-14*	2%	-4%	-5%

* sponsor did not report values from PND 15-20

Uterine content: On PND 21, F_0 females that delivered pups were euthanized and uterine implantation sites were counted and recorded. On Day 25 post coitum (PC), mated F_0 females that did not deliver a litter were euthanized. See Reproductive Assessment.

Necropsy observation: On PND 21, F_0 females that delivered pups were euthanized by carbon dioxide asphyxiation, a gross necropsy of the cranial (including pituitary), thoracic, abdominal and pelvic viscera was performed. There were no test-article related necropsy findings.

Mated F_0 females that did not deliver a litter were euthanized by carbon dioxide asphyxiation, on Day 25 PC examined for gross lesions, and a gross necropsy of the cranial (including pituitary), thoracic, abdominal and pelvic viscera was performed. There were no test-article related necropsy findings.

Toxicokinetics: On PND 20, blood samples (0.3 mL) were collected from three F_0 dams per dose group. Blood was collected from the lateral tail vein at 0.25, 0.75, 2, 4, 8, and 24 hours after dose administration On PND 10/11, blood was collected via cardiac puncture at 3, 8, and 22 hours after the maternal dose from 3 F_1 pups/sex/time point (non-littermates) from each group, and euthanized. Plasma concentrations of GSK573719 were determined by high performance liquid chromatography with MS/MS detection (HPLC MS/MS).

Test article was generally detectable in dams at PND 20 at the LD for up to 4 hrs after dosing, and at the MD/HD for up to 24 hrs after dosing. Exposure was approximately dose proportional based on AUC_{0-t} and C_{max}. In pups, the test article was only quantifiable in 2/54 dosed animals on PND 10/11 (see F_1 generation results for further information.)

Toxicokinetic Parameters from Dams on PND 20:

Parameter [a]	Dose of GSK573719A (mg/kg/day)		
	0.01	0.06	0.18
AUC_{0-t} (ng.h/mL)	0.966 [0.596 – 1.48]	8.07 [7.20 – 8.60]	24.9 [24.5 – 25.7]
C_{max} (ng/mL)	1.42 [1.02 – 1.83]	8.81 [8.06 – 9.50]	24.5 [21.3 – 29.5]
Median T_{max} (h)	0.25 [0.25 - 0.25]	0.25 [0.25 - 0.25]	0.25 [0.25 - 0.25]

a. Results are reported as mean unless stated otherwise and [range].

Sponsor's Table

Dosing Solution Analysis: GSK573719A at concentrations of 0.005, 0.006, and 0.018 mg/mL in 0.9% sodium chloride were stored refrigerated (2°C to 8°C) while protected from light, and were prepared once every week. The sponsor stated that solutions were stable for at least 8 days at room temperature, protected from light and at least 15

days when stored at refrigerated (2°C to 8°C), protected from light. The stability data were determined to be acceptable (study WD2010/00081), and were reviewed under the study titled "GSK573719A: Subcutaneous Male Fertility Study in Rats" (study #G09187; GSK Report #CD2010/00187") reviewed in this document.

Reproductive assessment: The date and approximate time parturition was initiated, adverse clinical signs, duration of gestation, litter size, pup viability, and the length of parturition were recorded. For dams that delivered pups, uterine implantation sites were counted and recorded at necropsy on PND 21. Uteri of the apparently nonpregnant rats were examined after staining with ammonium sulfide to confirm the absence of implantation sites on PC day 25. When naturally delivered F1 offspring were found dead when first counted, lungs were removed and immersed in water to determine whether the offspring were stillborn or born alive.

There were no test-article related findings with respect to reproductive parameters. There was a significant increase ($P \leq 0.01$) in pups found dead at post natal day (PND) 1 at the LD, but this was limited only to the LD. One control dam was unable to complete parturition and was euthanized on GD 24.

Table 31: Subcutaneous PPND study: Reproductive parameters of F_0 dams

	Dose (mg/kg/day)			
Parameter	0	0.01	0.06	0.18
No. of pregnant rats	23	24	22	21
No. of parturient rats	22	24	22	21
Parturition index (%)	95.6	100.0	100.0	100.0
Mean gestation period (days)	22.6	22.6	22.6	22.5
Litter mean no. of implant sites	15.3	15.1	15.1	14.8
Litter mean no. of total newborns	14.0	14.4	14.0	14.1
Litter mean no. of live newborns	13.7	14.3	13.7	14.0
Live males %	49.3	50.7	48.4	53
Viability of F_1 pups on Day 0 (%)[a]	90%	95%	91%	95%
Viability of F_1 pups on Day 4 (%)[b]	100%	97%	99%	99%
Viability of F_1 pups on Day 7 (%)[c]	100%	100%	100%	100%

[a]Viability of F_1 pups on Day 0 (%) = (No. live pups/Total No. implants) x 100
[b]Viability of F_1 pups on Day 4 (%) = (No. live pups on day 4/ No. live pups on day 0) x 100
[c]Viability of F_1 pups on Day 7(%) = (No. live pups on day 7/ No. live pups after adjustment on day 4) x 100

F_1 Generation

Survival: Viability checks were performed at least twice daily. After weaning (PND 22 and beyond), there were no test-article related mortalities.

Clinical signs: Clinical observations were performed at least once daily. There were no dose-dependent findings with an incidence greater than 3 over the control group. A sparse hair coat was observed on the limb (control 0/22, LD 1/23, MD 1/22, HD 2/21) and underside (control 0/20, LD 1/23, MD 0/19, HD 2/18).

Body weight: F_1 animals were individually weighed on PNDs 1 (birth), 4, 7, 14, 18, and 21. After weaning, body weights were recorded weekly and on the day of euthanasia. Beginning after PND 78, body weights for Subset 1 F1 mated females were recorded on GD 0, 7, 10, 14, 18, and 21 and Days 1, 4, and 7 post partum.

During PND 1-21, there were dose-dependent decreases in body weight compared to controls at the HD ($P < 0.05$) on PND 7, PND 14, PND 18 (not shown), and PND 21. After weaning of pups on PND 21 and cessation of dosing (on PND 20), there was a reversal in body weight loss.

F_1 generation pups: Body weight analysis (PND 1-21):

	Dose (mg/kg/day)			
Study Day	0	0.01	0.06	0.18
PND 1	6.6	6.8*	6.8*	6.5
PND 7	13.6	14	13.8	13.1*
ΔBW (g)	7	7.2	7	6.6
BW gain, % initial (GD 1)	106%	106%	103%	102%
BW gain, % control	100%	103%	100%	94%
PND 14	28	28.2	27.4	26.1*
ΔBW (g)	14.4	14.2	13.6	13
BW gain, % initial (GD 1)	218%	209%	200%	200%
BW gain, % control	100%	96%	92%	92%
PND 21	43.4	43.7	42.8	40.5*
ΔBW (g)	15.4	15.5	15.4	14.4
BW gain, % initial (GD 1)	233%	228%	226%	222%
BW gain, % control	100%	98%	97%	95%

F_1 generation pups: Body weight analysis (PND 22-78):

	Dose (mg/kg/day)			
Study Day	0	0.01	0.06	0.18
Males				
PND 22	48.4	49.3	47.3	45.2
PND 29	278	281.6	276.2	267.5
ΔBW (g)	229.6	232.3	228.9	222.3
BW gain, % initial (Day 22)	474%	471%	484%	492%
BW gain, % control	100%	101%	100%	97%
PND 78	467.5	470.3	466.6	457.5

ΔBW (g)	189.5	188.7	190.4	190
BW gain, % initial (Day 22)	392%	383%	403%	420%
BW gain, % control	100%	98%	103%	107%
Females				
PND 22	46.1	47.4	46.5	43
PND 29	194.4	196.8	195.4	188.9
ΔBW (g)	148.3	149.4	148.9	145.9
BW gain, % initial (Day 22)	322%	315%	320%	339%
BW gain, % control	100%	101%	100%	98%
PND 78	274.4	273.2	271.2	269.3
ΔBW (g)	80	76.4	75.8	80.4
BW gain, % initial (Day 22)	174%	161%	163%	187%
BW gain, % control	100%	96%	95%	101%

F_1 generation females, which were mated to produce F_2 generation pups, showed only a transient decrease in body weight gain from GD 0-21. There was an initial decrease in body weight gain (-10%) at the HD between GD 0-7, but this effect was reversed by GD 14. There were no test-article related changes in body weight gain during the remainder of the gestation period, or during PND 1-7.

F_1 generation maternal body weight assessment:

	Dose (mg/kg/day)			
Study Day	0	0.01	0.06	0.18
Gestation				
GD 0	278.8	279.8	270.7	266.6
GD 21	453.2	462.6	450.5	446.6
ΔBW (g)	174.4	182.8	179.8	180
BW gain, % initial (GD 0)	63%	65%	66%	68%
BW gain, % control	100%	104%	106%	108%

Feed consumption: Food consumption was measured weekly (PND 29-78) in both males and females to determine post-weaning feed consumption. For Subset 1 females, food consumption was recorded on GD 0, 7, 10, 14, 18, and 21 and Days 1, 4, and 7 pp.

There was a significant ($P \leq 0.01$) decrease in post-weaning feed consumption in F_1 males from PND 29-36 and PND 36-43 (-7%) compared to controls, but this was not dose dependent, and reversed starting at PND 43-50. F_1 females showed no affect in feed consumption from PND 29-78, and during mating (GD 0- 21). For F_1 females that were mated (Subset 1), there was no change in feed consumption during the gestation period (GD 0-21), or after weaning (PND 1-7).

Physical development: Subset 1 females were examined daily for the occurrence of vaginal opening beginning on PND 28; subset 1 males were examined daily for the occurrence of balano-preputial separation beginning on PND 39. Body weights were recorded on the day of vaginal patency or balano-preputial separation. There were no test-article related effects on sexual maturation.

Neurological assessment: The F_1 offspring were assigned to 2 subsets: Subset 1 animals were tested for acoustic startle habituation (PND 45 ± 2) and reproductive performance (see Physical Development and Reproduction). Subset 2 animals were tested for motor activity (PND 46 ± 2), acoustic startle habituation (PND 77 ± 3), and modified M-watermaze (PND 70 ± 2).

For motor activity, there was a slight decrease in number of movements in HD males (-8%) compared to controls, but this was not dose-dependent, and there were no notable differences in the time spent in movement. Based on the lack of severity and dose-dependence, this is not considered test-article related. There were no significant differences in acoustic startle habituation or the modified M-watermaze.

Reproduction: Estrous cyclicity of F_1 females was evaluated daily for a maximum of 10 days (from PNDs 65 to 74), and daily during the cohabitation period (from PNDs 78 to 84) until mated, or up to 14 days. Mated females were observed during parturition for adverse clinical signs, pup viability, and length of parturition.

There was no test-article related change in reproductive parameters (see table at end of F_1 evaluation.)

Necropsy: Pups were euthanized on PND 7 by intraperitoneal injection of sodium pentobarbital and discarded. Pups that died on or before PND 7 were examined for gross lesions to determine a cause of death. F2 offspring that were found dead shortly after delivery, when litters were first

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counted, were examined for gross lesions and the lungs were removed to determine if the offspring were stillborn or alive (based on if the lungs floated in water.) There were no test-article related visceral or external findings. The sponsor reported that all pups appeared normal at necropsy.

Toxicokinetics: See Toxicokinetics of F_0 generation for methods.

In F_1 generation pups, the test article was only quantifiable in 2/54 dosed animals on PND 10/11 (see F_1 generation results for further information.) Pup #2465 (maternal dose 0.06 mg/kg/day) at had a plasma concentration of 21.4 pg/mL at 3 hrs post dose. Pup 2481 (maternal dose 0.18 mg/kg/day), at had a plasma concentration of 29.2 pg/mL at 22 hrs post dose.

Table 32: Subcutaneous PPND study: Reproductive parameters for F_1 generation

	Dose (mg/kg/day)			
Parameter	0	0.01	0.06	0.18
F1 males: Mating index (%)	95.4 (21/22)	91.7 (22/24)	100.0 (22/22)	100 (21/21)
F1 males: Fertility index (%)	90.5 (19/21)	100.0 (22/22)	86.4 (19/22)	85.7 (18/21)
F1 females: Mating index (%)	100.0 (22/22)	95.8 (23/24)	100.0 (22/22)	100.0 (21/21)
F1 females: Fertility index (%)	90.9 (20/22)	100.0 (23/23)	86.4 (19/22)	85.7 (18/21)
Mean no. of corpora lutea	17.0	17.9	18.0	16.1
Mean litter no. of implantation	15.8	16.5	16.5	15.2
Mean litter no. of live fetuses	14.4	15.7	14.8	14.0
Mean litter no. of dead fetuses	0.3	0.0	0.3	0.3
Estrous stages/10 days	2.2	2	2.2	2
Females with 6+ days of diestrus	0	1	1	1
Females with 6+ days of estrus	0	0	0	0

F_2 Generation

Survival and sex: Viability checks were performed at least once daily. There were no test-article related effects on the mean number of live fetuses or viability index. Regarding the sex of pups, there was a statistically significant difference in the sex of the pups, but it was not dose-dependent and not considered test-article related.

	Dose (mg/kg/day)			
Parameter	0	0.01	0.06	0.18
Litters	20	23	19	18
Mean litter no. of live fetuses	14.4	15.7	15.3	14.3
Mean litter no. of dead fetuses	0.3	0.0	0.3	0.3
Live males %	55.6	44.8**	44.8**	47.1*
Viability of F_1 pups on Day 4 (%)[a]	98.3	97.5	94.3	98.8

[a] Viability of F1 pups on Day 4 (%) = (No. live pups on day 4/ No. live pups on day 1 post partum) x 100
* P ≤0.05
** P ≤0.01

Body weight: As soon as practical after completion of parturition, live pups were individually weighed. Live F2 offspring were individually weighed on PNDs 1, 4, and 7. There were no test-article related differences in individual pup weight.

External evaluation: When naturally delivered F2 offspring were found dead when first counted, the pups were examined for gross lesions using a magnifier, and lungs were removed and immersed in water to determine whether the offspring were stillborn or born alive As soon as practical after completion of parturition, the F2 pups were examined externally. Clinical observations were performed on PNDs 1, 4, and 7. F2 pups that died on or before PND 7 were examined for gross lesions (using a microscope) and the cause of death. F2 pups that survived until PND 7 were euthanized without further examination.

There were no test-article related clinical observations based on external evaluation. At the HD, 3/18 litters showed paleness, but this was not dose-dependent (control 0/20, LD 1/23, MD 0/19), and is not considered test-article related.

10 Special Toxicology Studies

See IND 104479 Pharmacology/Toxicology review dated 5/4/10 for review of study titled "*In Vitro* Haemolytic potential in Rat. Dog and Human Peripheral Blood" (study # WD2008/01499).

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11 Integrated Summary and Safety Evaluation

ANORO ELLIPTA is fixed dose combination, dry powder inhaler of umeclidinium and vilanterol for the treatment of airflow obstruction in chronic obstructive pulmonary diseases (COPD) in adults. ANORO ELLIPTA delivers a mixture of 62.5 mcg of umeclidinium bromide and 25 mcg of vilanterol trifenatate in each actuation. The formulation includes magnesium stearate and lactose as excipients. The maximum recommended human dose is one actuation, once daily, or 62.5 mcg of umeclidinium bromide and 25 mcg of vilanterol trifenatate per day.

The sponsor GlaxoSmithKline (GSK) has conducted adequate nonclinical safety evaluations to support the approval of ANORO ELLIPTA from the nonclinical perspective. Nonclinical studies included pharmacology, safety pharmacology, ADME, general toxicology, genotoxicity, carcinogenicity, and reproductive toxicology. Additional studies to support the use of magnesium stearate as an excipient were referenced by the sponsor. There are no outstanding nonclinical issues at this time.

The summary below is divided into sections for umeclidinium, umeclidinium and vilanterol in combination, vilanterol, and excipients. A complete summary for the nonclinical program of vilanterol is available under NDA 204275; a brief summary is provided below. A labeling evaluation is provided after these summaries.

11.1 Umeclidinium

The nonclinical studies to support the safety of umeclidinium adequately assessed the pharmacology, pharmacokinetics, and toxicology of umeclidinium in animals. The relevant species tested were primarily mice, rats and dogs. The following summary is based upon the referenced reviews, and the reviews of studies presented here.

Pharmacology: Umeclidinium is long-acting antimuscarinic antagonist (LAMA), which is often referred to under the more general term anticholinergic. The mechanism of action of umeclidinium is similar to that of tiotropium bromide and aclidinium bromide, which are currently marketed LAMAs.

In vitro pharmacology studies show that umeclidinium bromide is a high affinity, pan active muscarinic antagonist, similar to tiotropium bromide and aclidinium bromide. Umeclidinium was assessed for muscarinic activity, binding and calcium mobilization at cloned human receptors. In competitive binding assays, umeclidinium bromide bound to M1, M2, M3, M4 and M5 receptors with K_i values of 0.16, 0.15, 0.06, 0.05, and 0.13 nM, respectively. Umeclidinium bromide was assayed for muscarinic agonist activity in cells expressing human M1, M2, and M3 receptors, and there was no significant calcium mobilization response, indicating a lack of muscarinic agonist activity. Umeclidinium bromide was characterized as a competitive inhibitor at the M1, M2, and M3 receptors, expressed by CHO cells, with slopes of 0.829, 0.928 and 0.963, respectively, which were consistent with competitive kinetic assays. Reversibility of antagonism at the M3 receptor was assessed for umeclidinium bromide and tiotropium. Pretreatment and washout for tiotropium resulted in a 10 to 15-fold decrease in receptor affinity, while umeclidinium bromide showed a 5 to 20-fold decrease in receptor affinity.

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In vitro studies using human and animal models of contraction assessed the ability of umeclidinium bromide to induce bronchodilation. In human bronchial strips with carbachol-induced contraction, umeclidinium bromide caused a concentration-dependent rightward shift, illustrating suppression of the carbachol response. The recovery halftime for umeclidinium bromide-induced inhibition (10 nM) was determined to be 381 min. Similar results were reported for guinea pig trachea.

In vivo studies measured the effect of umeclidinium bromide on methacholine-induced bronchoconstriction in mice, and showed that intranasal administration inhibited bronchoconstriction for up to 7 days. Oral administration did not inhibit methacholine-induced bronchoconstriction, showing that this is a site-specific effect. Intratracheal administration on acetylcholine-induced bronchoconstriction in guinea pigs showed greater than 90% inhibition at 4 hr post dosing, 68.9% ± 11.3 inhibition at 24 hr, and 37.7% ± 10.2 at 48 hr. Intratracheal administration of umeclidinium bromide on acetylcholine-induced bronchoconstriction and bradycardia in guinea pigs showed 74.4% inhibition of bronchoconstriction, with no effect on acetylcholine-induced bradycardia.

Safety Pharmacology: Umeclidinium bromide was assessed for effects on CNS, cardiovascular, and respiratory functions. Effects on neurobehavior, body temperature, and motor activity were assessed in male rats following single doses of 36, 322, or 1994 mcg/kg administered by nose only inhalation. Moderately dilated pupil was observed for rats at 322 and 1994 mcg/kg, which is an expected pharmacological effect, but no other changes in CNS were observed.

Umeclidinium bromide inhibited the hERG tail current in a concentration-dependent manner with a nominal IC_{50} value of 9.41 μM. Effects of single intravenous doses of umeclidinium bromide at 0.3, 3, and 10 mcg /kg on arterial pressures, heart rate, and ECG parameters were evaluated in dogs. At 10 mcg/kg, there was an increase in heart rate up to 49 bpm, from a predose average of 65 bpm, which recovered 30 min after infusion. In agreement with increased heart rate, RR interval decreased, but returned to approximate predose values by 30 min post dose. Changes in heart rate and RR interval are associated with the tachycardia seen following dosing with 10 mcg/kg; second degree atrioventricular (AV) block was observed in 3 of 4 animals with 10 mcg/kg. Increased heart rate, decreased RR interval, and AV block can be attributed to the antimuscarinic effects of umeclidinium.

Effects of umeclidinium on respiratory function were assessed following single inhaled doses to rats of 36, 215, or 2660 mcg/kg by nose only inhalation. Inhaled doses of 215 or 2260 mcg/kg umeclidinium produced increases in respiratory rate (18 to 45%) and decreases in tidal volume (3 to 17%).

ADME: The absorption, distribution, metabolism, and excretion of umeclidinium have been examined in mice, rats, dogs, and humans. The half-lives of umeclidinium in rats and dogs following intravenous administration were 1.42 and 11.6 hr, respectively.

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Plasma protein binding in mouse, rat, rabbit, dog, and human ranged from 74.7 to 88.8% and was concentration-independent. Association with red blood cells in dog, human, rabbit, rat, and mouse was generally low. Studies in rats and dogs suggested extensive distribution into tissues and rapid clearance suggestive of metabolic clearance. Umeclidinium was a substrate for p-glycoprotein. Following intravenous administration in rats, the highest levels of radioactivity were present in the kidney cortex, kidney medulla, liver, pituitary gland, small intestine wall, thyroid, and pancreas.

Metabolism was examined *in vitro* using hepatocytes from mouse, rat, rabbit, dog and human. Studies with human hepatocytes and microsomes found that the main routes of metabolism of umeclidinium were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The main routes of metabolism in humans were also present in rat and dog, but not in mice or rabbits. Metabolism of umeclidinium in human liver microsomes was mediated primarily by CYP2D6, with some contribution from CYP3A4; umeclidinium was an inhibitor of both enzymes. Umeclidinium is also metabolized by the CYP1A1, which is known to be expressed extrahepatically.

Excretion was examined in rats and dogs. Following intravenous administration of 1 mg/kg umeclidinium to rats, 65.3 and 16.9% of the radioactive dose were found in the feces and urine, respectively. Following intravenous administration of umeclidinium to dogs, 61.8 and 11.9% of the dose were found in the feces and urine, respectively.

General Toxicology: The pivotal general toxicology studies supporting the safety of umeclidinium are chronic toxicology studies in rats and dogs. The common target organ between the two species was the lung. Importantly, the heart was identified as a target organ in the dog.

In the 26 week month inhalation toxicology study in rats, animals received estimated achieved doses of 0, 87.1, 289, or 987 mcg/kg/day. Pulmonary deposited doses were 0, 8.7, 28.9, or 98.7 mcg/kg/day. Target organs of toxicity were the lung, tracheal bifurcation, nasal cavity/sinuses, nasopharynx, and larynx. Findings in the nasal cavity/sinuses, nasopharynx, and larynx were judged to be irrelevant to human subjects as they will receive the drug by oral inhalation. In the lungs, alveolar macrophages were increased for all treatment groups. These findings were reversible by the end of the 6-week recovery period and there was no evidence of inflammation in surrounding tissues. At the tracheal bifurcation, squamous metaplasia was observed in the mid and high dose groups. These findings are indicative of irritant effects induced by the test article. The NOAEL was identified as the low dose (8.7 mcg/kg/day pulmonary deposited dose) based upon histopathological findings for the tracheal bifurcation (squamous metaplasia) in the mid and high dose groups. Doses for males and females in the low dose group, expressed on mcg per gram of lung weight, were 2.86 and 2.02 mcg/g/day, respectively. AUC values during week 26 for males and females in the low dose group were 8.88 and 10.1 ng•hr/mL (mean 9.49 ng•hr/mL), respectively.

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In a 39-week inhalation toxicology study, dogs received umeclidinium at estimated achieved doses of 0, 109, 421, and 1002 mcg/kg/day. Pulmonary deposited doses were 0, 27, 105, and 250 mcg/kg/day. Several clinical signs were evident for treated dogs (i.e., dry mouth, swollen neck, decreased incidences of unformed, watery, and mucoid stools, and increased incidences of vomit and vomit with food). Many of these findings were expected pharmacological effects of an M3 muscarinic antagonist. For ECG measurements conducted during week 39, increased heart rates were observed at all doses. Respiratory sinus arrhythmia was generally absent in males given ≥421 mcg/kg/day and females given ≥109 mcg /kg/day. On day 1, elevations of serum cardiac Troponin I (cTnI) concentrations were observed for dogs that received ≥421 mcg/kg/day.

The target organs of toxicity in the dog were the heart, lungs (intimal thickening with focal areas of inflammatory cell infiltrate, pleural fibrosis), larynx (epithelial erosion/ulceration in squamous epithelium at base of vocal folds; inflammation at mucosal glands adjacent to the arytenoid cartilage), and nasal turbinates (squamous metaplasia). Moderate subacute inflammation of the extramural coronary arteries was seen in one female given 1002 mcg/kg/day and in one male (Animal No. 3427) given 421 mcg/kg/day. The NOAEL was identified as the low dose of 109 mcg/kg/day based upon histopathological findings in the heart at the mid and high doses and the lung, larynx, and nose turbinates at the high dose. AUC values for males and females at 109 mcg/kg/day during week 39 were 4.64 and 7.13 ng•hr/mL (mean 5.89 ng•hr/mL), respectively. The deposited doses, expressed on mcg/g lung weight/day basis, for males and females were 3.14 and 2.86 mcg/g lung weight/day, respectively.

Safety margins for the proposed clinical dose of 62.5 mcg/day of umeclidinium are calculated based on the NOAELs identified in the 26 week rat and 39 week dog toxicology studies.

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Table 33: Safety margins for the proposed clinical dose of umeclidinium based on AUC

	NOAEL		Safety Margin for clinical dose of 62.5 mcg/day
Study	Achieved dose (mcg/kg/day)	AUC (ng•hr/mL)	AUC = 0.3124 ng•hr/mL
26 week rat	87.1	9.49	30
39 week dog	109	5.89	19

Values based on Pharm/Tox review dated 2/14/11 and 9/6/12 under INDs 104479/106616

Table 34: Safety margins for the proposed clinical dose of umeclidinium based on mcg/g lung weight

	NOAEL		AUC for clinical dose of 62.5 mcg/day
Study	Achieved dose (mcg/kg/day)	Deposited dose (mcg/g lung weight)	0.0625 mcg/g lung weight
26 week rat	87.1	2.44	39
39 week dog	109	3	48

Values based on Pharm/Tox review dated 2/14/11 and 9/6/12 under INDs 104479/106616

Genetic toxicology: Umeclidinium was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation assay, *in vitro* mouse lymphoma assay, and *in vivo* rat micronucleus test.

Carcinogenicity: The sponsor determined the carcinogenic potential of umeclidinium in traditional 2-year bioassays conducted in mice and rats. Both bioassays were negative for test-article related tumors.

The sponsor conducted a 2-year bioassay in CD-1 mice (75/sex/group) with GSK573719A by inhalation (nose only). Initial doses were based on MTD determined in a 13 week toxicology study. Female mice received nominal doses of 0 (1% magnesium stearate in lactose), 20, 60, and 200 mcg/kg/day throughout the study (60 minute exposures). Males initially received nominal doses of 0 (1% magnesium stearate in lactose), 60, 200, and 600 mcg/kg/day (60 minute exposures). Doses were decreased at Week 67 for males only to 0, 30, 100, and 300 mcg/kg/day (30 minute exposures), based on loss of body weight in dosed males with Executive Carcinogenicity Assessment Committee (ECAC) concurrence. There were no treatment-related neoplastic findings in the mouse study based on the lack of statistical significance for both trend and pair-wise statistical analysis.

The sponsor conducted a 2-year bioassay in Crl: CD (Sprague Dawley) rats (65/sex/group) with GSK573719A by inhalation (nose only). Initial doses were based on

the MTD determined from a 13-week inhalation toxicology study. The recommended doses were 0 (1% magnesium stearate in lactose), 30, 100, and 300 mcg/kg/day (60 minute exposures). Effective from Week 73, doses were reduced to target doses of 15, 50, and 150 mcg/kg/day (30 minute exposures) with ECAC concurrence. Based on data up to Week 70, the sponsor noted decreases in body weight gains and proposed lower doses. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD. There were no treatment-related neoplastic findings in the rat study based on the lack of statistical significance.

Table 35: Safety margins for the proposed clinical dose of umeclidinium based on AUC for carcinogenicity

		NOAEL		Safety Margin for clinical dose of 62.5 mcg/day
Study	Sex	Achieved dose* (mcg/kg/day)	AUC (ng•hr/mL)	AUC = 0.3124 ng•hr/mL
2-year mouse	Males	295	8.21	26
	Females	200	6.87	22
2-year rat	Both	137	6.745	22

*lower dose, after dose change

Reproductive and developmental toxicity: The standard battery of reproductive and developmental toxicity studies were completed with UMEC in rats and rabbits. UMEC did not affect fertility in rats, it was not teratogenic in rats or rabbits, and it did not have any pre- or postnatal effects in rats. A skeletal variation, ventral arch of the 1st cervical vertebra, was noted in the rat embryo fetal development study to be dose-dependent (see Table 40 under Section 11.5 Labeling Evaluation for summary of studies and findings).

Fertility studies were conducted in male and female rats. Males were dosed with 0, 30, 60, or 180 mcg/kg/day by the subcutaneous (SC) route for 2 weeks before mating with untreated females and through the mating period until termination (total of 50 to 54 days). Dosed males overall showed a decrease in body weight gain compared to controls (LD 85%, MD 87%, HD 92%), but the effect was not dose-dependent. Based upon the overall decrease in body weight gain, the dose selection was adequate. There were no notable test article related effects in the mating index for males, and there were no effects relating to female fertility or offspring malformations at doses up to 180 mcg/kg/day. In the female fertility study, rats were dosed with 0, 3.37, 29.1, 100, and 294 mcg/kg/day (estimated achieved dose) by the inhalation route. Females were treated for 2 weeks before pairing, throughout pairing, and until Day 7 after mating. There were no test-article related changes in body weight. Dose selection was based on a 4 week inhalation toxicology study in which animals received 0, 26.1, 243, and 1829 mcg/kg/day. Those that received 26.1 and 243 mcg/kg/day had reduction in body weight gain of 69.8 and 47.3%, and there was a loss in body weight at 1829

mcg/kg/day. Therefore, dose selection was deemed adequate. There were no effects on fertility or reproductive performance for female rats at doses up to 294 mcg/kg/day.

Embryofetal development (EFD) after dosing with UMEC was evaluated in two definitive studies. UMEC was not teratogenic in rabbits and rats, based on the lack of test article related malformations. Two embryofetal development studies (EFD) were conducted in rabbits, and the definitive study EFD study with higher drug exposure is described here. Pregnant rabbits were dosed with 0, 40, 100, 180 mcg/kg/day of UMEC via the subcutaneous route from gestation day (GD) 7 to 19, and sacrificed on GD 29. Rabbits at the MD and HD showed slight decreases in body weight gain (-8%) compared to controls from GD 7 to 29, but data were difficult to interpret as animals in the MD and HD groups started with less weight than those in the control and LD groups. Dose selection appeared adequate. There was a possible test-article related variation (lack of ossification at the hind limb talus; control 0, LD 0, MD 2.14%, HD 1.86%) not covered by historical control data, but there is lack of clear dose-response, and this may be an effect of maternal toxicity. Therefore, this finding is not clearly test-article related, and UMEC resulted in no test article related malformations in rabbits dosed up to 180 mcg/kg/day.

An EFD study was also conducted in rats via the inhalation route of administration. Pregnant rats received doses of 0, 31.7, 96.9, 278 mcg/kg/day (estimated achieved dose) of UMEC from GD 6 to 17. Body weight gains from GD 6 to 18 were unaffected. Dose selection was based on a 4 week inhalation toxicology study in which animals received 0, 26.1, 243, and 1829 mcg/kg/day. Those that received 26.1 and 243 mcg/kg/day had reduction in body weight gain of 69.8 and 47.3%, and there was a loss in body weight at 1829 mcg/kg/day. Therefore, dose selection was deemed adequate. Minor skeletal variations were observed, including ventral arch of the 1st cervical vertebra (increased for all treatment groups in a dose-related manner), and incomplete ossification/unossified of the metatarsals and phalanges of the bilateral hind limb (increased for all treatment groups with no dose-response.) Ventral arch of the 1st cervical vertebra, was determined to be test article related.
The effect of UMEC on pre- and post-natal development in rat pups was evaluated. Dams (F_0) were dosed with 0, 10, 60, or 180 mcg/kg/day during pregnancy and lactation (GD 6 to postnatal day (PND) 21). F_0 females that received ≥60 mcg/kg/day exhibited a slight decrease in body weight gain during the gestation period (GD 6-20), with those at the HD weighing 93% compared to controls. During the postnatal period (PND 1-20), HD animals weighted 92% compared to controls. Therefore, dosing appeared adequate. There were no dose-dependent changes in the number of viable litters, total pups delivered, or percent survival index across the dose groups. At weaning (PND 21), there was a slight but statistically significant ($P < 0.05$) decrease in body weight in the HD F_1 generation pups, seen as 95% body weight gain in the HD versus control. The decrease in body weight, however, was transient; at PND 78, both male and female HD F_1 generation pups showed no statistically significant difference in body weight, compared to controls. F_1 pups showed no clear test article related effects on development based on evaluation of motor activity, acoustic startle habituation or testing through the modified M-watermaze. There were no test article-related effects on

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F_2 fetuses based on survival, sex, body weight, or malformations (based on external examination). Therefore, there were no effects of UMEC on pre- and post-natal development with doses up to 180 mcg/kg/day. A quantifiable level of umeclidinium was present in 2 pups, which may indicate transfer of umeclidinium in milk.

Exposure ratios from reproductive and developmental studies are shown in Section 11.5 Labeling Evaluation under Table 38.

11.2 Umeclidinium and vilanterol

Safety Pharmacology: The combination of umeclidinium and vilanterol were tested in a single intravenous dose study in conscious beagle dogs. Using a Latin square design, all animals received doses over 1 min of vehicle, umeclidinium alone (0.3 mcg/kg), vilanterol alone (0.3 mcg/kg), or both umeclidinium (0.3 mcg/kg) and vilanterol (0.3 mcg/kg) in combination. Dosing with vilanterol, with or without umeclidinium resulted in transient increases in heart rate up to 111 beats per minute (bpm), starting 6 min from the commencement of infusion. As a result, there was a concurrent, small reduction in RR interval. A small reduction in QT interval (10-20%) was associated with the change in heart rate after dosing with vilanterol only. Infusion with both vilanterol and umeclidinium resulted in small increases (10% or less) in mean systolic and diastolic blood pressure between 126 min after dosing, to the end of the recording period at 3 hrs after dosing. It was noted that the doses used in the study were low compared to the proposed clinical dose, and that vilanterol was quantifiable for up to 30 min after dosing, but umeclidinium was only quantifiable up to 5 min after dosing.

General Toxicology: The sponsor conducted a 13 week repeat dose oropharyngeal tube inhalation study in beagle dogs. The dog was chosen as the most appropriate species for a combination toxicology study based on 28 day repeat dose studies in both rats and dogs. In the 13 week study, dogs received umeclidinium and vilanterol alone or in combination. Animals were treated with and without pretreatment for 3 days (Phase 1), followed by 13 week of treatment (Phase 2). For the 13 week dosing period, estimated achieved doses of umeclidinium/vilanterol for Groups 1, 2, 3, 4, 5, 6, and 7 were 0/0, 1070/7.5, 23/29, 60/72, 177/183, 1048/0, and 0/180 mcg/kg/day, respectively. For the 3-day pretreatment, Group 5 received umeclidinium/vilanterol at 44/47 mcg/kg/day, and Group 7 received vilanterol alone at 49 mcg/kg/day (i.e. target dose of 40 mcg/kg/day).

The use of a pretreatment period in the 13 week study was of concern to the Division. The sponsor contended that the pre-adaptation phase of dosing (Phase 1) was required to induce tachyphylaxis to the cardiovascular effects of vilanterol, allowing administration of higher doses than would normally be possible in untreated dogs. The Division communicated that pre-exposure of dogs to smaller doses may induce tolerance, providing protection against subsequent exposure to larger doses (see Toxicology and Applied Pharmacology 69: 199-205, 1983). To address this concern, the sponsor conducted a 4 week repeat dose oropharyngeal tube inhalation study in

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dogs with and without 3-day pre-adaptation (Phase I) with 40/40 mcg/kg/day of umeclidinium/vilanterol (target doses), and dosing in Phase 2 with 160/160 mcg/kg/day umeclidinium/vilanterol (target doses) for 4 weeks. Indeed, 3-day pretreatment with a lower dose of umeclidinium and vilanterol in combination led to tolerance to cardiac effects with higher combination doses, as measured by ECG parameters and cardiac troponin I (cTnI). No microscopic cardiac findings were induced in the study. Histopathological examination of other organs and tissues was not conducted based upon the limited focus of the study.

In the 13 week dog study, excessive salivation and bilateral swelling were observed in all treatment groups during dosing and/or immediately after dosing (IAD). IAD on day 91/92, heart rates increases were observed in dogs that received either umeclidinium or vilanterol alone or in combination. Both prior to and immediately after dosing on days 91/92, respiratory sinus arrhythmia (RSA) was absent in 15/16 animals from Groups 2 and 6 that received umeclidinium at a target dose of 1000 mcg/kg/day either in combination with vilanterol or alone, respectively, suggesting that the effect was persistent at this target dose of umeclidinium. RSA was absent immediately after dosing on days 91/92 for several male and female dogs in Groups 3, 4, and 5 that received the combination of umeclidinium and vilanterol (up to target doses of 160 mcg/kg/day for each component).

Elevations of serum cTnI concentrations were noted during Phase 1 in both groups that received 3-day pretreatment (Group 5: 44/47 mcg/kg/day; Group 7: 180 mcg/kg/day vilanterol only). During the main treatment period (Phase 2, 13 weeks), animals in Groups 3, 4, 5, and 7 had elevated levels of cTnI. It appeared that tolerance was induced by pretreatment in Group 5 based on the decreased number of animals in Phase 2 with elevated cTnI, compared to Phase 1.

Histopathological findings were observed in the lung (inflammation, bronchial ectasia with luminal cellular debris, plant/food material, bacterial colonies, pleural adhesions,), larynx (mixed inflammatory cell infiltrate), nose/turbinates (inflammatory cells), trachea (mixed inflammatory cell infiltrate) thyroid (C cell hyperplasia), testes (dilation of seminiferous tubules), thymus (involution/atrophy), and heart (inflammation of epicardium, epicardial proliferation) that could potentially be attributed to treatment. For the heart, each finding was limited to only 1 animal in each affected group: inflammation of the epicardium was observed for 1 male in Group 5 (177/183 mcg/kg/day) and epicardial proliferation was observed for 1 female in Group 7 (180 mcg/kg/day). In general, these findings were consistent with administration of the individual test articles alone, and there was no evidence of novel toxicity with the combination. There was no evidence of additive or synergistic toxicity with the combination of GSK573719 and GW642444. Observed toxic effects were consistent with the individual agents.

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Table 36: Safety margins for the proposed clinical doses of umeclidinium and vilanterol based on AUC

		NOAEL		Safety Margin for clinical dose
Umeclidinium (62.5 mcg/day)	Group	Achieved dose (mcg/kg/day)	AUC (ng•hr/mL)	UMEC 62.5 mcg/day: AUC = 0.3124 ng•hr/mL
	2	1070	72.25	231
	3	23	1.239	4
	4	60	6.15	20
	5	177	9.99	32
	6	1048	84.2	270
Vilanterol (25 mcg/day)	Group	Achieved dose (mcg/kg/day)	AUC (ng•hr/mL)	VI 25 mcg/day: AUC = 0.615 ng•hr/mL
	2	7.5	11.04	18
	3	29	67.85	110
	4	72	168.5	274
	5	183	183.5	298
	6	180	214	348

Values based on Pharm/Tox review dated 2/14/11 under INDs 104479/106616 and adjusted for applicable AUCs of UMEC and VI.

Table 37 Safety margins for the proposed clinical doses of umeclidinium and vilanterol based on mcg/g lung weight

		NOAEL		Safety Margin for clinical dose
Umeclidinium (62.5 mcg/day)	Group	Achieved dose (mcg/kg/day)	Deposited dose (mcg/g lung weight)	UMEC 62.5 mcg/day: 0.0625 mcg/g lung weight
	2	1070	28.11	449.76
	3	23	0.61	9.76
	4	60	1.71	27.36
	5	177	5.04	80.64
	6	1048	27.42	438.72
Vilanterol (25 mcg/day)	Group	Achieved dose (mcg/kg/day)	AUC (ng•hr/mL)	VI 25 mcg/day: AUC = 0.025 mcg/g lung weight
	2	7.5	0.2	8.00
	3	29	0.77	30.80
	4	72	2.06	82.40
	5	183	5.22	208.80
	6	180	5.17	206.80

Values based on Pharm/Tox review dated 2/14/11 under INDs 104479/106616 and adjusted for applicable dose of UMEC.

11.3 Vilanterol

The toxicological profile of vilanterol has been characterized previously under NDA 204275. Briefly, vilanterol is a long acting beta$_2$-agonist (LABA). Pivotal general toxicology studies to support the use of vilanterol were 26 and 39 week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. Target organs identified were the upper airways, lung, heart, liver and testes. Findings were consistent with other beta$_2$ agonists.

Two-year carcinogenicity studies were conducted in rats and mice. Rats showed a dose-dependent shortening of latency for pituitary neoplasms in both sexes, and increased incidence of mesovarian leiomyomas in females (at greater than or equal to 20 times the MRHD on an AUC basis). In mice, there were increases in tubulostromal carcinomas of the ovaries in females (at greater than or equal to 3800 times the MRHD on an AUC basis). No tumors were observed at approximately 1 time the MRHD on an AUC basis. Findings were typical of beta$_2$ agonists.

Reproductive and developmental studies showed that vilanterol caused dose-dependent skeletal variations in both rats and rabbits. There were no teratogenic effects in rats and rabbits at approximately 13,000 and 70 times, respectively, the MRHD in adults (on a mcg/m^2 basis in rats and on an AUC basis in rabbits). There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHD in adults (on a mcg/m^2 basis). Vilanterol did not have an affect on fertility in either males or females.\

11.4 Excipients

The drug product includes lactose and magnesium stearate as excipients (see Section 2.3 Formulation). There was no safety concern for either compound. Lactose is used as an excipient in a number of currently marketed inhalation drug products.

Magnesium stearate: To support the safety of magnesium stearate as an excipient, the sponsor submitted inhalation toxicity studies up to 26 week in rats (GSK Report# WD2004/00488 and WD2006/03154). These reports were reviewed by Dr. Luqi Pei (June 7, 2007). Briefly, no treatment-related effects were observed at pulmonary deposited doses up to 180 mcg/kg/day magnesium stearate. The NOAEL for inhaled magnesium stearate was 180 mcg/kg/day in rats. These studies did not reveal any safety concern about the inhalation administration of magnesium stearate. The expected exposure of patients to magnesium stearate was 3.3 mcg/kg/day (200 mcg/day for a 60 kg individual). The rat data provided a safety margin of 54 for the proposed clinical doses. The safety margin is considered adequate for the proposed use of magnesium stearate.

Conclusions: The applicant has a complete nonclinical pharmacology and toxicology program for UMEC under NDA 203975, and for VI as previously reviewed under NDA 204275, which support the safety of the proposed clinical dose of 62.5 mcg/day of UMEC and 25 mcg/day for VI.

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Unresolved toxicology issues (if any): None.

11.5 Labeling Evaluation

A review of the sponsor's proposed labeling and recommended labeling is provided here. The recommended established pharmacological classification is based on sponsor provided data, and consistency with products of the same class. The nonclinical sections presented below include the Section for Indication and Usage, 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.

The following labeling recommendation considers 62.5 mcg of UMEC as the MRHD, which is associated with an AUC of 0.3124 ng•hr/mL, based on AUC exposure in humans (email from Clinical Pharmacologist Dr. Jianmeng Chen, 3/13/2013). All doses from nonclinical studies reflect the dose administered by the subcutaneous route, or the estimated achieved dose for the inhalation route.

The recommended labeling text for vilanterol generally follows the recently approved label under NDA 204275. The dose multiples are changed to reflect the higher exposure to VI (AUC 0.615 ng•hr/mL) in combination with UMEC, versus VI (AUC 0.266 ng•hr/mL) in combination with fluticasone furoate; email from Clinical Pharmacologist Dr. Jianmeng Chen, 6/10/2013). To adjust for the higher exposure with UMEC, dose multiples for VI have been decreased by a factor of 2.31.

A deviation from the approved label under NDA 204275 is recommended under Section 8.1 Pregnancy. The following statement is recommended to be moved from "Teratogenic Effects" to "Nonteratogenic Effects" under Section 8.1:

> "There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHD in adults (on a mcg/m^2 basis at maternal oral doses up to 10,000 mg/kg/day)."

11.5.1 Labeling Recommendation

1 INDICATIONS AND USAGE

ANORO ELLIPTA is a combination anticholinergic/long-acting beta$_2$-adrenergic agonist (Anticholinergic/LABA) indicated for the long-term, once-daily, maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. (1)

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C: There are no adequate and well-controlled trials of ANORO ELLIPTA or its individual components, umeclidinium and vilanterol, in pregnant women. Because animal reproduction studies are not always

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predictive of human response, ANORO 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 ANORO ELLIPTA.

Umeclidinium: There was no evidence of teratogenic effects in rats and rabbits at approximately 110 and 290 times, respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 278 mcg/kg/day in rats and at maternal subcutaneous doses up to 180 mcg/kg/day in rabbits). However, a fetal skeletal variation, ventral arch of the first cervical vertebra, was observed at an increased incidence in rats at approximately 20 times the MRHD in adults (on an AUC basis at maternal inhaled doses of 31.7 mcg/kg/day and higher in rats).

Vilanterol: There were no teratogenic effects in rats and rabbits at approximately 13,000 and 70 times, respectively, the MRHD in adults (on a mcg/m^2 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 rats at approximately 450 times the MRHD 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.

Nonteratogenic Effects:
Umeclidinium: There were no effects on peri-natal and post-natal developments in rats at approximately 80 times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 180 mcg/kg/day).

Vilanterol: There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHD in adults (on a mcg/m_2 basis at a maternal oral doses up to 10,000 mcg/kg/day.)

8.3 Nursing Mothers

ANORO ELLIPTA: It is not known whether ANORO ELLIPTA is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when ANORO ELLIPTA is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of ANORO ELLIPTA by nursing mothers, based on the data for the individual components, a decision should be made whether to discontinue nursing or to discontinue ANORO ELLIPTA, taking into account the importance of ANORO ELLIPTA to the mother.

Umeclidinium: It is not known whether umeclidinium is excreted in human breast milk. However, administration to lactating rats at approximately 25 times the MRHD in adults resulted in measurable umeclidinium in the milk (on an AUC basis at a maternal subcutaneous dose of 60 mcg/kg/day).

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Vilanterol: It is not known whether vilanterol is excreted in human breast milk. However, other and beta$_2$-agonists have been detected in human milk.

12.1 Mechanism of Action

ANORO ELLIPTA: ANORO ELLIPTA contains both umeclidinium and vilanterol; the mechanisms of action described below for the individual components apply to ANORO ELLIPTA. The drugs represent 2 different classes of medications (an anticholinergic and a LABA) that have different effects on clinical and physiological indices.

Umeclidinium: Umeclidinium is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methacholine and acetylcholine-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of umeclidinium is predominantly a site-specific effect.

Vilanterol: Vilanterol is a LABA. *In vitro* tests have shown the functional selectivity of vilanterol was similar to salmeterol. The clinical relevance of this *in vitro* finding is unknown.

Although beta$_2$-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta$_1$-receptors are the predominant receptors in the heart, there are also beta$_2$-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$_2$-agonists may have cardiac effects.

The pharmacologic effects of beta$_2$-adrenoceptor agonists, including vilanterol, are at least in part attributable to stimulation of intracellular adenyl 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 on Fertility

ANORO ELLIPTA: No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with ANORO ELLIPTA; however, studies are available for individual components, umeclidinium and vilanterol, as described below.

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Umeclidinium: Umeclidinium produced no treatment-related increases in the incidence of tumors in 2-year inhalation studies in rats and mice at inhaled doses up to 137 mg/kg/day and 295/200 mcg/kg/day (male/female), respectively (approximately 20 and 25/20 times the MRHD in adults on an AUC basis, respectively).

Umeclidinium tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* mouse lymphoma assay, and *in vivo* rat bone marrow micronucleus assay.

No evidence of impairment of fertility was observed in male and female rats at subcutaneous doses up to 180 mcg/kg/day and inhaled doses up to 294 mcg/kg/day, respectively (approximately 100 and 110 times, respectively, the MRHD in adults on an AUC 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 3,800 times the MRHD in adults on an AUC basis). No tumors were seen at inhalation doses up to 615 mcg/kg/day (approximately 230 times the MRHD 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 20 times the MRHD in adults on an AUC basis). No tumors were seen at an inhalation dose of 10.5 mcg/kg/day (approximately 1 times the MRHD 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* rat unscheduled DNA synthesis (UDS) assay, and *in vitro* Syrian hamster embryo (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 MRHD in adults on a mg/m^2 basis).

11.5.2 Labeling Discussion

Introduction

The following review evaluates the nonclinical sections of the ANORO ELLIPTA labeling proposed by GSK, as submitted to this NDA. Recommendations include editing the proposed text for wording, and updating dose ratios between animals and humans to reflect the Agency's findings.

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9 Pages Of Draft Labeling Have Been Withheld In Full As b4 (CCI/TS) Immediately Following This Page

(b) (4)

This review recommends the following word, with the dose ratio based on AUC exposure:

> "Umeclidinium produced no treatment-related increases in the incidence of tumors in 2-year inhalation studies in rats and mice at inhaled doses up to 137 mg/kg/day and 295/200 mcg/kg/day (male/female), respectively (approximately 20 and 25/20 times the MRHD in adults on an AUC basis, respectively)."

Vilanterol

See NDA 204275 Pharmacology/Toxicology Labeling Review dated 4/22/2013.
The recommended labeling text for vilanterol generally follows the recently approved label under NDA 204275. The dose multiples for VI are changed to reflect the higher exposure to VI (AUC 0.615 ng•hr/mL) in combination with UMEC, versus VI (AUC 0.266 ng•hr/m; email from Clinical Pharmacologist Dr. Jianmeng Chen, 6/10/2013) in combination with fluticasone furoate. To adjust for the higher exposure with UMEC, dose multiples for VI have been decreased by a factor of 2.31.

A deviation from the approved label is recommended under Section 8.1 Pregnancy. The following statement is recommended to be moved to "Non-teratogenic Effects" under Section 8.1:

> "There were no effects on peri-natal and post-natal developments in rats at approximately 3,900 times the MRHD in adults (on a mcg/m2 basis at maternal oral doses up to 10,000 mg/kg/day)."

12 Appendix/Attachments

Appendix #	Content	Appendices Page#
1	Abbreviations	1
2	Executive CAC minutes, dated 6/3/09	3
3	Pharm/Tox review by Dr. Timothy Robison, dated 6/3/09: Special Protocol Assessments of 2 year carcinogenicity protocols in rat and mouse.	8
4	Pharm/Tox review by Dr. Timothy Robison, dated 5/25/10 under IND 104479.	105
5	Pharm/Tox review by Dr. Timothy Robison, dated 2/18/11 under IND 104479.	249
6	Pharm/Tox review by Dr. Timothy Robison, dated 2/10/10 under IND 106616	267
7	Pharm/Tox review by Dr. Timothy Robison, dated 2/14/11 under INDs 106616 and 104479	324
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9	Pharm/Tox review by Dr. Luqi Pei for magnesium stearate (redacted)	435

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Appendix 1

Abbreviations

API	active pharmaceutical ingredient
AUC	area under the curve
bmp	beats per minute
BW	body weight
BrW	brain weight
calc	calculated value for label
carc	carcinogenesis
C_{max}	maximum concentration
CMC	chemistry, manufacturing and controls
cTnI	cardiac troponin I
d	day
DPI	dry powder inhaler
ECG	electrocardiogram
EFD	embryofetal development
EOF2	end of phase 2
F	females
GD	gestation day
GSK573719A	umeclidinium bromide
GW642444M	vilanterol triphenylacetate salt
HD	high dose
HLQ	higher limit of quantification
hr(s)	hour(s)
IH	inhalation
IP	intraperitoneal
IC_{50}	half maximal inhibitory concentration
ICH	International Conference on Harmonisation
IV	intravenous
kg	kilograms
LABA	long-acting β2-agonist
LAMA	long-acting muscarinic antagonist
LD	low dose
LLQ	lower limit of quantification
M	males
mcg	micrograms
MD	mid dose
min	minutes
mg	milligrams
MRHD	maximum recommended human dose

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PC	post coitum
PND	post natal day
PPND	peri- and post-natal development
round	rounded value for label
SC	subcutaneous
SD	study day
sec	second(s)
T_{max}	time at maximum
UMEC	umeclidinium bromide
VI	vilanterol trifenatate
VPC	ventricular premature complex(es)

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Executive CAC
Date of Meeting: June 2, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Chuck Resnick, Ph.D., DCRP, Alternate Member
Molly Shea, Ph.D., DPAP, Acting Team Leader
Tim Robison, Ph.D., DPAP, Presenting Reviewer

Author of Draft: Tim Robison, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND# 104,479
Drug Name: GSK573719
Sponsor: GlaxSmithKline

Background:
GSK573719 is a quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist.

In vitro plasma protein binding of GSK573719 in mice, rats, and humans was comparable at 88, 86, and 88%, respectively. In vitro metabolism was examined with hepatocytes from mice, rats, and humans. Studies with human hepatocytes and microsomes found that the main routes of metabolism of GSK573719 were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The major routes of metabolism in mouse hepatocytes were O-dealkylation, hydroxylation followed by glucuronide conjugation, and formation of two hydrated glutathione conjugates. These represented approximately 18%, 10%, 47%, and 3% of total metabolism, respectively. All other metabolites were glucuronide conjugates of hydroxylated metabolites. The main route of metabolism in human hepatocytes (O-dealkylation) was also a major route of metabolism in rat (38% of total metabolism). For hydroxy metabolites in humans (M33/M34), M33 in rats was only a very minor route and M34 was absent in rats. The major metabolic routes in the rat were O-dealkylation, hydrated glutathione and cysteine conjugation, and hydroxy, O-glucuronide and cysteine conjugation.

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GSK573719A was negative in the in vitro bacterial reverse mutation assay, in vitro mouse lymphoma assay, and in vivo rat micronucleus assay.

Mouse Carcinogenicity Study Protocol and Dose Selection:
In a 13-week inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day. Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively. Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain. Incidences of slight or moderate retinal folds were increased for male and female treatment groups. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Potential treatment-related findings were observed in the liver and uterus. Histopathological findings were evident in the larynx at all doses and appeared to be nonspecific irritant effects associated with inhalation exposure. Histopathological findings were observed in the nasal turbinates at doses ≥0.287 mg/kg/day. Histopathological findings were observed in the nasopharynx at doses of 1.06 and 2.85 mg/kg/day. Histopathological findings in the tracheal bifurcation, liver, and uterus were observed at 2.85 mg/kg/day. A potential dose-limiting finding in the larynx was ventral cartilage necrosis observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of relatively low incidence (i.e., 1 of 10 and 2 of 12, respectively). Further, the finding for 1 female at 0.092 mg/kg/day was graded as minimal. A potential dose-limiting finding in the nasal turbinates was slight ulceration of the olfactory epithelium for 1 male at 2.85 mg/kg/day. Based upon findings of ventral cartilage necrosis in larynx for males at 1.06 and 2.85 mg/kg/day and females at 0.092, 0.287, 1.06, and 2.85 mg/kg/day, maximum tolerated doses were identified as 0.6 mg/kg/day for males and 0.2 mg/kg/day for females. Doses of 0.6 and 0.2 mg/kg/day for males and females, respectively, provide extrapolated 2.0 and 0.6 exposure margins on an AUC basis for the highest proposed clinical dose.

Rat Carcinogenicity Study Protocol and Dose Selection:
In a 13-week inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day. Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively. Body weight gains for males in the 102, 288, and 924 µg/kg/day groups were decreased to 84.8, 82.9, and 78.7% of the control, respectively. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study. Body weight gains for females in the 288 and 924 µg/kg/day groups were decreased to 89.3 and 81.3% of the control, respectively. The decrease for females in the 288 µg/kg/day group did not appear to be toxicologically significant. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. For the larynx, minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at doses ≥38 µg/kg/day. These changes were not reversible. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study. However, minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting. Given that minimal focal necrosis in the larynx observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study, identification of maximum tolerated doses for males and females were based primarily on decreases of body weight gain. Based upon

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decreased body weight gains for males and females at 924 µg/kg/day, the MTD was identified as 288 µg/kg/day. The dose of 288 µg/kg/day provides an approximate 1.7 exposure margin on an AUC basis for the highest proposed clinical dose.

Executive CAC Recommendations and Conclusions:

Mouse:

- The Committee recommended doses of 0.06, 0.2, and 0.6 mg/kg/day in males and 0.02, 0.06, and 0.2 mg/kg/day in females, by inhalation, based on MTD (effects in the larynx).

Rat:

- The Committee concurred with the sponsor's proposed doses of 30, 100, and 300 µg/kg/day, by inhalation, based on MTD (body weight effects).

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
(b) for statistically significant or otherwise remarkable findings in the high dose group, the sponsor will need to look at the affected tissues in all of the dose groups.
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

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

cc:\
/Division File, DPAP
/MShea, DPAP
/TRobison, DPAP
/MRaggio, DPAP
/ASeifried, OND IO

Reference ID: 3330354

Linked Applications	Sponsor Name	Drug Name / Subject
IND 104479	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	GSK573719 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.

/s/

DAVID JACOBSON KRAM
06/03/2009

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 104,479
Review number: #001
Sequence number/date/type of submission:
#006/April 30, 2009/Special Protocol Assessment
#007/April 30, 2009/Special Protocol Assessment
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: GlaxoSmithKline
P.O. Box 13398
Five Moore Drive
Research Triangle Park
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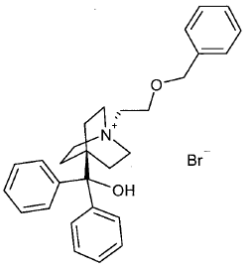
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Products
HFD #: 570
Review completion date: June 3, 2009

Drug:

Code name: GSK573719
Chemical name: 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide
Molecular formula/molecular weight: $C_{29}H_{34}NO_2 \cdot Br$
508.5 (GSKS73719A as quaternary ammonium bromide compound)
428.6 (as free cation, GSKS73719)
Structure:



Relevant INDs/NDAs/DMFs:
GW642444 Inhalation Powder (Long-acting β2 agonist, IND 74,696)
GSK233705 (Anticholinergic bronchodilator, (b) (4)

Drug class: Quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist

Reference ID: 3330354

Intended clinical population: Chronic obstructive pulmonary disease (COPD)

Clinical formulation: The drug product, GSK573719 Inhalation Powder, 62.5, 125, 250, and 500 µg/dose, comprises two strips contained within the Novel DPI. One strip contains the appropriate dose of GSK573719, as the bromide compound GSK573719A (micronized [redacted] (b) (4) with magnesium stearate and lactose monohydrate. The second strip contains a [redacted] (b) (4) magnesium stearate and lactose monohydrate.

The drug product GSK573719 Inhalation Powder, 1000 µg/dose comprises two strips contained within the Novel DPI. Each strip contains 500 µg of GSK573719, as the bromide compound GSK573719A (micronised [redacted] (b) (4) with magnesium stearate and lactose monohydrate.

The placebo drug product comprises two strips in the Novel DPI containing magnesium stearate and lactose.

Table 13 Composition of Novel DPI Active Strip for GSK573719 Inhalation Powder, 62.5, 125, 250, 500, and 1000 µg

Component	Quantity per blister[1]				Function	Reference to Standard
GSK573719A (micronised [redacted] (b) (4)	62.5 µg	125 µg	250 µg	500 µg[3]	Active	GlaxoSmithKline
Magnesium Stearate	[redacted] (b) (4)				(b) (4)	GlaxoSmithKline
Lactose Monohydrate						Ph. Eur. or USP/NF

Note:
1. [redacted] (b) (4)
2. [redacted] (b) (4)
3. The 1000 µg dose product consists of 2 x 500 µg strips in the Novel DPI [redacted] (b) (4)

Table 14 Composition of Novel DPI [redacted] (b) (4) Second Strip for GSK573719 Inhalation Powder, 62.5, 125, 250, and 500 µg

Component	Quantity per blister[1]	Function	Reference to Standard
Magnesium Stearate	125 µg	[redacted] (b) (4)	Ph. Eur. or USP/NF
Lactose Monohydrate	to 12.5 mg		Ph. Eur. or USP/NF

Note:
1. [redacted] (b) (4)

Route of administration: Oral inhalation

Reference ID: 3330354

Proposed clinical protocol: None

Previous clinical experience: Five clinical pharmacology studies have been conducted outside of the United States in a total of 91 healthy volunteers and 48 COPD subjects to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single and repeat dose administration of GSK573719. Doses of up to 1000 µg once daily have been examined. A total of 74 healthy volunteers and 42 COPD subjects have been dosed with GSK573719. Completed studies are comprised of two single dose studies in healthy volunteer subjects (AC4105209) and COPD subjects (AC4108123), and two repeat dose studies in healthy volunteer subjects (AC4106889 and AC4110106).

Two dry powder formulations of GSK573719 containing lactose plus (b) (4) delivered via DISKUS™ dry powder inhaler and lactose plus magnesium stearate by a Novel Dry Powder Inhaler (Novel DPI) have been used.

Table 1 Clinical Studies with GSK573719 Inhalation Powder

Protocol	Design	Diagnosis/No. of Subjects Treated	Treatments	Status	Formulation and Device
Studies in healthy subjects					
AC4105209	R, DB, PC, XO Single Dose Ascending	Healthy males aged 21-50 years / N=20	GSK573719 - 10, 20, 60, 100, 250 & 350 mcg, tiotropium 18mcg, or placebo	Completed	Lactose (b) (4) via DISKUS
AC4106869	R, DB, PC, PG, 14-Day Repeat Dose Ascending	Healthy males and females aged 20-53 years / N=36	GSK573719 - 250 and 750, 1000mcg QD or placebo	Completed	Lactose via DISKUS
AC4110106	R, DB, PC Dose Ascending, Single and 7-day Repeat dose	Healthy males and females aged 18-64 years normal (N=20) and poor (N=16) CYP2D6 activity	Part 1: Normal CYP2D6 subjects: GSK573719 – 100, 500, and 1000 mcg or placebo single dose then 500 or 1000mcg or placebo QD 7-days Part 2: Poor CYP2D6 activity subjects: GSK573719 – Cohort 1) 100mcg or placebo single dose then100mcg or placebo QD 7-days, then 500mcg or placebo single dose then 500mcg or placebo QD 7 days. Cohort 2) 500mcg or placebo single dose, then 500mcg or placebo QD 7 days, then 1000mcg or placebo single dose, then 1000mcg or placebo QD 7 days	Completed	Lactose/MgSt via Novel DPI
Studies in COPD subjects					
AC4108123	R, DB, PC, XO, Single Dose Ascending	COPD subjects aged 48-67 years / N=24	GSK573719 - 250, 500 & 1000mcg, tiotropium 18mcg, or placebo	Completed	Lactose (b) (4) powder via DISKUS
AC4105211	R, DB, PC, PG,7-Day, Repeat Dose Ascending	COPD subjects aged 48-75 years / N=26 (to date)	GSK573719 – 250mcg QD, or placebo[1]	Ongoing	Lactose/MgSt via Novel DPI

1. Due to a protocol violation, a cohort of subjects scheduled to receive GSK573719 1000mcg were administered GSK573719 250mcg. The study has been amended to repeat the cohort with 1000mcg (n=12)

R=randomised, DB=double-blind, PC=placebo-controlled, PG=parallel group, XO=crossover, QD=once daily, (b) (4) MgSt=magnesium stearate, CDY2P6=cytochrome P450 isoenzyme 2D6; DPI=dry powder inhaler

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

Studies reviewed within this submission:

STUDY:	STUDY NUMBER:
PHARMACOKINETICS/TOXICOKINETICS:	
Distribution:	
In Vitro Plasma Protein Binding of GSK573719 and Blood Cell Association of [^{14}C]GSK573179 in Mouse, Rat, Rabbit, Dog, and Human	WD2008/00503/00
Metabolism:	
A Preliminary Study To Estimate the Intrinsic Clearance of GSK573179A in Rat, Dog, and Human Hepatocytes	CH2006/00011/00
An In Vitro Investigation of the Metabolism of [^{14}C]GSK573719 in Human, Rat, and Dog	WD2006/00147/00
An In Vitro Investigation of the Metabolism of [^{14}C]GSK573719 in Human, Female	WD2007/01370/00

Rabbit, and Mouse Hepatocytes	
Quantification and Identification of the Metabolites of GSK573719 in the Male Sprague-Dawley Rat Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide at 1 mg free base/kg	WD 2006/00172/00
Preliminary Characterization of Metabolites of GSK573719 in Human Plasma and Urine	WD2009/00030/00
Excretion:	
Elimination of Drug-Related Material Following a Single Oral or Single Intravenous Administration of [^{14}C]GSK573719 Bromide to Male Sprague-Dawley Rats at a Target Dose Level of 1 mg free base/kg	FD2005/00208/00
TOXICOLOGY:	
Repeat Dose Toxicity:	
Preliminary Toxicity Study by Inhalation Administration to CD-1 Mice for 2 Weeks	WD2007/00074/00
Toxicity Study by Inhalation Administration to CD-1 Mice for 13 Weeks	WD2007/01600/00
A 28-Day Inhalation Toxicity Study of a Powdered Aerosol Formulation in the Rat	WD2005/01422/00
A 13-Week Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat with a 4-Week Recovery Period	WD2007/02012/00
GENETIC TOXICOLOGY:	
Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli*	WD2005/00750/00
In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus	WD2005/00751/00
Intravenous Bone Marrow Micronucleus Assay in Rats	WD2005/01079/00
CARCINOGENICITY:	
DRAFT PROTOCOL FOR AN INHALATION CARCINOGENICITY STUDY OF A POWDER AEROSOL FORMULATION IN THE MOUSE	
PROTOCOL FOR AN INHALATION CARCINOGENICITY STUDY OF A POWDER AEROSOL FORMULATION IN THE RAT	

Studies not reviewed within this submission: None

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

GSK573719 demonstrated potent muscarinic receptor antagonist activity in a range of in vitro binding and cellular functional activity assays in Chinese hamster ovary cells stably expressing individual muscarinic receptor subtypes and human bronchial tissues. The in vivo efficacy of GSK573719 was demonstrated after intranasal or intratracheal administration to mice and guinea pigs by providing bronchoprotection in response to methacholine- or acetylcholine-induced bronchoconstriction, respectively.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Oral bioavailability of GSK573719 was extremely low. Plasma protein binding in mouse, rat, rabbit, dog, and human ranged from 74.7 to 88.8% and was concentration-independent. Red blood cell associations of GSK573719 in dog, human, rabbit, rat, and mouse were generally low.

In vitro metabolism of GSK573719 was examined with hepatocytes from mouse, rat, rabbit, dog, and human. Studies with human hepatocytes and microsomes found that the main routes of metabolism of GSK573719 were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The major routes of metabolism in humans were also present in rat and dog. O-dealkylation (as observed in human) was a major pathway in the mouse, but only a minor pathway in the rabbit. Hydroxylation (at the position observed in M33 in human) was also a major pathway in the mouse (M21) but a minor pathway in rabbit hepatocytes (M22).

Following intravenous administration of 1 mg/kg GSK573719 to rats, 65.3 and 16.9% of the radioactive dose were found in the feces and urine, respectively. A further 10% of the administered dose was recovered in the carcass. Elimination of GSK573719 was primarily by excretion of parent into the feces (53% of the dose) and renal excretion (14% of the dose). Metabolism accounted for 4.4% dose with the major routes of metabolism being O-dealkylation to form a cleavage product and hydroxylation to form several hydroxylation products.

Following repeat inhalation of GSK573719A at a dose of 1 mg/day for 7 days to healthy male subjects and to a healthy male population of CYP2D6 poor metabolizers, GSK573719 and GSK339067 (M14, product of O-dealkylation) were the only GSK573719-related components detected in 5 min, 1 hr, and 0-24 hr pooled human plasma extracts and in 0-4 hr pooled human urine. The proportion of GSK339067 (relative to the parent drug, GSK573719) in CYP2D6 poor metabolizers compared to normal metabolizers was similar in the 5 min pooled plasma samples, but decreased over 3-fold in the 1 hr pooled plasma samples. In the 0-24 hr pooled plasma samples, the proportion of GSK339067 was approximately 18% in 2D6 poor metabolizers and

35% in healthy subjects. In 0-4 hr pooled urine samples, the proportion of GSK339067 relative to the parent drug, GSK573719, was comparable in both populations at 6 to 7%. CYP2D6 appears to play a significant role in the metabolism of GSK573719 in humans.

2.6.4.2 Methods of Analysis

Plasma samples were analyzed for GSK573719 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) for GSK573719 was 0.1 ng/mL, using a 50 µL aliquot of rat plasma with a higher limit of quantification (HLQ) of 100 ng/mL.

2.6.4.4 Distribution

In Vitro

In Vitro Plasma Protein Binding of GSK573719 and Blood Cell Association of [^{14}C]GSK573179 in Mouse, Rat, Rabbit, Dog, and Human (WD2008/00503/00)

Methods: In vitro plasma protein binding and red blood cell association of GSK573719 were examined in mouse, rat, rabbit, dog, and human. Plasma protein binding of GSK573179 was examined in mouse, rat, rabbit, dog, and human plasma at a concentration range of 5 to 200 ng/mL using an equilibrium dialysis method. The in vitro red blood cell association of [^{14}C]GSK573719 was examined with mouse, rat, rabbit, dog, and human blood at concentration range of 50 to 500 ng/mL. Radioactivity in blood and plasma were determined.

Results: Plasma protein binding in mouse, rat, rabbit, dog, and human ranged from 74.7 to 88.8% and was concentration-independent. Blood to plasma ratios of GSK573719 in mouse, rat, rabbit, dog, and human ranged from 0.520 to 0.778 and were concentration-independent. Red blood cell associations of GSK573719 in dog, human, rabbit, rat, and mouse were 2.00-4.40%, 6.35-7.62%, 6.70-9.01%, 25.4-27.6%, and 26.2-30.5%, respectively, and were concentration-independent.

2.6.4.5 Metabolism

In Vitro

A Preliminary Study to Estimate the Intrinsic Clearance of GSK573719A in Rat, Dog, and Human Hepatocytes (CH2006/00011/00):

Methods: The in vitro intrinsic clearance of 0.5 µM GSK573719A was examined during incubations up to 4 hr with fresh, suspended rat, dog, or human hepatocytes at a concentration of 0.2 x 10^6 cells/mL. Fresh male Sprague-Dawley rat and beagle dog hepatocytes were obtained from (b) (4) Fresh male hepatocytes from one donor were also obtained from (b) (4). Concentrations of GSK 573719 were quantified using an LC/MS/MS method.

Results: In vitro intrinsic clearance of GSK573719A in rat, dog, and human hepatocytes are listed in the table below. Intrinsic clearance was greatest for the dog. Clearance values for human and rat hepatocytes were relatively comparable.

Table 2 In vitro Intrinsic Clearance of GSK573719A in Hepatocytes

Species	Rate Constant (minute^{-1})	CL_i (mL/minute/g liver)
	0.2 x 10^6 cells/mL	0.2 x 10^6 cells/mL
Rat	0.0014	0.840
Dog	0.0015	1.800
Human (Donor # Hu355)	0.0018	1.080

An In Vitro Investigation of the Metabolism of [^{14}C]GSK573719 in Human, Rat, and Dog (WD2006/00147/00):

Methods: In vitro metabolism of [^{14}C]GSK573719 at concentrations of 10 and 50 µM was examined with human, rat, and dog hepatocytes. [^{14}C]GSK573719 was incubated with hepatocytes for 0, 4, and 24 hr. In vitro metabolism of [^{14}C]-GSK573719 at concentrations of 0.01, 0.1, and 1 µM was also examined with human liver microsomes. The incubation period was 1 hr. Hepatocytes were prepared in-house from fresh liver samples taken from a male Sprague-Dawley rat and a male beagle dog. Human, rat, and dog cryopreserved hepatocytes were obtained from [redacted] (b) (4) Human liver microsomes (Batch Number: 0410014) were purchased from [redacted] (b) (4) Human liver microsomes were pooled from a mixed gender pool of 50 preparations and were validated against CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, CYP4A11, and flavin monooxygenase. Radiometabolite profiles were determined using on- and off-line radio-HPLC. Representative samples (10 µM, 24hours) were analyzed by HPLC-MS. During these analyses, radio-HPLC detection was used in parallel with mass spectrometry to assist in the identification of radiolabeled metabolites and to confirm assignments.

Results: Based upon metabolism studies with human hepatocytes and microsomes, the main routes of metabolism of GSK573719 in humans were determined to be O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The major routes of metabolism in humans were also present in rat and dog.

Human hepatocytes: The extent of metabolism of GSK573719 in human hepatocytes was variable ranging from 19 to 84%. Metabolites represented, on average, 43% of the radiochromatogram after the 24 hr hepatocytes incubations.

HPLC-MS of selected human hepatocyte samples revealed major peaks corresponding to O-dealkyl (M14), hydroxy (M33), and hydroxy methoxy (M34) GSK5737I9. Other metabolites detected were a hydroxy glucuronide (M21), a hydroxy methoxy

glucuronide (M22), a hydroxy dimethoxy metabolite (M54), hydrated glutathione conjugates (M13/M45), two cysteine conjugates (M52/M53) and two hydrated cysteine conjugates (M59/M60), a methoxy O-sulfate conjugate (M49) and a dihydrodiol (M5l). Minor amounts of M14 were also detected in the drug only control.

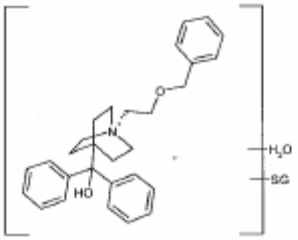
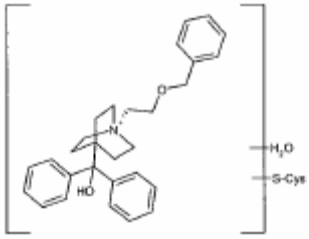
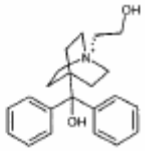
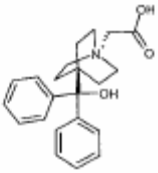
Radiometabolite profiles in each of the human hepatocyte samples varied considerably, although they were qualitatively similar for many of the components. The major metabolites were M14, representing 20% of the total metabolism, and M33 plus M34, which co-eluted, representing 23% of the total metabolism. Radiolabeled peaks M13/M60, M22/M51, and M59 represented 9, 8, and 5% of the total metabolism, respectively. All other metabolites were present at <5% of total metabolism. Metabolites M21, M22, M45, M49, and M54 were only detected in one of the five human hepatocyte preparations.

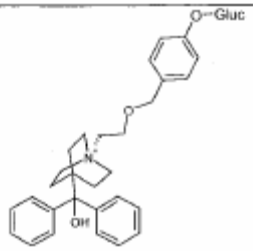
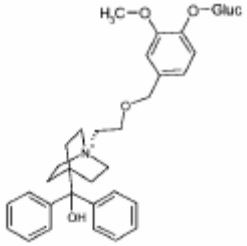
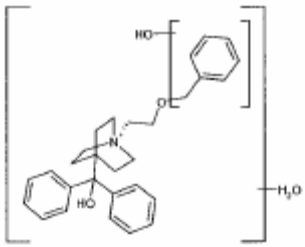
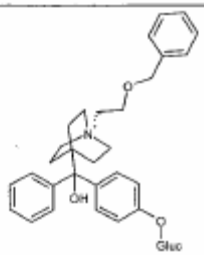
Rat and Dog Hepatocytes: The metabolism of GSK573719 in rat and dog was 26% and 28%, respectively, with the parent compound as the predominant component in the hepatocytes. The main route of metabolism in human hepatocytes (M14) was also a major route of metabolism in rat and dog (38% and 26% of total metabolism, respectively). The other major routes (M33/M34) were present in dog (18% of total metabolism), but in rat, M33 was only a very minor route and M34 was absent. All of the metabolites identified in human hepatocytes were present in one or both of the nonclinical species, with the exception of M45 (a glutathione conjugate) and M49 (a sulphate conjugate). However, there were several minor unidentified components in some of the human preparations which appeared to be absent in rat and dog. The major metabolites formed in the rat were M14, M13/M60, and M27/M53. The major metabolites formed in dog were M14, M22/M51 and M33/M34. Additional metabolites, not seen in human hepatocytes, were a hydroxy O-glucuronide conjugate (M27) and an acid (M18) of the O-dealkyl (only observed in rat).

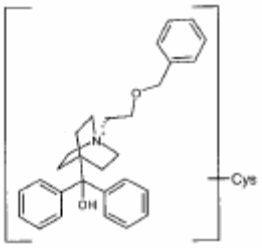
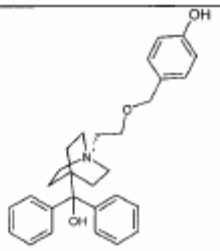
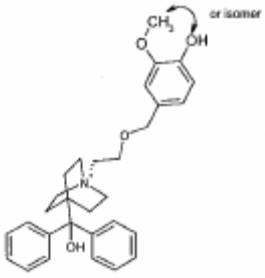
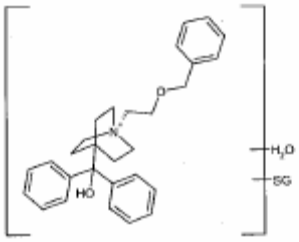
Human Liver Microsomes: Two metabolites, M14 and M33, were formed by human microsomes. Minor amounts of M14 and M33 were also detected in the no cofactor control.

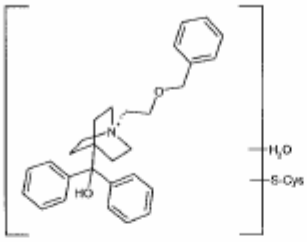
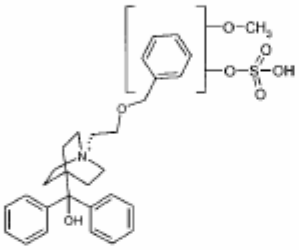
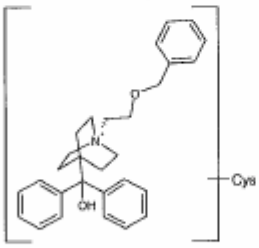
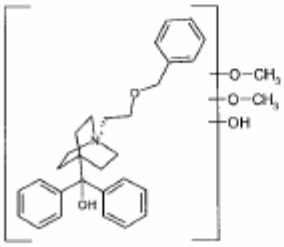
Metabolic Activation of [^{14}C]-GSK573719 in Human Liver Microsomes: Following incubation of [^{14}C]-GSK573719 with human liver microsomes followed by exhaustive solvent extraction the amount of extraction-resistant radioactivity (i.e. extent of binding) was 114 pmoles/mg protein/hour. This was similar to that of acetaminophen, which was 122 pmoles/mg protein/hour. The binding observed was predominately cofactor dependent. The significance of these findings is unclear at this time.

Table 1 Summary of the HPLC Analyses of Hepatocytes Incubated with 10 Micromolar [^{14}C]-GSK573719 for 24 hours

Peak ID	Metabolite structure	% Total Metabolism		
		Human (n=5)	Rat (n=2)	Dog (n=2)
M13/M60	 M13, hydrated glutathione conjugate, isomer of M45  M60, hydrated cysteine conjugate, isomer of M59	8.9	**16.4**	4.8
M14	 O-dealkyl	**22.9**	**37.9**	**25.9**
M18	 O-dealkyl, acid	ND	7.8	ND

Peak ID	Metabolite structure	% Total Metabolism		
		Human (n=5)	Rat (n=2)	Dog (n=2)
M21/M22 (rat) M22/M51 (dog) M21/M22/M51 (human)	 M21,hydroxy, O-glucuronide conjugate  M22,methoxy, hydroxy, O-glucuronide conjugate  M51, diydrodiol	7.5	9.0	**30.8**
M27*/M53	 M27, hydroxy, O-glucuronide conj.	4.5	**16.2**	BQL

Peak ID	Metabolite structure	% Total Metabolism		
		Human (n=5)	Rat (n=2)	Dog (n=2)
	 M53, Cysteine conj.			
M33/M34	 M33, hydroxy  M34 methoxy, hydroxy	**18.4**	BQL	**18.0**
M45/M59	 M45, hydrated glutathione conjugate, isomer of M13	5.2	BQL	BQL

Peak ID	Metabolite structure	% Total Metabolism		
		Human (n=5)	Rat (n=2)	Dog (n=2)
	 M59, hydrated cysteine conjugate, isomer of M60			
M49	 methoxy, O-sulfate	BQL	ND	ND
M52	 Cysteine conj.	3.5	ND	BQL
M54	 hydroxy dimethoxy	BQL	ND	8.0
A		BQL	ND	ND

Peak ID	Metabolite structure	% Total Metabolism		
		Human (n=5)	Rat (n=2)	Dog (n=2)
B		BQL	ND	ND
C		BQL	ND	ND
D		BQL	ND	ND
E		BQL	ND	ND
% run chromatogram#		43	26	28
Quantification limit		2.6	4.1	3.6

Notes:
BQL = Peak detected by MS and radiodetection but below quantification limit.
ND = Peak not detected by MS or radiodetection
*M27 not detected in human
%Total metabolism was calculated by expressing each metabolite as a percentage of the sum of the detected metabolites (i.e. not including unchanged GSK573719).
Figures in bold type indicate peaks which contribute >10% of total metabolism for this species.
Represents % of the total chromatogram accounted for by metabolites (i.e. % turnover)
Quantification Limit (QL) was assigned as 1% of run chromatogram which is then represented as % total metabolism.

An In Vitro Investigation of the Metabolism of [^{14}C]GSK573719 in Human, Female Rabbit, and Mouse Hepatocytes (WD2007/01370/00):

Methods: In vitro metabolism of [^{14}C]GSK573719 at concentrations of 10 and 50 µM was examined with human, rabbit, and mouse hepatocytes. Pooled male and female human, female rabbit, and male mouse cryopreserved hepatocytes were obtained from [redacted] (b) (4) [^{14}C]GSK573719 was incubated with hepatocytes for 0, 0.5, 1.5, 3, 4, and 24 hr. Radiometabolite profiles were determined for sample extracts (10 µM, 3 hr) using off-line radio HPLC. Representative samples were analyzed by HPLC. Radio-HPLC detection was used in parallel with mass spectrometry to assist in the identification of radiolabeled metabolites and confirm assignments.

Results:

Human hepatocytes: The extent of GSK573719 metabolism in human hepatocytes was low given that metabolites represented only 13% of the radiochromatogram after a 3-hr incubation with 10 µM. HPLC-MS analysis identified major metabolites as O-dealkylation (M14), hydroxylation (M33), and hydroxylation and methylation (M34) of GSK573719. These major metabolites represented 33, 27, and 11% of the total metabolism, respectively. A minor metabolite observed was a dihydrodiol (M51).

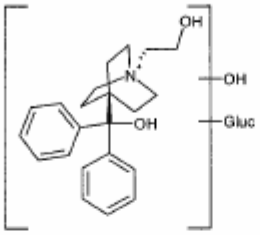
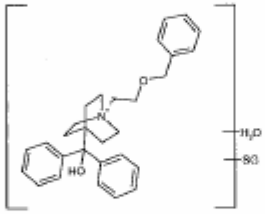
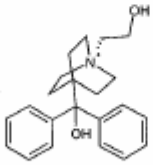
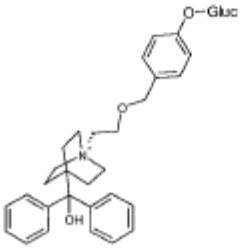
Rabbit and Mouse hepatocytes: The extent of metabolism in rabbit and mouse hepatocytes was high such that metabolites represented >84% of the radiochromatogram after a 3-hr incubation at 10 µM.

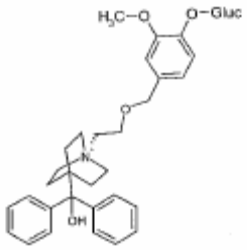
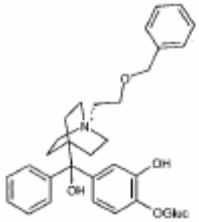
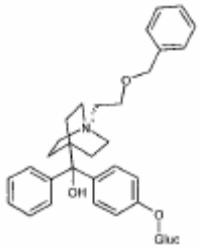
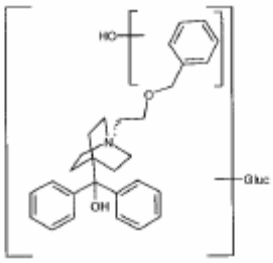
O-dealkylation to M14 (as observed in human) was a major pathway in the mouse, but only a minor pathway in the rabbit (M64, M1). Hydroxylation (at the position observed in M33 in human) was also a major pathway in the mouse (M21) but a minor pathway in rabbit hepatocytes (M22). Methoxylation (to M34 in human) was a minor pathway in rabbit (M22) and absent in the mouse.

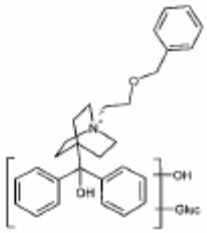
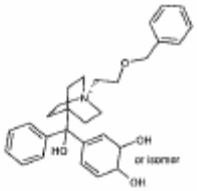
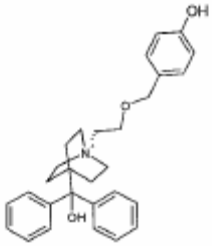
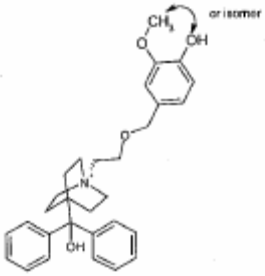
The major routes of metabolism identified in the rabbit were hydroxylation (M37) and subsequent glucuronidation (M27), representing 5% and 17% of the total metabolism, respectively. Other notable metabolites were M62 (O-dealky1ation and hydroxylation), M22 (methoxylation, hydroxylation and glucuronide conjugation) and M63 (dihydrodiol formation).

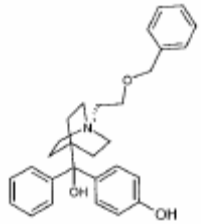
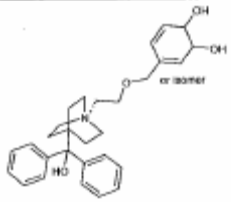
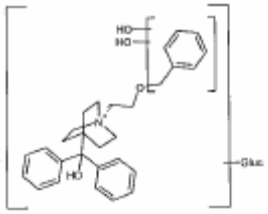
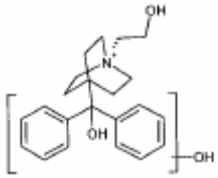
The major routes of metabolism in mouse hepatocytes were O-dealkylation (M14), hydroxylation followed by glucuronide conjugation (M21), and formation of hydrated glutathione conjugates (M13 and M45). These represented approximately 18%, 10%, 47%, and 3% of total metabolism, respectively. All other metabolites (M21, M26, M27, and M29) were glucuronide conjugates of hydroxylated metabolites.

Table 1 **Summary of the HPLC Analyses of Hepatocytes Incubated with 10 Micromolar [14C]GSK573719 for 3 hours.**

ID	Proposed Structure	% Total Metabolism [1]		
		Human	Rabbit	Mouse
M64 M1	 Hydroxylation & glucuronide conjugation of M14	ND ND	1.73 4.79	ND ND
M45 M13	 Hydrated glutathione conjugate	ND ND	ND ND	2.33 **47.1**
M14	 O-dealkylation	**33.4**	ND	**17.7**
M21	 Hydroxylation & glucuronide conjugation	ND	ND	**10.2**

ID	Proposed Structure	% Total Metabolism [1]		
		Human	Rabbit	Mouse
M22	 Dihydroxylation, methylation, glucuronide conjugation	ND	1.68	ND
M26	 Dihydroxylation, glucuronide conjugation	ND	6.26	0.87
M27	 Hydroxylation, glucuronide conjugation	ND	**16.7**	5.01
M29	 Hydroxylation, glucuronide conjugation	ND	ND	

ID	Proposed Structure	% Total Metabolism [1]		
		Human	Rabbit	Mouse
M31	 Dihydroxylation, glucuronide conjugation	ND	5.33	ND
M63	 Dihydrodiol	ND		ND
M33	 Hydroxylation	**26.9**	ND	ND
M34	 Dihydroxylation, methylation	**11.2**	ND	ND

ID	Proposed Structure	% Total Metabolism [1]		
		Human	Rabbit	Mouse
M37	 Hydroxylation	ND	5.27	ND
M51	 Dihydrodiol	**10.8**	ND	ND
M61	 Dihydroxylation & glucuronide conjugation	ND	1.10	ND
M62	 Hydroxylation of M14	ND	4.00	ND
% Run Chromatogram [2]		13.4	84.3	85.8
Quantification Limit		0.067	0.422	0.429

1. Calculated by expressing each metabolite as a percentage of the sum of the detected metabolites (i.e. not including unchanged GSK573719). Peaks which contribute > 10% of total metabolism for this species are presented in bold type.
2. Represents % of the total chromatogram accounted for by metabolites (i.e., % turnover).

ND - Peak not detected by MS or radiodetection

QL - Quantification Limit (QL) was assigned as 0.5% of run chromatogram.

Rats

Quantification and Identification of the Metabolites of GSK573719 in the Male Sprague-Dawley Rat Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide at 1 mg free base/kg (WD 2006/00172/00):

Methods: Major metabolites of GSK573719 were identified in the plasma, liver, lung, urine, and feces following administration of [^{14}C]GSK573719 by the oral or intravenous route to male Sprague-Dawley rats at a dose of 1 mg/kg. Plasma, liver, and lung from intact rats were collected at 0.5 and 2 hr postdose. Following intravenous administration, urine and feces were collected from 0-24 and 0-48 hr postdose, respectively. Following oral administration, feces were collected 0-24 hr postdose. Radiometabolite profiles were determined by an analysis of appropriate aliquots of plasma, liver, lung, urine, and fecal extracts using radio-HPLC. Representative samples of plasma, liver, lung, urine, and feces were analyzed by HPLC-MS. Radiochemical detection was used in parallel with mass spectrometry to assist in the identification of radiolabeled metabolites.

Results: Following oral administration, greater than 93% of the radioactive dose was excreted in feces and less than 0.1% was found in the urine, which appeared to be indicative of poor oral bioavailability. Results from oral dosing are not discussed. Following intravenous administration, 65.3 and 16.9% of the radioactive dose were found in the feces and urine, respectively. A further 10% of the administered dose was recovered in the carcass. Elimination of GSK573719 was primarily by excretion of parent into the feces (53% of the dose) and renal excretion (14% of the dose). Metabolism accounted for 4.4% dose with the major routes of metabolism being O-dealkylation to form a cleavage product (M14) and hydroxylation to form the hydroxylation products, M33, M34, and M37. The total radioactivity accounted for was 71% of the administered dose or 92% of the radioactivity eliminated in 0-24 h urine and 0-48 h feces.

Plasma: Following intravenous administration, the only radiolabeled component found in plasma samples at 0.5 hr postdose was unchanged GSK573719 (267 ng eq./mL).

Lung: Following intravenous administration, the only radiolabeled component found in extracts of pooled lung homogenates at 0.5 and 2 hr was unchanged GSK573719 that corresponded to 552 and 315 ng eq./g lung homogenate, respectively.

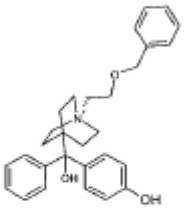
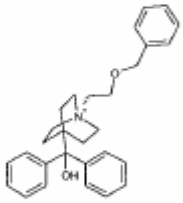
Liver: Following intravenous administration, metabolite profiles in extracts of pooled liver homogenates at 0.5 and 2 hr were qualitatively similar. The principal radiolabeled component in liver extracts was unchanged GSK573719, which represented 88 and 70% of liver radioactivity, respectively (4290 and 545 ng eq./g of liver homogenate, respectively). The only other notable radiolabeled component observed at each time point was M37 (hydroxyl) which accounted for approximately 4 and 9% of liver radioactivity, respectively. Liver to plasma ratios of radioactive drug-related material at 0.5 and 2 hours post-dosing were approximately 18:1 and 87:1, respectively.

Urine: Following intravenous administration, radioactivity in the 0 to 24 hr urine samples represented 15% of the administered dose, which equated to approximately 90% of the material excreted by this route. The predominant radioactive drug-related component in rat urine was unchanged GSK573719, which represented 90% of the urinary

radioactivity (14% of the dose). The only metabolites identified were M14 (cleavage product), accounting for 3% of the urinary radioactivity (0.5% of the dose) and M33 which could not be quantified.

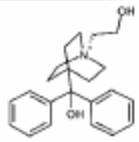
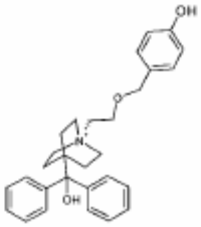
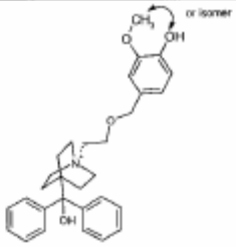
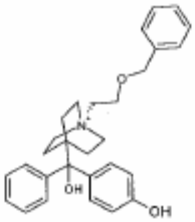
Feces: Following intravenous administration, radioactivity in the 0 to 48 hr fecal samples represented 62% of the administered dose, which equated to approximately 95% of the material excreted by this route. The primary radiolabeled component in rat feces following intravenous dosing was unchanged GSK573719 which represented 86% of the fecal radioactivity (53% of the administered dose). Other minor radiolabeled components were M14 (cleavage product) and M37 (hydroxyl), each representing approximately 3% of the fecal radioactivity. Additionally, M33 and M34 were detectable but could not be quantified.

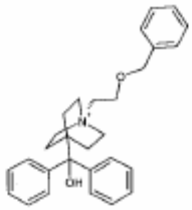
Table 1 Summary of the HPLC Analyses of Plasma, Liver and Lung Following a Single Intravenous Administration of [^{14}C]-GSK573719 bromide to Male Sprague Dawley Rats at a Target Dose Level of 1 mg free base/kg

Peak ID	Metabolite Structure	Plasma[1] (0.5h)	Liver (0.5h)	Liver (2h)	Lung (0.5h)	Lung (2h)
		% sample radioactivity (ng equivalents [^{14}C]-GSK573719/g)				
M37	 Hydroxy	ND	3.8 (186)	8.5 (66.5)	ND	ND
P	 GSK573719	98.2 (262)	88.2 (4290)	69.8 (545)	99.6 (552)	92.3 (315)
Total radioactive material assigned		98.2 (262)	92.0 (4470)	78.3 (611)	99.6 (552)	92.3 (315)
Non-extracted radioactive material		1.0 (2.7)	2.6 (126)	10.4 (81.2)	0.6 (3.3)	1.0 (3.4)
Quantification limit		4.2 (11.2)	2.1 (101)	5.5 (43.2)	8.6 (47.8)	6.9 (23.6)
Total in sample (ng equivalents [^{14}C]-GSK573719)		267	4860	781	554	341

ND: Not Detected
[1] 2h plasma sample could not be quantified due to insufficient radioactivity

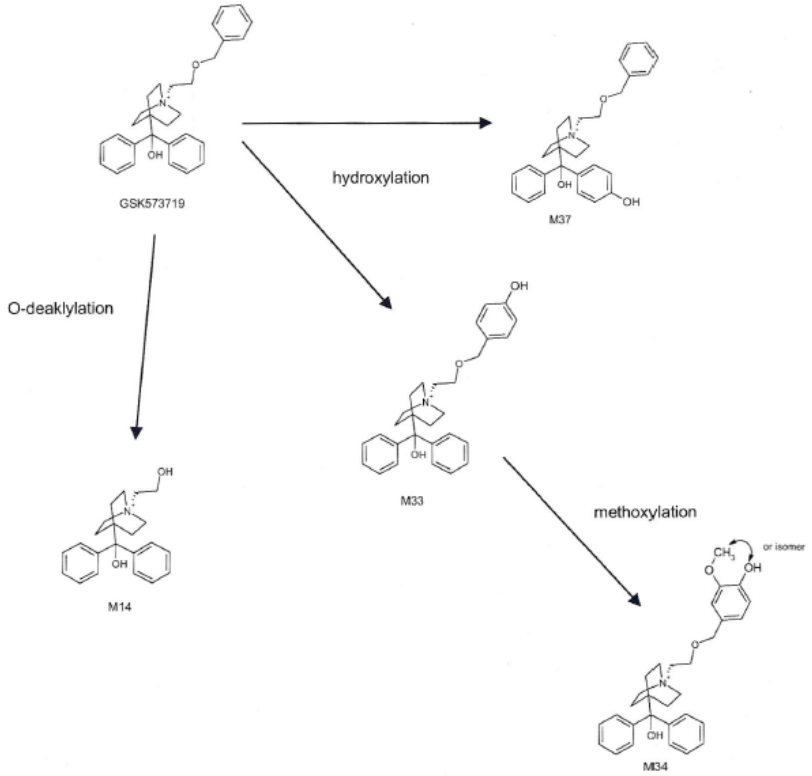
Table 2 Summary of HPLC Analyses of Urine and Faeces Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide to Male Sprague Dawley Rats at a Target Dose Level of 1 mg free base/kg

Peak ID	Metabolite Structure	Faeces (Oral)	Faeces (Oral BDC)	Urine (IV)	Faeces (IV)
		Mean % matrix radioactivity (mean % dose)			
M14	 Cleavage product	BQL	BQL	3.5 (0.5)	3.2 (2.0)
M33	 Hydroxy	ND	ND	BQL	BQL
M34	 Methoxy, Hydroxy	ND	ND	ND	BQL
M37	 Hydroxy	ND	ND	ND	3.1 (1.9)

Peak ID	Metabolite Structure	Faeces (Oral)	Faeces (Oral BDC)	Urine (IV)	Faeces (IV)
P	 GSK573719	99.2 (94.9)	96.0 (86.8)	90.2 (13.7)	85.7 (53.2)
Total radioactive material assigned		99.2 (94.9)	96.0 (86.8)	93.7 (14.2)	92.0 (57.1)
Non-extracted radioactive material		0 (0)	3.0 (2.4)	NA	0 (0)
Quantification limit		0.6 (0.6)	0.8 (0.7)	2.2 (0.3)	2.6 (1.6)
% dose in matrix pool analysed		95.7	90.4	15.2	62.1
Total % dose excreted in matrix		96.4	92.9	16.9	65.3

ND: Not Detected
BQL: Below Limit of Quantification
NA: Not Applicable

Figure 1 Putative Metabolic Scheme for GSK573719 in the Rat



Humans

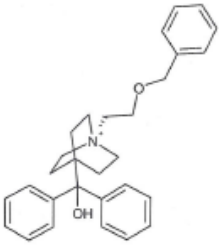
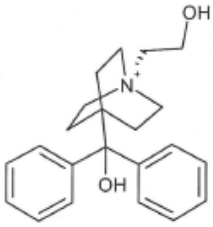
Preliminary Characterization of Metabolites of GSK573719 in Human Plasma and Urine (WD2009/00030/00):

Methods: Preliminary structural information on the metabolites of GSK573719 in human plasma and urine was obtained following repeated inhalation administration of 1 mg/day GSK573719A (bromide salt) for 7 days in healthy male subjects and in a healthy male population of cytochrome P450 isoenzyme 2D6 poor metabolizers. Blood samples for determination of metabolites present in plasma were collected at 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 hr postdose. Plasma samples from study subjects were pooled in equal proportions at 5 min, 1 hr, and 0-24 hr resulting in a single plasma sample for each sample time. Urine pools were created by combining equal volumes from the 0-4 hr collection from the same subjects as for plasma to give a single representative sample for analysis. Representative samples of pooled plasma extract and urine were analyzed by HPLC-MS.

Results:
Following repeat inhalation of GSK573719A at a dose of 1 mg/day for 7 days to healthy male subjects and to a healthy male population of CYP2D6 poor metabolizers, GSK573719 and GSK339067 (M14, product of O-dealkylation) were the only GSK573719-related components detected in 5 min, 1 hr, and 0-24 hr pooled human plasma extracts and in 0-4 hr pooled human urine. GSK1761002 (M33, hydroxylated metabolite) was not detected in any of the pooled samples, but was detected in plasma sample spiked at 0.1 ng/mL.

The proportion of GSK339067 (relative to the parent drug, GSK573719) in CYP2D6 poor metabolizers compared to normal metabolizers was similar in the 5 min pooled plasma samples, but decreased over 3-fold in the 1 hr pooled plasma samples. In the 0-24 hr pooled plasma samples, the proportion of GSK339067 was approximately 18% in 2D6 poor metabolizers and 35% in healthy subjects. In 0-4 hr pooled urine samples, the proportion of GSK339067 relative to the parent drug, GSK573719, was comparable in both populations at 6 to 7%.

Table 1 GSK573719-Related Material Identified in a Pooled Plasma Extract and in a Pooled Urine Sample Following Repeat Inhaled Administration of GSK573719A to Healthy Volunteers at a Dose of 1 mg for 7 Days

Peak ID	P GSK573719	M14 GSK339067 (% relative to GSK573719)[3]
Proposed Structure		
5 min (normal[1]) plasma	✓	4%
5 min (poor[2]) plasma	✓	3%
1 h (normal[1]) plasma	✓	10%
1 h (poor[2]) plasma	✓	3%
0-24 h (normal[1]) plasma	✓	35%
0-24 h (poor[2]) plasma	✓	18%
0-4 h (normal[1]) urine	✓	7%
0-4 h (poor[2]) urine	✓	6%

1. Normal: healthy subjects
2. Poor: healthy population of CYP2D6 poor metabolisers
3. Percentage are the mean of three measurements, determined by LC/MS peak area ratios with reference to a control human plasma sample spiked with known amount of authentic standards (GSK573719 and GSK339067)

2.6.4.6 Excretion

Elimination of Drug-Related Material Following a Single Oral or Single Intravenous Administration of [^{14}C]GSK573719 Bromide to Male Sprague-Dawley Rats at a Target Dose Level of 1 mg free base/kg (FD2005/00208/00):

Methods: Excretion of drug-related radioactivity was examined following a single oral or intravenous administration (infusion over 30 min) of [^{14}C]GSK573719 bromide at a dose of 1 mg/kg to intact and bile duct-cannulated male Sprague-Dawley rats. In addition, plasma, bile, excreta, and tissue samples were provided to the sponsor for metabolite pattern determinations to be conducted under a separate protocol.

Results: Following oral administration, the major route of elimination was by the feces (96.4% of the administered dose). Urinary and biliary elimination were minimal. These results are attributed to poor oral bioavailability. Further results from oral administration are not discussed.

Following intravenous infusion administration to intact male rats, the predominant route of elimination was by the feces (mean of 65.3% of the dose) with urinary elimination accounting for a mean of 16.9% of the dose. Elimination of radioactivity was initially relatively rapid following intravenous administration, with a mean of 78.8% of the dose recovered by 48 hr postdose. However, overall elimination was incomplete by 96 hr postdose, with a mean of 11.0% of the dose remaining in the carcass (mean of 10.1% of the dose) and tissues. The mean total recovery of radioactivity (including cage washings and tissues) was 93.9% of the dose.

Following intravenous administration, mean liver to plasma concentration ratios were 18.4, 90.5, and 92.3 at 0.5, 0.75, and 2 hr, respectively. Mean lung to plasma concentration ratios were 2.11, 12.4, and 40.8 at 0.5, 0.75, and 2 hr, respectively.

Table 3: Elimination of radioactivity following a single intravenous infusion administration of [^{14}C]GSK573719 bromide to intact male Sprague Dawley rats at a target dose level of 1 mg free base/kg (Phase C)

Sample and collection period (hours)	% Administered dose				
	9M	10M	11M	Mean	sd
Urine					
0 - 24	15.9	16.4	13.2	15.2	1.7
24 - 48	0.7	0.9	1.1	0.9	0.2
48 - 72	0.4	0.5	0.6	0.5	0.1
72 - 96	0.2	0.3	0.4	0.3	0.1
Total	17.2	18.1	15.3	16.9	1.4
Faeces					
0 - 24	50.5	55.0	48.5	51.3	3.3
24 – 48	13.1	6.7	12.7	10.8	3.6
48 – 72	2.7	1.7	1.6	2.0	0.6
72 – 96	1.1	1.1	1.1	1.1	0.0
Total	67.4	64.5	63.9	65.3	1.9
Aqueous Cage Wash					
24	0.3	0.6	0.7	0.5	0.2
48	0.0	0.1	0.1	0.1	0.1
72	0.0	0.0	0.1	0.0	0.1
96	0.1	0.1	0.1	0.1	0.0
Methanol Cage Wash					
96	0.1	0.1	0.1	0.1	0.0
Total	0.5	0.9	1.1	0.8	0.3
G.I.T and contents	0.7	0.7	0.7	0.7	0.0
Liver	0.0	0.1	0.1	0.1	0.1
Lungs	0.1	0.1	0.1	0.1	0.0
Carcass	9.1	9.7	11.5	10.1	1.2
Total	9.9	10.6	12.4	11.0	1.3
Total Recovery 0 - 96	95.0	94.1	92.7	93.9	1.2

sd Standard deviation
G.I.T Gastrointestinal tract
Note: All times given above were measured from the start of the infusion

2.6.4.9 Discussion and Conclusions

In vitro metabolism of GSK573719 with hepatocytes from mouse, rat, dog, and human was found to be relatively comparable. In vitro metabolism with rabbit hepatocytes

appeared to differ. Studies with human hepatocytes and microsomes found that the main routes of metabolism of GSK573719 were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. Some minor metabolites observed with human hepatocytes were not observed in other species. CYP2D6 appears to play a significant role in the metabolism of GSK573719 in humans.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

In a 14-day non-GLP inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.299, 1.08, 2.69, and 8.11 mg/kg/day. Pulmonary deposited doses were 0, 0.03, 0.1, 0.27, and 0.81 mg/kg/day, respectively. Treatment-related mortality was observed for the high dose of 8.11 mg/kg/day. Body weight losses were evident for most male and female control and treatment groups. Losses were most pronounced for males and females in the 8.11 µg/kg/day group. A limited set of tissues was submitted to histopathological examination. Treatment-related histopathological findings were observed in tracheal bifurcation, nasal cavities, larynx, and nasopharynx. Potentially, treatment-related histopathological findings were also observed in the stomach and uterus. Histopathological findings were evident in the larynx and nasal turbinates at all doses. Findings in the nasopharynx were evident at doses ≥1.08 mg/kg/day. Findings were observed in tracheal bifurcation and stomach at 8.11 mg/kg/day. Findings were observed in the uterus at 2.69 and 8.11 mg/kg/day. Dose-limiting findings in the larynx consisted of ventral cartilage necrosis for males and female at doses ≥1.08 mg/kg/day, ventral region erosion/ulceration for males and females at 8.11 mg/kg/day, and ventrolateral epithelium erosion/ulceration for males at 2.69 and 8.11 mg/kg/day and females at 8.11 mg/kg/day. Dose-limiting findings in the nasal turbinates consisted of olfactory epithelium ulceration for males and females at doses ≥2.69 mg/kg/day and respiratory epithelium ulceration for males at 8.11 mg/kg/day and females at 2.69 and 8.11 mg/kg/day. The MTD was less than 1.08 mg/kg/day based upon findings of ventral cartilage necrosis in the larynx for males and females at doses ≥1.08 mg/kg/day.

In a 13-week inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day. Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively. Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain. Incidences of slight or moderate retinal folds were increased for male and female treatment groups. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Potential treatment-related findings were observed in the liver and uterus.

Histopathological findings were evident in the larynx at all doses and appeared to be nonspecific irritant effects associated with inhalation exposure. Histopathological findings were observed in the nasal turbinates at doses ≥0.287 mg/kg/day. Histopathological findings were observed in the nasopharynx at doses of 1.06 and 2.85 mg/kg/day. Histopathological findings in the tracheal bifurcation, liver, and uterus were observed at 2.85 mg/kg/day. A potential dose-limiting finding in the larynx was ventral cartilage necrosis observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of relatively low incidence (i.e., 1 of 10 and 2 of 12, respectively). Further, the finding for 1 female at 0.092 mg/kg/day was graded as minimal. A potential dose-limiting finding in the nasal turbinates was slight ulceration of the olfactory epithelium for 1 male at 2.85 mg/kg/day. Based upon findings of ventral cartilage necrosis in larynx for males at 1.06 and 2.85 mg/kg/day and females at 0.092, 0.287, 1.06, and 2.85 mg/kg/day, maximum tolerated doses were identified as 0.6 mg/kg/day for males and 0.2 mg/kg/day for females.

In a 28-day inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 25.1, 242.8, and 1828.5 µg/kg/day. Pulmonary deposited doses were 0, 2.5, 24.3, and 182.9 µg/kg/day, respectively. Body weight gains were significantly decreased for all male treatment groups and the female low dose and mid dose groups. Body weight loss was evident for the female high dose group. Treatment-related histopathological findings were observed in the nasal cavity/sinuses, larynx, nasopharynx, lung, tracheobronchial bifurcation, and prostate. Treatment-related histopathological findings were observed in the nasal cavity/sinuses at doses ≥242.8 µg/kg/day. Erosion/ulceration observed for males and females in the 1828.5 µg/kg/day group was judged to be dose-limiting. Treatment-related histopathological findings were observed in the larynx at all doses. Potential dose-limiting minimal to slight necrosis of the ventral pouch cartilage was observed for males in the 242.8 and 1828.5 µg/kg/day groups and females in the 1828.5 µg/kg/day group. The minimal necrosis of the ventral pouch cartilage for females in the 242.8 µg/kg/day group might be judged to be acceptable. Histopathological findings in the nasopharynx, lung, and prostate were confined to the high dose group. Histopathological findings in the tracheal bifurcation were observed in the 242.8 and 1828.5 µg/kg/day groups. The MTD in male and female rats was less than 25.1 µg/kg/day based upon decreased body weight gains or body weight losses.

In a 13-week inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day. Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively. Body weight gains for males in the 102, 288, and 924 µg/kg/day groups were decreased to 84.8, 82.9, and 78.7% of the control, respectively. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study. Body weight gains for females in the 288 and 924 µg/kg/day groups were decreased to 89.3 and 81.3% of the control, respectively. The decrease for females in the 288 µg/kg/day group did not appear to be toxicologically significant. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. For the larynx,

minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at doses ≥38 µg/kg/day. These changes were not reversible. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study. However, minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting. Given that minimal focal necrosis in the larynx observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study, identification of maximum tolerated doses for males and females were based primarily on decreases of body weight gain. Based upon decreased body weight gains for males and females at 924 µg/kg/day, the MTD was identified as 288 µg/kg/day.

Genetic toxicology:
GSK573719A was negative in the in vitro bacterial reverse mutation assay, in vitro mouse lymphoma assay, and in vivo rat micronucleus assay.

2.6.6.3 Repeat-dose toxicity

Mice

Study title: **Preliminary Toxicity Study by Inhalation Administration to CD-1 Mice for 2 Weeks**

Key study findings:

● In a 14-day non-GLP inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.299, 1.08, 2.69, and 8.11 mg/kg/day. Pulmonary deposited doses were 0, 0.03, 0.1, 0.27, and 0.81 mg/kg/day, respectively.

● Treatment-related mortality was observed for the high dose of 8.11 mg/kg/day.

● Clinical signs of toxicity were observed in males and females in the 1.08, 2.69, and 8.11 mg/kg/day groups. Clinical signs evident following exposure for males and females in the 8.11 mg/kg/day group included partially closed eyes, animal cold to touch, piloerection, and/or wet fur. Underactivity, unsteady gait, and hunched posture were additionally seen for females in the high dose group.

● Body weight losses were evident for most male and female control and treatment groups. Losses were most pronounced for males and females in the 8.11 µg/kg/day group.

● A limited set of tissues was submitted to histopathological examination. Treatment-related histopathological findings were observed in tracheal bifurcation, nasal cavities, larynx, and nasopharynx. Potentially, treatment-related histopathological findings were also observed in the stomach and uterus.

● Histopathological findings were evident in the larynx and nasal turbinates at all doses. Findings in the nasopharynx were evident at doses ≥1.08 mg/kg/day. Findings were observed in tracheal bifurcation and stomach at 8.11 mg/kg/day. Findings were observed in the uterus at 2.69 and 8.11 mg/kg/day.

● Dose-limiting findings in the larynx consisted of ventral cartilage necrosis for males and females at doses ≥1.08 mg/kg/day, ventral region erosion/ulceration for males and females at 8.11 mg/kg/day, and ventrolateral epithelium erosion/ulceration for males at 2.69 and 8.11 mg/kg/day and females at 8.11 mg/kg/day.

● Dose-limiting findings in the nasal turbinates consisted of olfactory epithelium ulceration for males and females at doses ≥2.69 mg/kg/day and respiratory epithelium ulceration for males at 8.11 mg/kg/day and females at 2.69 and 8.11 mg/kg/day.

● The MTD was less than 1.08 mg/kg/day based upon findings of ventral cartilage necrosis in the larynx for males and female at doses ≥1.08 mg/kg/day.

Study no.: WD2007/00074/00
Volume #, and page #: Volume 1, Pages 1 to 214
Conducting laboratory and location: (b) (4)

Date of study initiation: August 21, 2006
GLP compliance: No; however, the study was conducted in a GLP compliant facility.
QA report: yes ( ) no (X)
Drug, lot #, and % purity: The test material, GSK573719, was formulated as a 40% w/w blend with lactose (inhalation grade). Magnesium stearate (1 % w/w), was included in the GSK573719/lactose powder formulation to aid chemical and physical stability of the dry powder formulation. The lot number and purity were not provided.

Methods
Doses: Target inhaled doses were 0, 0.3, 1, 3, and 10 mg/kg/day (estimated achieved doses were 0, 0.299, 1.08, 2.69, and 8.11 µg/kg/day, respectively).

GSK573719

Group Number	Target Dose[1] (mg/kg/day)	Estimated Achieved Dose[1] (mg/kg/day)	Target Chamber Aerosol Concentration (µg/L)	Achieved Chamber Aerosol Concentration[1,2] (µg/L)
1	0	0	0	0
2	0.3	0.299	5.1	5.16
3	3	2.69	51.3	46.5
4	10	8.11	171	137
5	1.0	1.08	17.1	18.8

1. Doses are expressed in terms of parent compound, GSK573719
2. As determined by chemical analysis

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BWx1000)

D	dose (mg/kg)
RMV(L/min)	$0.499 \times BW(kg)^{0.809}$ (Bide et al, 2000)
T	Duration of exposure/day (minutes)
C	Aerosol GSK573719 concentration (µg/L)
BW	Group average body weight for study (kg)

Pulmonary deposited doses

Group	Pulmonary deposited dose			
	µg/kg/day		µg/g LW/day	
	Males	Females	Males	Females
1	0	0	0	0
2	0.03	0.03	0.005	0.0037
3	0.26	0.27	0.044	0.0345
4	0.79	0.84	0.121	0.0935
5	0.10	0.11	0.018	0.0143

Lung weight (LW) = 0.216 g

Magnesium Stearate

Group Number	Estimated Achieved Dose[1] (µg/kg/day)	Achieved Chamber Aerosol Concentration[1,2] (µg/L)
1	486	8.34
2	4.00	0.0690
3	46.4	0.801
4	150	2.53
5	18.7	0.323

1. Doses are expressed in terms of Magnesium Stearate
2. As determined by chemical analysis

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)

D	dose (µg/kg)
RMV(L/min)	$0.499 \times BW(kg)^{0.809}$ (Bide et al, 2000)
T	Duration of exposure/day (minutes)
C	Aerosol Magnesium Stearate concentration (µg/L)
BW	Group average body weight for study (kg)

Summary of Group Particle Size Distribution Results for GSK573719 Aerosols

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
2	5.16	(b) (4)	
3	46.5		
4	137		
5	18.8		

1. Concentrations are expressed in terms of parent compound, GSK573719

Summary of Group Particle Size Distribution Results for Magnesium Stearate Aerosols

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
1	8.34	(b) (4)	
2	0.0690		
3	0.801		
4	2.53		
5	0.323		

1. Concentrations are expressed in terms of Magnesium Stearate

Species/strain: CD-1 mice were used in this study although the source was not provided.
Number/sex/group or time point (main study): 6 mice/sex/group
Route, formulation, volume, and infusion rate: Mice were exposed to the vehicle (lactose + magnesium stearate) or test article by nose-only inhalation for 60 min per

day. An aerosol was produced by dispersing in compressed air either vehicle or test powder formulations using a Wright Dust Feed Mechanism (WDF). Aerosols were conducted into ADG nose-only inhalation exposure chambers via an aerosol conditioning pre-chamber upon which the WDF was mounted. The inhalation exposure system also included restraining tubes, air supplies attached to the top of the chamber and the WDF and an extract line incorporating a filtration system at the bottom of the chamber. Five individual exposure systems were used in the study.
Satellite groups used for toxicokinetics or recovery: For toxicokinetic assessments, there were 3 mice/sex/group in the control group and 18 mice/sex/group in each treatment group.
Age: Not provided.
Weight: Body weight ranges on day 1 of treatment were 30.7 to 39.9 g for male mice and 24.4 to 32.8 g for female mice.
Unique study design or methodology (if any): This was a non-GLP study.

Observation and Times:
Clinical signs: Animals were observed daily during the treatment period as follows: pre-exposure, immediately after dosing, on return to home cage, and late as possible in the working day.
Body weights: Body weights were measured daily.
Food consumption: Food consumption was reported for weeks -1, 1, and 2. Data were not recorded for males in Groups 1, 2, 3, and 4 during week 2.
Ophthalmoscopy: Not performed.
EKG: Not performed.
Hematology: Not performed.
Clinical chemistry: Not performed.
Urinalysis: Not performed.
Gross pathology: Mice were sacrificed and submitted to a macroscopic pathologic examination. Specific details were not provided.
Organ weights: Organ weights were measured for the brain, heart, kidneys, liver, lungs + bronchi, spleen, testes, thymus, and ovaries.
Histopathology: The larynx, nasopharynx, tracheal bifurcation, and nasal turbinates were examined for all animals. Examination of the lungs + bronchi, kidneys, spleen, thymus, thyroids, adrenals, mandibular LN, stomach, skin, eyes, sternum + bone marrow, testes, ovaries, uterus, vagina, and bronchial LN was limited to the control and high dose groups. Gross lesions in these tissues were examined for lower dose groups. Specific details were not provided.
Toxicokinetics: Blood was collected on day 14 for measurement of plasma concentrations of GSK573719. No further details were provided.

Results:

Mortality: Treatment-related mortality was observed for the high dose of 8.11 mg/kg/day.

One male in the high dose group was killed on day 7 due to body weight loss (10.7 g, 27% of start weight was lost from days 1 to 7). Microscopic examination of this mouse revealed test article related findings in the nasal cavity, larynx, and nasopharynx that were similar to those recorded in other mice from this dose group (see below).

Two toxicokinetic mice in the high dose group were killed due to body weight losses (Male, 9.5 g on day 4, 26% of start weight was lost; and Female, 10.2 g on day 8, 36% of start weight was lost).

Clinical signs: Clinical signs of toxicity were observed in males and females in the 1.08, 2.69, and 8.11 mg/kg/day groups.

Clinical signs evident following exposure for males and females in the 8.11 mg/kg/day group included partially closed eyes, animal cold to touch, piloerection, and/or wet fur. Underactivity, unsteady gait, and hunched posture were additionally seen for females in the high dose group. The duration of these signs generally increased during the course of the treatment period. Most clinical signs resolved prior to treatment on the next day.

For males and females in the 2.69 µg/kg/day group, partially closed eyes, cold to touch, fast breathing, pallor, and wet fur were observed.

For males in the 1.08 µg/kg/day group, two animals were observed with partially closed eyes on day 1. For females in the 1.08 µg/kg/day, there were single observations of partially closed eyes on day 2 and wet fur on day 13.

Body weight: Body weight losses were evident for most male and female control and treatment groups. Losses were most pronounced for males and females in the 8.11 µg/kg/day group.

Body weight changes from days 1 to 14

	Males					Females				
mg/kg	0	0.299	1.08	2.69	8.11	0	0.299	1.08	2.69	8.11
BW(g), Day 1	35.6	37.6	37.7	36.4	36.4	26.8	26.1	28.4	27.3	26.0
BW(g), Day 14	35.3	37.8	36.5	36.1	33.2	26.5	25.5	27.6	27.1	24.1
Δ (g)	-0.2 (-5.6%)	0.3 (+0.8%)	-1.2 (-3.2%)	-0.3 (-0.8%)	-2.2 (-6.0%)	-0.3 (-1.1%)	-0.5 (-1.9%)	-0.9 (-3.2%)	-0.2 (-0.7%)	-1.9 (-7.3%)

Histopathology: Treatment-related histopathological findings were observed in tracheal bifurcation, larynx, nasal cavities, and nasopharynx. Potentially, treatment-related histopathological findings were also observed in the stomach and uterus.

Epithelial hyperplasia in the tracheal bifurcation was observed for a single male and single female in the 8.11 mg/kg/day group.

In the larynx at all doses, squamous hyperplasia/metaplasia of the ventral epithelium was observed. Erosion/ulceration and vesiculation were also evident in the ventral epithelium at only 8.11 mg/kg/day. Subacute/chronic inflammation in the ventral region, and ventrolateral/lateral epithelial squamous hyperplasia/metaplasia were evident for mice receiving 1.08, 2.69, or 8.11 mg/kg/day with exudates seen at 2.69 and 8.11mg/kg/day. Erosion/ulceration of the ventrolateral epithelium was confined to mice receiving 2.69 or 8.11 mg/kg/day. Squamous hyperplasia of the arytenoids and of the ventral pouch epithelium, and necrosis of the ventral cartilage were recorded at 1.08, 2.69, or 8.11 mg/kg/day. Generally, there was a dose-related increase in severity and incidence of findings in the larynx at all doses. In addition findings associated with treatment were generally more pronounced in males than females. Findings of necrosis and ulceration were judged to be dose-limiting.

For the nasal turbinates, histopathological findings were evident in the olfactory, respiratory and transitional epithelia of mice in all treatment groups with a dose-related severity and incidence. Findings in the olfactory and respiratory epithelia of ulceration, erosion, eosinophilic inclusions and degeneration/regeneration, sometimes with squamous metaplasia in the respiratory epithelium, were evident for mice receiving 2.69 or 8.11 mg/kg/day. The ulceration was more evident in females. In the transitional epithelium, degeneration/regeneration and erosion were observed at 2.69 and 8.11 mg/kg/day. The squamous epithelium was affected at only 8.11 mg/kg/day, where degeneration/erosion was evident in one male. Exudate was evident in the nasal passages in mice receiving 2.69 or 8.11 mg/kg/day. In mice receiving 1.08 mg/kg/day, findings in the nasal cavities were similar, but were generally less extensive, and did not include epithelial ulceration or exudates in the nasal passages. The transitional and squamous epithelia were not affected at 1.08 mg/kg/day. Atrophy of olfactory nerve fibers and vomeronasal organ degeneration/regeneration and/or inflammatory cells occurred in mice receiving 1.08, 2.69, or 8.11 mg/kg/day, with a dose-relationship. Only minimal findings were evident in males receiving 0.299 mg/kg/day, where eosinophilic inclusions were evident in olfactory or respiratory epithelia in 1 male, and minimal degeneration/regeneration was evident in the respiratory epithelium in two males. Findings of ulceration were judged to be dose-limiting.

Epithelial degeneration/regeneration in the nasopharynx was evident for mice receiving 1.08, 2.69, or 8.11 mg/kg/day with a dose-relationship. Epithelial eosinophilic inclusions were also evident for females at these doses.

Hyperkeratosis in the nonglandular stomach was observed for females in the high dose group. Luminal dilatation was observed in the 2.69 and 8.11 mg/kg/day groups.

Histopathological findings following treatment for 2 weeks

Organ/Tissue	Males					Females				
	0	0.299	1.08	2.69	8.11	0	0.299	1.08	2.69	8.11
Tracheal bifurcation										
-epithelial hyperplasia	0/6	0/5	0/5	0/6	1/4	0/5	0/5	0/6	0/6	1/4
Larynx										
-arytenoid, squamous hyperplasia	0/6	0/6	1/6	5/6	4/6	0/6	0/6	0/6	3/6	6/6
-dilated ducts laryngeal gland	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6
-exudate	0/6	0/6	0/6	1/6	1/6	0/6	0/6	0/6	0/6	1/6
-foreign body reaction, ventral pouch	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6
-ventral cartilage **necrosis**	0/6	0/6	1/6	5/6	6/6	0/6	0/6	2/6	4/6	5/6
-ventral epithelium, squamous hyperplasia/metaplasia	0/6	5/6	6/6	6/6	6/6	0/6	5/6	6/6	6/6	6/6
-ventral epithelium, vesiculation	0/6	0/6	0/6	0/6	3/6	0/6	0/6	0/6	0/6	1/6
-ventral pouch epithelium, squamous hyperplasia	0/6	0/6	2/6	5/6	5/6	0/6	0/6	2/6	4/6	5/6
-ventral region, **erosion/ulceration**	0/6	0/6	0/6	0/6	2/6	0/6	0/6	0/6	0/6	1/6
-ventral region, subacute/chronic inflammation	0/6	0/6	3/6	6/6	6/6	0/6	0/6	3/6	6/6	6/6
-ventrolateral epithelium, **erosion/ulceration**	0/6	0/6	0/6	1/6	1/6	0/6	0/6	0/6	0/6	1/6
-ventrolateral/lateral epithelial squamous hyperplasia/metaplasia	0/6	0/6	3/6	6/6	6/6	0/6	0/6	4/6	6/6	6/6
Nasal turbinates										
-dilated/cystic Bowman's gland	0/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6
-exudate in nasal passages	0/6	0/6	0/6	2/6	4/6	0/6	0/6	0/6	4/6	3/6
-olfactory epithelium, degeneration/regeneration	0/6	0/6	2/6	6/6	6/6	0/6	0/6	5/6	6/6	6/6
-olfactory epithelium, **erosion**	0/6	0/6	2/6	4/6	6/6	0/6	0/6	1/6	6/6	6/6
-olfactory epithelium, **ulceration**	0/6	0/6	0/6	3/6	4/6	0/6	0/6	0/6	6/6	4/6
-olfactory, epithelium, eosinophilic inclusions	0/6	1/6	2/6	2/6	4/6	0/6	0/6	4/6	5/6	5/6
-olfactory nerve fibers, atrophy	0/6	0/6	2/6	5/6	6/6	0/6	0/6	5/6	6/6	6/6
-respiratory epithelium, goblet cell hyperplasia	1/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
-respiratory epithelium, eosinophilic inclusions	0/6	1/6	1/6	4/6	2/6	0/6	0/6	4/6	4/6	4/6
-respiratory epithelium, **erosion**	0/6	0/6	0/6	2/6	2/6	0/6	0/6	1/6	3/6	2/6
-respiratory epithelium, squamous metaplasia	0/6	0/6	1/6	1/6	0/6	0/6	0/6	0/6	1/6	4/6
-respiratory epithelium, **ulceration**	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	1/6	1/6
-respiratory epithelium, degeneration/regeneration	0/6	2/6	5/6	6/6	5/6	0/6	0/6	6/6	6/6	6/6
-squamous epithelium, degeneration/**erosion**	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6
-transitional epithelium, degeneration/regeneration	0/6	0/6	0/6	3/6	5/6	0/6	0/6	0/6	4/6	6/6
-transitional epithelium, **erosion**	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	1/6	2/6
-vomeronasal organ, degeneration/regeneration/ inflammatory cells	0/6	0/6	1/6	0/6	4/6	0/6	0/6	0/6	4/6	5/6

Nasopharynx										
-epithelial degeneration/regeneration	0/6	0/6	0/5	3/6	3/6	0/6	0/6	1/6	3/6	6/6
-epithelial eosinophilic inclusions	0/6	0/6	0/5	0/6	0/6	0/6	0/6	2/6	1/6	1/6
Stomach										
-hyperkeratosis, nonglandular region	1/6	-	-	-	1/6	1/6	-	-	1/1	3/6
Uterus										
-luminal dilatation						0/6	1/6	-	2/3	2/5

Toxicokinetics: Plasma C_{max} and AUC values for GSK573719 increased in a generally dose proportional manner. There were no differences in these values between males and females.

Dose-Normalised Parameter	Day	Male Estimated Achieved Dose			
		0.299 mg/kg/day	1.08 mg/kg/day	2.69 mg/kg/day	8.11 mg/kg/day
AUC_{0-t} (ng.h/mL)	Day 14	14.4	96.9	175.4	884.0
C_{max} (ng/mL)	Day 14	2.06	45.1	129.4	365.8

Dose-Normalised Parameter	Day	Female Estimated Achieved Dose			
		0.299 mg/kg/day	1.08 mg/kg/day	2.69 mg/kg/day	8.11 mg/kg/day
AUC_{0-t} (ng.h/mL)	Day 14	16.8	91.0	167.9	776.1
C_{max} (ng/mL)	Day 14	3.29	63.2	84.5	207.6

Study title: **Toxicity Study by Inhalation Administration to CD-1 Mice for 13 Weeks**

Key study findings:

● In a 13-week inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day. Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively.

● Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain.

● Incidences of slight or moderate retinal folds were increased for male and female treatment groups.

● Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Potential treatment-related findings were observed in the liver and uterus.

● Histopathological findings were evident in the larynx at all doses and appeared to be nonspecific irritant effects associated with inhalation exposure. Histopathological

findings were observed in the nasal turbinates at doses ≥0.287 mg/kg/day. Histopathological findings were observed in the nasopharynx at doses of 1.06 and 2.85 mg/kg/day. Histopathological findings in the tracheal bifurcation, liver, and uterus were observed at 2.85 mg/kg/day.

● A potential dose-limiting finding in the larynx was ventral cartilage necrosis observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of relatively low incidence (i.e., 1 of 10 and 2 of 12, respectively). Further, the finding for 1 female at 0.092 mg/kg/day was graded as minimal.

● A potential dose-limiting finding in the nasal turbinates was slight ulceration of the olfactory epithelium for 1 male at 2.85 mg/kg/day.

● Based upon findings of ventral cartilage necrosis in larynx for males at 1.06 and 2.85 mg/kg/day and females at 0.092, 0.287, 1.06, and 2.85 mg/kg/day, maximum tolerated doses were identified as 0.6 mg/kg/day for males and 0.2 mg/kg/day for females.

Study no.: WD2007/01600/00
Volume #, and page #: Volumes 1 and 2, Pages 1 to 521
Conducting laboratory and location: (b) (4)

Date of study initiation: December 19, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: GSK573719A from GSK batch number R230166 was blended with lactose powder BP/USNF, containing a nominal 1% (w/w) magnesium stearate, at a nominal concentration of 4% (w/w) (as base), blend batch BVR/H/1083/06).

The vehicle comprise lactose (batch number 612435) blended with nominal 1% (w/w) magnesium stearate (batch number C512923). The blend number was BVR/H/1082/06.

Group 5 on 4 dosing occasions received a blend of GSK573719A (0.5% w/w) with magnesium stearate (1% w/w) in lactose.

Methods
Doses: GSK573719 was administered at nominal inhaled doses of 0, 0.1, 0.3, 1, or 3 mg/kg/day (estimated achieved doses were 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day, respectively).

GSK573719

Group	Average achieved aerosol chamber concentration (µg/L)[1]	Time of exposure (mins)	Body weight[2] (g)		Estimated achieved dose (mg/kg/day)[1]	
			Male	Female	Males	Females
1	0	60	36.2	27.3	0	0
					Average = 0	
2	1.60	60	38.1	26.8	0.0895	0.0957
					Average = 0.0923	
3	4.99	60	37.4	27.7	0.280	0.296
					Average = 0.287	
4	18.3	60	37.9	26.6	1.02	1.10
					Average = 1.06	
5	49.2	60	35.6	26.9	2.78	2.94
					Average = 2.85	

1. Expressed in terms of GSK573719 (the parent compound)
2. Average of the group mean weekly body weight results

Calculation of Estimated Inhaled Doses

$$\text{Estimated dose to animals (mg/kg/day)} = \frac{RMV \times T \times C}{BW \times 1000}$$

Where
T = Duration of exposure/day (minutes)
C = Chamber concentration (µg GSK573719/L)
BW = Group average body weight for study (kg)
RMV = Respired minute volume (L/min) (0.499 x BW (kg) $^{0.809}$) [Bide et al, 2000]

Pulmonary deposited doses of GSK573719 (using a pulmonary deposition factor of 0.10)

Group	Pulmonary deposited dose			
	mg/kg/day		mg/g lung weight/day	
	Males	Females	Males	Females
1	0	0	0	0
	Average = 0		Average = 0	
2	0.009	0.0096	0.0015	0.0013
	Average = 0.009		Average = 0.0014	
3	0.0280	0.0296	0.0046	0.0042
	Average = 0.0287		Average = 0.0044	
4	0.102	0.110	0.0170	0.0149
	Average = 0.106		Average = 0.016	
5	0.278	0.294	0.0434	0.0404
	Average = 0.285		Average = 0.0419	

Lung weight = 0.228 g for males and 0.196 g for females

Estimated achieved doses of magnesium stearate:

Magnesium Stearate

Group	Average achieved aerosol chamber concentration (µg/L)[1]	Time of exposure (mins)	Body weight[2] (g)		Estimated achieved dose (mg/kg/day)[1]	
			Male	Female	Males	Females
1	11.9	60	36.2	27.3	0.0674	0.0712
					Average = 0.0691	
2	0.371	60	38.1	26.8	0.207	0.221
					Average = 0.214	
3	1.02	60	37.4	27.7	0.0570	0.0604
					Average = 0.0586	
4	3.96	60	37.9	26.6	0.222	0.237
					Average = 0.229	
5	11.9	60	35.6	26.9	0.675	0.712
					Average = 0.692	

1. Expressed in terms of magnesium stearate
2. Average of the group mean weekly body weight results

Summary of Group Particle Size Distribution Results for GSK573719 Aerosols

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
2	1.60	(b) (4)	
3	4.99		
4	18.3		
5	49.2		

1. Expressed in terms of GSK573719 (the parent compound)
MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Summary of Group Particle Size Distribution Results for Magnesium Stearate Aerosols

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
1	11.9	(b) (4)	
2	0.371		
3	1.02		
4	3.96		
5	11.9		

1. Expressed in terms of magnesium stearate
MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Species/strain: CD-1 mice were obtained from (b) (4)
Number/sex/group or time point (main study): 12 mice/sex/group
Route, formulation, volume, and infusion rate: Vehicle and GSK573719 were administered by nose-only administration for 60 min/day. Aerosols were generated

using a Wright Dust Feed (WDF) mechanism by suspending material scraped from the surface of a reservoir of compressed test article powder blend in a stream of oil-free compressed air. The inhalation exposure system comprised an ADG nose-only inhalation exposure chamber, restraining tubes, a dust generator, air supply, and extract lines, which attached to the top and bottom of the chamber, respectively, and an air supply to the dust generator. The nose-only inhalation chamber was a modular apparatus of aluminum alloy construction comprising a base unit, a variable number of animal exposure sections, and a top section incorporating a central aerosol inlet surrounded by a tangential air inlet. During dosing, each chamber was housed in an enclosed ventilated cabinet. The WDF mechanism was mounted on the top of the aerosol conditioning pre-chamber and the aerosol passed through it into the top of the exposure chamber.

For measurements of chamber aerosol concentrations of GSK573719 and magnesium stearate, a measured volume of air was drawn from the exposure chambers at a rate of 5 liters/min through a Whatman GF/A glass microfiber filter for GSK573719 and a quartz filter for magnesium stearate, mounted in an open face filter holder. Samples were collected daily from each dose group for gravimetric analysis. Samples were collected daily from each dose group for chemical analysis, for week 1 of the study, 3 times per week for weeks 2 to 4 and twice weekly from week 5 onward. Additional samples were sent for chemical analysis for Group 5 due to incorrect blend strength being used. Filters were analyzed for GSK573719 content using UPLC with UV detection. Filters were analyzed for magnesium stearate content using atomic absorption.

For determination of particle size distribution, a measured volume of air was drawn at a rate of 3 liters/min from inhalation exposure chambers containing GSK573719 and magnesium stearate through a Marple Model 296 Personal Cascade Impactor attached to the chamber. Samples were collected during weeks 1, 4, 8, and 12 for GSK573719 and during weeks 2, 5, 9, and 13 for magnesium stearate. Samples were subject to gravimetric analysis prior to chemical analysis.

Satellite groups used for toxicokinetics or recovery: For toxicokinetic assessments, there were an additional 3 mice/sex/group in the vehicle-control group and 18 mice/sex/group in each treatment group.
Age: Mice were 6-7 weeks old on day 1 of treatment.
Weight: Body weight ranges were 29.7 to 39.7 g for male mice and 22.5 to 30.3 g for female mice.
Unique study design or methodology (if any): Dose selection for the 13-week inhalation toxicology study was based upon a 2-week dose range finding inhalation toxicology study with mice.

Observation and Times:
Clinical signs: A viability check was performed twice daily. Clinical observations were recorded daily immediately before dosing, immediately after dosing on return of the animal to its cage, and as late as possible in the working day.
Body weights: Body weights were measured weekly.

Food consumption: Food consumption was recorded weekly.
Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of dosing and during week 13.
EKG: Not performed.
Hematology: Blood samples for measurement of hematology parameters were collected during week 13.
Clinical chemistry: Blood samples for measurement of clinical chemistry parameters were collected during week 13. Analysis was limited to ALT, AST, urea, creatinine, glucose, total protein, and albumin.
Urinalysis: Not performed.
Gross pathology: After week 13 of treatment, surviving toxicology animals were killed. A gross necropsy examination was conducted for each animal. Organs and tissues were fixed and transported to (b) (4) for processing.
Organ weights: Organ weights were measured for the brain, heart, kidneys, liver, lung, ovaries, spleen, testes, and thymus.
Histopathology: Samples of tissues were processed to paraffin wax, sectioned, stained with hematoxylin and eosin and microscopically examined for all control and high dose animals killed at the end of the dosing period and macroscopic abnormalities from all toxicology animals. Tissues with test article-related findings in the high dose group were examined in lower dose groups.
Toxicokinetics: Blood samples for measurement of plasma concentrations of GSK573719 were obtained at 1 (immediately after dosing), 1.5, 3, 5, 8, and 24 hr after the start of dosing. After blood collection, animals were killed and discarded without examination. Samples were shipped to the Department of Drug Metabolism and Pharmacokinetics (DMPK), GlaxoSmithKline for analysis of GSK573719.

Results:

Mortality: Seven main study animals died before the scheduled termination of the study; however, none of the deaths were judged to be attributable to treatment with GSK573719. Several of these animals found dead had attempted to turn in the exposure tube. All animals in the 2.85 mg/kg/day (high dose) group that were found dead had attempted to turn in the exposure tube.

Animal 25M (Group 2, 0.092 mg/kg/day) was found dead on day 9 in the exposure tube having turned in the tube.

Animal 77M (Group 4, 1.06 mg/kg/day) was found dead on day 91 in the holding cage at the morning check. Histopathological findings included minimal congestion in the lungs + bronchi, slight plasmacytosis in the cervical LN, minimal erosion/ulceration in the ventral region and moderate necrosis in the ventral cartilage of the larynx, minimal degeneration/regeneration/inflammatory cells in the vomeronasal organ of the nasal turbinates, minimal hemosiderosis in the spleen, and moderate lymphocyte necrosis in the thymus. The cause of death was not identified.

Animal 112M (Group 5, 2.85 mg/kg/day) was found dead on day 29 in the exposure tube having turned in the tube.

Animal 114M (Group 5, 2.85 mg/kg/day) was found dead on day 12 in the exposure tube having turned in the tube.

Animal 140F (Group 1, Control) was found dead on day 47 in the exposure tube.

Animal 158F (Group 2, 0.092 mg/kg/day) was found dead on day 9 in the exposure tube.

Animal 159F (Group 2, 0.092 mg/kg/day) was killed on day 60 for welfare reasons due to weight loss and observation of a swollen head. Pyogranulomatous inflammation of the head was identified as the factor contributory to death for this animal.

Test article-related findings similar to those seen in animals killed after 13 weeks of treatment were seen in the larynx of animals 77M, 112M and 114M, and in the nasal turbinates of Animal 112M.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain.

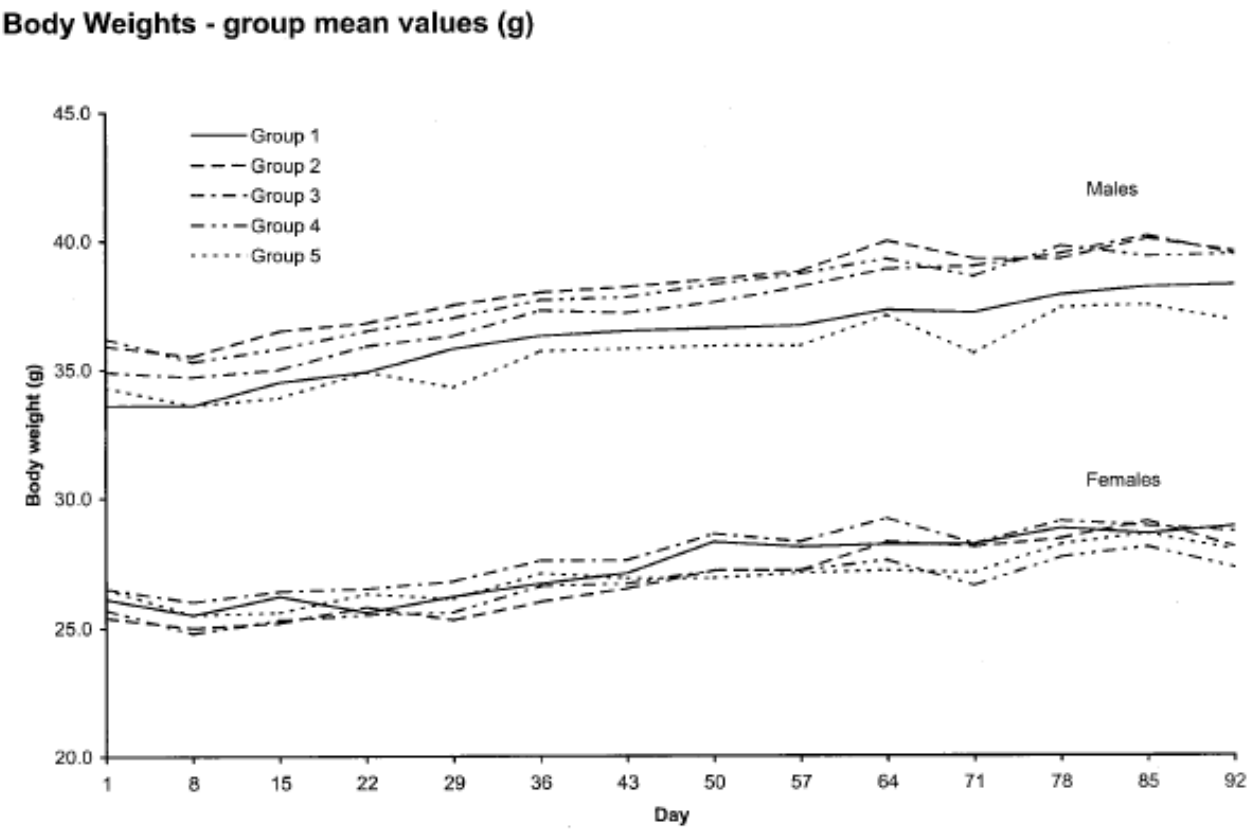
Calculations of body weight gain relative to control found significant decreases for males in the 0.092, 1.06, and 2.85 mg/kg/day groups and females in the 0.287, 1.06, and 2.85 mg/kg/day groups (see table below). Decreases for male treatment groups were not dose-related as body weight gain for males in the 0.287 mg/kg/day group was calculated to be 94.2% of the control.

Examination of body weight curves over the 13-week dosing period found that female control and treatment groups were indistinguishable. Curves for males in the 0.092, 0.287, and 1.06 mg/kg/day groups were higher than the male control; however, the curve for males in the 2.85 mg/kg/day group was less than or equal to the male control.

Body weight changes from week 0 to 13

	Males					Females				
mg/kg	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
BW (g) Wk 0	33.6	35.9	34.9	36.2	34.3	26.1	25.4	26.5	25.7	26.5
BW (g) Wk 13	38.3	39.6	39.5	39.5	36.9	28.9	28.1	28.7	27.3	28.0
Δ (g)	4.7	3.7	4.6	3.3	2.9	2.8	2.7	2.2	1.6	1.5
% of initial BW	13.99	10.31	13.18	9.12	8.45	10.73	10.63	8.30	6.23	5.66

% of control	100.0	73.7	94.2	65.2	60.4	100.0	99.1	77.4	58.0	52.8



Food consumption: Food consumption was unaffected for male treatment groups over the 13-week dosing period. Food consumption was reduced for females in the 0.287, 1.06, and 2.85 mg/kg/day groups although the significance of these differences was unclear based upon body weight curves.

Dose, µg/kg/day	Mean Food Consumption (Weeks 0 to 13), g/animal/week	
	Male	Female
0	37	36
0.092	39	34
0.287	38	33 (91.7%)
1.06	36	31 (86.1%)
2.85	38	31 (86.1%)

Ophthalmoscopy: Incidences of slight or moderate retinal folds were increased for male and female treatment groups. These findings were not evident prior to the start of dosing. The sponsor should be requested to provide the historical control incidences of these findings from the testing laboratory as well as provide an assessment of the toxicological significance of these findings.

Structure/observation	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
Fundus -slight retinal folds			47 49	85	116				216 218	251
Fundus -moderate retinal folds		18	48	78 83	109 111 115L		161	191	212	242

Hematology: Increased leukocyte counts were generally evident for males and females at doses ≤1.06 mg/kg/day although increases were either not present or the magnitude of the increase was reduced for the high dose group. The significance of these findings was unclear.

Increased white blood cell counts, as reflected by elevated lymphocyte and neutrophil counts, were observed for males and females in the 0.092, 0.287, and 1.06 mg/kg/day groups; however, these increases were either not evident or the magnitude of the increase was reduced for the high dose (2.85 mg/kg/day) group. Eosinophil counts were increased for male treatment groups and females in the 0.287, 1.06, and 2.85 mg/kg/day groups although dose-response relationships were not present. Basophil and monocyte counts were increased for females in the 0.092, 0.287 and 1.06 mg/kg/day groups although no changes were evident for the high dose group. Large unstained cell counts were increased for female treatment groups although a dose-response relationship was not present.

Hematology parameters – values in parentheses are percent of the control

Parameter	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
White blood cells x 10^9/L	3.99	5.49* (138%)	5.51* (138%)	6.33* (159%)	3.51 (88%)	2.82	4.97 (176%)	6.67* (237%)	6.24* (221%)	3.86 (137%)
Neutrophils x 10^9/L	0.89	1.10 (124%)	1.08 (121%)	0.96 (108%)	0.59 (66%)	0.68	1.02 (150%)	1.33* (196%)	1.08 (159%)	0.76 (112%)
Lymphocytes x 10^9/L	2.84	4.05* (143%)	4.12* (145%)	4.93* (174%)	2.65 (93%)	1.96	3.71 (189%)	4.85* (247%)	4.79* (244%)	2.84 (145%)
Eosinophils x 10^9/L	0.11	0.15 (136%)	0.14 (127%)	0.20* (182%)	0.16* (145%)	0.11	0.10	0.24* (218%)	0.16 (145%)	0.16 (145%)
Basophils x 10^9/L	-	-	-	-	-	0.00	0.01	0.02*	0.01*	0.00
Monocytes x 10^9/L	-	-	-	-	-	0.06	0.10	0.18* (300%)	0.13* (217%)	0.07
LUC x 10^9/L	-	-	-	-	-	0.01	0.03* (300%)	0.05* (500%)	0.07* (700%)	0.03 (300%)

- = no difference

Clinical chemistry: Glucose levels were increased for males and females in the 1.06 mg/kg/day group; however, no change was evident for the high dose (2.85 mg/kg/day) group. The significance of this finding was not clear.

Clinical chemistry parameters

Parameter	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
Glucose mmol/L	9.41	9.33	9.59	13.49* (143%)	9.17	10.65	11.08	11.32	13.40 (126%)	9.69

Gross pathology: Observed gross pathological findings had no correlations to treatment-related histopathological findings.

Organ weights: There were no treatment-related changes of absolute or relative organ weights.

Histopathology: Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Potential treatment-related findings were observed in the liver and uterus.

Minor changes (minimal epithelial hyperplasia or loss of cilia) at the tracheal or bronchial bifurcation were seen in a small number of animals, including a single female control. A marginal increase in incidence was seen in males and females dosed at 2.85 mg/kg/day, compared with controls.

Several treatment-related histopathological findings were observed in the larynx of mice in all groups treated with GSK573719. Incidences and severities of these findings were generally dose-related. These findings in the larynx appeared to be nonspecific irritant effects generally associated with inhalation exposure in rodents and probably not relevant to humans. Females were generally affected to a greater extent than males. The findings observed in animals dosed at ≥0.092 mg/kg/day included necrosis of the ventral cartilage, increased incidences of mucous cell hypertrophy of the ventral gland, subacute/chronic inflammation in the ventral region, and squamous hyperplasia/metaplasia. Changes seen in animals dosed at ≥0.287 mg/kg/day included squamous hyperplasia/metaplasia of the ventrolateral/lateral epithelium, and increased incidences and/or severities of squamous hyperplasia of the epithelium over the arytenoid cartilage and of the ventral pouch. Keratinization of the ventral epithelium was seen in animals dosed at ≥1.06 mg/kg/day and a marginally increased incidence of ductal dilatation in the ventral gland was seen in females dosed at 2.85 mg/kg/day. A potential dose-limiting finding of ventral cartilage necrosis for observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of low incidence. Further, the finding was minimal for females at 0.092 mg/kg/day.

Findings of ventral cartilage necrosis in the larynx

Larynx	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
Ventral cartilage necrosis										
-number examined	12	11	12	11	10	11	10	12	12	12
-minimal	0	0	0	5	3	0	1	0	6	1
-slight	0	0	0	2	4	0	0	1	1	5
-moderate	0	0	0	0	2	0	0	1	0	1
-marked	0	0	0	0	0	0	0	0	0	1
-total effected	0	0	0	7	9	0	1	2	7	8

For the nasal turbinates, treatment-related histopathological findings were seen in the olfactory and respiratory regions, and, to a lesser extent, in the transitional region for animals dosed at 1.06 or 2.85 mg/kg/day. Incidences and severities of these findings were generally dose-related. These findings do not appear to be relevant to humans that will receive the drug by oral inhalation. These findings do not appear to be dose-limiting for a 2-year inhalation carcinogenicity study. The principal findings in the olfactory region were degeneration/regeneration of the epithelium, atrophy of olfactory nerve fibers, and increased incidences and severities of olfactory epithelial atrophy and eosinophilic inclusions. Erosion and ulceration of olfactory epithelium were each seen in a single male dosed at 2.85 mg/kg/day. Lesions of Bowman's glands (atrophy, inflammation, epithelial hyperplasia, and hypertrophy and eosinophilic inclusions) were seen in a few animals. The principal findings in the respiratory region were increased incidences and severities of degeneration/regeneration and eosinophilic inclusions and squamous metaplasia in animals dosed at 2.85 mg/kg/day only. Eosinophilic inclusions in the respiratory epithelium of the maxillary sinus were also seen. Degeneration/regeneration, inflammation, and eosinophilic inclusions in the transitional epithelium, degeneration/regeneration/inflammatory cells in the vomeronasal organ, and exudate in the nasal passages were seen in a few animals dosed at 1.06 or 2.85 mg/kg/day. Test article-related changes were also seen in the olfactory and respiratory epithelia of several animals dosed at 0.287 mg/kg/day. These were generally graded as minimal or slight, and comprised increased incidences and/or severities of respiratory epithelial degeneration/regeneration and eosinophilic inclusions, and of olfactory epithelial atrophy, compared with controls; and respiratory epithelial ulceration, and eosinophilic inclusions in the respiratory epithelium of the maxillary sinus, each in a single female. A potential dose-limiting finding was slight ulceration of olfactory epithelium for 1 male in the high dose group.

For the nasopharynx, epithelial degeneration/regeneration was seen in a few males and females given 2.85 mg/kg/day and in two females dosed at 1.06 mg/kg/day. Epithelial eosinophilic inclusions, dose-related in incidence and severity, were seen in males and females dosed at 1.06 or 2.85 mg/kg/day. These findings do not appear to be relevant to humans that will receive the drug by oral inhalation. These findings do not appear to be dose-limiting for a 2-year inhalation carcinogenicity study.

Incidences of focal inflammation in the liver were increased for males and females in the 2.85 mg/kg/day group. The incidence of luminal dilatation was increased for females in the 2.85 mg/kg/day group.

Histopathological findings following exposure for 13 weeks

Organ/Tissue	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
Tracheal bifurcation										
-epithelial hyperplasia at bifurcation, minimal	0/12	0/11	0/12	0/11	1/10	0/11	0/10	0/12	0/12	0/12
-loss of cilia at bifurcation	0/12	0/11	1/12	1/11	2/10	1/11	0/10	1/12	0/12	2/12
-loss of cilia at bronchial bifurcation, minimal	0/12	0/11	0/12	1/11	0/10	0/11	0/10	0/12	0/12	0/12
Larynx										
-arytenoid, squamous hyperplasia, minimal-slight	1/12	2/10	6/12	7/11	10/10	1/11	1/10	3/12	7/12	9/12
-ventral cartilage **necrosis**, minimal-marked	0/12	0/10	0/12	7/11	9/10	0/11	1/10	2/12	7/12	8/12
-ventral epithelium, keratinization, minimal-moderate	0/12	0/10	0/12	0/11	7/10	0/11	0/10	0/12	2/12	6/12
-ventral epithelium, squamous hyperplasia/metaplasia, minimal-marked	0/12	6/10	11/12	11/11	10/10	1/11	4/10	9/12	12/12	12/12
-ventral gland, ductal dilatation, minimal-moderate	0/12	2/10	0/12	1/11	2/10	1/11	0/10	0/12	2/12	5/12
-ventral gland, mucous cell hypertrophy, minimal-moderate	3/12	7/10	9/12	9/11	9/10	2/11	8/10	5/12	7/12	9/12
-ventral pouch epithelium, squamous hyperplasia, minimal-marked	1/12	0/10	0/12	5/11	10/10	0/11	2/10	6/12	10/12	12/12
-ventral region, subacute/chronic inflammation, minimal	3/12	0/10	4/12	5/11	10/10	1/11	8/10	6/12	7/12	9/12
-ventrolateral/lateral epithelial squamous hyperplasia/ metaplasia, minimal-moderate	0/12	0/10	0/12	3/11	10/10	0/11	0/10	1/12	2/12	12/12
Nasal turbinates										
-Bowman's glands, atrophy, slight	0/12	0/11	0/12	0/11	0/10	0/11	0/10	0/12	0/12	3/12
-Bowman's glands, epithelial hyperplasia, minimal-slight	0/12	0/11	0/12	2/11	1/10	0/11	0/10	0/12	2/12	2/12
-Bowman's glands, hypertrophy and eosinophilic inclusions, minimal-slight	0/12	0/11	0/12	0/11	0/10	0/11	0/10	0/12	0/12	2/12
-Bowman's glands, inflammation, minimal-	0/12	0/11	0/12	1/11	3/10	0/11	0/10	0/12	0/12	3/12

Organ/Tissue	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
slight										
-exudate in nasal passages	0/12	0/11	0/12	0/11	5/10	0/11	0/10	0/12	2/12	2/12
-maxillary sinus, respiratory epithelium, eosinophilic inclusions, minimal-moderate	0/12	0/11	0/12	2/11	7/10	0/11	0/10	1/12	0/12	12/12
-olfactory epithelium, atrophy, minimal-marked	1/12	2/11	2/12	7/11	10/10	1/11	2/10	5/12	12/12	12/12
-olfactory epithelium, degeneration/regeneration, minimal-moderate	0/12	0/11	0/12	1/11	6/10	0/11	3/10	3/12	5/12	10/12
-olfactory epithelium, eosinophilic inclusions, minimal-marked	4/12	5/11	5/12	5/11	10/10	4/11	3/10	4/12	8/12	12/12
-olfactory epithelium, **erosion**, minimal	0/12	0/11	0/12	0/11	1/10	0/11	0/10	0/12	0/12	0/12
-olfactory epithelium, **ulceration**, slight	0/12	0/11	0/12	0/11	1/10	0/11	0/10	0/12	0/12	0/12
-olfactory nerve fibers, atrophy, slight-marked	0/12	0/11	0/12	2/11	9/10	0/11	0/10	0/12	3/12	12/12
-olfactory/respiratory junction, dilated gland(s), minimal-moderate	1/12	3/11	4/12	4/11	2/10	2/11	3/10	6/12	2/12	9/12
-respiratory epithelium, degeneration/regeneration, minimal-moderate	0/12	0/11	1/12	7/11	10/10	1/11	0/10	3/12	12/12	12/12
-respiratory epithelium, eosinophilic inclusions, minimal-moderate	0/12	0/11	3/12	3/11	9/10	2/11	1/10	3/12	9/12	12/12
-respiratory epithelium, inflammation in lamina propria, minimal-slight	0/12	0/11	0/12	1/11	1/10	2/11	0/10	0/12	2/12	2/12
-respiratory epithelium, squamous metaplasia, minimal-moderate	0/12	0/11	0/12	0/11	5/10	0/11	0/10	0/12	0/12	6/12
-Steno's gland, epithelial hyperplasia, slight	0/12	0/11	0/12	0/11	1/10	0/11	0/10	0/12	0/12	0/12
-transitional epithelium, degeneration/regeneration, minimal-slight	0/12	0/11	0/12	0/11	2/10	0/11	0/10	0/12	0/12	3/12
-transitional epithelium, eosinophilic inclusions, minimal-moderate	0/12	0/11	0/12	1/11	1/10	0/11	0/10	0/12	0/12	2/12
-transitional epithelium, inflammation, minimal-slight	0/12	0/11	0/12	0/11	0/10	0/11	0/10	0/12	0/12	2/12
-vomeronasal organ, degeneration/regeneration/ inflammatory cells, minimal	0/12	0/11	0/12	2/11	1/10	0/11	0/10	0/12	2/12	1/12
Nasopharynx										
-epithelial degeneration/ regeneration, minimal-	0/12	0/11	0/12	0/11	4/10	0/11	0/10	0/12	2/12	3/12

Organ/Tissue	Males					Females				
	0	0.092	0.287	1.06	2.85	0	0.092	0.287	1.06	2.85
slight -epithelial eosinophilic inclusions, minimal-slight	0/12	0/11	0/12	3/11	5/10	0/11	0/10	0/12	4/12	11/12
Liver -inflammation, focal, minimal	2/12	-	-	-	5/10	4/11	-	-	-	8/12
Uterus -luminal dilatation						1/11	1/2	1/1	0/1	3/12

Toxicokinetics: Plasma AUC and C_{max} values for GSK573719 generally increased with elevating dose. Increases appeared to be generally less than dose proportional. There were no significant differences in exposure between male and female mice. There was no evidence of drug in plasma from control animals.

Table 1 Summary of the Toxicokinetic Parameters for GSK573719 Derived from Composite Plasma Concentration-Time Results from Male and Female CD-1 Mice Following Inhaled Administration of GSK573719A for 13 Weeks During a 13 Week Study

Sex	Estimated Achieved Dose [1] (mg/kg/day)	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} [2] (h)
Male	0.104	6.06	0.773	1.5
	0.298	4.52	1.76	1.0
	1.01	29.5	9.28	1.0
	1.75	67.1	24.8	1.0
Female	0.111	5.99	1.18	1.0
	0.319	7.82	3.22	1.0
	1.06	42.5	18.5	1.0
	1.85	77.6	45.9	1.0

1. Estimated achieved dose on the toxicokinetic sampling day
2. T_{max} values were derived from the averaged composite profile

N= 3 males and 3 females per time-point per dose group

Rats

Study title: **A 28-Day Inhalation Toxicity Study of a Powdered Aerosol Formulation in the Rat**

Key study findings:

● In a 28-day inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 25.1, 242.8, and 1828.5 µg/kg/day. Pulmonary deposited doses were 0, 2.5, 24.3, and 182.9 µg/kg/day, respectively.

● Body weight gains were significantly decreased for all male treatment groups and the female low dose and mid dose groups. Body weight loss was evident for the female high dose group.

● Treatment-related histopathological findings were observed in the nasal cavity/sinuses, larynx, nasopharynx, lung, tracheobronchial bifurcation, and prostate.

● Treatment-related histopathological findings were observed in the nasal cavity/sinuses at doses ≥242.8 µg/kg/day. Erosion/ulceration observed for males and females in the 1828.5 µg/kg/day group was judged to be dose-limiting.

● Treatment-related histopathological findings were observed in the larynx at all doses. Potential dose-limiting minimal to slight necrosis of the ventral pouch cartilage was observed for males in the 242.8 and 1828.5 µg/kg/day groups and females in the 1828.5 µg/kg/day group. The minimal necrosis of the ventral pouch cartilage for females in the 242.8 µg/kg/day group might be judged to be acceptable.

● Histopathological findings in the nasopharynx, lung, and prostate were confined to the high dose group. Histopathological findings in the tracheal bifurcation were observed in the 242.8 and 1828.5 µg/kg/day groups.

● The MTD in male and female rats was less than 25.1 µg/kg/day based upon decreased body weight gains or body weight losses. Dose-limiting histopathological findings were also observed in the larynx at doses ≥242.8 µg/kg/day and the nasal cavity/sinuses at 1828.5 µg/kg/day.

Study no.: WD2005/01422/00
Volume #, and page #: Volume 2, Pages 1-448
Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: September 22, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:
Micronized GSK573719A from Input batch number 70683AA001, Lot number 051093955 (83.8% purity as free base) was supplied by the sponsor. GSK573719A powder was blended with lactose powder BP/USNF (batch number B143232, Lot number 051089255) at a nominal concentration of 4% (w/w) with 8% (w/w) micronized [redacted] (b) (4) GSK573719A/ [redacted] (b) (4) lactose formulations were stable at RT/Dark/Dessicant for the duration of the study and were dispensed daily.

Reference ID: 3330354

The vehicle consisted of 8% (w/w) (b) (4) in lactose from the same batches as those used for test article preparation

The use of (b) (4) was discontinued in subsequent studies due to issue of pulmonary macrophage accumulation in rats under another IND.

Methods

Doses: Nominal inhaled doses were 0, 30, 200, and 2000 µg/kg/day (estimated achieved doses were 0, 26.1, 242.8, and 1828.5 µg/kg/day, respectively).

Inhaled doses of GSK573719

Group	Average achieved aerosol chamber concentration (µg/L)[a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day)	
			Male	Female	Male	Female	Males	Females
2	0.7	60	0.238	0.164	0.400	0.253	25.0	27.2
							Average = 26.1	
3	6.5	60	0.237	0.165	0.398	0.254	232.2	253.3
							Average = 242.8	
4	48.5	60	0.229	0.158	0.381	0.241	1749.1	1907.8
							Average = 1828.5	

a. Expressed in terms of GSK573719 (the parent compound)

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/(BW)

D Dose (µg/kg/day)

RMV $0.499 \times BW(kg)^{0.809}$ (L/min) [Bide, 2000]

T Duration of exposure/day (minutes)

C Aerosol GSK573719 concentration (µg/L)

BW Group average body weight for study (Kg) Days 1-28

Pulmonary deposited doses (using pulmonary deposition factor of 0.1)

Group	µg/kg/day		µg/g lung weight/day	
	Males	Females	Males	Females
1	0	0	0	0
2	2.5	2.72	0.74	0.63
3	23.2	25.3	6.84	5.84
4	174.9	190.7	49.4	41.8

Lung weight = 1.35 g for males 1.10 g for females

Inhaled doses of (b) (4)

Group	Average achieved aerosol chamber concentration (µg/L)[a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day)	
			Male	Female	Male	Female	Males	Females
1	121.1	60	0.241	0.165	0.406	0.256	4313.1	4701.5
							Average = 4507.3	
2	1.5	60	0.238	0.164	0.400	0.253	53.6	58.3
							Average = 56.0	
3	15.2	60	0.237	0.165	0.398	0.254	543.1	592.4
							Average = 567.8	
4	110.4	60	0.229	0.158	0.381	0.241	3981.4	4342.7
							Average = 4162.1	

a. Expressed in terms of (b) (4)

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/(BW)

D Dose (µg/kg/day)

RMV $0.499 \times BW(kg)^{0.809}$ (L/min) [Bide, 2000]

T Duration of exposure/day (minutes)

C Aerosol (b) (4) concentration (µg/L)

BW Group average body weight for study (Kg) Days 1-28

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Reference ID: 3330354

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolized GSK573719A are presented below.

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	MMAD (µm)	GSD
2	0.7	(b) (4)	
3	6.5		
4	48.5		

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolized (b) (4) are presented below.

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	MMAD (µm)	GSD
1	121.1	(b) (4)	
2	1.5		
3	15.2		
4	110.4		

Species/strain: Sprague-Dawley Crl:CD(SD) rats were obtained from (b) (4) (b) (4)
Number/sex/group or time point (main study): 10 rats/sex/group
Route, formulation, volume, and infusion rate: Rats received the vehicle or test article by nose-only inhalation for 60 min per day. Aerosols were generated using an extended duration powder delivery system (EDPDS), which allowed the powdered test article to be introduced into the flow-through chamber by pre-dried compressed air. Aerosols were conducted into standard nose-only inhalation exposure chambers. Forty exposure ports were available on each exposure chamber (20 ports/exposure section).

For determination of chamber aerosol concentration, a measure volume of air was drawn from exposure chambers through an open-face glass fiber filter fitted to a sampling holder attached to the sampling port. Samples were collected twice during the pre-study period, daily during the first week of treatment, and 3 times weekly thereafter. Filters were analyzed for GSK573719 and (b) (4) ((b) (4) content using HPLC with a quantification range of (b) (4), respectively.

For determination of particle sizes, a measured volume of air was drawn from each inhalation exposure chamber through a cascade impactor attached to a sampling port. Samples were collected prior to the start of treatment and during weeks 1 and 4. The glass fiber filters from stages 0 to 9 of the cascade impactor and glass fiber terminal filter were weighed. Filters were analyzed for GSK573719 and (b) (4)

content using HPLC with quantification ranges of (b) (4) mg/filter, respectively.

Satellite groups used for toxicokinetics or recovery: 3 rats/sex/group
Age: On day 1 of treatment, rats were approximately 12 weeks old.
Weight: Body weight ranges on day 1 of treatment were 351 to 404 g for males and 220 to 261 g for females.
Unique study design or methodology (if any): None

Observation and Times:
Clinical signs: A viability check was performed twice daily. During the treatment period, animals were examined and mortality and clinical signs were recorded at least once daily (during in-life, recurring clinical signs were recorded pre and immediately after dosing and 1-4 hr after dosing). Detailed clinical examinations were performed prior to the start of treatment, at the end of week 4, and on the day of necropsy.
Body weights: Body weights were measured twice during the pretreatment period, on day 1 and twice weekly thereafter during the treatment period, and on the day of necropsy.
Food consumption: Food consumption was measured weekly.
Ophthalmoscopy: Ophthalmic examinations were performed prior to the initiation of dosing and prior to dosing during week 4.
EKG: Not performed.
Hematology: Blood samples for measurement of hematology parameters were collected during week 4.
Clinical chemistry: Blood samples for measurement of clinical chemistry parameters were collected during week 4.
Urinalysis: Urine samples were collected overnight (approximately 16 hr) during week 4.
Gross pathology: On day 29, toxicology animals were killed and submitted to detailed external and internal examinations.
Organ weights: Organ weights were measured for the brain, heart, kidneys, liver, lungs, ovaries, prostate, spleen, testes, and thymus.
Histopathology: Samples of tissues were processed to paraffin wax, sectioned, and stained with hematoxylin and eosin. Light microscopic examinations of tissue samples were performed for all rats in the control and high dose groups and all unscheduled deaths (toxicology animals). Macroscopic abnormalities were examined from all animals. Tissues with treatment-related findings in the high dose group were examined in lower dose groups.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on days 1 and 28 immediately postdose and at 0.5, 1, 2, 4, 8, and 23 hr postdose from 3 rats/sex/group. On completion of sampling, surviving TK animals were killed, the liver was collected for determination of hepatic cytochrome p450, and carcasses were discarded without examination. Plasma samples were shipped to the sponsor for analysis.
Other: Stage-dependent evaluation of spermatogenesis was conducted. Sections of testes from all animals were prepared and stained with Periodic Acid Schiff's-Hematoxylin. A qualitative examination of spermatogenic states was made for normal

progression of the stages of the spermatogenic cycle, cell associations, and proportions expected to be present during normal spermatogenesis for animals from the control and high dose groups.

At termination, the liver from each TK rat was removed, weighed, washed, and divided into 2 pieces. Liver portions taken for microsome preparation were frozen and shipped to the sponsor for analysis. Results will be reported separately.

Results:

Mortality: There were no apparent treatment-related deaths. Observed deaths appeared to be associated with blood sampling on day 27.

One male and one female given 242.8 µg/kg/day (Numbers 3002 and 3503; Group 3) were found dead immediately after the clinical pathology bleed upon return to the home cage on day 27 and one female given 1828.5 µg/kg/day (Number 4508; Group 4) was found dead in the home cage after clinical pathology bleeding and prior to dosing (a period of approximately 2.5 hr), also on Day 27. Female numbers 3503 and 4508 had hemorrhage in the axillary/thoracic area (i.e., focal skeletal muscle hemorrhage Grade 3 for #3503 and hemorrhage in thorax Grade 3 for #4508) that may have contributed to deaths and could be attributed to the experimental procedure (blood sampling). The cause of death of the male treated with 242.8 µg/kg/day could not be determined at necropsy or following microscopic examination.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Body weight gains were significantly decreased for all male treatment groups and the female low dose and mid dose groups. Body weight loss was evident for the female high dose group. Body weight curves appear to parallel calculated decreased of body weight gain or body weight loss.

Body weight gains

Parameter	Males				Females			
	0	25.1	242.8	1828.5	0	25.1	242.8	1828.5
BW (g), day 1	337.6	379.2	375.0	375.9	241.2	243.1	245.3	240.5
BW (g), day 28	427.5	410.6	410.8	385.8	257.4	254.5	253.1	233.6
Δ (g)	49.9	31.4	35.8	9.9	16.2	11.4	7.8	-6.9
% of BW day1	13.2150	8.2806	9.5467	2.6337	6.7164	4.6894	3.1798	-2.8690
% of Control	100.0	62.7	72.2	19.9	100.0	69.8	47.3	-

Figure 1 Body Weights (g) - Males

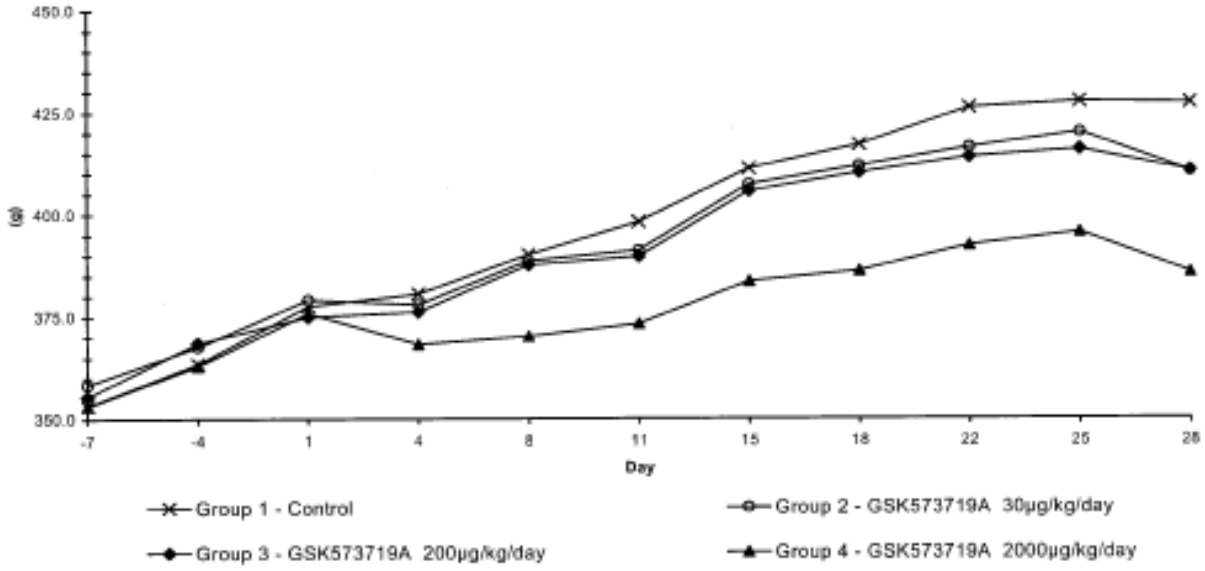
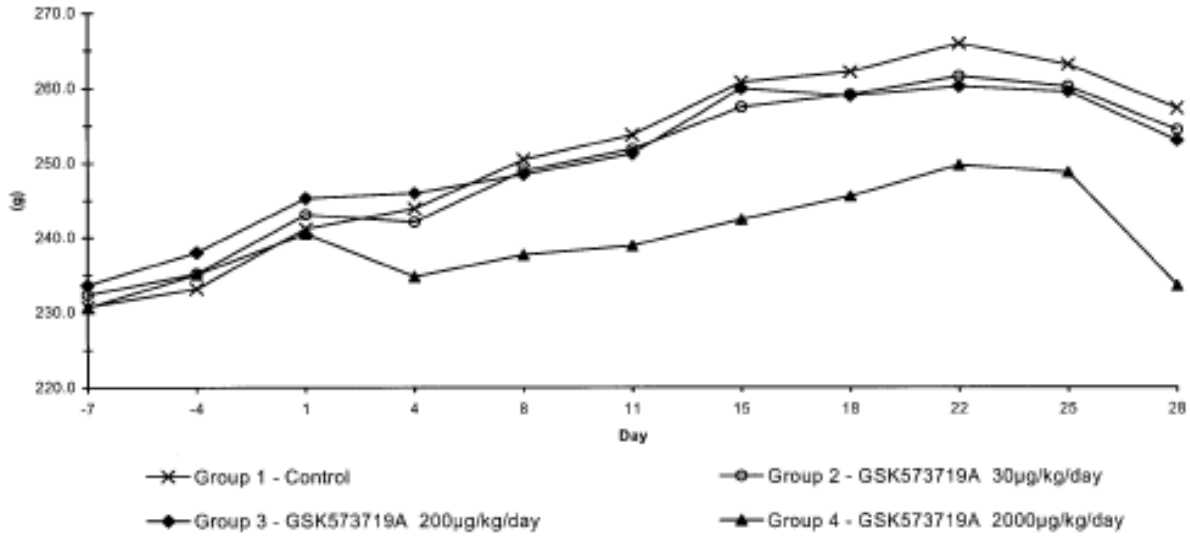


Figure 2 Body Weights (g) - Females



Food consumption: Food consumption was reduced for females in the 1828.5 µg/kg/day group from days 15-22 (20 g/animal/day) and 22-28 (16 g/animal/day) versus 21 and 18 g/animal/day for the control group, respectively.

Ophthalmoscopy: There were no treatment-related ophthalmic effects.

Hematology: There were no treatment-related changes of hematology parameters.

Clinical chemistry: Triglyceride levels were reduced for male and female treatment groups although dose-response relationships were not present.

Triglyceride levels, Week 4 (relative to control level)

Dose µg/kg/day	Triglyceride, mmol/L	
	Males	Females
0	0.662	0.582
25.1	0.535 (0.81x)	0.521 (0.89x)
242.8	0.412 (0.62x)	0.443 (0.76x)
1828.5	0.488 (0.74x)	0.538 (0.92x)

Urinalysis: Urinary protein collected from females in the 1828.5 µg/kg/day group was increased to 2.3 mg/collection period as compared to 0.9 mg/collection period for the control group. There were no treatment-related histopathological findings in the kidneys.

Gross pathology: Observed gross pathological findings did not correlate with treatment-related histopathological findings.

Organ weights: There were no treatment-related changes of organ weights.

Histopathology: Treatment-related histopathological findings were observed in the nasal cavity/sinuses, larynx, nasopharynx, lung, tracheobronchial bifurcation, and prostate.

Nasal cavity/sinuses: In the nasal cavities, minimal to marked degeneration of the olfactory epithelium and minimal to moderate degeneration/regeneration of the respiratory epithelium was seen in all animals given 1828.5 µg/kg/day. Additional changes in some animals included minimal to moderate erosion/ulceration (all three levels of the cavity), minimal or slight squamous metaplasia (olfactory and respiratory epithelium), minimal or slight respiratory metaplasia (olfactory epithelium), slight dilatation/atrophy of the Bowman's gland and minimal luminal exudates. In the transitional epithelium, minimal or slight hyperplasia was seen in most animals and two females showed minimal or slight epithelial degeneration in the vomeronasal organ. In animals given 242.8 µg/kg/day the only change seen was a minimal increase in the incidence of goblet cell hyperplasia of the respiratory epithelium. Erosion/ulceration observed for males and females in the 1828.5 µg/kg/day group was judged to be dose-limiting.

Larynx: In the larynx, minimal to moderate epithelial hyperplasia/squamous metaplasia and minimal or slight subacute and/or chronic inflammation of the submucosa of the ventral region of the larynx was seen at all doses. At doses ≥242.8 µg/kg/day, minimal to moderate necrosis of the ventral pouch cartilage was seen. Minimal to slight squamous metaplasia of the glandular duct was seen in both sexes given 1828.5 µg/kg/day and in males given 242.8 µg/kg/day with minimal to moderate amounts of

exudates in the laryngeal lumen in some animals given 1828.5 µg/kg/day. Dose-limiting minimal to slight necrosis of the ventral pouch cartilage was observed for males in the 242.8 and 1828.5 µg/kg/day groups and females in the 1828.5 µg/kg/day group. The minimal necrosis of the ventral pouch cartilage for females in the 242.8 µg/kg/day group might be judged to be acceptable.

Larynx: necrosis of the ventral pouch cartilage

Larynx	Males				Females			
	0	25.1	242.8	1828.5	0	25.1	242.8	1828.5
necrosis of the ventral pouch cartilage								
-number examined	10	10	10	10	10	10	10	10
-minimal	0	0	3	4	0	0	3	2
-slight	0	0	1	1	0	0	0	6
-total affected	0	0	4	5	0	0	3	8

Nasopharynx: Goblet cell hyperplasia/hypertrophy and degeneration/regeneration of the respiratory epithelium were observed for males and females in the 1828.5 µg/kg/day group.

Lung: There were findings of hemorrhage for males and females in the 1828.5 µg/kg/day group, and interstitial inflammation and mixed cell infiltration for females in the 1828.5 µg/kg/day group.

Tracheobronchial bifurcation: Degeneration/regeneration of the epithelium at the tracheal bifurcation was observed for males in the 242.8 and 1828.5 µg/kg/day groups and females in the 1828.5 µg/kg/day group.

Prostate: Inflammation was observed in the prostate for males in the 1828.5 µg/kg/day group.

Histopathological findings after the 4-week exposure period

Organ/Tissue	Males				Females			
	0	25.1	242.8	1828.5	0	25.1	242.8	1828.5
Nasal cavity/sinuses								
-degeneration, olfactory epithelium, Grade 2-4	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
-degeneration/regeneration, respiratory epithelium, Grade 1-3	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
-erosion/**ulceration**, Grade 1-3	0/10	0/10	0/10	7/10	0/10	0/10	0/10	9/10
-exudate, Grade 1	0/10	0/10	0/10	3/10	0/10	0/10	0/10	5/10
-hyperplasia/hypertrophy, goblet cells, Grade 1	1/10	1/10	4/10	0/10	0/10	0/10	4/10	0/10
-hyperplasia, transitional epithelium, Grade 1-2	0/10	0/10	0/10	7/10	0/10	0/10	0/10	8/10
-metaplasia, respiratory olfactory epithelium, Grade 1-2	0/10	0/10	0/10	5/10	0/10	0/10	0/10	6/10
-metaplasia squamous olfactory epithelium, Grade 1-2	0/10	0/10	0/10	6/10	0/10	0/10	0/10	6/10
-degeneration/regeneration, olfactory epithelium, Grade 2	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
-degeneration/regeneration, vomeronasal organ, Grade 1-2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
-metaplasia, squamous, respiratory epithelium, Grade 2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
-dilatation/atrophy, Bowman's atrophy, Grade 2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	8/10
Larynx								
-hyperplasia/squamous metaplasia, Grade 1-3	0/10	3/10	10/10	10/10	0/10	3/10	7/10	10/10
-inflammation, ventral, Grade 1-2	0/10	3/10	5/10	10/10	0/10	2/10	9/10	8/10
-**necrosis**, ventral pouch cartilage, Grade 1-3	0/10	0/10	4/10	10/10	0/10	0/10	3/10	8/10
-squamous metaplasia, glandular duct, Grade 1-2	0/10	0/10	4/10	8/10	0/10	0/10	0/10	4/10
-exudate, Grade 1-3	0/10	0/10	0/10	5/10	0/10	0/10	0/10	6/10
-hemorrhage, Grade 2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
Nasopharynx								
-hyperplasia/hypertrophy, goblet cells, Grade 1-2	0/10	0/10	0/10	4/10	0/10	0/10	0/10	3/10
-degeneration/regeneration epithelium, Grade 1-2	0/10	0/10	0/10	6/10	0/10	0/10	0/10	6/10
Lung								
-hemorrhage, Grade 1	0/10	0/1	0/1	2/10	0/10	-	0/1	1/10
-inflammation, interstitial, Grade 1	0/10	0/10	0/10	0/10	0/10	-	0/1	1/10
-infiltration, mixed cell, Grade 1	0/10	0/10	0/10	0/10	0/10	-	0/1	3/10
Tracheal bifurcation								
-degeneration/regeneration, epithelium, Grade 1	0/10	0/10	2/10	5/10	0/10	0/10	0/10	6/10
Prostate								
-inflammation, Grade 1-2	0/10	-	0/1	5/10	-	-	-	-

Toxicokinetics: Plasma C_{max} and AUC values for GSK573179 increased with elevating doses on both days 1 and 28. C_{max} and AUC values for males on days 1 and 28 appeared to increase in dose proportional manner. Plasma C_{max} and AUC values for

females on days 1 increased in a less than dose proportional manner from 25.1 top 242.8 µg/kg/day and a greater than dose proportional manner from 242.8 to 1828.5 µg/kg/day. Plasma C_{max} and AUC values for females on days 28 increased in a less than dose proportional manner. There were no significant differences of C_{max} and AUC values between males and females on days 1 and 28.

Table 1 Summary of the Toxicokinetic Parameters for GSK573719 Derived from Serial Plasma Concentration-Time Results from Male and Female Rats Following Nose-Only Inhalation of GSK573719A for 28 Days.

Day	Estimated Achieved Dose[1] (µg/kg/day)	AUC_{0-t} (ng.h/mL)		C_{max} (ng/mL)		T_{max}[2] (h)	
		Male	Female	Male	Female	Male	Female
Day 1	41.4	2.51	6.23	1.06	1.45	1.00	1.05
	200	11.3	13.7	4.01	4.65	1.02	1.02
	1990	257	313	237	235	1.02	1.02
Day 28	22.1	2.65	6.95	0.824	3.08	1.02	1.02
	328	32.7	33.8	10.4	11.6	1.02	1.02
	1690	183	187	74.2	55.2	1.02	1.02

1. Estimated achieved dose on the relevant toxicokinetic sampling day for male and female rats combined
2. T_{max} value expressed as a median
n = 3 males and 3 females per timepoint per dose group

Plasma samples collected from vehicle-control animals, sampled at the same time points as the GSK573719-treated animals, were analyzed for GSK573719. It was determined that 8 samples (out of 84) contained quantifiable levels of GSK573719 above the lower limit of quantification (0.1 ng/mL). The control samples with quantifiable levels of GSK573719 were collected on Day 28 and were in the range of 0.102 to 0.388 ng/mL with one animal having a plasma concentration of 4.22 ng/mL at one time point. Quantifiable levels of GSK573719 were also detected in 1 of 4 naïve samples (0.198 and 3.73 ng/mL) with the remaining two samples being insufficient for analysis. The quantifiable levels of GSK573719 in control samples were judged to be the result of external contamination because of the relatively low levels of quantifiable GSK573719 in the samples, lack of any corresponding toxicokinetic profile, and evidence of similar concentrations of GSK573719 in naïve samples. GSK573719 was not detected in the control exposure chamber on Day 28.

Other: Seminiferous tubules were evaluated with respect to their stage in the spermatogenic cycle and the integrity of the various cell types present within the different stages. No cell- or stage-specific abnormalities were noted. In the female reproductive tract, there was a slight increased number of females in dioestrus stage of the estrous cycle at 242.8 and 1828.5 µg/kg/day compared to the control group. However, these treated females showed evidence of normal cyclical activity and synchrony of activity between reproductive tract organs (ovaries, uterus, and vagina) according to their stage of the estrous cycle. It did not appear that this data was provided in the study report. The sponsor should be asked to provide this data or direct the reviewer to where it is located in the study report.

Study title: **A 13-Week Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat with a 4-Week Recovery Period**

Key study findings:

● In a 13-week inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day. Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively.

● Body weight gains for males in the 102, 288, and 924 µg/kg/day groups were decreased to 84.8, 82.9, and 78.7% of the control, respectively. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study. Body weight gains for females in the 288 and 924 µg/kg/day groups were decreased to 89.3 and 81.3% of the control, respectively. The decrease for females in the 288 µg/kg/day group did not appear to be toxicologically significant.

● Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx.

● For the larynx, minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at doses ≥38 µg/kg/day. These changes were not reversible. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study. However, minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting.

● For the nasal cavity/sinuses, degeneration/regeneration and hyperplasia were generally observed in the respiratory, olfactory and transitional epithelia in both sexes at doses ≥288 µg/kg/day. Goblet cell hyperplasia/hypertrophy was observed for all male treatment groups. These findings for females were not dose-related. These findings appeared to be reversible

● In the nasopharynx, minimal, focal squamous metaplasia was seen in one female at 288 µg/kg/day. There were no findings for the high dose group.

● Given that minimal focal necrosis in the larynx observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study, identification of maximum tolerated doses for males and females were based primarily on decreases of body weight gain. Based upon decreased body weight gain for males and females at 924 µg/kg/day, the MTD was identified as 288 µg/kg/day.

Study no.: WD2007/02012/00
Volume #, and page #: Volumes 3 and 4, Pages 1 to 935
Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: January 15, 2007
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:
Concentrations of test article and excipients in the formulation are expressed in terms of parent and are nominal. GSK573719A powder was blended with lactose monohydrate with 1% (w/w) magnesium stearate at a nominal concentration of 25% (w/w). GSK573719/lactose/magnesium stearate blends were stable at room temperature, in the dark, with desiccant for the duration of the study and were dispensed as needed. Blends were deemed acceptable for use on the study only after blend sample analysis results were approved by the Sponsor. The vehicle was lactose with 1% (w/w) magnesium stearate.

Material	Input Material Batch Number	Input Material Purity	Blend Batch Number	Blend Concentration (w/w)	Content Uniformity	Blend Expiry	Blend Storage
GSK573719A	R230164	99.6%	79189-16-01-07-02 79189-27-03-07-03	25%	97.7% 99.6%	16 July 07 27 Sept. 07	RT, Dark, Desiccate
Magnesium Stearate	C512923		79189-16-01-07-01	1%		16 July 07	RT, Dark, Desiccate
Lactose Monohydrate	612436		79189-16-01-07-01	99%		16 July 07	RT, Dark, Desiccate

RT Room Temperature

Methods
Doses: Nominal inhaled doses were 0, 30, 100, 300, and 1000 µg/kg/day (estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day, respectively).

Study Design					
Group Number	Target Dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day)[a, c]	Target Chamber Aerosol Concentration (µg/L)[a]	Achieved Chamber Aerosol Concentration (µg/L)[a, b]	Number/Sex[d, e]
1	0	0	0	0	21
2	30	38	0.8	1.0	15
3	100	102	2.7	2.7	15
4	300	288	8.2	7.6	15
5	1000	924	27.4	24.3	21

D = (RMVxTxC)/(BW)
D Dose (µg/kg/day)
RMV 0.499xBW(kg)$^{0.809}$ (L/min) [Bide, 2000]
T Duration of exposure/day (minutes)
C Active Aerosol concentration (µg/L)
BW Group average body weight (kg) for study from Days -1 to Day 91
a. Expressed in terms of parent compound, GSK573719.
b. As determined by chemical analysis.
c. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract.
d. 3 of 15 or 21 animals were assigned to the toxicokinetic study.
e. 6 of 21 animals were assigned to the recovery groups from Groups 1 and 5.

Pulmonary deposited doses

Group	Pulmonary deposited doses		
	µg/kg/day	µg/g LW/day	
		Males	Females
1	0	0	0
2	3.8	1.3	0.8
3	10.2	3.3	2.2
4	28.8	9.0	6.0
5	92.4	28.3	18.6

Lung weight = 1.5 g for males and 1.2 g for females

Average achieved chamber GSK573719 aerosol concentrations and resulting estimated achieved doses (males and females combined) of GSK573719 are presented below.

Group	Average Achieved Aerosol Chamber Concentration (µg/L)[a,b]	Estimated Achieved Dose (µg/kg/day)[a,c]		MMAD (µm)	GSD
2	1.0	Males	36	(b) (4)	
		Females	40		
		Average	38		
3	2.7	Males	97		
		Females	107		
		Average	102		
4	7.6	Males	274		
		Females	302		
		Average	288		
5	24.3	Males	877		
		Females	970		
		Average	924		

MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation
D = (RMVxTxC)/(BW)
D Dose (µg/kg/day)
RMV $0.499 \times BW(kg)^{0.809}$ (L/min) [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol GSK573719 concentration (µg/L)
BW Group average body weight for study (Kg) Days -1 to 91
a. Expressed in terms of GSK573719.
b. As determined by chemical analysis.
c. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract.

Average achieved chamber Magnesium Stearate aerosol concentrations and resulting estimated achieved doses (males and females combined) of Magnesium Stearate are presented below.

Group	Average Achieved Aerosol Chamber Concentration (µg/L)[a, b]	Estimated Achieved Dose (µg/kg/day)[a, c]		MMAD (µm)	GSD
1	0.6	Males Females Average	21 24 23	[redacted] (b) (4)	
2	0	Males Females Average	0 0 0		
3	0.1	Males Females Average	4 4 4		
4	0.2	Males Females Average	7 8 8		
5	0.7	Males Females Average	25 28 27		

MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation
D = (RMVxTxC)/(BW)

D Dose (µg/kg/day)
RMV $0.499 \times BW(kg)^{0.809}(L/min)$ [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol Magnesium concentration (µg/L)
BW Group average body weight for study (Kg) Days -1 to 91
a. Expressed in terms of Magnesium Stearate.
b. As determined by chemical analysis.
c. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract.

Species/strain: Sprague-Dawley Crl:CD(SD) rats were obtained from [redacted] (b) (4) [redacted] (b) (4)

Number/sex/group or time point (main study): 12 rats/sex/group

Route, formulation, volume, and infusion rate: Rats were exposed to the vehicle or test article by nose-only inhalation for 60 min per day. Aerosols were generated using an extended duration powder delivery system (EDPDS) that allowed powder test article to be introduced into the flow-through chamber by pre-dried compressed air. Five individual exposure chambers, one for each dose group, were used in the study. There were 60 to 80 exposure ports available on each exposure chamber with 20 ports/exposure section.

The magnesium stearate/lactose vehicle control group was exposed to a dry powder blend of magnesium stearate blended in lactose at a nominal concentration of 1% (w/w). The concentration of magnesium stearate in the aerosol given to vehicle control group was targeted to be the same as that given to the high dose (924 µg/kg/day) group.

For determination of chamber aerosol concentrations, filters were fitted to a sampling device attached to an animal breathing port. Samples were collected and analyzed prestudy, daily during the first week of treatment, 3 times a week during weeks 2 to 4, and twice weekly thereafter. Filters were analyzed for GSK573719 and magnesium stearate content using HPLC or atomic absorption methods, respectively.

For determination of particle size, measured volumes of air were drawn through inhalation exposure chambers containing GSK573719 and/or vehicle into a cascade

impactor attached to an animal breathing port. Samples were collected pretreatment and on days 2, 30, 58, and 86. The glass fiber or Teflon filters from Stages 0 to 8 of the cascade impactor were weighed. Filters were analyzed for GSK573719 and magnesium stearate content using HPLC or atomic absorption methods, respectively.

Satellite groups used for toxicokinetics or recovery: For toxicokinetic assessments, 3 rats/sex/group were added to each group. For a 4-week recovery period, 6 rats/sex/group were added to the control and high dose groups.
Age: On day 1 of treatment, rats were approximately 6 weeks old
Weight: On day 1 of treatment, body weight ranges were rats were 173 to 223 g for males and 130 to 163 g for females.
Unique study design or methodology (if any): Dose selection was based upon a 4-week inhalation toxicology study with rats.

Observation and Times:
Clinical signs: Animals were observed for viability twice per day. Animals were monitored within the restraint tube during exposure. Animals were observed daily before and after dosing. Detailed physical examinations were conducted periodically.
Body weights: Body weights were measured weekly.
Food consumption: Food consumption was measured weekly.
Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of dosing and in week 13 (predose). In Week 13, animals given 38 µg/kg/day (Animal #2508), 102 µg/kg/day (Animal #3006), and 924 µg/kg/day, (Animal numbers 5016, 5017, 5018, 5513, 5514, 5515, 5516, 5517 and 5518) were not examined, due to technical oversight.
EKG: Not performed.
Hematology: Blood samples for measurement of hematology parameters were collected during weeks 4 and 13 and at the end of the recovery period.
Clinical chemistry: Blood samples for measurement of clinical chemistry were collected during weeks 4 and 13 and at the end of the recovery period.
Urinalysis: Urine samples were collected overnight (over approximately 16 hr) during weeks 4 and 13 and at the end of recovery period.
Gross pathology: Animals were sacrificed at the end of the 13-week dosing period and 4-week recovery period and submitted to external and internal examinations.
Organ weights: Organ weights were measured for the adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, spleen, testes, and thymus.
Histopathology: Tissue samples were processed to paraffin wax, sectioned, and stained with hematoxylin and eosin. Microscopic examinations of all tissues were performed for all animals found dead, all animals in the 0 (vehicle-control) and 924 µg/kg/day (high dose) groups, all recovery animals, and macroscopic abnormalities from all main animals. Tissues with treatment-related findings in the 924 µg/kg/day group were examined in the 38, 102, and 288 µg/kg/day groups (i.e., larynx and nasal cavities/sinuses).
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected during weeks 4 and 13 at 1, 1.5, 2, 3, 5, 9, and 24 hr after the start of dosing. Samples were shipped to the sponsor for analysis. Plasma samples were analyzed for

GSK573719 using a HPLC/MS/MS method with a lower limit of quantitation at 0.1 ng/mL.

Animals given GSK573719 at doses of 924 µg/kg/day were inadvertently dosed due to technical oversight before the 24 hr bleed in Week 13. The animals were removed from the dosing chamber after 19 min of exposure and blood samples were collected. The animals given GSK573719 at a dose of 924 µg/kg/day were bled again 24 hr after the following day's dosing (24 hr) in order to have valid samples for analysis. Although both sets of samples were shipped to the Sponsor, and the second set should have been analyzed, the original 24 hr samples were analyzed by technical error. The results were not used in the toxicokinetic evaluation.

Results:

Mortality: Male (Animal #3001) that received 102 µg/kg/day and one female (Animal #4501) that received 288 µg/kg/day were found dead on study Days 74 and 27, respectively. After pathological examinations, the causes of death for these two animals remained undetermined. There were no deaths for rats in the high dose (924 µg/kg/day). These two deaths are assumed to be unrelated to treatment.

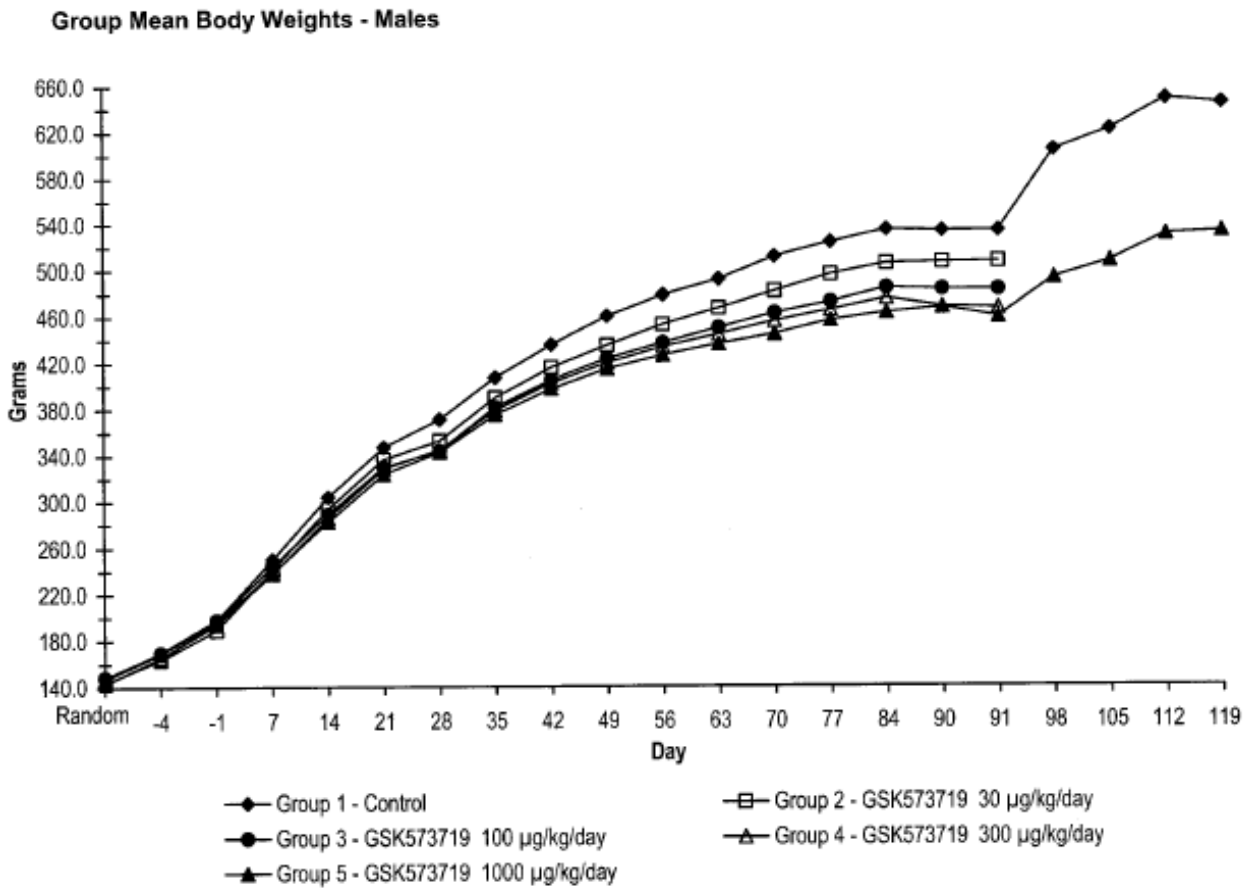
Clinical signs: There were no treatment-related clinical signs.

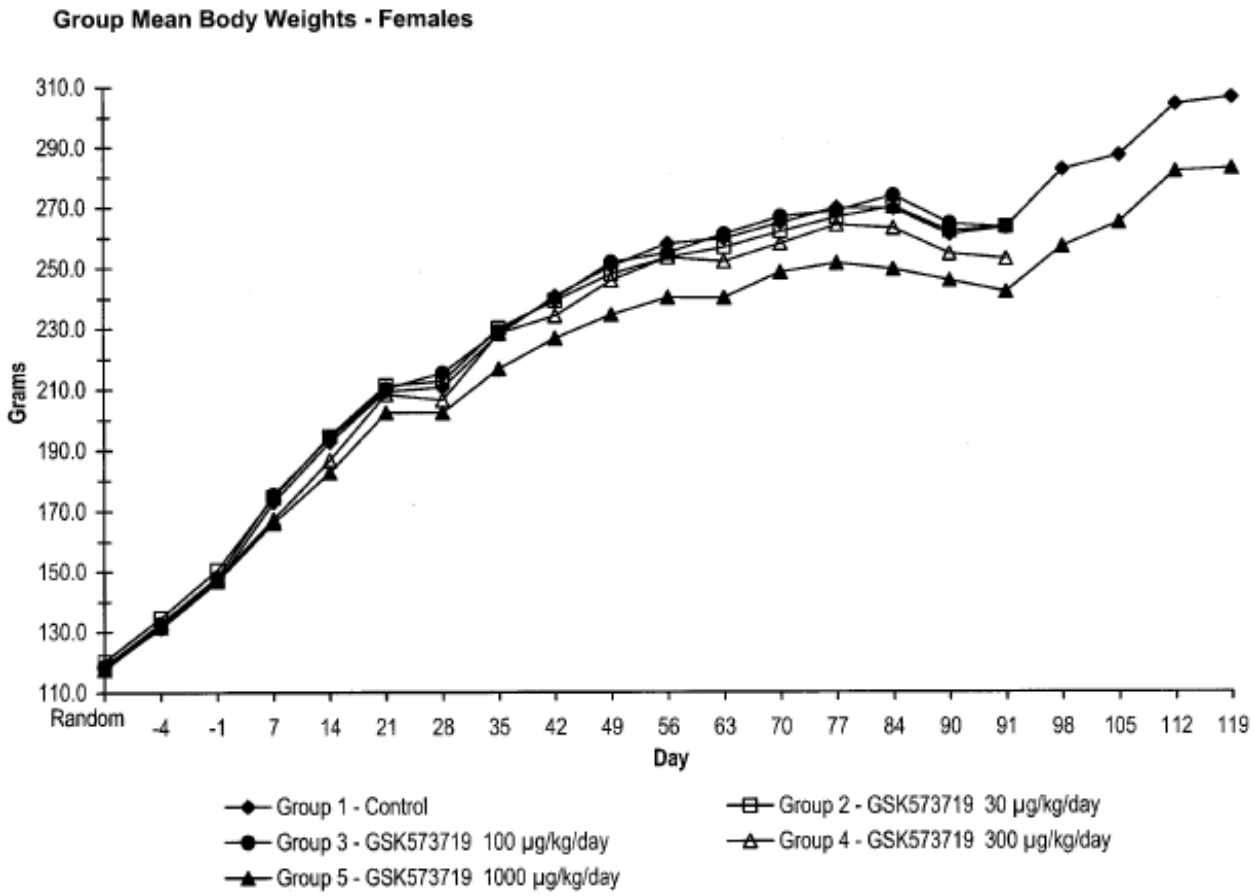
Body weights: Significant decreases of body weight gain were evident for males in the 100, 300, and 1000 µg/kg/day groups and females in the 1000 µg/kg/day group. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study.

At the end of the 4-week recovery period, body weight gains for males and females in the high dose group were increased to 120.4 and 118.7% of the controls, respectively.

Body weight gains from days 1 to 91

Parameter	Males					Females				
	0	38	102	288	924	0	38	102	288	924
BW, day 1	197.4	189.2	197.6	193.8	196.3	146.6	150.6	148.1	147.8	146.9
BW, day 91	534.2	507.5	483.4	467.8	460.0	263.6	263.9	263.5	253.1	242.2
Δ	336.8	318.3	285.8	274	263.7	117.0	113.3	115.4	105.3	95.3
% of BW day1	170.62	168.23	144.64	141.38	134.34	79.81	75.23	77.92	71.2449	64.87
% of Control	100.0	98.6	84.8	82.9	78.7	100.0	94.3	97.6	89.3	81.3


Group Mean Body Weights - Males
Grams
660.0
620.0
580.0
540.0
500.0
460.0
420.0
380.0
340.0
300.0
260.0
220.0
180.0
140.0
Random
-4
-1
7
14
21
28
35
42
49
56
63
70
77
84
90
91
98
105
112
119
Day
Group 1 - Control
Group 2 - GSK573719 30 µg/kg/day
Group 3 - GSK573719 100 µg/kg/day
Group 4 - GSK573719 300 µg/kg/day
Group 5 - GSK573719 1000 µg/kg/day



Food consumption: Food consumption was lower for male treatment groups although a dose-response relationship was not observed. Food consumption for female treatment group appeared to be unaffected.

Mean food consumption (g/animal/day) from days 1 to 91

Dose µg/kg/day	Males	Females
0	29.05	19.31
38	27.66 (0.952x)	18.31 (0.948x)
102	25.92 (0.893x)	18.56 (0.961x)
288	27.48 (0.946x)	18.48 (0.957x)
924	26.02 (0.896x)	20.37 (1.055x)

Ophthalmoscopy: There were no treatment-related ophthalmic effects.

Hematology: Differences of hematology parameters were evident between female control and treatment groups although the toxicological significance of these differences was questionable. There were no comparable changes for male treatment groups.

Week 4: White blood cell and lymphocyte counts for females in the 102, 288, and 924 µg/kg/day groups were increased to 112.8 to 122.6% of control values (6.276 and 5.469

x 10^9/L, respectively). Monocyte counts for females in the 102, 288, and 924 µg/kg/day groups were increased to 122.9 to 141.7% of the control (0.096 x 10^9/L). Basophil and LUC counts for females in the 102, 288, and 924 µg/kg/day groups were increased to 143 to 159% of control values (0.014 and 0.051 x 10^9/L, respectively). Reticulocyte counts for females in the 924 µg/kg/day group were decreased to 77.5% of the control (162.81 x 10^9/L).

Week 13: Neutrophil counts for female treatment groups were decreased 57.1 to 69.8% of the control (0.953 x 10^9/L). APTT values for female treatment groups were decreased to 77.8 to 86.7% of the control (27.15 sec). The basophil and LUC counts for males in the 924 µg/kg/day group were decreased to 65 and 74% of control values (0.40 and 0.076 x 10^9/L), respectively.

End of Recovery Period: Lymphocyte and monocyte counts for females in the 924 µg/kg/day group were decreased to 74.8 and 47.2% of control values (4.242 and 0.123 x 10^9/L), respectively. Eosinophil and basophil counts for males in the 924 µg/kg/day group were increased to 169 and 132% of control values (0.162 and 0.038 x 10^9/L), respectively.

Clinical chemistry: Some differences of clinical chemistry parameters were evident between control and treatment groups although any toxicological significance of these differences was not clear.

Triglyceride levels were increased for all female treatment groups during week 4 and females in the 924 µg/kg/day group during week 13. Triglyceride levels were still slightly elevated for females in the 924 µg/kg/day group at the end of the recovery period. There were no comparable changes for male treatment groups.

ALT and ALP values for females in the 924 µg/kg/day group at the end of the recovery period were elevated to 211 and 150% of control values, respectively. No differences were observed during the dosing period.

Clinical chemistry parameters during weeks 4 and 13 and at the end of the recovery period

Parameter	Time	Males					Females				
		0	38	102	288	924	0	38	102	288	924
Triglyceride mmol/L	Wk4	NC	NC	NC	NC	NC	0.310	0.455* (147%)	0.444 (143%)	0.433 (140%)	0.659* (213%)
	Wk13	NC	NC	NC	NC	NC	0.356	0.398	0.339	0.309	0.480* (135%)
	R	NC				NC	0.553				0.650 (118%)
ALT U/L	R	NC				NC	43.2				91.2 (211%)
ALP U/L	R	NC				NC	34.2				51.3 (150%)

NC = no change

Urinalysis: There were no treatment-related changes of urinalysis parameters.

Gross pathology: There were no correlations between observed gross pathological findings and treatment-related histopathological findings.

Organ weights: There were no treatment-related changes of organ weights.

Histopathology: Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx.

Larynx: Minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at ≥38 µg/kg/day. Both changes showed dose-dependant increases of incidence and/or severity. Necrosis appeared to be focal at all doses. Necrosis was graded as minimal at doses ≤288 µg/kg/day; however, gradings of minimal to slight were observed for the high dose (924 µg/kg/day). The ventral cartilage is a small superficial cartilage beneath the ventral epithelium. Additional laryngeal changes seen in both sexes at ≥288 µg/kg/day were squamous metaplasia of the submucosal glands often seen in combination with inflammation. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting. Minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting.

Larynx: necrosis of the ventral pouch cartilage at the end of the dosing period

Larynx	Males					Females				
	0	38	102	288	924	0	38	102	288	924
Necrosis										
-number examined	12	12	11	12	12	12	12	12	11	12
-minimal	0	6	10	12	10	0	6	9	9	9
-slight	0	0	0	0	2	0	0	0	0	3
-total effected	0	6	10	12	12	0	6	9	9	12

At the end of the recovery period, microscopic findings in the larynx included squamous metaplasia in the ventrolateral aspect of the larynx, necrosis of the ventral pouch cartilage, squamous metaplasia of the submucosal glands and inflammation that were morphologically comparable to those observed at the end of the dosing period.

Nasal cavity/sinuses: Degeneration/regeneration and hyperplasia were observed in the respiratory, olfactory and transitional epithelia and were often seen concurrently in affected animals. Minimal to moderate degeneration/regeneration was present in respiratory epithelium in both sexes at ≥288 µg/kg/day. This change was dose-related in terms of incidence and severity. Moderate grading of degeneration/regeneration in the respiratory epithelium was observed for 1 male and 1 female at 924 µg/kg/day. There was an increased incidence of degeneration/regeneration in the olfactory epithelium for females at 924 µg/kg/day. Incidences for males at 288 and 924 µg/kg/day were not dose-related.

Minimal to slight hyperplasia of the transitional epithelium was seen in two females at 288 µg/kg/day and in three males at 924 µg/kg/day. Goblet cell hyperplasia/hypertrophy was observed for all male treatment groups. The incidence and severity showed some relation to dose. Findings for females were not dose-related in terms of incidence or severity. This is a well established adaptive response to irritant compounds. A minor effect of treatment with GSK573719 appears to be evident. Minimal degeneration/regeneration of the vomeronasal organ was seen in one female (Animal # 5507) at 924 µg/kg/day. There was a low incidence of minimal to slight inflammation and/or minimal luminal exudate in treated animals at ≥102 µg/kg/day with no clear dose response.

At the end of the recovery period, findings in the nasal cavity/sinuses were significantly reduced suggesting reversibility. Degeneration/regeneration in the respiratory epithelium was only present at the minimal grade in one female animal at 924 µg/kg/day. Degeneration/regeneration of the olfactory epithelium was observed for 2 of 6 males at 924 µg/kg/day. Minimal hyperplasia/ hypertrophy of goblet cells in the nasal cavities was observed in one female rat at 924 µg/kg/day.

Nasopharynx: In the nasopharynx, minimal, focal squamous metaplasia was seen in one female (Animal #4511) at 288 µg/kg/day. There were no findings for the high dose group.

Tracheal bifurcation: There were no histopathological findings for the tracheal bifurcation after dosing for 13 weeks.

Histopathological findings at the end of the dosing and recovery periods

Organ/Tissue	Sex	Dosing Period					Recovery Period	
		0	38	102	288	924	0	924
Larynx								
-metaplasia, squamous, Grade 1-2	M	0/12	9/12	10/11	12/12	12/12	0/6	6/6
	F	0/12	4/12	9/12	10/11	12/12	0/6	6/6
-squamous, metaplasia, submucosal glands, Grade 1-2	M	0/12	0/12	0/11	3/12	5/12	0/6	0/6
	F	0/12	0/12	0/12	3/11	3/12	0/6	3/6
-inflammation, Grade 1-2	M	0/12	0/12	0/11	1/12	5/12	0/6	1/6
	F	0/12	0/12	0/12	2/11	1/12	0/6	1/6
-necrosis, Grade 1-2	M	0/12	2/12	10/12	12/12	12/12	0/6	6/6
	F	0/12	6/12	9/12	9/11	12/12	0/6	6/6
Nasal cavity/sinuses								
-hyperplasia, transitional epithelium, Grade 1-2	M	0/12	0/12	0/11	0/12	3/12	0/6	0/6
	F	0/12	0/12	0/12	2/11	0/12	0/6	0/6
-degeneration/regeneration, respiratory epithelium, Grade 1-3	M	0/12	0/12	0/11	1/12	5/12	0/6	0/6
	F	0/12	0/12	0/12	3/11	4/12	0/6	1/6
-degeneration/regeneration,	M	0/12	0/12	0/11	2/12	1/12	0/6	2/6

Organ/Tissue	Sex	Dosing Period					Recovery Period	
		0	38	102	288	924	0	924
olfactory epithelium, Grade 1-2	F	1/12	0/12	0/12	0/11	6/12	0/6	0/6
-degeneration/regeneration, vomeronasal organ, Grade 1	M	0/12	0/12	0/11	0/12	0/12	0/6	0/6
	F	0/12	0/12	0/12	0/11	1/12	0/6	0/6
-hyperplasia/hypertrophy, goblet cells, Grade 1	M	0/12	1/12	2/11	4/12	3/12	0/6	0/6
	F	0/12	0/12	1/12	3/11	1/12	0/6	1/6
-inflammation, Grade 1-2	M	0/12	0/12	1/11	0/12	1/12	0/6	0/6
	F	0/12	0/12	0/12	2/11	2/12	1/6	0/6
-exudate, Grade 1	M	0/12	0/12	0/11	0/12	1/12	0/6	0/6
	F	0/12	0/12	0/12	1/11	0/12	0/6	0/6
Nasopharynx								
-metaplasia, squamous, nasopharynx, Grade 1	M	0/12	0/12	0/11	0/12	0/12	0/6	0/6
	F	0/12	0/12	0/12	1/11	0/12	0/6	6/6

Toxicokinetics: Plasma C_{max} and AUC values for GSK573719 increased with elevating doses during weeks 4 and 13. AUC values for female treatment groups during weeks 4 and 13 generally increased in a dose proportional manner. Dose proportionality was not generally evident for male AUC values during weeks 4 and 13. C_{max} values during weeks 4 and 13 for male and female treatment groups generally increased in a dose proportional manner. AUC and C_{max} values during week 13 were generally lower than values during week 4. AUC and C_{max} values for females during weeks 4 and 13 were generally greater than values for males.

Table 1 Mean Toxicokinetic Parameters for GSK573719 from Male and Female Rats Following Inhalation of GSK573719A for 13 Weeks

Sex	Week	Target Dose Group (µg/kg/day)	Estimated Achieved Dose[1] (µg/kg)	AUC_{0-t}[2] (ng.h/mL)	C_{max}[2] (ng/mL)	T_{max}[3] (h)
Male	4	30	21.8	NC	NC	NC
		100	110	10.2	1.71	1.0
		300	286	13.2	3.96	1.0
		1000	997	50.5	21.4	1.0
	13	30	41.0	4.09	0.363	1.0
		100	117	5.93	1.06	1.0
		300	215	15.8	3.00	1.0
		1000	801	22.6	9.18	1.0
Female	4	30	24.0	0.879	1.24	1.0
		100	120	5.02	2.40	1.0
		300	314	15.9	3.58	1.0
		1000	1116	61.3	16.7	1.0
	13	30	45.8	3.88	0.918	1.5
		100	132	7.04	1.57	1.0
		300	242	13.5	3.28	1.0
		1000	908	36.2	21.4	1.0

1. Estimated achieved dose of GSK573719 on the relevant toxicokinetic sampling day
2. AUC0-t and Cmax are the mean of n=3 rats
3. Tmax values are the median of n=3

NC: Not calculated. Data and resultant profiles for male rats week 4 were variable and concentrations were similar to those quantifiable in the control group and have therefore not been used in the analysis or subsequently reported

Plasma samples collected from vehicle-control and naïve-control rats plasma were analyzed for GSK573719. GSK573719 was not quantifiable in any of the naïve samples analyzed (total of 5 samples confirmed, 1 sample, NR- not reportable). In vehicle-control animals, GSK573719 was quantifiable in 13 samples from week 4 (out of 42) with a range of 0.141-27.9 ng/mL (limit of quantification 0.1 ng/mL) and 2 samples from week 13 (out of 42) with a range of 0.119 and 1.47 ng/mL (limit of quantification 0.084 ng/mL). This contamination of vehicle-control samples was judged to have occurred ex-vivo. Filter samples taken from exposure chambers on the toxicokinetic sampling days were analyzed and the test material was not detected, confirming that vehicle-control animals had not been exposed to GSK573719 during dosing. Further, only sporadic samples contained test material and no profile was evident in the values reported.

Histopathology inventory (optional)

Study	14-day non-GLP inhalation study	13-week inhalation study	4-week inhalation study	13-week inhalation study
Species	Mouse	Mouse	Rat	Rat
Adrenals	X	X	X*	X*

Aorta	NE	X	X	X
Bone Marrow smear				
Bone (femur)	NE	X	X	X
Brain	X*	X*	X*	X*
Cecum	NE	X	X	X
Cervix	NE	X	X	X
Colon	NE	X	X	X
Duodenum	NE	X	X	X
Epididymis	NE	X	X	X
Esophagus	NE	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder	NE	X	NA	NA
Gross lesions	X	X	X	X
Harderian gland	NE	NE	NE	NE
Heart	X*	X*	X*	X*
Ileum	NE	X	X	X
Injection site				
Jejunum	NE	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx	X	X	X	X
Liver	X*	X*	X*	X*
Lungs	X*	X*	X*	X*
Lymph nodes, bronchial	NE	X	X	X
Lymph nodes, cervical	NE	X		
Lymph nodes mandibular	X		X	X
Lymph nodes, mesenteric	NE	X	X	X
Mammary Gland	NE	X	X	X
Nasal cavity	X	X	X	X
Optic nerves	NE	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas	NE	X	X	X
Parathyroid	NE	X	X	X
Peripheral nerve	NE	(Sciatic)	(Sciatic)	(Sciatic)
Pharynx (Naso)	X	X	X	X
Pituitary	NE	X	X	X
Prostate	NE	X	X*	X*
Rectum	NE	NE	NE	NE
Salivary gland, mandibular	NE	X	X	X
Salivary gland, sublingual	NE	X	X	X
Salivary gland, parotid			X	X
Sciatic nerve	NE	X	X	X
Seminal vesicles	NE	X	X	X
Skeletal muscle	NE	X	X	X
Skin	X	X	X	X
Spinal cord	NE	X	X	X
Spleen	X*	X*	X*	X*
Sternum	X (w/BM)	X (w/BM)	X (w/BM)	X (w/BM)

Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid	X	X	X	X
Tongue	NE	X	X	X
Trachea	X	X	X	X
Tracheal bifurcation	X	X	X	X
Urinary bladder	NE	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X
Zymbal gland				

X, histopathology performed
*, organ weight obtained
NE, not examined
NA, not applicable

2.6.6.4 Genetic toxicology

Study title: **Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli***

Key findings:

● GSK573719 at doses up to 2500 µg/plate ± S9 did not produce any increases of revertant colony counts for TA98, TA100, TA1535, and TA1537. GSK573719 at doses up to 5000 µg/plate ± S9 did not produce any increases of revertant colony counts for WP2uvrA pKM101. GSK573719 was negative in the in the in vitro bacterial reverse mutation assay.

Study no.: WD2005/00750/00

Volume #, and page #: Volume 3, Pages 1 to 50
Conducting laboratory and location: (b) (4)

Date of study initiation: March 2, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:

Test compound:	GSK573719A
Batch number:	70683AA001
Purity:	83.8% (active moiety)
Molecular weight:	508.50 as 'A' salt, 428.60 as parent
Correction factor:	1.193 g contains 1.000 g of GSK573719 content
Salt form:	Bromide
Storage conditions:	Room temperature in the dark
Retest date:	(b) (4)

Methods

Strains: *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* strain WP2 *uvrA* (pKM101) were used in this study.

Doses used in definitive study: In the first and second definitive studies, plates were treated with vehicle control, positive control and GSK573719A at concentrations of 5, 15, 50, 150, 500, 1500 and 2500 µg/plate for strains TA98, TA100, TA1535 and TA1537 in the presence and absence of S9-mix and at concentrations of 5, 15, 50, 150, 500, 1500, 2500 and 5000 µg per plate for strain WP2 uvrA pKM101 in the presence and absence of S9-mix.

Basis of dose selection: Plates were treated with vehicle control, positive control and GSK573719A at concentrations of 50, 150, 500, 1500, 2500 and 5000 µg/plate both in the presence and absence of S9-mix. For the vehicle control, positive control and compound treatments, 1 plate per group was used. For TA98, TA100, TA1535, and TA1537 at 1500 µg/plate in the absence of S9, slight toxicity to the background lawn and/or reduction of revertant colony counts were observed. At 2500 µg/plate in the absence of S9, toxicity with no revertant colonies was observed for TA100 and TA1537, slight toxicity to background lawn and/or reduced colony counts were observed for TA98, TA1535, and WP2uvrA (pKM101). At 5000 µg/plate in the absence of S9, toxicity with no revertant colony counts was observed for TA98, TA100, TA1535, and TA1537 and slight toxicity to the background lawn with reduced revertant colony counts was observed for WP2uvrA (pKM101). At 1500 µg/plate in the presence of S9, slight toxicity to the background lawn was observed for TA100. At 2500 µg/plate in the presence of S9, slight toxicity to the background lawn and/or reduced revertant colony counts were observed for TA98, TA100, TA1535, and TA1537. At 5000 µg/plate in the presence of S9, toxicity to background lawn ranging from slight to severe and no or reduced revertant colony counts were observed for all strains. The sponsor's dose selection for the definitive studies appears appropriate.

Negative controls: DMSO

Positive controls:

3.2.2.2. Positive Controls in the Presence of S9-mix

Strain	Positive Control[1]	Batch	Final Conc. µg/plate	Vehicle	Batch
TA98	Benzo[a]pyrene (B[a]P)	044K1110	10	DMSO[2]	03155HC-474 and 08034EO-121
TA100, TA1535, TA1537	2-Aminoanthracene (AAN)	S11804-483	5	DMSO[2]	04046PB-254 or 03155HC-474
WP2*uvrA* pKM101	2-Aminoanthracene (AAN):	S11804-483	10	DMSO[2]	03155HC-474

1. The positive controls were supplied by Sigma-Aldrich Company Ltd.
2. DMSO was supplied by Sigma-Aldrich Company Ltd.

3.2.2.3. Positive Controls in the Absence of S9-mix

Strain	Positive Control[1]	Batch	Final Conc. µg/plate	Vehicle	Batch
TA98	2-Nitrofluorene (2NF)	9110924227	5	DMSO[2]	03155HC-474 03155HC-015
TA100, TA1535	Sodium Azide (NaN_3)	101K2502	2	Purified water[3]	04J15B27
TA1537	9-Aminoacridine (AAC)	07673-037	50	DMSO[2]	03155HC-474
WP2*uvrA* pKM101	4-Nitroquinoline 1-Oxide (NQO)	10425HO	2	DMSO[2]	03155HC-474

1. The positive controls were supplied by Sigma-Aldrich Company Ltd.
2. DMSO was supplied by Sigma-Aldrich Company Ltd.
3. Sterile water was supplied by Baxter Healthcare

Metabolic activation: An Aroclor 1254 induced rat liver post-mitochondrial fraction (S9) was obtained from Molecular Toxicology Incorporated (MolTox™). Each batch of rat liver S9 fraction was checked by the manufacturer for sterility, protein content, ability to convert known promutagens to bacterial mutagens and cytochrome P450 enzyme (alkoxyresorufin-O-dealkylase) activity. The concentration of S9 in the S9 mix was 10%.

Incubation and sampling times: The plate incorporation method was used in these studies. Plates were incubated for approximately 72 hr at 37°C. Following a preliminary toxicity test, two definitive mutation tests were conducted in the presence and absence of S9-mix. For definitive studies, 6 plates per group were used for the vehicle controls and 3 plates per group were used for the treated groups and the positive controls. Plates were scored with an automated counter or by manual count. Toxicity was assessed according to one or more of the following criteria: reduced growth (diminution) of the background lawn; the presence of pin-dot (pseudorevertant) colonies; a reduction in colony numbers.

If the data for any treatment level show a response ≥2 times the concurrent vehicle control value (TA98, TA100 and WP2 uvrA (pKM101), or ≥3 times the concurrent vehicle control value (TA1535 and TA1537), in conjunction with a dose-related response, the result was considered positive.

Results

Study validity: Positive controls produced expected increases in the frequency of revertant colony counts in the presence and absence of S9. TA98, TA100, TA1535, and TA1537 were treated with GSK573719 at levels up to 2500 µg/plate ± S9. Toxicity to the background lawn and/or reduced revertant colony counts were evident at 1500 and 2500 µg/plate ± S9. WP2uvrA pKM101 was treated with GSK573719 at levels up to 2500 µg/plate ± S9. Toxicity to the background lawn and/or reduced revertant colony counts were evident at 2500 and 5000 µg/plate ± S9. The dose of 5000 µg/plate is the limit dose. Dose selection for tester strains appeared to be adequate.

Study outcome: GSK573719 at doses up to 2500 µg/plate ± S9 did not produce any increases of revertant colony counts for TA98, TA100, TA1535, and TA1537. GSK573719 at doses up to 5000 µg/plate ± S9 did not produce any increases of revertant colony counts for WP2uvrA pKM101. GSK573719 was negative in the in the in vitro bacterial reverse mutation assay.

Study title: **In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus**

Key findings:

- GSK573719A was negative in the mouse lymphoma L5178Y TK locus assay in the presence and absence of S9.

- The maximum concentration tested was limited by solubility to 200 µg/mL for the 3-hr treatment in the presence of S9 and toxicity to 225 and 110 µg/mL for the 3- and 24-hr treatments in the absence of S9, respectively.

Study no.: WD2005/00751/00
Volume #, and page #: Volume 3, Pages 1 to 39
Conducting laboratory and location: (b) (4)

Date of study initiation: February 24, 2005
GLP compliance: Yes
QA reports: yes (X) no (X)
Drug, lot #, and % purity:

Test compound:	GSK573719A
Batch number:	70683AA001
Purity:	99.4% (83.8% as active moiety)
Molecular weight:	508.50 as 'A' salt, 428.60 as parent
Correction factor:	1.193 g contains 1.000 g of GSK573719 content
Salt form:	Bromide salt
Storage conditions:	Room temperature in the dark
Retest date:	(b) (4)

Methods

Cell line: Mouse lymphoma L5178Y ($TK^{+/-}$) cells

Doses used in definitive study: Cultures were treated with vehicle control, positive control and GSK573719A at concentrations of 20, 40, 60, 80, 100, 125, 150, 175, 200, 250 and 300 µg/mL for the 3 hr treatment in the presence of S9-mix, at concentrations of 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 µg/mL for the 3 hr treatment in the absence of S9-mix, and at concentrations of 10, 20, 40, 60, 80, 90, 100, 110, 125 and 150 µg/mL for the 24 hr treatment in the absence of S9-mix.

Basis of dose selection: GSK573719A was tested at concentrations ranging from 62.5 to 2000 µg/mL in the presence and absence of S9 (3 hr treatment) and from 7.813 to 2000 µg/mL in the absence of S9 (24 hr treatment). For the vehicle control and compound treatments, a single culture per group was used. For the 3-hr treatment, the RTG at 125 µg/mL in the presence and absence of S9 was reduced to 5 and 9%, respectively. For the 24-hr treatment, the RTG at 31.25 and 62.5 µg/mL was reduced to 55 and 12%, respectively. The sponsor's dose selection for definitive studies appears acceptable.

Negative controls: DMSO

Positive controls:

Positive Control[1]	Batch	Final Conc. µg/mL	Vehicle	Batch
Methyl Methanesulphonate	074K1110 074K3720	5[3], 15 and 20[4]	DMSO	03155HC-0474 03155HC-015
Benzo[a]pyrene[2]	044K1110	2 and 3[4]	DMSO	03155HC-474

1. The positive controls were supplied by Sigma-Aldrich Company Ltd.
2. Benzo[a]pyrene solutions prepared in anhydrous analytical grade dimethyl sulphoxide (DMSO). BP solutions not used immediately, were stored as frozen aliquots at −80°C in the dark.
3. Concentration tested for the 24 hour treatment
4. Concentration not analysed for mutagenicity as an acceptable response was observed at the lower concentration analysed.

Metabolic activation: An Aroclor 1254 induced rat liver post-mitochondrial fraction (S9) from Molecular Toxicology Incorporated, USA (MolTox™) was used as an exogenous oxidative metabolizing system. Each batch of rat liver S9 fraction was checked by the manufacturer for sterility, protein content, ability to convert known promutagens to bacterial mutagens and cytochrome P450 enzyme (alkoxyresorufin-O-dealkylase) activity. The final concentration of S9 in culture was 2% v/v.

Incubation and sampling times: At least 1 x 10^7 cells (3-hr treatment) or 4 x 10^6 cells (24 hr treatment) were placed in 50 mL centrifuge tubes (3 hr treatment) or tissue culture flasks (24 hr treatment) and the appropriate volume of vehicle, test compound, or positive control solution added. All treatments were in duplicate except for the vehicle control, for which quadruplicate cultures were used. For 3 hr treatments, the medium contained a reduced level (5% v/v) of horse serum and 1.0 mL of S9-mix or 1.0 mL (150 mM) KCl solution for treatments in the presence and absence of S9-mix, respectively. Following treatment, cultures were centrifuged and cells were washed and resuspended.

Cells were incubated for a 2-day expression period to allow for $TK^{-/-}$ mutations to be expressed. Cell viability was determined at the end of the expression period. For determination of mutant frequency, cultures were adjusted to 1 x10^4/mL with RPMI 20 containing TFT (3 µg/mL) and transferred to four 96-well microtitre plates (0.2 mL/well at 2 x 10^3 cells per well). The plates were placed in a humidified incubator and maintained for 1 to 2 weeks. Wells containing mutant clones were then identified by visual inspection and counted. Only colonies of >50 cells were scored.

After 6 to 7 days (cytotoxicity test viability plates), 7 to 11 days (mutation assay viability plates), or 11 to 13 days (mutant plates), the plates were removed from the incubator and scored by visual inspection for the number of colony-bearing wells for each microtitre plate.

If increases in mutant frequency compared with the vehicle control were observed, the distribution of wells containing large and small colonies were determined for the vehicle and positive controls and for those concentrations of test compound showing increases in mutant colony frequency.

If the MF of any test concentration exceeds the sum of the mean control mutant frequency plus GEF (126), a linear trend test should be applied.
a) If the linear trend test is negative, the result is regarded as negative.
b) If the linear trend test is positive, this will indicate a positive, biologically relevant response.

Results

Study validity: Positive control produced expected increases of mutant frequencies in the presence and absence of S9. For the 3-hr treatment in the absence of S9, the RTG at 225 µg/mL was reduced to 24%. For the 3-hr treatment in the presence of S9,

precipitate was evident at 200 µg/mL. The RTG at 200 µg/mL was 91%. For the 24-hr treatment in the absence of S9, the RTG at 110 µg/mL was reduced to 19%.

Study outcome: There were no significant increases of the mutant frequency for the 3-hr treatment ± S9 or 24-hr treatment – S9. GSK573719 was negative in the mouse lymphoma assay.

Study title: **Intravenous Bone Marrow Micronucleus Assay in Rats**

Key findings:

● In the in vivo micronucleus study, GSK573719A was administered by intravenous infusion at doses of 10 and 20 mg/kg/day on days 1 and 2. The dose of 20 mg/kg/day was determined to be the maximum feasible dose based upon solubility of GSK573719A in 0.9% NaCl and using a dose volume of 10 mL/kg for intravenous infusion.

● MPCE/2000 PCE for male rats treated with GSK573719A at 10 and 20 mg/kg/day were similar to the concurrent vehicle-control and fell within the ranges determined from the laboratory historical control data. GSK573719A was not genotoxic in the rat bone marrow micronucleus assay.

Study no.: WD2005/01079/00
Volume #, and page #: Volume 3, Pages 1-40
Conducting laboratory and location: (b) (4)

Date of study initiation: April 14, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:

Test compound:	GSK573719A
Batch number:	70683AA001
Purity:	99.4% (83.8% active moiety)
Molecular weight	508.50 as 'A' salt, 428.60 as parent
Correction factor:	1.193 g of GSK573719A contains 1.000 g GSK573719 content
Salt form	Bromide
Storage conditions:	Room temperature in the dark
Expiry date:	31 January 2006

Methods

Species: Sprague Dawley (Crl:CD®(SD)IGS BR) rats were obtained from (b) (4) (b) (4) For the dose range finding study, rats were 7-10 weeks old

and had body weight ranges of 210-326 g for males and 199-230 g for females. For the micronucleus assay, male rats were approximately 8 weeks old and had a body weight range of 265 to 329 g.

Doses used in definitive study: In the micronucleus study, GSK573719A was administered by intravenous infusion at doses of 10 and 20 mg/kg/day on days 1 and 2. The dose of 20 mg/kg/day was determined to be the maximum feasible dose based upon solubility of GSK573719A in 0.9% NaCl (see below) and using a dose volume of 10 mL/kg for intravenous infusion. The standard intravenous dose volume with bolus administration is 1 to 2 mL/kg.

Treatment	Dose [1] (mg/kg/day)	Dose [1] Concentration (mg/mL)	No. of Animals
Micronucleus assay animals:			
Vehicle [2]	0	0	6Male
GSK573719A	10	1.0	6Male
GSK573719A	20	2.0	6Male
Positive Control[3]	20	2	6Male
Satellite animals for bioanalysis (see section 3.3.3)			
GSK573719A [4]	20	2.0	6Male

1. Expressed as active moiety GSK573719
2. Vehicle was 0.9% NaCl
3. Positive control animals dosed once only, on Day 2
4. Satellite animals dosed for bionalysis of plasma samples once only, and sampled immediately after dosing.

Basis of dose selection: GSK573719A was determined to be soluble in 0.9% NaCl at concentrations up to 3 mg/mL. However, during dose preparation for the dose range-finding study, it was determined that concentrations of 2.5 and 3.0 mg/mL could not be administered by a 30 min intravenous infusion due to precipitation. Therefore, 2.0 mg/mL was considered to be the maximum dose concentration that could be practically administered by a 30 min intravenous infusion. Formulations of GSK573719A were prepared as a solution in 0.9% NaCl at 2.5 hours prior to dosing.

Frequency of formulation:	Daily, 2.5 hours prior to dosing
Formulation stability:	Formulations of GSK573719A in the range 0.003 to 3.0 mg/mL were stable for 7 days when stored at either ambient temperatures or approximately 4°C both protected from light (GSK Document No. FD2005/00065/00).
Concentration of formulations:	Dose Range Finding Test: 1.0, 2.0 and 2.5 mg/mL Micronucleus Assay: 1.0 and 2.0 mg/mL
Dose volume:	Dose Range Finding Test: 5 mL/kg Micronucleus Assay: 10 mL/kg

In the dose range finding studies, 3 rats/sex/group received GSK573719 by intravenous infusion at doses of 5 or 10 mg/kg/day on days 1 and 2 in the first study and doses of 10 or 12.5 mg/kg/day on days 1 and 2 in the second study. The dose volume was 5 mL/kg. Animals were observed for 4 hr following each dose and prior to the second dose. Clinical signs of toxicity were observed in male rats dosed at 5 mg/kg/day or greater. Clinical signs included dilated pupils observed immediately or 0.5 hr postdose and continuing throughout the observation period at all doses tested. Increased rate of breathing was observed on day 2 in males and females treated at 5 mg/kg/day. Clinical signs at 10 mg/kg/day ranged from dilated pupils alone in males and females to increased rate of breathing, eyelid closure, lethargy, and piloerection in males and increased rate of breathing and eyelid closure in females. Animals were also seen to be excessively lip licking and eating the bedding. At 12.5 mg/kg/day piloerection was observed in males and females, 4 hr postdose on day 2. There were no apparent differences in toxicity between male and female rats.

Negative controls: 0.9% Sodium chloride

Positive controls: 20 mg/kg cyclophosphamide

Incubation and sampling times: GSK573719A and the vehicle control were administered by intravenous infusion on days 1 and 2 (approximately 24 hr apart). The positive control was administered on day 2 only. In addition bioanalysis of plasma samples from satellite animals was conducted to confirm in vivo exposure to the test compound. A dose volume of 10 mL/kg was used for all treatments. There were 6 male rats per group. Postdose observations were performed immediately after each dose administration, at least twice in the 4 hr following each dose and prior to the second administration. Observations were also recorded at least once on the day of bone marrow sampling.

Test compound and vehicle treated rats were killed in groups, approximately 24 hr after the second dose. Positive control rats were killed 24 hr after their single dose of cyclophosphamide. One femur from each animal was removed and bone marrow was collected. Bone marrow cells were placed on slides. At least 4 slides were prepared from each animal. Two slides were stained with were stained with acridine orange. The relative proportions of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were determined from the analysis of at least 1000 erythrocytes (PCE plus NCE) per animal. The numbers of micronucleated polychromatic erythrocytes (MPCE) were determined from a minimum of 2000 PCE analyzed per animal.

Blood samples for measurement of plasma drug concentrations were collected from satellite animals immediately after dosing. Plasma samples were analyzed for GSK573719 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) for GSK573719 was 0.1 ng/mL, using a 50 µL aliquot of rat plasma with a higher limit of quantification (HLQ) of 100 ng/mL.

If any treatment group shows a response (mean frequency of MPCE) which is >4 times the concurrent vehicle control value, the result is considered positive. If, after additional analysis, one or more treatment groups show a response <4 times the concurrent vehicle control but this exceeds the historical control range (observed range), the data are subjected to the following statistical analysis. If statistical significance is not observed, the result is considered negative. If statistical significance is observed with at least one dose group and the frequency of MPCE for animals in that dose group is not consistent with or exceeds the historical vehicle-control range, the result is considered positive.

Results

Study validity: The positive control, cyclophosphamide, produced the expected increases in MPCE in the positive control group. The dose of GSK573719 at 20 mg/kg/day was determined to be the maximum feasible dose based upon solubility in 0.9% NaCl and using a dose volume of 10 mL/kg for intravenous infusion.

Study outcome:
Rats treated with GSK573719 at intravenous doses of 10 or 20 mg/kg/day displayed dilated pupils, an expected pharmacological effect, immediately postdose onwards. Individual animal values, and group mean values for MPCE/2000 PCE for male rats treated with GSK573719A at 10 and 20 mg/kg/day, were similar to the concurrent vehicle-control and fell within the ranges determined from the laboratory historical control data. Analysis of plasma from rats dosed once with GSK573719A at 20 mg/kg demonstrated detectable circulating levels of test material immediately after dosing (range 537 to 580 ng/mL). GSK573719A was not genotoxic in vivo, in the rat bone marrow micronucleus assay, following two intravenous doses of 10 and 20 mg/kg/day administered 24 hours apart.

2.6.6.5 Carcinogenicity

DRAFT PROTOCOL FOR AN INHALATION CARCINOGENICITY STUDY OF A POWDER AEROSOL FORMULATION IN THE MOUSE

Outline of Sponsor's Draft Protocol: The carcinogenic potential of GSK5737I9A will be assessed in Crl:CD1(ICR) mice following daily nose-only inhalation exposure for a minimum of 104 consecutive weeks. The proposed high dose of 1.0 mg/kg/day is based on toxicokinetic and toxicity endpoints that were established in 2-week and 13-week inhalation toxicology studies with CD-1 mice. The proposed mid and low doses are 0.3 and 0.1 mg/kg/day, respectively. The high dose of 1.0 mg/kg/day was selected as the high dose based upon reductions of food consumption and body weight gain observed in the 13-week study with mice that received 2.85 mg/kg/day.

The study is scheduled to be conducted at (b) (4)

H9X 3R3. Mice will be obtained from [redacted] (b) (4) (b) (4). Mice will be 6 weeks old and approximately 20-30 g for males and 15-25 g for females at the onset of treatment. For the carcinogenicity evaluation, there will be four groups of 60 mice/sex/group. For the toxicokinetic assessment, there will be four groups of 48 mice/sex/group. The test material will be administered by nose-only inhalation since this is comparable to the intended route of administration in humans (oral inhalation). Vehicle-control mice will receive lactose blended with a nominal 1 % w/w magnesium stearate. Treated mice will receive 25% w/w GSK573719A blended in lactose with a nominal 1 % w/w magnesium stearate.

Group No. Identification	Targeted Aerosol Concentration* (mg/L)	Targeted Dose* (mg/kg/day)	Animal Number[a]			
			Main Study		Toxicokinetic@	
			Males	Females	Males	Females
1 Control**	**	0	1001-1060	1501-1560	1061-1126	1561-1626
2 Low Dose	*[Low]*	*[Low Dose]*	2001-2060	2501-2560	2061-2126	2561-2626
3 Mid Dose	*[Mid]*	*[Mid Dose]*	3001-3060	3501-3560	3061-3126	3561-3608
4 High Dose	*[High]*	*[High Dose]*	4001-4060	4501-4560	4061-4126	4561-4608
5 Health Screen	----	----	5001-5010	5501-5510	-	-

* Expressed in terms of parent compound, GSK573719.

** The control group will be exposed to the vehicle formulation at the equivalent Magnesium Stearate concentration targeted for the high dose group (Group 4).

@ Toxicokinetic animals will be used for blood toxicokinetic evaluation only. Data relating to clinical examination and body weight collected from these animals will be retained, but not reported. Numbers to be confirmed when TK profile established

[a] An additional 6 animals/sex/group will be assigned to the study and will be used as dosed spares to replace toxicokinetic animals or main study animals in case of tube deaths. Any animal number replaced during the study will be documented in the study file and will be included in the final report.

Standard cylindrical flow-through nose-only inhalation chambers will be utilized in this experiment. Each chamber is of modular design with 20 separate ports in each row into which can be inserted the conical front section of a polycarbonate restraint tube. The top section of the inhalation chamber has an opening for inlet air into which the test or control articles can be introduced. The bottom section of the chamber has a corresponding air extraction port. The powder test article or vehicle control atmosphere (Groups 1 to 4) will be generated using an extended duration powder delivery system (EDPDS) with a Venturi powered T section supplied with a stream of pre-dried compressed air. The resulting atmosphere then travels through an air inlet located at the top of the inhalation chamber. The test article or vehicle control concentration will be achieved by varying the exposure duration (up to 60 min) and/or rate of powder introduction into the feed nozzle of the Venturi T-section. Chamber concentrations of GSK573719 and magnesium stearate will be monitored during the study. Particle size determinations will also be conducted.

Mice will be observed twice daily for mortality and clinical signs. A cageside observation will be conducted once daily. Mice will be examined weekly for palpable masses. Body weights will be measured weekly for the first 16 weeks and monthly thereafter. Food

consumption will be recorded on a monthly basis. A hematology assessment will be conducted at necropsy. Ophthalmic examinations will be conducted once prior to the start of treatment (all animals) and during weeks 13, 52, 78, and 104. Toxicokinetic assessments will be conducted at weeks 4 and 26 using 8 time points and 3 animals/time point. Concentrations of GSK573719 in plasma from all animals administered GSK573719 will be determined using currently approved methods. Samples from control and naïve animals will also be analyzed. All animals found dead or sacrificed in a moribund condition during the study will be subjected to necropsy and tissue samples will be preserved. At terminal necropsy, a full list of tissues will be examined from main study animals. A limited examination of toxicokinetic animals will be conducted with no retention of tissues. All specified tissues from all animals will be prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin and examined.

15.2. Tissue Preservation

On completion of the necropsy of each animal, the following tissues and organs will be retained. Neutral buffered 10% formalin will be used for fixation and preservation unless otherwise indicated:

	abnormalities
a	animal identification
	adrenals
	aorta (thoracic)
	brain (cerebrum, cerebellum, midbrain and medulla oblongata)
	cecum
	colon
h	drainage lymph node of clinically observed external mass
	duodenum
	epididymides
	esophagus
cf	eyes with optic nerves
e	femur (femoro-tibial joint)
	gall bladder
	harderian glands
	heart (including section of aorta)
	ileum
	jejunum
	kidneys
	larynx (3 levels)
	liver (sample of 2 lobes)
g	lungs (all lobes)
	lymph nodes (mandibular, mesenteric and tracheobronchial)
b	mammary gland (inguinal)
cg	nasal cavities/skull (3 levels)
ag	nasopharynx
	ovaries
	pancreas
	pituitary
	preputial/clitoral gland
	prostate
	rectum
	salivary gland (mandibular, sublingual, parotid)
	sciatic nerve
	seminal vesicles
	skeletal muscle (hind-limb)
	skin (inguinal)
	spinal cord (cervical[a], thoracic[a] and lumbar)
	spleen

e	sternum (bone marrow)
	stomach
d	testes
	thymus/thymic area
f	thyroid lobes (and parathyroids)
	tongue
	trachea
	tracheal bifurcation (with mainstem bronchi)
	urinary bladder
	uterus (horns, body and cervix)
	vagina

[a] Retained but not processed.
[b] Examined only if present in routine section of skin (mammary gland).
[c] Fixed in Davidson's fluid (all animals).
[d] Fixed in Bouin's fluid (all animals).
[e] Bone decalcified prior to sectioning.
[f] At least one parathyroid and one optic nerve should be examined. One recut and/or recheck of wet tissues will be performed to try to find missing tissues.
[g] Infused with neutral buffered 10% formalin (all animals).
[h] Retained only if gross lesion is present.

Evaluation: General aspects of the study design appear acceptable; however, the dose selection is not considered appropriate. In a 13-week inhalation toxicology study, mice received GSK573719 at estimated achieved doses up to 2.85 mg/kg/day. Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain with the possible exception of males in the high dose (2.85 mg/kg/day) group. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. A potential dose-limiting finding in the larynx was ventral cartilage necrosis observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of relatively low incidence (i.e., 1 of 10 and 2 of 12, respectively). Further, the finding for 1 female at 0.092 mg/kg/day was graded as minimal. A potential dose-limiting finding in the nasal turbinates was slight ulceration of the olfactory epithelium for 1 male at 2.85 mg/kg/day. Based upon findings of ventral cartilage necrosis in larynx for males at 1.06 and 2.85 mg/kg/day and females at 0.092, 0.287, 1.06, and 2.85 mg/kg/day, maximum tolerated doses were identified as 0.6 mg/kg/day for males and 0.2 mg/kg/day for females.

PROTOCOL FOR AN INHALATION CARCINOGENICITY STUDY OF A POWDER AEROSOL FORMULATION IN THE RAT

Outline of Sponsor's Draft Protocol: The carcinogenic potential of GSK5737I9A will be assessed in Sprague-Dawley rats following daily nose-only inhalation exposure for a minimum of 104 consecutive weeks. The proposed high dose of 300 µg/kg/day is based on toxicokinetic and toxicity endpoints that were established in 4-week and 13-week inhalation toxicology studies with rats. Proposed mid and low doses are 100 and 30 µg/kg/day, respectively. The high dose of 300 µg/kg/day was selected as the high dose for the carcinogenicity study based on reductions of food consumption and bodyweight gain observed in the 13-week inhalation toxicology study.

The study is scheduled to be conducted at [redacted] (b) (4) (b) (4). For the carcinogenicity evaluation, there will be four groups of 60 rats/sex/group. For the toxicokinetic assessment, there will be four groups of 6 rats/sex/group. Rats will be obtained from [redacted] (b) (4) At the start of treatment, rats will be 5 to 6 weeks old and body weight ranges will be 79-97 g for males and 73-87 g for females. The test material will be administered by nose-only inhalation since this is comparable to the intended route of administration in humans (oral inhalation). Vehicle-control rats will receive lactose blended with a nominal 1 % w/w magnesium stearate. Treated rats will receive 25% w/w GSK573719A blended in lactose with a nominal 1 % w/w magnesium stearate.

Group	Treatment	Dose (mg/kg/day)* #	Number of animals			
			Main study		Satellite study†	
			Male	Female	Male	Female
1	Control	0	60	60	6	6
2	GSK573719A	Low	60	60	6	6
3	GSK573719A	Intermediate	60	60	6	6
4	GSK573719A	High	60	60	6	6

†Satellite animals used for Toxicokinetic sampling only.

\# Expressed in terms of parent compound GSK573719. A conversion factor will be used, and documented in a file note.

* Dose (mg/kg/day) to be selected by the Sponsor and documented in an amendment to the protocol.

The vehicle and test substance blends (nominal 25% w/w in lactose with 1 % w/w magnesium stearate) will be formulated in the [redacted] (b) (4) under Study Number BVR/222, under which no formal claim of GLP compliance will be made. A Certificate of Analysis for the blend will be provided and lodged in the study data. Data concerning the identity, purity and stability of the blend will be generated within a GMP environment.

The test atmosphere will be generated using a Wright Dust Feed Mechanism. The mechanism is designed to produce and maintain test atmospheres containing dust by suspending material scraped from the surface of a compressed powder in a stream of dry air. The concentration of dust in the air may be altered by selecting one of two sizes of dust container and by changing the gear ratio, and hence the speed of rotation against the scraper blade. A regulated flow of compressed air will conduct the aerosol to the inhalation chamber. ADG exposure chambers, having a variable number of sections with 20 exposure ports each and a top section incorporating a central aerosol inlet with a tangential air inlet, will be used for this study. A separate chamber will be used for

each group. During exposure, the rats will be held in restraining tubes with their snouts protruding from the ends of the tubes into the exposure chambers. Each exposure system will be housed in a separate extract cabinet to avoid possible cross-contamination between groups. The duration of daily exposure will be 60 min. Chamber concentrations of GSK573719 and magnesium stearate will be monitored during the study. Particle size determinations will also be conducted.

Animals will be observed at least twice daily for evidence of reaction to treatment or ill-health. Physical examination will be conducted once weekly. Rats will be examined weekly for palpable masses. Body weights and food consumption will be measured weekly for the first 16 weeks and every 4 weeks thereafter. Ophthalmic examinations will be conducted during pretreatment and weeks 13 (or 26), 52, 78, and 103. Blood samples for measurement of hematology parameters will be obtained at week 104. Blood samples for measurement of plasma drug concentrations will be collected during weeks 4 and 26 at 0, 0.5, 1, 2, 4, 4, 8, and 24 hr after completion of dosing. Concentrations of GSK573719 in plasma from all animals, including controls, will be determined by HPLC-MS/MS. All animals found dead or sacrificed in a moribund condition during the study will be subjected to necropsy and tissue samples will be preserved. At terminal necropsy, a full list of tissues will be examined from main study animals. All specified tissues from all animals will be prepared for histopathological examination by embedding in paraffin wax, sectioning, and staining with hematoxylin and eosin followed by examination by light microscopy.

TABLE 1 - Pathology procedures

Tissue and regions to be examined	Necropsy Fix	Histology	Pathology Light microscopy
Abnormalities	*	*	*
Adrenals	*	*	*
Aorta - thoracic	*	*	*
Brain (cerebellum including medulla, cerebrum, midbrain)	*	*	*
Caecum	*	*	*
Cervix	*	*	*
Colon	*	*	*
Duodenum	*	*	*
Epididymides	*	*	*
Eyes**	*	*	*
Femur (femoro-tibial joint)	*	*	*
Harderian glands	*	*	*
Heart, 3 levels anterior, mid and posterior portions	*	*	*
Ileum	*	*	*
Jejunum	*	*	*
Kidneys	*	*	*
Larynx (2 levels)	*	*	*
Liver (section from all main lobes)	*	*	*
Lungs including bronchi (sections from all major lobes) ‡	*	*	*
Lymph nodes – cervical	*	*	*
– mesenteric	*	*	*
– tracheobronchial	*	*	*
Nasal cavity incorporating skull (3 levels)	*	*	*
Nasopharynx (1 level)	*	*	*
Oesophagus	*	*	*
Optic nerves	*	*	*
Ovaries	*	*	*
Pancreas	*	*	*
Pituitary	*	*	*
Preputial/clitoral glands	*	*	*
Prostate	*	*	*
Rectum	*	*	*
Salivary glands (mandibular, sublingual and parotid)	*	†	†
Sciatic nerves	*	†	†
Seminal vesicles	*	*	*
Skeletal muscle (Gastrocnemius muscle)	*	†	†
Skin with mammary glands (inguinal area)	*	*	*
Spinal cord (transverse and longitudinal sections at the lumbar level)	*	*	*
Spinal cord (whole cord)	*	#	#
Spleen (transverse section)	*	*	*
Sternum with bone marrow	*	*	*
Stomach (keratinised, glandular and pyloric)	*	*	*
Testes	*	*	*
Thymus	*	*	*
Thyroid with parathyroids	*	*	*
Tongue	*	*	*
Trachea (including bifurcation)	*	*	*
Urinary bladder	*	*	*
Uterus	*	*	*
Vagina	*	*	*

* Samples fixed or sections examined microscopically.
** Eyes will be retained separately with left and right identified and the north pole of each eye marked for orientation at histopathology.
\# Examined if effects suspected during the study. † Only one examined. Animal ID retained.
‡ At least 4 lobes examined, preferably left and right, to include proximal and distal areas.

Evaluation: General aspects of the study design appear acceptable; however, the dose selection is not considered appropriate. In the 13-week inhalation toxicology study, rats received GSK573719 at estimated achieved doses up to 924 µg/kg/day. Body weight gains for males in the 102, 288, and 924 µg/kg/day groups were decreased to 84.8, 82.9, and 78.7% of the control, respectively. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study. Body weight gains for females in the 288 and 924 µg/kg/day groups were decreased to 89.3 and 81.3% of the control, respectively. The decrease for females in the 288 µg/kg/day group did not appear to be toxicologically significant. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. For the larynx, minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at doses ≥38 µg/kg/day.

These changes were not reversible. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study. However, minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting. Given that minimal focal necrosis in the larynx observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study, identification of maximum tolerated doses for males and females were based primarily on decreases of body weight gain. Based upon decreased body weight gains for males and females at 924 µg/kg/day, the MTD was identified as 288 µg/kg/day.

2.6.6.9 Discussion and Conclusions

In inhalation toxicology studies with mice and rats, toxicity was generally confined to the respiratory tract. Target organs of toxicity for both mice and rats were the nasal cavity/sinuses, nasopharynx, larynx, and tracheobronchial bifurcation. Potential findings in the lung were only observed in the 4-week inhalation study with rats. Findings in the tracheal bifurcation and lung were not observed in the 13-week inhalation study with rats. Potential findings of systemic toxicity were unclear.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:
The sponsor has proposed to evaluate the carcinogenic potential of GSK573719 in mice and rats by daily nose-only inhalation exposure for a minimum of 104 weeks. Based upon in vitro studies conducted with mouse, rat, and human hepatocytes, metabolism of GSK573719 appears to be relatively comparable between these three species. GSK573719 does not possess a genotoxic potential based upon results from the in vitro bacterial reverse mutation assay, in vitro mouse lymphoma assay, and in vivo rat micronucleus test. The sponsor has proposed inhalation doses of 0.1, 0.3, and 1.0 mg/kg/day for the 2-year carcinogenicity study with mice and inhalation doses of 30, 100, and 300 µg/kg/day for the 2-year carcinogenicity study with mice. Dose selection was primarily based upon 13-week inhalation toxicology studies conducted with mice and rats. The sponsor also conducted 2-week and 4-week inhalation toxicology studies with mice and rats, respectively that provide supportive information.

Design and dose selection for the 2-year inhalation carcinogenicity with mice:
General aspects of the study design appear acceptable; however, the dose selection is not considered appropriate. In the 13-week inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day. Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively. Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain. Incidences of slight or moderate retinal folds were increased for male and female treatment groups. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Potential treatment-related findings were observed in the liver and uterus. Histopathological findings were evident in the larynx at

all doses and appeared to be nonspecific irritant effects associated with inhalation exposure. Histopathological findings were observed in the nasal turbinates at doses ≥0.287 mg/kg/day. Histopathological findings were observed in the nasopharynx at doses of 1.06 and 2.85 mg/kg/day. Histopathological findings in the tracheal bifurcation, liver, and uterus were observed at 2.85 mg/kg/day. A potential dose-limiting finding in the larynx was ventral cartilage necrosis observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of relatively low incidence (i.e., 1 of 10 and 2 of 12, respectively). Further, the finding for 1 female at 0.092 mg/kg/day was graded as minimal. A potential dose-limiting finding in the nasal turbinates was slight ulceration of the olfactory epithelium for 1 male at 2.85 mg/kg/day. Based upon findings of ventral cartilage necrosis in larynx for males at 1.06 and 2.85 mg/kg/day and females at 0.092, 0.287, 1.06, and 2.85 mg/kg/day, maximum tolerated doses were identified as 0.6 mg/kg/day for males and 0.2 mg/kg/day for females. Recommended doses in the 2-year carcinogenicity study for male mice are 0.06, 0.20, and 0.6 mg/kg/day. Recommended doses for female mice are 0.02, 0.06, and 0.2 mg/kg/day.

Design and dose selection for the 2-year inhalation carcinogenicity study with rats: General aspects of the study design and dose selection appear acceptable. In the 13-week inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day. Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively. Body weight gains for males in the 102, 288, and 924 µg/kg/day groups were decreased to 84.8, 82.9, and 78.7% of the control, respectively. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study. Body weight gains for females in the 288 and 924 µg/kg/day groups were decreased to 89.3 and 81.3% of the control, respectively. The decrease for females in the 288 µg/kg/day group did not appear to be toxicologically significant. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. For the larynx, minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at doses ≥38 µg/kg/day. These changes were not reversible. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study. However, minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting. Given that minimal focal necrosis in the larynx observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study, identification of maximum tolerated doses for males and females were based primarily on decreases of body weight gain. Based upon decreased body weight gain for males and females at 924 µg/kg/day, the MTD was identified as 288 µg/kg/day. Recommended doses in the 2-year carcinogenicity study for male and female rats are 30, 100, and 300 µg/kg/day.

Systemic Exposure Comparisons between Nonclinical Species and Humans: Systemic exposures in the 13-week inhalation toxicology studies with mice and rats are compared to the exposure at the highest anticipated clinical dose of 1000 µg/day in the

table below. The sponsor proposed maximum doses of 1.0 mg/kg/day for mice and 300 µg/kg/day for rats in 2-year inhalation carcinogenicity studies. These doses do provide exposure margins >1 relative to the highest proposed clinical dose; however, the sponsor's proposed maximum dose for mice was judged to be generally inappropriate. For the 2-year inhalation carcinogenicity study with mice, the reviewer recommends maximum doses of 0.6 and 0.2 mg/kg/day for males and females, respectively, which provide extrapolated 2.0 and 0.6 exposure margins for the highest proposed clinical dose. For the 2-year inhalation carcinogenicity study with rats, the reviewer concurs with the sponsor's proposed doses.

Study	Sex	Estimated achieved dose, mg/kg/day	AUC_{0-t} ng·hr/mL	Exposure margins for clinical dose of 1000 µg/day (8.64 ng·hr/mL[a])
13-week inhalation study with mice	Males	0.0923	6.06	0.70
		0.287	4.52	0.52
		1.06[b]	29.5	3.41
		2.85	67.1	7.77
	Females	0.0923	5.99	0.69
		0.287	7.82	0.91
		1.06[b]	42.5	4.92
		2.85	77.6	8.98
13-week inhalation study with rats	Males	0.038	4.09	0.47
		0.102	5.93	0.69
		0.288[b,c]	15.8	1.83
		0.924	22.6	2.62
	Females	0.038	3.88	0.45
		0.102	7.04	0.81
		0.288[b,c]	13.5	1.56
		0.924	36.2	4.19

a. The steady-state AUC_T achieved following an inhaled dose of 1000 µg/day is 8.64 ng·hr/mL (estimated based on day 14 in healthy subjects).
b. Exposure at sponsor's recommended high dose for the 2-year inhalation carcinogenicity study.
c. Exposure at reviewer's recommended high dose for the 2-year inhalation carcinogenicity study.

Internal comments: The ECAC will convey comments regarding design and dose selection for the 2-year inhalation carcinogenicity studies with mice and rats. Information is being requested from the sponsor regarding findings in the 4-week study with rats and 13-week study with mice.

Recommendation:
1. Recommended doses in the 2-year inhalation carcinogenicity study with mice are as follows:
Male mice: 0.06, 0.20, and 0.6 mg/kg/day
Female mice: 0.02, 0.06, and 0.2 mg/kg/day

2. Recommended doses in the 2-year inhalation carcinogenicity study with rats are as follows:
Male and Female rats: 30, 100, and 300 µg/kg/day

External comments (to sponsor): The following comments should be conveyed to the sponsor.

Draft Letter:
Your nonclinical submissions provided on April 30, 2009 are currently under review. The following information is requested to facilitate this review.

1. With reference to the 13-week inhalation toxicology study with CD-1 mice (WD2007/01600/00), incidences of slight or moderate retinal folds were increased for male and female treatment groups as compared to concurrent control. Provide the historical control mean and range of these findings from the testing laboratory over the past 5 years.

2. With reference to the 4-week inhalation toxicology study with rats (WD2005/01422/00), stage-dependent evaluation of spermatogenesis was conducted in male rats and estrous cyclicity was assessed in female rats. It appears that only summary information was provided in the study report. Provide the actual data or direct us to where this information is provided in the study report.

Signatures (optional):

Reviewer Signature _____
Timothy W. Robison, Ph.D.

Supervisor Signature _____
Molly E. Shea, Ph.D.
Concurrence Yes ___ **No** ___
cc:
IND 104,479, HFD-570 Division Files
RaggioM, HFD-570
SheaM, HFD-570
RobisonT, HFD-570

Linked Applications	Sponsor Name	Drug Name / Subject
IND 104479	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	GSK573719 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.

/s/

TIMOTHY W ROBISON
06/03/2009

MOLLY E SHEA
06/03/2009
I concur.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 104,479
Review number: #004
Sequence number/date/type of submission: #009/July 14, 2009/Initial Submission
#010/July 14, 2009/Amendment
#014/September 17, 2009/Amendment

Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: GlaxoSmithKline
P.O. Box 13398
Five Moore Drive
Research Triangle Park
North Carolina 27709-3398
Manufacturer for drug substance: SmithKline Beecham (Cork) Ltd
Currabinny
Carrigaline
Co. Cork
Ireland

Glaxo Wellcome Manufacturing Pte Ltd
1 Pioneer Sector 1
Jurong
Singapore 628413

SmithKline Beecham Corporation d/b/a*
GlaxoSmithKline
Five Moore Drive
Research Triangle Park
North Carolina 27709
USA

Glaxo Operations UK Ltd (trading as
Glaxo Wellcome Operations)
Priory Street
Ware
Hertfordshire SG 12 ODJ
UK

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Products
HFD #: 570
Review completion date: May 25, 2010

Reference ID: 3330354

Drug:

Code name: GSK573719

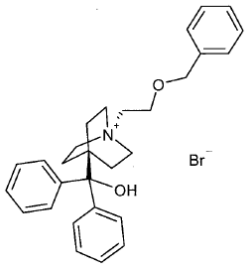
Chemical name: 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide

Molecular formula/molecular weight: $C_{29}H_{34}NO_2 \cdot Br$

508.5 (GSK573719A as quaternary ammonium bromide compound)

428.6 (as free cation, GSK573719)

Structure:



Relevant INDs/NDAs/DMFs:

IND 74,696 (GSK, GW642444 Inhalation Powder, Long-acting β_2 agonist)

IND [redacted] (b) (4)

IND 77,855 (GSK, GW642444 + Fluticasone)

IND [redacted] (b) (4)

IND 106,616 (GSK, GSK573719 + GW642444)

Drug class: Quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist

Intended clinical population: Chronic obstructive pulmonary disease (COPD)

Clinical formulation:

Table 1 Composition of the GSK573719 Blister Strip for GSK573719 Inhalation Powder, 62.5, 125, 250, 500, and 1000 µg

Component	Quantity per blister[1]				Function	Reference to Standard
GSK573719A (micronised (b) (4)	74.1 µg	148.3 µg	296.5 µg	593.0 µg[3]	Active	GlaxoSmithKline
Magnesium Stearate	(b) (4)				(b) (4)	USP/NF or Ph Eur
Lactose Monohydrate						USP/NF or Ph Eur

Note:
1. (b) (4)
2.
3. The 1000 µg dose product consists of two 500 µg blister strips in the Novel DPI.

Table 2 Composition of (b) (4) Blister Strip for GSK573719 Inhalation Powder, 62.5, 125, 250, and 500 µg

Component	Quantity per blister[1]	Function	Reference to Standard
Magnesium Stearate	125 µg	(b) (4)	USP/NF or Ph Eur
Lactose Monohydrate	to 12.5 mg		USP/NF or Ph Eur

Note:
1. (b) (4)

Route of administration: Inhalation

Proposed clinical protocol:
The sponsor has proposed a multicenter, randomized, double-blind, double-dummy, placebo-controlled, three-way cross-over, incomplete block design study to evaluate 5 doses of GSK573719 administered once-daily and 3 doses of GSK573719 administered twice-daily via a Novel DPI over a 14 day treatment period in subjects (M or F, 40 to 80 years old) with COPD. The study includes an open-label active control, tiotropium. Treatment with GSK573719 and placebo will be by the Novel DPI and will be double-

blind. A double-dummy technique will be used to maintain the blind between once- and twice-daily doses of GSK573719 and placebo. Treatment with tiotropium (18 µg/day) will be via the HandiHaler and will be open label.

Subjects will be randomized to receive placebo and 2 of the 9 active treatments in a random order (total of 28 days of treatment with GSK573719).

A total of 170 patients will be enrolled in this clinical trial.

Once daily doses: 62.5, 125, 250, 500, and 1000 µg/day
Twice daily doses: 62.5, 125, and 250 mg BID

The primary endpoint will be trough FEV1 defined as the 24 hour postdose assessment obtained at the end of each 14 day treatment period.

Table 1 Possible Study Treatments

Treatment Arm	Inhaler A (morning)	Inhaler B (evening)
GSK573719 1000mcg once-daily	1000mcg	Placebo
GSK573719 500mcg once-daily	500mcg	Placebo
GSK573719 250mcg once-daily	250mcg	Placebo
GSK573719 125mcg once-daily	125mcg	Placebo
GSK573719 62.5mcg once-daily	62.5mcg	Placebo
GSK573719 250mcg twice-daily	250mcg	250mcg
GSK573719 125mcg twice-daily	125mcg	125mcg
GSK573719 62.5mcg twice-daily	62.5mcg	62.5mcg
Tiotropium once-daily	18mcg	Placebo
Placebo (all subjects)	Placebo	Placebo

A female is eligible to enter and participate in this study if she is of non-childbearing potential (i.e., physiologically incapable of becoming pregnant), including any female who is post-menopausal. If indicated, menopause can be confirmed by serum follicle stimulating hormone (FSH) levels >40 mIU/mL and estradiol <40pg/mL (<140pmol/L).

Previous Clinical Experience: Five clinical pharmacology studies have been conducted in a total of 91 healthy volunteers and 48 COPD subjects to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single and repeat dose administration of GSK573719. Doses of up to 1000 µg once daily have been examined. A total of 74 healthy volunteers and 42 COPD subjects have been dosed with

GSK573719. Completed studies comprise two single dose studies in healthy volunteer subjects (AC4105209) and COPD subjects (AC4108123), and two repeat dose studies in healthy volunteer subjects (AC4106889 and AC4110106).

In these completed studies, GSK573719 was generally well tolerated. The most commonly reported AE across studies has been headache, although this has been reported at no higher rates in active treatment groups than with placebo. No SAEs have been reported during these studies. There have been no consistent changes in vital signs, ECG or laboratory parameters that suggest a causal association with treatment. Since gall bladder distension had been identified in a 14 day dog toxicological study, an exploratory ultrasound examination of the gall bladder was performed in study AC4106889 to determine if a similar effect was seen in man. Four patients were judged to have gallbladder enlargement (1 in the placebo group, 3 at the top dose of GSK573719 (1000 µg)). There is no recognized normal value for gallbladder size, so these results were of uncertain significance.

Table 1 Summary of Completed GSK573719 Clinical Studies

Study Number	Design	Number of Subjects	Treatments	Formulation and Device
Studies in Healthy Volunteers				
AC4105209	R, DB, PC, XO Single Dose Ascending	19	GSK573719 - 10, 20, 60, 100, 250 & 350 mcg, tiotropium 18 mcg, or placebo	Lactose (b) (4) via DISKUS
AC4106889	R, DB, PC, PG, 14-Day Repeat Dose Ascending	36	GSK573719 - 250 and 750, 1000 mcg QD or placebo	Lactose (b) (4) via DISKUS
AC4110106	R, DB, PC Dose Ascending, Single and 7-day Repeat dose	36	Part 1: Normal CYP2D6 subjects: GSK573719 – 100, 500, and 1000 mcg or placebo single dose then 500 or 1000 µg or placebo QD 7-days Part 2: Poor CYP2D6 activity subjects: GSK573719 – Cohort 1) 100 mcg or placebo single dose then100 mcg or placebo QD 7-days, then 500 mcg or placebo single dose then 500 mcg or placebo QD 7 days. Cohort 2) 500 mcg or placebo single dose, then 500 mcg or placebo QD 7 days, then 1000 mcg or placebo single dose, then 1000 mcg or placebo QD 7 days	Lactose/MgSt via Novel DPI
Studies in COPD subjects				
AC4108123	R, DB, PC, XO, Single Dose Ascending	24	GSK573719 - 250, 500 & 1000 mcg, tiotropium 18 mcg, or placebo	Lactose (b) (4) via DISKUS
AC4105211	R, DB, PC, PG,7-Day, Repeat Dose Ascending	38	GSK573719 – 250mcg QD, 1000mcg QD, or placebo	Lactose/MgSt via Novel DPI

R=randomised, DB=double-blind, PC=placebo-controlled, PG=parallel group, XO=crossover, QD=once daily, (b) (4) MgSt=magnesium stearate, CDY2P6= cytochrome P450 Isoenzyme 2D6; DPI=dry powder inhaler

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

Studies reviewed within this submission:

STUDY TITLE:	STUDY NUMBER:
PHARMACOLOGY:	
SAFETY PHARMACOLOGY:	
Neurological effects:	
GSK573719A: Acute Neurobehavioral Effects Following Inhalation Administration in the Conscious CD™ Rat	VD2005/00625/00
Cardiovascular effects:	
In Vitro hERG Channel Selectivity Screen	CH2006/00029/00
Effect on hERG Tail Current Recorded from Stably Transfected HEK293 Cells	FD2005/00109/00
The Effects of Single Intravenous Administration of a Range of Doses on Cardiovascular Function in the Conscious Beagle Dog	FD2005/00167/00
Pulmonary effects:	
Acute Effects on Respiratory Function Following Inhalation Administration in the Conscious CD™ Rat	CD2005/01385/02
PHARMACOKINETICS/TOXICOKINETICS:	
Absorption	
In Vitro	
An In Vitro Investigation of Both the Transport via Heterologously Expressed Human P-Glycoprotein and the Passive Membrane Permeability of [^{14}C]GSK573719 in MDCKII-MDR1 Cells	WD2006/02657/00
An In Vitro Investigation of the Inhibition by GS573719A of Xenobiotic Transport via Human P-Glycoprotein, Heterologously Expressed in MDCKII Cells	WD2006/02596/00
Mice	
A Study to Determine the Oral Absorption and Disposition of [^{14}C]GSK573719 following a Single Administration of [^{14}C]GSK573719 (trifluoroacetate) at a Target Dose Level of 40 µg free base/kg in the FVBn and mdr1a/1b Mouse using the Short Oral Absorption Model	WD2008/00001/00
Rats	
Pharmacokinetics of GSK573719A in the Male Sprague-Dawley Rat	CH2006/00004/00
Pharmacokinetics of GSK573719A Following Intravenous Infusion, Portal Infusion, and Oral Administration in Male Sprague-Dawley Rats	CH2006/00012/00
A Study to Investigate the Pharmacokinetics for GSK573719 in Male CD/SD Rats Following Intravenous Infusion at Target Dose Level of 0.5 mg/kg	WD2006/00073/00
Dogs	
Pharmacokinetics of GSK573719A in the Male Beagle Dog	CH2006/00001/00
A Study to Investigate the Pharmacokinetics of GSK573719 in Male Beagle Dogs Following Intravenous Infusion at Target Dose Levels of 0.5 mg free base/kg	WD2006/00075/00
Distribution	
Rats	
Quantitative Whole-Body Autoradiography Following a Single Oral or a Single Intravenous Administration of [^{14}C]GSK573719 bromide to Male Lister Hooded Pigmented Rats at a Target Dose Level of 1 mg free base/kg	FD2005/00236/00
A Study to Estimate the Lung Retention and Systemic Exposure of GSK573719A Following Intranasal Administration in the Male Balb/c Mouse	CH2006/00002/00
Investigation of the Brain to Plasma Ratio of GSK573719A in the Conscious Male Sprague-Dawley Rat	CH2006/00013/00
Metabolism	
In Vitro	
A Preliminary In Vitro Investigation into the Human Oxidative Enzymology of GSK573719	WD2006/00367/00

A Preliminary Screen of the In Vitro Concentration-Dependent Inhibition of Human Cytochrome P450 Enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by GSK573719A	CH2005/00950/00
The Effect of GSK573719A on the mRNA Levels of Cytochrome P450 Genes in Rat Livers Obtained Following Inhaled Administration at 30, 200, and 2000 µg/kg/day for 28-Days	WD2005/01627/00
Rats	
A Study to Investigate the Biotransformation of [^{14}C]-GSK573719 in the Isolated Perfused Rat Liver Model	WD2005/01195/00
Quantification and Identification of the Metabolites of GSK573719 in the Male Beagle Dog Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide at 1 mg free base/kg	WD2006/00250/00
Excretion	
Dogs	
Elimination of Drug-Related Material Following a Single Oral or a Single Intravenous Administration of [^{14}C]GSK573719 Bromide to Male Beagle Dogs at a Target Dose Level of 1 mg free base/kg	FD2005/00164/00
Elimination of Drug-Related Material Following Intravenous Administration of [^{14}C]GSK573719 Trifluoroacetate to Male Bile Duct-Cannulated Beagle Dogs over Various Time Intervals and at Various Target Dose Levels	WD2007/01907/00
TOXICOLOGY:	
Rats	
14-Day Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat (Magnesium Stearate Bridging Study)	WD2006/03225/00
Dogs	
A 14-Day Study to Examine the Influence of Magnesium Stearate on the Toxicity and Toxicokinetics of GSK573719A by Inhalation Administration to Beagle Dogs	WD2006/03669/00
A 28-Day Inhalation Toxicity Study of a Powder Aerosol Formulation in the Beagle Dog	WD2005/01423/00
A 28-Day Investigative Toxicity of a Powder Aerosol Formulation in the Beagle Dog	WD2006/03294/00
Toxicity Study by Inhalation Administration to Beagle Dogs for 13 Weeks Followed by a 4-Week Recovery Period	WD2007/01512/00
Dose Range Finding Study by Inhalation Administration to Beagle Dogs for 4 Weeks (Non-GLP Study)	WD2008/01504/00
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
Fertility and early embryonic development	
Inhaled Female Fertility and Early Embryonic Development Study in Rats	WD2007/00763/00
Embryofetal development	
Inhaled Embryo-Fetal Development Study in Rats	WD2007/00764/00
Inhaled Embryo-Fetal Development Study in Rabbits	WD2007/00762/00

The following studies were reviewed in Review #01 dated June 3, 2009

STUDY:	**STUDY NUMBER:**
PHARMACOKINETICS/TOXICOKINETICS:	
Distribution:	
In Vitro Plasma Protein Binding of GSK573719 and Blood Cell Association of [^{14}C]GSK573179 in Mouse, Rat, Rabbit, Dog, and Human	WD2008/00503/00
Metabolism:	
A Preliminary Study To Estimate the Intrinsic Clearance of GSK573179A in Rat, Dog, and Human Hepatocytes	CH2006/00011/00
An In Vitro Investigation of the Metabolism of [^{14}C]GSK573719 in Human, Rat, and Dog	WD2006/00147/00

An In Vitro Investigation of the Metabolism of [^{14}C]GSK573719 in Human, Female Rabbit, and Mouse Hepatocytes	WD2007/01370/00
Quantification and Identification of the Metabolites of GSK573719 in the Male Sprague-Dawley Rat Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide at 1 mg free base/kg	WD 2006/00172/00
Preliminary Characterization of Metabolites of GSK573719 in Human Plasma and Urine	WD2009/00030/00
Excretion:	
Elimination of Drug-Related Material Following a Single Oral or Single Intravenous Administration of [^{14}C]GSK573719 Bromide to Male Sprague-Dawley Rats at a Target Dose Level of 1 mg free base/kg	FD2005/00208/00
TOXICOLOGY:	
Repeat Dose Toxicity:	
Preliminary Toxicity Study by Inhalation Administration to CD-1 Mice for 2 Weeks	WD2007/00074/00
Toxicity Study by Inhalation Administration to CD-1 Mice for 13 Weeks	WD2007/01600/00
A 28-Day Inhalation Toxicity Study of a Powdered Aerosol Formulation in the Rat	WD2005/01422/00
A 13-Week Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat with a 4-Week Recovery Period	WD2007/02012/00
GENETIC TOXICOLOGY:	
Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli*	WD2005/00750/00
In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus	WD2005/00751/00
Intravenous Bone Marrow Micronucleus Assay in Rats	WD2005/01079/00
CARCINOGENICITY:	
DRAFT PROTOCOL FOR AN INHALATION CARCINOGENICITY STUDY OF A POWDER AEROSOL FORMULATION IN THE MOUSE	
PROTOCOL FOR AN INHALATION CARCINOGENICITY STUDY OF A POWDER AEROSOL FORMULATION IN THE RAT	

The following studies are reviewed under IND 106,616

STUDY:	**STUDY NUMBER**:
SAFETY PHARMACOLOGY:	
Cardiovascular effects:	
GW642444M and GSK573719A: The Effect of Single Intravenous Administration on Cardiovascular Function in the Conscious Beagle Dog	FD2008/00365/00
TOXICOLOGY:	
GW642444M and GSK573719A: Toxicity Study by Inhalation Administration to Rats for 4 Weeks	FD2009/00392/00
GSK573719A and GW642444M: A 4-Week Combination Inhalation Toxicity Study in Dogs	FD2009/00391/00

Studies not reviewed within this submission:

1. A dose range finding study by inhalation administration to rats for 7 days (WD2004/01556/00).
2. A 7-day dose range-finding inhalation toxicity study of a nebulized aerosol formulation in the beagle dog (WD2005/00370/00).
3. A 7-day inhalation dose range finding toxicity study of a powder aerosol formulation in the beagle dog (WD2005/01061/00).
4. A 7-day inhalation dose range finding toxicity study of a powder aerosol formulation in the rat (WD2005/01063/01).

5. A dose range finding study by inhalation administration to beagle dogs (WD2006/03228/00).
6. Single and 4-day intravenous (infusion) irritancy and toxicity study in male Sprague-Dawley rats (FD2006/00339/00).
7. In vitro hemolysis testing in rat and dog blood (CD2005/00038/00).
8. In vitro hemolytic potential in rat, dog, and human peripheral blood (WD 2008/01499/00).
9. The abbreviated validation of a method for the determination of GSK573719 in mouse plasma (range 0.1 to 100 ng/mL) using HPLC-MS/MS (WD2006/03558/00).
10. Method for the determination of GSK573719 (range 0.10 to 100 ng/mL) in rat plasma using HPLC-MS/MS (WD2004/01614/00).
11. The abbreviated validation of a method for the determination of GSK573719 in rat plasma (range 0.1 to 100 ng/mL) using HPLC-MS/MS (WD2005/00426/00).
12. The abbreviated validation of a method for the determination of GSK573719 and GW642444 (ranges 0.1 to 100 ng/mL) in rat plasma using HPLC-MS/MS (WD2008/00583/00).
13. The abbreviated validation of a method for the determination of GSK573719 in rabbit plasma (range 0.1 to 50 ng/mL) using HPLC-MS/MS (WD2006/03559/00).
14. The abbreviated validation of a method for the determination of GSK573719 (range 0.1 to 100 ng/mL) in dog plasma using HPLC-MS/MS (WD2005/00427/00)
15. The validation of a method for the determination of GSK573719 and GW642444 (range 0.1 to 100 ng/mL) in dog plasma using HPLC-MS/MS (WD2008/00560100).
16. The validation of a method for the determination of GSK573719 in human plasma (range 0.02 - 10 ng/mL) using HPLC-MS/MS (WD2006/00081/00).
17. The validation of a method for the determination of GSK573719 in human urine (range 0.1 - 50 ng/mL) using HPLC-MS/MS (WD2006/03251/00).
18. The abbreviated validation of a method for the determination of GSK573719 in human urine (range 0.1 to 50 ng/mL) using HPLC-MS/MS (WD2008/00425/00).

Drug History: A Pre-IND meeting was held with the sponsor on May 26, 2009. Nonclinical issues discussed during the meeting are listed below.

Nonclinical

***Question 1**: Nonclinical Data Supporting Studies up to 28 Days Duration*

Does the Division agree that the current nonclinical package is adequate to support dosing for up to 28 days in patients with COPD?

Division Response: Pending review of the second 4-week investigative dog and the 13-week rat and dog inhalation toxicology studies, your proposed clinical dosing up to 28-days appears to be supported.

Confirm that the second 4-week investigative inhalation toxicology study with dogs (1130 and 2390 µg/kg/day) was a GLP study. The NOAEL appears to be at 1130 µg/kg/day; however, this group was composed of only three male dogs. Additionally, justify the lack of inclusion of female dogs in this group for determination of a NOAEL.

NOAELs were not identified in the 4-week toxicology study with dogs (16.2, 208, or 2758 µg/kg/day) or first 4-week investigative toxicology study with dogs (22, 2254, and 1835 µg/kg/day). Based on local toxicity observed in these 4-week studies and the absence of local toxicity in the 13-week dog study, adequate characterization of the local toxicity in the 13-week study is in question (see below).

Discussion: GSK stated that the second 4-week investigative study was not a GLP study. The 1130 µg/kg/day group was composed of only 3 male dogs although the 2390 µg/kg/day group was composed of 3 dogs/sex/group.

GSK contended that results of the first GLP 4-week investigative dog study established that findings of lung granulomas in the GLP 4-week toxicology dog study were due to the feeding regime.

GSK noted that exposure to the test article in the GLP 13-week dog study, both on a local and systemic basis, was lower than expected in comparison to toxicokinetic results obtained from other studies. However, systemic exposure at the NOAEL provided safety margins >1 on an AUC basis for the proposed clinical doses. There were no treatment-related histopathological findings in the gall bladder from dogs in this study.

The Division stated that a NOAEL was not identified in 4-week toxicology study with dogs due to findings in the lung (i.e., granulomas and subacute interstitial inflammation) and thymus (atrophy) at all doses. A NOAEL was not identified in the first 4-week investigative study due to findings of acute inflammation in the bronchi at all doses. Characterization of local toxicity in the lung from the 13-week toxicology study with dogs was in question due to unexpected lower drug exposures. The second 4-week investigative study was not GLP compliant. The Division questioned how these studies would support the proposed total of 28 days of drug treatment.

Reference ID: 3330354

GSK stated that the studies would support the proposed 28-day treatment period although the path was somewhat convoluted. GSK reiterated that findings of lung granulomas were shown to be unrelated to drug treatment. GSK stated that the 13-week dog study was inadequate and would not be used to support clinical trials exceeding the 4-weeks duration. Further, the 9-month dog study would be available before the duration of clinical trials exceeded 28 days.

The Division indicated that the studies would be reviewed; however, the findings of lung granulomas in the 4-week toxicology study with dogs could not simply be set aside with respect to the review of this study.

***Question 2**: Nonclinical Data Supporting Progression to Phase IIb Study*

Does the Division agree that the inhalation studies conducted with magnesium stearate, and the bridging toxicology studies using GSK573719 with magnesium stearate, will be adequate to support initiation of the planned Phase IIb study?

Division Response: There appear to be no safety issues regarding the clinical use of magnesium stearate as an excipient by the inhalation route, pending review of these studies.

Concerns regarding the 4- and 13-week inhalation toxicology studies with dogs need to be addressed before initiation of the planned Phase IIb study, as discussed in our responses to Questions 1 and 3.

***Question 3**: Summary of Granuloma Findings*

Does the Division agree that the induction of granulomas in the dog has been adequately explained and resolved?

Division Response: The first and second 4-week investigative studies with dogs appear to explain the induction of granuloma observed in the 4-week dog study; however, granulomas should not be evident in longer duration inhalation toxicology studies with dogs in order to conclude that the observation has been adequately explained and resolved.

We have concerns with the 13-week inhalation dog toxicology study given that systemic exposures were significantly lower as compared to similar doses used in the 4-week and/or 14-day studies. In particular, there was no evidence of the histopathological findings in nasal cavities/sinuses, larynx, trachea, and/or bronchi as observed in the 4-week and 14-day studies with dogs. The nominal inhalation doses (i.e., 40.7, 187, and 1070 µg/kg/day) used in the 13-week study may not accurately reflect what was actually delivered. The study may not have accurately characterized GSK573719 with respect to local toxicity in the respiratory tract. This study may not support dosing beyond four weeks.

Provide justification that the 13-week inhalation toxicology study with dogs supports clinical dosing beyond four weeks with particular respect to local toxicity in the respiratory tract.

Refer to our response to Question 1 regarding concerns about the second 4-week investigative toxicology study with dogs.

Provide the historical control incidences of lung granulomas in dogs from the testing laboratory that received the old and new feeding regimens.

Reference ID: 3330354

Question 4: Study Proposal to Support 12 Months of Dosing

GSK intend to conduct a 9-month dog study to support dosing in phase III for up to 12 months. Does the Division agree?

Division Response: Pending review, we agree that a 9-month dog study, in addition to a 6-month rat inhalation study, is adequate to support dosing up to 12 months.

We remind you that per the M3 Guidance, the duration of the animal toxicity studies conducted in two mammalian species (one non-rodent) should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies (six months for rats and nine months for dogs).

As included in our responses to Questions 1 and 3, the summary data you provided for the 4- and 13-week dog studies may not be adequate assessments due to GLP status/number of animals and characterization of local toxicity, respectively, of your product in dogs. These concerns might impact the design of longer duration studies.

Question 5: Proposed Carcinogenicity Studies

Does the Division agree that, in support of NDA registration, a mouse carcinogenicity study conducted by the oral route, rather than the inhaled dose route, would be acceptable provided that the oral route achieved adequate systemic exposure (i.e. equivalent to that achieved by the inhaled route) and the rat carcinogenicity study was conducted using the inhaled route?

Division Response: Based upon pharmacokinetic studies conducted with rats and dogs, there is concern that oral bioavailability is extremely low. This is not unexpected for a quaternary ammonium compound. Further, the special protocol assessment for the mouse carcinogenicity study submitted under a different cover (April 30, 2009) proposes administration by the inhalation route. The supporting 2- and 13-week dose range finding studies with mice were conducted using the inhalation route.

However, if adequate systemic exposure by the oral route can be established in the mouse and a 13-week oral dose range finding study with mice is conducted, it might be acceptable to conduct the mouse carcinogenicity study using the oral route of administration. This assumes that the rat carcinogenicity study is conducted using the inhalation route of administration.

Additional Comments:

Nonclinical

3. We noted in your briefing package that your proposed future clinical studies include dosing with a combination of your product in this IND (GSK573719) and one from another IND (GW642444). To support clinical dosing with the combination of GSK573719 and GW642444, a bridging study with the combination in the most appropriate species should be conducted. The duration should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies for a combination of two NMEs (three months). Short-term studies with the combination in both a rodent and non-rodent may be needed to identify the most appropriate species. We recommend that the combination be tested at doses that produce exposure ratios that are relevant to the intended clinical use. Prior to clinical dosing with the combination exceeding three months, toxicology studies with each NME should be complete (i.e., six months in a rodent and nine months in a nonrodent). Further, a fertility and reproductive performance study in rats and embryofetal studies in rats and rabbits for each NME should be completed. We recommend an embryofetal development study with the combination in the most appropriate species prior to enrollment of women of childbearing potential.

4. In your IND submission, provide structures of impurities and degradants of the drug substance and drug product. All impurities and degradation products of active ingredients should be monitored throughout development. Impurities or degradants that are identified as structural alerts should be at or below acceptable qualification thresholds for genotoxic and carcinogenic impurities as described in the draft FDA Guidance for Industry " *Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (December 2008)*" for assessment of impurities to support clinical studies for an IND and NDA. In addition, refer to the ICH Guidance for qualification of drug impurities in drug substances [ICH Q3A(R)] and degradants in drug products [ICH Q3B(R)]. If applicable, conduct the appropriate toxicity studies to qualify impurities and degradants.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

GSK573719A is a high affinity, pan active muscarinic antagonist. The compound competes with [^{3}H]-N-methyl-scopolamine binding for human recombinant receptor membranes from CHO cells stably expressing the M1, M2, M3, M4 and M5 receptors with K_i values of 0.16, 0.15, 0.06, 0.05, 0.13 nM, respectively. Intranasal administration of GSK573719A to mice resulted in a time and dose-related inhibition of methacholine-induced bronchoconstriction. The ED50 at 5-hr post-administration for GSK573719A was 0.02 µg/mouse. GSK573719A had a long, dose-related duration of bronchoprotection. A single intranasal dose of 0.05 µg/mouse inhibited methacholine-induced bronchoconstriction for up to 7 days. GSK573719A dose-dependently inhibited acetylcholine-induced bronchoconstriction and exhibited a dose-related duration of action. At doses of ≥2.5 µg/guinea pig, GSK573179A, caused inhibition ≥50% for more than 48 hr. The duration of inhibition for GSK573719A and tiotropium was comparable.

Effects of GSK573719 on CNS, cardiovascular, and respiratory functions were assessed. Effects of GSK573719 on neurobehavior effects, body temperature, and motor activity were assessed in male rats following single doses of 36, 322, or 1994 µg/kg administered by nose only inhalation. Moderately dilated pupil was observed for rats at 322 and 1994 µg/kg, which is an expected pharmacological effect, but no other changes in CNS were observed. GSK573719A inhibited the hERG tail current in a concentration-dependent manner with a nominal IC50 value of 9.41 µM. Effects of single intravenous doses of GSK573719A at 0.3, 3, and 10 µg/kg on arterial pressures, heart rate, and ECG parameters were evaluated in dogs. At 10 µg/kg GSK573719A, there was an increase in heart rate with a maximum change of 49 bpm, from predose average of 65 bpm, which returned towards the predose average approximately 30 min after infusion. In agreement with increased heart rate, RR interval decreased to 0.545 sec from a predose average of 0.974 sec following infusion of 10 µg/kg GSK573719A. RR interval returned to approximate predose values by 30 min after the end of dosing. The changes in RR interval are associated with the tachycardia seen following dosing with GSK573719A at 10 µg/kg. Following dosing with GSK573719A at 10 µg/kg, incidences of second degree atrioventricular (AV) block were observed in 3 of 4 animals. Observed effects of increased heart rate and corresponding decreased RR interval as well as AV block can be attributed to the antimuscarinic effects of GSK573719. Effects of GSK573719 on respiratory function were assessed following single inhaled doses to rats of 36, 215, or 2660 µg/kg by nose only inhalation. Single achieved inhaled doses of 215 or 2260 µg/kg GSK573719 produced increases in respiratory rate (18 to 45%) and decreases in tidal volume (3 to 17%).

The sponsor plans to combine GSK573719 with GW642444, a long-acting β2 agonist, in future clinical trials. Effects of GW642444 and GSK573719 administered by the intravenous route alone or in combination, on arterial pressure, heart rate, and ECG parameters were assessed in dogs. Increased heart rate was observed following infusion of 0.3 µg/kg GW642444 alone, 0.3 µg/kg GSK573719 alone, and 0.3 µg/kg

GW642444 with 0.3 µg/kg GSK573719. There were also small reductions of RR interval following infusion concomitant to the increase in heart rate. Infusion of 0.3 µg/kg GW642444 with 0.3 µg/kg GSK573719 resulted in a small non-statistically significant prolongation in rate corrected QT interval. Increased heart rate and corresponding decreases of RR interval can be attributed to the β_2-adrenergic agonist properties of GW642444 and antimuscarinic properties of GSK573719.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

GSK573719A: In vitro Muscarinic Antagonist Activity, Binding, and Calcium Mobilization at Cloned Human Receptors (CH2006/00020/00): GSK573719A is a high affinity, pan active muscarinic antagonist. In classical kinetic studies, it appeared to show competitive kinetics in both binding and calcium mobilization. The compound competes with [^{3}H]-N-methyl-scopolamine binding for human recombinant receptor membranes from CHO cells stably expressing the M1, M2, M3, M4 and M5 receptors with K_i values of 0.16, 0.15, 0.06, 0.05, 0.13 nM, respectively. GSK573719A was assayed for agonist activity in CHO-M1, CHO-M2, and CHO-M3 cells and there was no significant calcium mobilization response, indicating a lack of muscarinic agonist activity. GSK573719 was characterized as a competitive inhibitor at the M1, M2, and M3 receptors with slopes of 0.829, 0.928 and 0.963, respectively, which were consistent with competitive kinetics. Reversibility of antagonism at the M3 receptor was assessed for GSK573719 and tiotropium. M3 ACh receptors were pretreated with GSK573719A (0, 3.3, 33, and 330 nM) for 30 min and then washed for 180 min. Tiotropium (0, 3.3, 10, or 33 nM) was evaluated in similar studies with a 30 min pretreatment and then washed for 90 min. Pretreatment and washout for tiotropium resulted in a 10 to 15-fold decrease in receptor affnity, while GSK573719A showed a 5 to 20-fold decrease in receptor affinity.

GSK1761002A and GSK339067A: In vitro Antagonist Potency Against Cloned Human Muscarinic ACh Receptors (CH2009/00016/00): The objectives of this study were to determine the inhibitory potency and direct agonist potential of GSK1761002A and GSK339067A, identified as metabolites of GSK573719A, against the cloned hM1, hM2+Gqi5, and hM3 ACh receptors. GSK1761002A was shown to be a functional inhibitor of cloned hM1 ACh receptors (pIC50>8.0) and cloned hM3 receptors, pA2 of 9.87 ± 0.08. GSK339067A was a much less potent functional antagonist of hM1, hM2, and hM3 ACh receptors showing pIC50s of 5.92, 5.78, and 6.25, respectively. GSK1761002A and GSK339067A were shown to have no direct stimulatory affect at any of the mACh receptors tested.

GSK573719A: In vitro Muscarinic Antagonist Activity on Human Bronchus in a Static Organ Bath (CH2006/00014/00): Antagonist of carbachol-induced contraction of isolated human bronchus by GSK573719A was assessed. Bronchial strips were isolated from human airway and suspended in static tissue baths. Cumulative carbachol concentration-response curves were obtained in the presence and absence of

GSK573719A. Ipratropium, tiotropium, and atropine were also tested as comparators. GSK573719A (1, 10, or 100 nM) caused a concentration-dependent rightward shift of carbachol concentration-response curves with suppression of the maximal carbachol response (pA2 = 9.5, slope = 1.5). Ipratropium (1, 10, or 100 nM) was a potent inhibitor of carbachol-induced contraction with a pA2 of 9.2 (Schild slope = 1.4). Ipratropium and atropine did not suppress the maximal carbachol response. Tiotropium had no effect at 0.1 nM, yet suppressed the maximal carbachol response by 68% and 66% at 1 and 10 nM, respectively. Atropine (10 nM) induced a parallel-shift of the carbachol response, yielding a pK_B of 9.6.

GSK573719A: Functional In vitro Muscarinic Antagonist Reversibility Profile in Human Bronchus and Guinea Pig Trachea using Superfusion Organ Bath with Carbachol Challenge (CH2006/00015/00): GSK573719A inhibited carbachol-induced contraction of isolated strips of human bronchus in an all-or-none manner, with minimal effect at 0.1 and 0.3 nM, but yielding maximal inhibition at concentrations ≥1 nM. Due to the lack of a defined inhibition curve, it was not possible to determine an IC_{50}. Consequently, the profile for GSK573719A, including percent inhibition, onset halftime, and offset halftime, was determined at 1, 10 and 100 nM and compared with ipratropium and tiotropium. GSK573719A, ipratropium, and tiotropium showed concentration-dependence for both onset of activity and recovery. The onset halftimes for GSK573719A at 1, 10, or 100 nM were 63, 27, and 14 min and the corresponding offset halftimes were 119, 145, and 299 min, respectively.

In separate studies, GSK573719A, ipratropium, and tiotropium were pre-incubated for 6 hr at 1, 10, or 100 nM each. The offset halftime for GSK573719A at 1 nM was 122 min. A recovery halftime of 381 min for 10 nM GSK573719A was extrapolated from plots of mean tension recovery vs. time. After 10 hr of washout, recovery of 100 nM GSK573719A-induced inhibition was limited to 5%. The offset halftimes for ipratropium at 1, 10 and 100 nM were 11, 47, and 214 min, respectively. The offset halftimes for tiotropium at 1 and 10 nM were 190 and 384 min, respectively, with recovery of 100 nM tiotropium-induced inhibition limited to 5%.

After recovery and subsequent washout of carbachol to baseline tension, tissues responded to carbachol in a concentration-dependent manner, with an EC50 of 0.4 μM and maximum contraction of 99.1% of the 1 mM post-histamine reference. Ipratropium had no effect on the carbachol response with an EC50 of 0.3 μM and maximum contraction of 99.2%. GSK573719A and tiotropium induced rightward shift of the carbachol response curve with EC50s of 11.5 and 4.6 μM and maximal contractions of 73.4 and 63.1 %, respectively. Similar results were obtained using guinea pig trachea.

Drug activity related to proposed indication:

GSK573719A: In vivo Effect on Methacholine-induced Bronchoconstriction in Balb/C Mice (CH2006/00018/00): Intranasal administration of GSK573719A to mice resulted in a time and dose-related inhibition of methacholine-induced bronchoconstriction. The ED50 at 5-hr post-administration for GSK573719A was 0.02

µg/mouse. GSK573719A had a long, dose-related duration of bronchoprotection. A single intranasal dose of 0.05 µg/mouse inhibited methachocline-induced bronchoconstriction for up to 7 days. The level of inhibition was maintained between 47-80% from 5 to 72 hr after treatment. Administration of GSK573719A for 5 consecutive days (once daily) to mice did not increase the level of bronchoprotection observed providing no evidence of drug accumulation in the lung. Additionally, following drug washout, there was no evidence of muscarinic receptor tolerance. Oral administration of GSK573719A did not inhibit methacholine-induced bronchoconstriction.

Effect of Intratracheal GSK573719 on Acetylcholine-Induced Bronchoconstriction in Guinea Pigs (CH2005/00954/00): The effect of intratracheal administration of GSK573719A on acetylcholine aerosol-induced bronchoconstriction in conscious male Dunkin-Hartley guinea pigs was determined. Four, 24, 48, 72, 96 and 120 hours after drug or vehicle administration, guinea pigs were placed into a whole body plethysmograph box. Animals were exposed to an aerosol of acetylcholine (ACh) produced by an ultrasonic nebulizer. Collected values were retained and Penh (enhanced pause) was calculated. Penh has previously been shown as an index of airway obstruction and correlates with increased intrapleural pressure. GSK573719A (0.25, 2.5, or 25 µg/guinea pig) dose-dependently inhibited acetylcholine-induced bronchoconstriction and exhibited a dose-related duration of action. At doses of ≥2.5 µg/guinea pig, GSK573179A, caused inhibition ≥50% for more than 48 hr. The highest dose of GSK573719A, 25 µg/guinea pig, caused inhibition ≥50% up to five days post-treatment. The duration of inhibition for GSK573719A and tiotropium was evaluated after administration of 2.5 µg/animal. Greater than 90% inhibition was observed at 4 hr post dosing for both compounds. At 24 hr, GSK573719A and tiotropium exhibited 68.9%±11.3 and 87.6%±6.5 inhibition, respectively, and at 48 hr, 37.7%±10.2 and 65.9%±9.4, respectively.

Effect of Intratracheal GSK573719A on Acetylcholine-Induced Bronchoconstriction and Bradycardia in Guinea Pigs (CH2005/00953/00): The effect of intratracheal administration of GSK573719A on acetylcholine-induced bronchoconstriction and bradycardia in guinea pigs was determined. GSK573719A demonstrated a dose-related inhibition of acetylcholine-induced increases in bronchoconstriction as measured by airway resistance compared to the vehicle-treated group. At the highest dose of acetylcholine (100 µg/kg), GSK573719A (0.25 µg) showed significant inhibition of bronchoconstriction (74.4%) and also at a ten-fold higher dose (2.5 µg). In vehicle-treated animals, acetylcholine caused a dose-related reduction in heart rate (bradycardia) and treatment with GSK573719A (0.25 or 2.5 µg) had no effect on the highest two doses (50 or 100 µg/animal) of acetylcholine-induced bradycardia. These data indicate that after intratracheal administration of GSK573719A, maximal inhibition of acetylcholine-induced bronchoconstriction is achieved at a dose that does not inhibit acetylcholine-induced bradycardia.

2.6.2.3 Secondary pharmacodynamics

GSK573719A: In Vitro Receptor and Ion Channel Selectivity Screening (CH2006/00030/00): GSK573719A at 1 µM was screened against a panel of receptors, ion channels, and transporters using radioligand binding. The percentage inhibition at each target was then determined. For those targets that were not muscarinic receptors and where greater than 50% inhibition of radioligand binding was obtained a further concentration response study was performed to determine the IC50 and K_i. GSK573719A (1 µM) inhibited radioligand binding by less than 50% at 41 of the 50 receptors, ion channels and transporters screened. The K_i values for the five targets that were not muscarinic M1-M4 receptors were Kappa opioid receptor (69 nM), Sigma (non-selective) receptor (220 nM), Ca^{2+} channel (L, verapamil site) (330 nM), Na+ channel (site 2) (170 nM), and Dopamine transporter (780 nM).

2.6.2.4 Safety pharmacology

Neurological effects:

GSK573719A: Acute Neurobehavioral Effects Following Inhalation Administration in the Conscious CD™ Rat (VD2005/00625/00)

Methods: Effects of GSK573719 on neurobehavior effects, body temperature, and motor activity were assessed following single inhaled doses to male CD™ rats. Eight male CD™ rats/group were given single doses of vehicle (8% w/w (b) (4) in Lactose) or GSK573719A (Lot No. 051093955) at 36, 322, or 1994 µg/kg by nose only inhalation. Body temperature and both peripheral and central nervous system activities were monitored before dosing, and subsequently at 1.25, 3, 9, and 25 hours from the start of the 1-hour aerosol exposure. Motor activity was evaluated prior to dosing and at 1.25, 9, and 25 hr from the start of the aerosol exposure.

Results: Moderately dilated pupil was observed at 1.25 and 3 hr after the start of treatment for rats at 322 and 1994 µg/kg, which is an expected pharmacological effect. Motor activity and body temperature were unaffected. CNS function was generally unaffected. Incidences of lying on side/curled up were increased (62.5, 37.5, and 62.5% at 36, 322, or 1994 µg/kg, respectively, versus 0% for the control) for all treatment groups at 25 hr after the start of treatment. Incidences of sitting/standing were reduced (37.5, 62.5, and 37.5% at 36, 322, or 1994 µg/kg, respectively, versus 100% for the control) for all treatment groups at 25 hr after the start of treatment.

Cardiovascular effects:

GSK573719A: In Vitro hERG Channel Selectivity Screen (CH2006/00029/00)

Methods: The effect of GSK573719A was evaluated on human ether-a-go-go related gene (hERG) tail currents in HEK-293 cells. Patch clamping was used to measure currents across the cell membrane and the concentration-related effect of GSK573719A was measured to determine an IC_{50} value for inhibition of the current.

Results: GSK573719A weakly inhibited the hERG tail current in a concentration-dependent manner with an IC_{50} estimated to be 8.0 µM. This data suggests some in vitro potential to inhibit the hERG channel; however, there was no significant evidence of in vivo QTc prolongation that might result from inhibition of the channel.

GSK573719A: Effect on hERG Tail Current Recorded from Stably Transfected HEK293 Cells (FD2005/00109/00)

Methods: The effect of GSK573719A (batch no. R12751/70/1) on hERG tail current by examining the effect of the test substance on human embryonic kidney (HEK-293) cells that were stably transfected with hERG cDNA.

Results: The maximum soluble nominal concentration of 48.997 µM GSK573719A (equivalent to 21 µg/mL active moiety) produced a 76.5% inhibition of the hERG tail current. Therefore, the effects of nominal concentrations of 1, 3, and 10 µM (equivalent to 0.4286, 1.286, and 4.286 µg/mL active moiety, respectively) were examined. The nominal IC25, IC50 and IC75 values were estimated to be 2.65, 9.41, and 33.4 µM (equivalent to 1.136, 4.033, and 14.315 µg/mL active moiety), respectively. This data suggests some in vitro potential to inhibit the hERG channel; however, there was no significant evidence of in vivo QTc prolongation that might result from inhibition of the channel.

GSK573719A: The Effects of Single Intravenous Administration of a Range of Doses on Cardiovascular Function in the Conscious Beagle Dog (FD2005/00167/00)

Methods: Effects of single intravenous doses of GSK573719A (Batch Number, 70683AA001, 99.4% pure, 83.8% pure as free base) on arterial pressures, heart rate, and electrocardiographic parameters were evaluated in conscious restrained male beagle dogs. Four male dogs, obtained from (b) (4), were given an intravenous infusion over 1 min with either vehicle (0.9% (w/v) aqueous sodium chloride) or GSK573719A at 0.3, 3, and 10 µg/kg on separate test days with 7 days between treatments. Cardiovascular function and ECG parameters were monitored for 30 min prior to dosing, during the 1 min infusion, and for 4 hours following the end of infusion. The following were evaluated: systolic, diastolic, and mean blood pressure, pulse pressure, heart rate, and ECG parameters. ECG waveforms were observed during data capture and reviewed during predose and for 1 hour following infusion for any abnormal changes in rhythm or morphology.

Results: At 10 µg/kg GSK573719A, there was an increase in heart rate with a maximum change of 49 bpm, from predose average of 65 bpm, which returned towards the predose average approximately 30 min after infusion. In agreement with increased heart rate, RR interval decreased to 0.545 sec from a predose average of 0.974 sec following infusion of 10 µg/kg GSK573719A. RR interval returned to approximate predose values by 30 min after the end of dosing. The changes in RR interval are associated with the tachycardia seen following dosing with GSK573719A at 10 µg/kg.

At 10 µg/kg GSK573719A, there was a small reduction in pulse pressure of up to 7 mmHg which returned to the predose average approximately 35 min after the end of infusion. GSK573719A at 0.3 and 3 µg/kg had no effects on systolic, diastolic, or mean blood pressure.

There was an increase in PR interval at 10 µg/kg GSK573719A, following infusion to a maximum of 0.118 sec, from a predose average of 0.0985 sec, returning to predose values approximately 35 minutes after dosing. Following treatment with vehicle and GSK573719A at 0.3, 3, and 10 µg/kg, there were no treatment-related changes of QRS, QT, R wave amplitude or QTcL.

Following dosing with GSK573719A at 10 µg/kg, incidences of second degree atrioventricular (AV) block indicated by the occurrence of isolated P-waves in the absence of QRS complexes, were observed in 3 out of 4 animals with an incidence ranging between 7-93 (up to 1 hour post dose). AV block was not observed during predose or following treatment with vehicle or GSK573719A at 0.3 or 3 µg/kg. However, arrhythmias (ventricular ectopic beats) were observed in dog 2N4932 following dosing with vehicle (incidence of 1, up to 1 hr post dose) and GSK573719A at 0.3 µg/kg (incidence of 8, up to 1 hour post dose) during predose and following dosing with GSK573719A at 0.3 µg/kg.

GSK573719 was quantifiable in the plasma of all animals up to at least 6 min following 0.3 µg/kg and up to at least 30 min following 10 µg/kg dose administration. T_{max} was observed in the sample taken immediately after dose administration, at approximately 1 min. The systemic exposure to GSK573719 (as measured by AUC_{0-t} and C_{max}) increased with increasing dose between 3 and 10 µg/kg with no major deviations from proportionality.

Observed effects of increased heart rate and corresponding decreased RR interval as well as AV block can be attributed to the antimuscarinic effects of GSK573719.

Pulmonary effects:

Acute Effects on Respiratory Function Following Inhalation Administration in the Conscious CD™ Rat (CD2005/01385/02)

Methods: Effects of GSK573719 on respiratory function were assessed following single inhaled doses to male CD™ rats. Six male CD™ rats/group were given single doses of vehicle (8% w/w (b) (4) in Lactose) or GSK573719A (Lot No. 051093955) at 36, 215, or 2660 µg/kg by nose only inhalation. Ventilatory measurements (tidal volume, respiratory rate, and minute volume) were recorded in conscious rats prior to dosing, continuously during the 1 hour exposure to evaluate effects during pulmonary exposure, and around the known and/or estimated times of plasma C_{max} and for approximately 1 hr at approximately 24 hr post-exposure to evaluate reversibility or delayed effects.

Results: A single achieved inhaled dose of 215 or 2260 µg/kg GSK573719 produced increases in respiratory rate during the 1-hr pulmonary exposure from approximately 45 to 60 and 30 to 60 min, respectively, following the start of exposure. The mean baseline-adjusted increases in respiratory rate relative to vehicle control were approximately 18 to 45% of the mean absolute vehicle control values. A single achieved inhaled dose of 215 or 2260 µg/kg GSK573719 also produced decreases in tidal volume from approximately 45 to 60 and 30 to 60 min, respectively, following the start of exposure. The mean baseline-adjusted decreases in tidal volume relative to vehicle control were approximately 3 to 17% of the mean absolute vehicle control values. These effects were not evident at other time points during exposure or at 24 hours post-exposure. Because the increases in respiratory rate were associated with concurrent decreases in tidal volume, there were no apparent changes in minute volume (total pulmonary ventilation). The absence of a change in total pulmonary ventilation suggests that the effect on ventilatory function was minimal.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Half-lives of GSK573719 in rats and dogs following intravenous administration were 1.42 and 11.6 hr, respectively. The terminal elimination half-lives (t½) of GSK573719 in humans calculated following 14-day repeat dose inhalation administration of 750 and 1000 µg/day were estimated to be 26.3 and 27.7 hr, respectively. Studies in rats and dogs suggested extensive distribution into tissues and rapid clearance suggestive of a metabolic clearance. GSK573719 was a substrate for p-glycoprotein. Oral bioavailability of GSK573719 was minimal in both rats and dogs. Following intravenous administration in rats, the highest levels of radioactivity were present in the kidney cortex, kidney medulla, liver, pituitary gland, small intestine wall, thyroid, and pancreas. Metabolism of GSK573719 in human liver microsomes was mediated primarily by CYP2D6, with some contribution from CYP3A4. GSK573719 is also metabolized by the CYP1A1, which is known to be expressed extrahepatically. GSK573719A was a marked direct inhibitor of CYP2D6 activity (IC50 = 0.1 µM) and CYP3A4 activities (IC50 = 1.0 µM for DEF and 8.0 for 7BQ). The primary route of elimination of GSK573719 in dogs was biliary excretion.

2.6.4.2 Methods of Analysis

Plasma samples were analyzed for GSK573719 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) for GSK573719 was 0.1 ng/mL, using a 50 µL aliquot of rat plasma with a higher limit of quantification (HLQ) of 100 ng/mL.

2.6.4.3 Absorption

In Vitro

An In Vitro Investigation of Both the Transport via Heterologously Expressed Human P-Glycoprotein and the Passive Membrane Permeability of [^{14}C]GSK573719 in MDCKII-MDR1 Cells (WD2006/02657/00)

Methods: The primary objective of this study was to determine whether [^{14}C]GSK573719 is a substrate of human P-glycoprotein and to determine the passive membrane permeability of [^{14}C]GSK573719 at pH 7.4, pH 6.5, and pH 5.5 in MDCKII-MDR1 cells. The potential for human P-glycoprotein (P-gp) to transport [^{14}C]GSK573719 was investigated using stable transfected MDCKII-MDR1 cells cultured as monolayers on 24-well PET membrane inserts. Directional transport was determined by measurement of apical to basolateral ([A→B]) and basolateral to apical ([B→A]) rates of transport using 3 µM [^{14}C]GSK573719 in the absence and presence of 2 µM GF120918, a potent P-gp inhibitor. The passive membrane permeability of [^{14}C]GSK573719 was determined in the presence of GF120918 at pH 7.4, pH 6.5, and pH 5.5.

Results: The apical efflux ratio of [^{14}C]GSK573719 at 3 µM was determined as being in the range of 7-17 in the absence of 2 µM GF120918 and decreasing to 0.8 in the presence of 2 µM GF120918. These results indicate that, under the assay conditions used, [^{14}C]GSK573719 is a substrate of human P-gp.

In the presence of GF120918, [^{14}C]GSK573719 demonstrated low passive membrane permeability in the MDCKII-MDR1 cell line with an average $P_{7.4}$ of 2.4 ± 0.8 nm/s.

The passive membrane permeability of [^{14}C]-GSK573719 in the A→B direction only at pH 7.4, pH 6.5 and pH 5.5 was 2.6 ± 1.0, 3.6 (n=2), and 1.3 ± 1.4 nm/s (mean ± SD), respectively. These results indicate that pH did not affect the passive membrane permeability of [^{14}C]GSK573719 over the range tested.

An In Vitro Investigation of the Inhibition by GS573719A of Xenobiotic Transport via Human P-Glycoprotein, Heterologously Expressed in MDCKII Cells (WD2006/02596/00)

Methods: The potential of GSK573719 to inhibit transport by human P-glycoprotein (Pgp) in vitro was assessed. The effect of GSK573719 (target concentrations of 0.1, 0.3, 1, 3, 10, 30, and 100 µM) on the Pgp-mediated transport of digoxin (30 nM) was assessed by determining the basolateral to apical ([B→A] transport of [^{3}H]-digoxin by MDCKII-MDR1 cells transfected with the human MDR1 gene, which produces the Pgp protein.

Results: GSK573719 did not inhibit transport of digoxin via human P-glycoprotein in vitro at concentrations up to 100 µM.

Mice

A Study to Determine the Oral Absorption and Disposition of [^{14}C]GSK573719 following a Single Administration of [^{14}C]GSK573719 (trifluoroacetate) at a Target Dose Level of 40 µg free base/kg in the FVBn and mdr1a/1b Mouse using the Short Oral Absorption Model (WD2008/00001/00)

Methods: The absorption and disposition of GSK573719 radioactive drug-related material was examined following a single oral dose of [^{14}C]GSK573719 (trifluoroacetate) at 40 µg free base/kg to mdr1a/1b (knockout, KO) and FVBn (wild type, WT) mice.

Results: Following a single oral administration of [^{14}C]GSK573719 (trfluoroacetate) at a dose level of 40 µg free base/kg, the levels of total radioactivity in the hepatic portal vein and systemic plasma of the KO mice were increased compared with WT mice (20 and 18-fold, respectively). The hepatic extraction ratios in both WT and KO mice were similar (0.7). This would indicate that P-glycoprotein (Pgp) at the level of the gut plays a role in determining the extent of absorption of GSK573719.

Rats

GSK573719A: Pharmacokinetics of GSK573719A in the Male Sprague-Dawley Rat (CH2006/00004/00)

Methods: Intravenous pharmacokinetic parameters and oral bioavailability of a solution formulation of GSK573719A were assessed in the male Sprague-Dawley rat. This study was carried out using a crossover design on 2 separate study days using 3 male rats. On day 1, each rat received GSK573719A as a 30-min intravenous infusion (1.0 mg free base equivalent/kg target dose), and on day 2 each rat received a solution formulation of GSK573719A as an oral gavage (2.0 mg free base equivalent/kg target dose). Blood samples were collected from the lateral tail vein at various times up to 24 hr following administration of GSK573719 A.

Results: GSK573719A demonstrated high plasma clearance and volume of distribution with an elimination half-life of 1.42 hr in the male Sprague-Dawley rat. Clearance was comparable to or greater than liver blood flow (54 mL/min/kg). The volume of distribution was large suggesting extensive distribution into tissues. Oral bioavailability of a solution formulation of GSK573719A, in the rat, was not determined since all plasma concentrations following oral administration were non-quantifiable (oral bioavailability is extremely low).

Plasma Pharmacokinetic Parameters of GSK573719 during and Following a 30 min Intravenous Infusion of GSK573719A (1.0 mg Free Base Equivalent/kg Target Dose) in the Male Sprague-Dawley Rat

Parameter	Mean
$DNAUC_{(0-t)}$ ng·hr/mL	164
CL mL/min/kg	102
Vss L/kg	2.57
MRT hr	0.43
T½ hr	1.42
C_{max} ng/mL	348

GSK573719A: Pharmacokinetics of GSK573719A Following Intravenous Infusion, Portal Infusion, and Oral Administration in Male Sprague-Dawley Rats (CH2006/00012/00)

Methods: The absorption and hepatic extraction ratio of a solution formulation of GSK573719A (the bromide salt of GSK573719) were determined following IV (femoral vein) infusion (target dose - 0.500 mg/kg), portal vein infusion, (target dose - 0.500 mg/kg), and oral administration (target dose - 2.00 mg/kg) in male Sprague-Dawley rats. Three animals each received GSK573719A as a 30 min femoral vein infusion (Group 1), 30 min portal vein infusion (Group 2), or by oral gavage (Group 3). Following each dose administration, serial blood samples were obtained through 12 hr postdose.

Results: GSK573719A demonstrated poor absorption and systemic exposure following portal infusion and oral administration in the rat.

A Study to Investigate the Pharmacokinetics for GSK573719 in Male CD/SD Rats Following Intravenous Infusion at Target Dose Level of 0.5 mg/kg (WD2006/00073/00)

Methods: Pharmacokinetic parameters of GSK573719 were assessed in male rats following a 1 hr continuous intravenous infusion at a target dose of 0.5 mg/kg.

Results: The concentration-time profile of GSK573719 showed a bi-exponential decline of plasma concentrations. The apparent terminal half-life (T½) was approximately 3.4 hours, although additional analysis showed that this terminal phase represented only 15% of the total dose, with the majority of GSK573719 being eliminated with a half-life of 1.3 min. The volume of distribution (Vdss) was high, 15 L/kg, and was far in excess of body water indicating substantial distribution of GSK573719 into tissues. Plasma clearance was shown to be 329 mL/min/kg, which exceeded liver blood flow (54 mL/min/kg). The large clearance values suggest a metabolic clearance.

Table 1 **Pharmacokinetics of GSK573719 in Plasma Following a 1 hour Continuous Intravenous Infusion of GSK573719A at a target dose level of 0.5 mg/kg to Male Rats.**

Compound	Rat	Rat weight kg	Actual Dose mg/kg	$AUC_{(0-t)}$ ng.h/mL	C_{max} ng/mL	$T_{1/2}$ h	$AUC_{(inf)}$ ng.h/mL	AUC Extrap %	$MRT_{(0-t)}$ h	Cl_p mL/min/kg	Vd_{ss} L/kg
GSK573719	1	0.322	0.501	23.2	28.5	4.22	23.9	2.58	0.537	350	20.5
	2	0.333	0.494	26.0	31.1	3.57	27.0	3.68	0.408	305	15.7
	3	0.287	0.491	24.4	31.3	2.26	24.8	1.38	0.259	330	7.47
	Mean			24.6	30.3	3.35	25.2	2.55	0.401	329	14.6

Dogs

Pharmacokinetics of GSK573719A in the Male Beagle Dog (CH2006/00001/00)

Methods: Intravenous pharmacokinetic parameters and the oral bioavailability of a solution formulation of GSK573719A were determined in the male beagle dog. On day 1 each dog received a solution formulation of GSK573719A as an oral gavage (2.0 mg free base equivalent/kg target dose) and on day 2 each dog received GSK573719A as a 60 min intravenous infusion (1.0 mg free base equivalent/kg target dose). Blood samples were collected from a cephalic vein at various times up to 24 hr following administration of GSK573719A. Plasma concentrations of GSK573719 were quantified by LC/MS/MS (LLOQ = 1.00 ng/mL).

Results: GSK573719A demonstrated high plasma clearance with an elimination half-life of approximately 12 hr. GSK573719 was widely distributed in tissues based upon a large volume of distribution value. Clearance was comparable to or greater than liver blood flow (31 mL/min/kg). Oral bioavailability of a solution formulation of GSK573719A was not determined since plasma concentrations at all time points were non-quantifiable.

Plasma Pharmacokinetic Parameters of GSK573719 during and following a 30 min Intravenous Infusion of GSK573719A (1.0 mg Free Base Equivalent/kg Target Dose) in the Male Beagle Dog

Parameter	Mean
$DNAUC_{(0-t)}$ ng·hr/mL	502
CL mL/min/kg	32.5
Vss L/kg	4.67
MRT hr	2.37
T½ hr	11.6
C_{max} ng/mL	651

A Study to Investigate the Pharmacokinetics of GSK573719 in Male Beagle Dogs Following Intravenous Infusion at Target Dose Levels of 0.5 mg free base/kg (WD2006/00075/00)

Methods: The pharmacokinetics of GSK573719 were examined in 3 male beagle dogs following a 1 hr intravenous infusion at a target dose of 0.5 mg/kg. Blood samples were collected at time points up to 48 hr after the start of the infusion. Plasma concentrations of GSK573719 were quantified by LC/MS/MS (LLOQ = 0.1 ng/mL).

Results: Following a 1 hour continuous intravenous infusion of GSK573719 at a target dose of 0.5 mg/kg, the concentration-time profile showed a bi-exponential decline in GSK573719 plasma concentrations. The apparent terminal half-life (T½) was approximately 16 hr although further analysis showed that this terminal phase represented only 28% of the total dose, with the majority of GSK573719 being eliminated with a half-life of 1.5 min. This was reflected in the substantially shorter mean residence time (MRT) of 2.7 hours. Volume of distribution (Vdss) was high, 14 L/kg, and was far in excess of body water indicating substantial distribution of GSK573719 into the tissues. Plasma clearance was shown to be 49 mL/min/kg, which was comparable to or greater than liver blood flow (31 mL/min/kg).

Table 1 Pharmacokinetics of GSK573719 in Plasma Following a 1 hour Continuous Intravenous Infusion of GSK573719A at a target dose level of 0.5 mg/kg to Male Beagle Dogs.

Compound	Dog	Rat weight kg	Actual Dose mg/kg	$AUC_{(0-t)}$ ng.h/mL	C_{max} ng/mL	$T_{1/2}$ h	$AUC_{(inf)}$ ng.h/mL	AUC Extrap %	$MRT_{(0-t)}$ h	Cl_p mL/min/kg	Vd_{ss} L/kg
GSK573719	1	12.3	0.470	183	176	16.5	190	3.42	2.76	41.2	12.6
	2	12.4	0.477	136	132	17.1	141	3.43	2.83	56.3	17.6
	3	12.3	0.477	162	152	13.6	165	2.22	2.56	48.1	11.5
	Mean			160	153	15.8	165	3.02	2.72	48.5	13.9

2.6.4.4 Distribution

Rats

Quantitative Whole-Body Autoradiography Following a Single Oral or a Single Intravenous Administration of [14C]GSK573719 bromide to Male Lister Hooded Pigmented Rats at a Target Dose Level of 1 mg free base/kg (FD2005/00236/00)

Methods: The tissue distribution of radioactivity was investigated in male pigmented (Crl:LIS BR: Lister Hooded) rats, using quantitative whole body autoradiography, following a single oral administration or a single 30-min intravenous infusion of [^{14}C]GSK573719 bromide at a target dose of 1 mg free base/kg. Tissues were collected at 0.5 (for IV dosing) and 1.5 hr and 1, 3, 10, and 35 days.

Results: GSK573719 has a very low oral bioavailability and results of this portion of the study are not discussed due to minimal absorption of radioactivity.

Tissue distribution following IV administration:
At 30 min after the start of an intravenous infusion administration of [^{14}C]GSK573719 to male pigmented rats at a target dose of 1 mg free base/kg, radioactivity was widely distributed with the majority of tissues containing concentrations greater than that observed in blood (0.348 µg equiv/g). The highest levels of radioactivity were present in the kidney cortex (radioactivity concentration greater than the upper limit of quantification [>ULQ; >7.03 µg equiv/g], kidney medulla (>ULQ), liver (6.94 µg equiv/g), pituitary gland (3.83 µg equiv/g), small intestine wall (3.76 µg equiv/g), thyroid (2.13 µg equiv/g), and pancreas (2.07 µg equiv/g). In various components of the CNS and the testes, concentrations of radioactivity were only just above the limit of quantification. Approximately half of the total number of tissues examined reached their maximal concentration at this time.

At 1.5-hr postdose, radioactivity was quantifiable in all tissues sectioned with the exception of brain and eye (lens). Most of the remaining tissues examined reached their maximal concentrations at this time. Radioactivity levels were declining for most tissues that had reached maximal concentrations at 0.5 hr with the exception.

At 1 day postdose, the meninges, brown fat, and the walls of the large intestine and rectum attained their highest observed concentrations. Concentrations of radioactivity in all other tissues had generally declined but were quantifiable in all tissues sectioned with the exception of blood, brain, spinal cord and eye (lens).

At 3-days postdose, concentrations of radioactivity in all tissues had declined further but were still quantifiable in all tissues sectioned with the exception of blood, brain, spinal cord, testes, and eye (lens).

By 10-days post-dose, concentrations of radioactivity were below the limit of quantification in approximately one third of the total number of tissues analyzed. By 35 days postdose, quantifiable concentrations of radioactivity were only observed in the kidney medulla, mucous gland, muscle, tongue, and uveal tract (choroid, iris and ciliary body)/retina. The presence of quantifiable concentrations of radioactivity in the uveal tract/retina suggests some association of drug-related material with melanin.

GSK573719A: A Study to Estimate the Lung Retention and Systemic Exposure of GSK573719A Following Intranasal Administration in the Male Balb/c Mouse (CH2006/00002/00)

Methods: The lung retention and systemic exposure of GSK573719A were assessed in the male Balb/c mouse following a single intranasal administration of a 2.0 mg free base equivalent/kg target dose in a saline solution formulation. Terminal blood samples were collected at 15, 60, 180, 360, and 1440 min postdose from 4 mice per time point. Following collection of each blood sample, the lungs were removed and homogenized.

Mouse plasma and lung homogenate concentrations of GSK573719 were quantified using an LC/MS/MS method (LLOQ = 2.00 ng/mL; Linear range 2.00-100.00 ng/mL).

Results: Concentrations of GSK573719 in the lung, were much higher than those observed in plasma at the same time point postdose (12915 ng/g vs. 191 ng/mL at 15 min, 10612 ng/g vs. 72 ng/mL at 60 min, and 2771 ng/g vs. 5 ng/mL at 24 hr, in lung vs. plasma, respectively).

GSK573719A: Investigation of the Brain to Plasma Ratio of GSK573719A in the Conscious Male Sprague-Dawley Rat (CH2006/00013/00)

Methods: CNS penetration of GSK573719A was assessed following a 24-hr continuous intravenous infusion of GSK573719A administered to three conscious male Sprague-Dawley rats at a target dose of 14.69 mg free base equivalent/kg. Blood samples were collected at 1380, 1395, 1410, 1425, 1440, and 1445 min following initiation of the infusion. Brain tissue was collected after the 1445-min blood sample and homogenized. Plasma and brain concentrations of GSK573719 were quantified using an LC/MS/MS method (LLOQ = 1.00 ng/mL; Linear range 1.00 to 2500 ng/mL).

Results: The sponsor claimed a brain-to-plasma ratio of 1.1.

2.6.4.5 Metabolism

In Vitro

A Preliminary In Vitro Investigation into the Human Oxidative Enzymology of GSK573719 (WD2006/00367/00)

Methods: An assessment of human cytochrome P450 enzymes involved in the in vitro metabolism of GSK573719 was conducted. [^{14}C]GSK573719 was incubated at 0.075 µM with human liver microsomes or bactosomes overexpressing individual cytochrome P450 enzymes: CYP1A1, 1A2, 2AB, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5. Further incubations with human liver microsomes were performed in the presence and absence of the selective cytochrome P450 inhibitors furafylline (CYP1A2), montelukast (CYP2C8), sulphaphenazole (CYP2C9), benzylnirvanol (CYP2C19), quinidine (CYP2D6), and azamulin (CYP3A4). Metabolites were quantified by HPLC with radiometric detection and identified by mass spectrometry.

Results: Incubation of 0.075 µM [^{14}C]GSK573719 with human liver microsomes produced 5 metabolite peaks quantifiable by radio-HPLC. Four of these peaks were identified by LC/MS analysis to be M14 (O-dealkylation), M33 (parahydroxylation of the phenylmethyl ether) and M56 and M61 (dihydroxylation of phenylmethyl ether). The quantifiable metabolites accounted for a mean total of approximately 50% of total radioactivity, with M14 and M33 being the major metabolites, representing 19% and 20% of total radioactivity, respectively. Production of metabolites was NADPH dependent, indicating the involvement of cytochrome P450 enzymes.

In incubations with human liver microsomes in the presence of quinidine (a selective inhibitor of CYP2D6), the production of M33 was reduced to non-quantifiable levels. Inhibition of the production of M33 was not observed with any of the other specific inhibitors investigated. M33 was the major metabolite quantified in incubations with overexpressed CYP2D6. It was not detected in incubations with any of the other CYPs investigated. These data indicate that CYP2D6 is the major cytochrome P450 enzyme responsible for production of M33.

The production of M14 in incubations with [^{14}C]GSK573719 and human liver microsomes in the presence of quinidine or azamulin (a selective inhibitor of CYP3A4) was reduced by 90% and 52%, respectively. Inhibition of the production of M14 was not observed with any of the other specific inhibitors investigated. M14 was quantified in incubations with overexpressed CYP2D6 and overexpressed CYP1A1 and, to a lesser extent, with overexpressed CYP3A4. The presence of M14 was detected by LC/MS in incubations with overexpressed CYP2E1, but was not quantified by radio-HPLC. These data indicate that CYP2D6 and CYP1A1 are the major cytochrome P450 enzymes responsible for the production of M14, with CYP3A4 playing a minor role in its production.

Low levels of M56 and M61 were produced in incubations with [^{14}C]GSK573719 and human liver microsomes. These metabolites were probably formed by secondary metabolism of M33. It was not possible to quantify inhibition of their formation using selective inhibitors of cytochrome P450 enzymes. Small amounts of both M56 and M61 were quantified in incubations with overexpressed CYP2D6. Neither of the metabolites was quantified in incubations with any of the other CYPs investigated.

Metabolism of GSK573719 in human liver microsomes was mediated primarily by CYP2D6, with some contribution from CYP3A4. GSK573719 is also metabolized by the CYP1A1, which is known to be expressed extrahepatically.

A Preliminary Screen of the In Vitro Concentration-Dependent Inhibition of Human Cytochrome P450 Enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by GSK573719A (CH2005/00950/00)

Methods: The in vitro concentration-dependent inhibition of human cytochrome P450 enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, by GSK573719A was determined. The ability of GSK573719 to inhibit the activities of these CYPs was determined by monitoring the metabolism of probe substrates in the presence and absence of GSK573719 (0-33 µM).

Results: GSK573719A demonstrated a marked direct inhibition of CYP2D6 activity (IC50 = 0.1 µM) and CYP3A4 (IC50 = 1.0 µM for DEF and 8.0 for 7BQ) activities. GSK573719A did not demonstrate inhibition of CYP1A2, CYP2C19, and CYP2C9.

Table 2 In Vitro Concentration-Dependent Inhibition of Recombinant Human Cytochrome P450 Isozymes by GSK573719A

CYP450 Isozyme	Probe Substrate	Study No.	IC_{50} (µM)
CYP1A2	ER	RI04088	>33
CYP2C9	FCA	RI04088	>33
CYP2C19	BMC	RI04088	14
CYP2D6	MMC	RI04088	0.1
CYP3A4	DEF	RI04088	1.0
	7BQ	RI04088	8.0

The Effect of GSK573719A on the mRNA Levels of Cytochrome P450 Genes in Rat Livers Obtained Following Inhaled Administration at 30, 200, and 2000 µg/kg/day for 28-Days (WD2005/01627/00)

Methods: The effects of GSK573719A on the messenger RNA (mRNA) levels of liver cytochrome P450 genes were evaluated in male and female Sprague-Dawley rats treated with doses of 0, 30, 200, and 2000 µg/kg/day in a 28-day inhalation toxicity study (WD2005/01422/00). Liver homogenates were prepared, total RNA was extracted and quantified, and cDNA templates were synthesized. The levels of mRNA expression for each specific cytochrome P450 were determined using quantitative real-time polymerase chain reaction technology (TaqMan™). Specific mRNA levels were quantitatively detected for the following genes: CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2E1, CYP3A2 (males only, as it is a male specific gene), CYP3A23, and CYP4A1 and the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Results: GSK573719A did not cause any increase in the levels of mRNA of the following CYPs: CYP1A1 (in male animals only), CYP1A2, CYP2B1, CYP2B2, CYP2E1, CYP3A2, CYP3A23 and CYP4A1 (in female animals only).

An increase in the levels of CYP1A1 mRNA (a mean ratio of treated over control of 8) was observed in the female livers from the 2000 µg/kg/day dose group. This increase appeared to be due to one animal. Increases in the levels of CYP4A1 mRNA (mean ratio of treated over control of 2 and 4) were observed in the male livers from the 30 and 200 µg/kg/day dose groups, respectively.

Decreases in the level of CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2E1, CYP3A2, CYP3A23 and CYP4A1 mRNA were observed (<50% of control value) in the male animals from the 2000 µg/kg/day dose group. Decreases in the level of CYP1A1 and CYP2B2 mRNA were observed (<50% of control value) in the female animals from the 200 and 2000 µg/kg/day, respectively.

Table 1 Effect of GSK573719A on CYP mRNA in Male Sprague Dawley Rat Livers

Cytochrome P450	The Ratio of mRNA Expression in Treated Over Control Group											
	Vehicle			30 μg/kg/day			200 μg/kg/day			2000 μg/kg/day		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
CYP1A1[1]	1.0	±	0.73	0.13	±	0.19	1.1	±	1.3	0.24	±	0.14
CYP1A2[2]	1.0	±	0.18	0.92	±	0.079	1.0	±	0.056	0.00096	±	0.00035
CYP2B1[1]	1.0	±	0.37	1.1	±	0.52	2.4	±	1.4	0.14	±	0.0087
CYP2B2[1]	1.0	±	1.1	0.34	±	0.25	0.62	±	0.72	0.15	±	0.042
CYP2E1[2]	1.0	±	0.16	0.74	±	0.15	1.2	±	0.24	0.000042	±	0.000022
CYP3A2[2]	1.0	±	0.25	1.6	±	0.53	1.6	±	0.58	0.000088	±	0.000039
CYP3A23[2]	1.0	±	0.25	0.86	±	0.24	1.6	±	0.59	0.032	±	0.016
CYP4A1[2]	1.0	±	0.59	2.3	±	0.73	3.6	±	0.27	0.0076	±	0.0056

Values are expressed as a Mean ± Standard Deviation of 3 animals. Data presented to 2 sig figs
1. Increase in mRNA level of ≥ 5 in treated over control is considered notable based on previous datasets.
2. Increase in mRNA level of ≥ 2 in treated over control is considered notable based on previous datasets.

Table 2 Effect of GSK573719A on CYP mRNA in Female Sprague Dawley Rat Livers

Cytochrome P450	The Ratio of mRNA Expression in Treated Over Control Group											
	Vehicle			30 μg/kg/day			200 μg/kg/day			2000 μg/kg/day		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
CYP1A1[1]	1.0	±	0.58	0.62	±	0.23	0.44	±	0.22	7.7	±	11
CYP1A2[2]	1.0	±	0.13	1.0	±	0.18	0.51	±	0.45	0.72	±	0.23
CYP2B1[1]	1.0	±	0.20	0.77	±	0.24	4.2	±	2.6	1.6	±	0.89
CYP2B2[1]	1.0	±	0.94	0.52	±	0.26	1.2	±	0.79	0.29	±	0.21
CYP2E1[2]	1.0	±	0.13	1.2	±	0.19	0.87	±	0.77	1.1	±	0.27
CYP3A23[2]	1.0	±	0.23	1.3	±	0.094	1.5	±	1.2	1.1	±	0.56
CYP4A1[2]	1.0	±	0.27	1.1	±	0.26	0.76	±	0.66	0.91	±	0.22

Values are expressed as a Mean ± Standard Deviation of 3 animals. Data presented to 2 sig figs
CYP3A2 is a male specific gene and therefore was not analysed in the female livers.
1. Increase in mRNA level of ≥ 5 in treated over control is considered notable based on previous datasets.
2. Increase in mRNA level of ≥ 2 in treated over control is considered notable based on previous datasets.

Rats

A Study to Investigate the Biotransformation of [^{14}C]-GSK573719 in the Isolated Perfused Rat Liver Model (WD2005/01195/00)

Methods: Structural characterization of identification of metabolites of GSK573719 produced by male and female isolated perfused rat livers (IPRL) was performed. Radiolabeled GSK573719A was added to the circulating perfusate for male and female preparations. Structural characterization of metabolites was carried out on selected samples of bile, β-glucuronidase hydrolyzed bile, perfusate supernatant, and liver homogenate by mass spectrometry (HPLC-MS) and nuclear magnetic resonance spectroscopy (NMR).

Results: The major radiolabeled peak observed in rat bile from the isolated perfused liver was unchanged parent. The major metabolites observed included a methoxy

hydroxy glucuronide conjugate (M22), hydroxy glucuronide conjugates (M21, M27), and thiol conjugates (M11, M13). Other notable metabolites observed included dehydroxy glucuronide conjugates (M20, M26) and products of O-dealkylation (M14, M18). The remaining identified radiocomponents each represented minor routes including sulfation (M3, M4), hydroxylation (M33, M34, M35, M37, M38, M41), and hydration (M9). β-glucuronidase hydrolysis of bile resulted in an increase in hydroxylated metabolites (M33 and M37).

The major radio-peak(s) observed in the perfusate supernatant were unchanged P, M1, M13, M14, M21, M22, and M27, while in the liver homogenate extract they were unchanged P, M4, M13, M14, and M18. The metabolic profiles in perfusate supernatants and liver extracts were similar to those in bile. See Review #01 dated June 3, 2009 for structures of metabolites.

Quantification and Identification of the Metabolites of GSK573719 in the Male Beagle Dog Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide at 1 mg free base/kg (WD2006/00250/00)

Methods: Major metabolites of GSK573719 in plasma, urine, and feces following a single oral or single intravenous administration to the male beagle dog at 1 mg free base/kg [^{14}C]GSK573719 were identified and quantified. Plasma samples from intact dogs (1 and 3 hr), urine (0-48 hr after IV dosing), and feces (0-48 hr after oral dosing and 0-72 or 24-72 hr after IV dosing) were collected. Samples were processed for radio-HPLC and HPLC-MS analyses.

Results: Oral bioavailability of GSK573719 was determined to be low and no metabolite data was available.

Following IV administration, drug-related radioactivity was primarily detected in the feces. Approximately 25.8% of the dose was not recovered.

Route and Status	Excretion Balance (% dose)	
	Urine	Faeces
Oral	0.4	95.1
IV	11.9	62.3

Plasma: The levels of radioactive drug-related material in pooled 1 and 3 hour plasma following intravenous dosing were ca. 274 and 19 ng eq./mL, respectively. The only quantifiable radiolabeled component in plasma samples at 1 hr postdose was unchanged GSK573719 (P) which represented 94.7% of plasma radioactivity. Several metabolites were detectable by MS including M14 (O-dealkylation product), M33 (hydroxy), M34 (methyl catechol), and M51 (dihydrodiol), but all were below the limit of

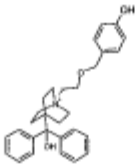
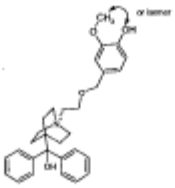
quantification. Unchanged GSK573719 was the major component in plasma samples at 3 hr postdose.

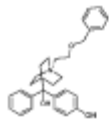
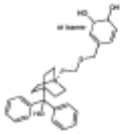
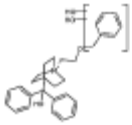
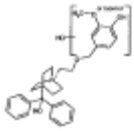
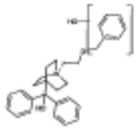
Urine: The radioactivity in the 0 to 48 hr urine represented 9.6% of the administered dose, which equated to approximately 81% of the material excreted by this route. Several radiolabeled components were present in male urine including, M14 (O-dealkylation product), M51 (dihydrodiol), M33 (hydroxy), and M34 (methyl catechol) in addition to unchanged GSK573719. The predominant components were M33 and unchanged parent, representing 29.8% and 18.6% of the total radiochromatogram, respectively (2.9% and 1.8% of the administered dose). Other notable peaks included M14 and M51, each corresponding to approximately 11.8% of the total radioactivity (1.1% of the dose) and M34 (0.1 % of the dose). Two components could not be identified.

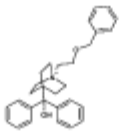
Feces: The radioactivity in the 0-72 hr intravenous male dog fecal samples examined represented 55% of the administered dose, which equated to 62% of the material excreted by this route. Several metabolites were detected in feces from intravenously dosed dogs. The predominant radiolabeled species were M14 (O-dealkylation) and unchanged GSK573719, representing 25.9% and 21.9% of the total radioactivity, respectively (14.2 and 12% of the total dose). Other notable metabolites representing 5% of the total radioactivity (3% of administered dose) were M51 (dihydrodiol), M33 (hydroxy), M34 (methyl catechol), and M37 (hydroxy). Additional metabolites, detectable by MS, but not quantifiable were M57, M56, and M58. The principal radiolabeled component in oral dog feces was unchanged GSK573719 (P), which represented 83.3% of the oral radioactivity equating to 77.9% of the administered dose. The only other radiolabeled component detectable in oral dog feces was M14, which represented 3.3% of the total radioactivity (3.1% of the dose).

In dogs, the major metabolic routes were O-dealkylation to produce M14 and hydroxylation of either the ethyl benzene ring to form M33 or of the phenyl group to form M37. Other notable routes were epoxidation to form a dihydrodiol (M51) which may be an intermediate for the formation of the methyl catechol M34. Minor routes included hydroxylation to form M58 and subsequently M56, which may be an intermediate for the formation of the methyl catechol product M57.

Table 2 Summary of HPLC Analyses of Urine and Faeces Following a Single Oral or Single Intravenous Administration of [^{14}C]-GSK573719 bromide to Male Beagle Dogs at a Target Dose Level of 1 mg Free Base/kg

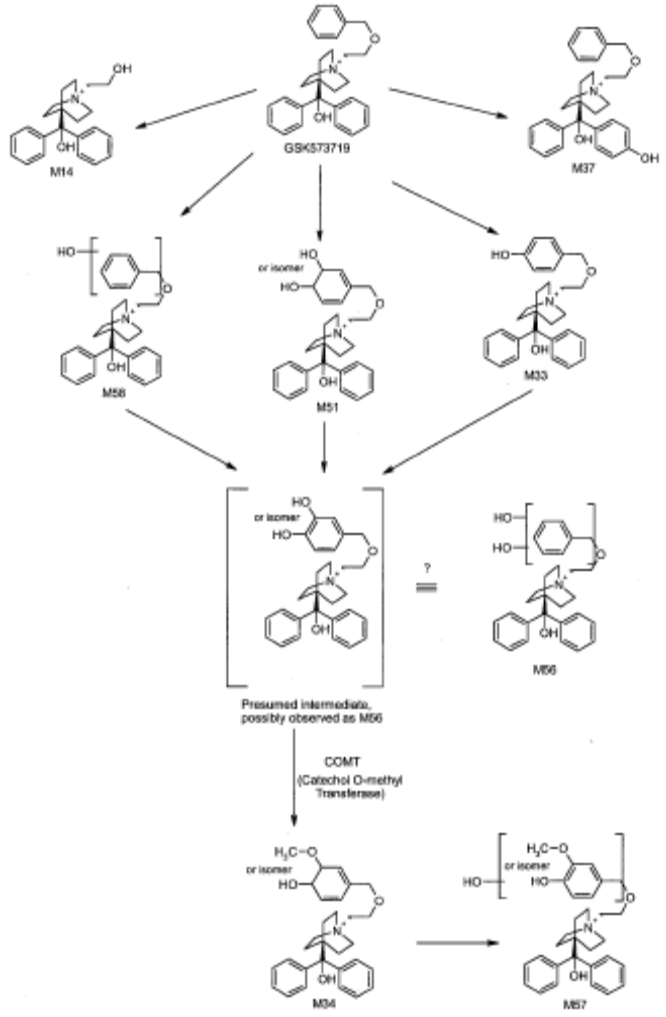
Peak ID	Metabolite Structure	Faeces (Oral)	Urine (IV)	Faeces (IV)
		Mean % matrix radioactivity (mean % dose)		
A	NA	NA	5.8 (0.6)	6.4 (3.5)
B	NA	NA	5.0 (0.5)	NA
M14	O-dealkylation product	3.3 (3.1)	11.8 (1.1)	25.9 (14.2)
M33	 Hydroxy metabolite	ND	29.8 (2.9)	4.4 (2.4)
M34	 Methoxy, Hydroxy metabolite	ND	1.0 (0.1)	4.9 (2.7)

Peak ID	Metabolite Structure	Faeces (Oral)	Urine (IV)	Faeces (IV)
		Mean % matrix radioactivity (mean % dose)		
M37	 Hydroxy metabolite	ND	ND	4.7 (2.6)
M51	 Dihydrodiol	ND	11.8 (1.1)	6.0 (3.3)
M56	 Dihydroxy	ND	ND	BQL
M57	 Methoxy, dihydroxy	ND	ND	BQL
M58	 Hydroxy	ND	ND	BQL

Peak ID	Metabolite Structure	Faeces (Oral)	Urine (IV)	Faeces (IV)
		Mean % matrix radioactivity (mean % dose)		
P	 GSK573719	83.3 (77.9)	18.6 (1.8)	21.9 (12.0)
Total radioactive material assigned		86.6 (81.0)	73.0 (7.0)	67.8 (37.1)
Non-extracted		1.0 (0.8)	NA	10 (8.1)
Quantification limit		1.8 (1.7)	0.9 (0.1)	4.3 (2.4)
% dose in matrix pool analysed		93.5	9.60	54.7
Total % dose excreted in matrix		95.1	11.9	61.8

BQL: Below limit of quantification
ND: Not detected
NA: Not applicable

Figure 1 Putative Metabolic Scheme for GSK573719 in the Dog



Best Available Copy

2.6.4.6 Excretion

Dogs

Elimination of Drug-Related Material Following a Single Oral or a Single Intravenous Administration of [^{14}C]GSK573719 Bromide to Male Beagle Dogs at a Target Dose Level of 1 mg free base/kg (FD2005/00164/00)

Methods: The elimination of radioactivity was investigated following a single oral or a single intravenous (l h infusion) administration of rI4CJGSK573719 bromide to male beagle dogs at a target dose level of 1 mg free base/kg.

Results: Following oral administration, bioavailability was low and 95.1% of drug-related material was eliminated by the feces. Urinary excretion was a minor route of elimination (mean of 0.4% of the dose).

Following intravenous administration, the major route of elimination of drug-related material was by the feces (61.8% of the dose). Urinary excretion accounted for 11.9% of the dose. Following intravenous administration, elimination was slow with approximately 1% of the dose being eliminated during each of the 24 hr periods between 96 and 168 hr postdose. This slow elimination may be the cause of the low total recovery of radioactivity (74.5% of the dose).

Elimination of Drug-Related Material Following Intravenous Administration of [^{14}C]GSK573719 Trifluoroacetate to Male Bile Duct-Cannulated Beagle Dogs over Various Time Intervals and at Various Target Dose Levels (WD2007/01907/00)

Methods: The elimination of radioactivity over 48 hr was determined in two male bile duct cannulated beagle dogs following single intravenous doses of [^{14}C]GSK573719 trifluoroacetate at target dose levels of either 10 µg free base/kg (Phase A, slow bolus over 30 sec) or 200 µg free base/kg (Phases B, 1 hr infusion and Phase C, 10 min infusion). There was a 14-day washout period between each dose. Urine and feces were collected in 8-hr periods up to 48-hr postdose. Bile was collected in 15-min intervals up to 4-hr postdose and at 1-hr intervals up to 48-hr postdose. Blood samples were collected at time points up to 8 hr in Phases A and C and up to 9 hr in Phase B. Samples were processed for measurement of radioactivity.

Results: Following all three dosing regimens, the major route of elimination of radioactivity was by the bile. Biliary elimination accounted for means of approximately 57% of the dose following all three dosing regimes. Urinary elimination was also similar following the three regimens, accounting for means of approximately 14% of the dose. Excretion in feces was a minor elimination pathway, accounting for means of no more than 4% of the dose. Total recoveries of radioactivity (including cage washes) ranged between 70% and 74% of the dose. The low recoveries were most likely due to the short collection period. The majority of the biliary elimination occurred within the first 8 hr postdose for all three dosing regimens. Overall, between 58% and 66% of the dose were recovered in the urine, feces, bile, and cage washes by 24 hr postdose.

2.6.4.9 Discussion and Conclusions

Half-lives of GSK573719 in rats and dogs were 1.42 and 11.6 hr, respectively, following intravenous administration. The terminal elimination half-lives (t½) of GSK573719 in humans calculated following 14-day repeat dose administration of 750 and 1000 µg/day by the inhalation route were estimated to be 26.3 hours and 27.7 hours, respectively. GSK573719 was a substrate for p-glycoprotein. Oral bioavailability of GSK573719 was minimal rats and dogs.

Plasma protein binding in mouse, rat, rabbit, dog, and human ranged from 74.7 to 88.8% and was concentration-independent. Red blood cell associations of GSK573719 in dog, human, rabbit, rat, and mouse were generally low. Studies in rats and dogs suggested extensive distribution into tissues and rapid clearance suggestive of a metabolic clearance. Following intravenous administration in rats, the highest levels of

radioactivity were present in the kidney cortex, kidney medulla, liver, pituitary gland, small intestine wall, thyroid, and pancreas.

In vitro metabolism of GSK573719 was examined with hepatocytes from mouse, rat, rabbit, dog, and human. Studies with human hepatocytes and microsomes found that the main routes of metabolism of GSK573719 were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The major routes of metabolism in humans were also present in rat and dog. O-dealkylation (as observed in human) was a major pathway in the mouse, but only a minor pathway in the rabbit. Hydroxylation (at the position observed in M33 in human) was also a major pathway in the mouse (M21) but a minor pathway in rabbit hepatocytes (M22). Metabolism of GSK573719 in human liver microsomes was mediated primarily by CYP2D6, with some contribution from CYP3A4. GSK573719 is also metabolized by the CYP1A1, which is known to be expressed extrahepatically. GSK573719A was a marked direct inhibitor of CYP2D6 activity (IC50 = 0.1 μM) and CYP3A4 activities (IC50 = 1.0 μM for DEF and 8.0 for 7BQ).

Following repeat inhalation of GSK573719A at a dose of 1 mg/day for 7 days to healthy male subjects and to a healthy male population of CYP2D6 poor metabolizers, GSK573719 and GSK339067 (M14, product of O-dealkylation) were the only GSK573719-related components detected in 5 min, 1 hr, and 0-24 hr pooled human plasma extracts and in 0-4 hr pooled human urine. The proportion of GSK339067 (relative to the parent drug, GSK573719) in CYP2D6 poor metabolizers compared to normal metabolizers was similar in the 5 min pooled plasma samples, but decreased over 3-fold in the 1 hr pooled plasma samples. In the 0-24 hr pooled plasma samples, the proportion of GSK339067 was approximately 18% in 2D6 poor metabolizers and 35% in healthy subjects. In 0-4 hr pooled urine samples, the proportion of GSK339067 relative to the parent drug, GSK573719, was comparable in both populations at 6 to 7%. CYP2D6 appears to play a significant role in the metabolism of GSK573719 in humans.

Following intravenous administration of 1 mg/kg GSK573719 to rats, 65.3 and 16.9% of the radioactive dose were found in the feces and urine, respectively. Following intravenous administration of GSK573719 to dogs, the major route of elimination of drug-related material was by the feces (61.8% of the dose). Urinary excretion accounted for 11.9% of the dose. The primary route of elimination of GSK573719 in dogs was biliary excretion.

2.6.4.10 Tables and figures to include comparative TK summary

Pharmacokinetic parameters of GSK573719 in rats, dogs, and humans

Parameter	Rat (IV dose of 0.5 mg/kg)	Dog (IV dose of 1 mg/kg)	Human (Inhalation)
T½, hr	1.42	11.6	26.3-27.7
CL, mL/min/kg	102	32.5	-
Vss, L/kg	2.57	4.67	-

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The sponsor has conducted inhalation toxicology studies with GSK573719A administered to mice, rats, and dogs for exposure periods up to 13 weeks. Early toxicology studies up to 4 weeks in duration were conducted using excipients, lactose and [redacted] (b) (4) However, safety concerns were identified regarding the use of (b) (4) (See Summary and Evaluation). The sponsor discontinued the use of [redacted] (b) (4) Magnesium stearate is an approved excipient for use by the inhalation route. Bridging studies with duration of 14 days were conducted in rats and dogs to compare the toxicity profile and toxicokinetics of GSK573719 formulated with (b) (4) or magnesium stearate. There were no significant changes in the toxicity profile and toxicokinetics for GSK573719A formulated in (b) (4) or magnesium stearate. Thirteen (13)-week inhalation toxicology studies with GSK573719A administered to mice, rats, and dogs were conducted using excipients, lactose and magnesium stearate.

Mice

In a 13-week inhalation toxicology study (WD2007/01600/00; submitted in support of dose selection for a 2-year carcinogenicity study), mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day (see Review #01 dated June 3, 2009). Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Additional findings were evident in the liver and uterus. Histopathological findings were observed in the larynx at all doses, nasal turbinates at doses ≥0.287 mg/kg/day, and nasopharynx at doses of 1.06 and 2.85 mg/kg/day; however, findings in the larynx were judged to be nonspecific irritant effects with no relevance to humans and findings in the nasal turbinates and nasopharynx were judged to be not relevant to oral inhalation used in a clinical setting. Histopathological findings in the tracheal bifurcation (epithelial hyperplasia and loss of cilia), liver (focal inflammation), and uterus (luminal dilatation) were observed at 2.85 mg/kg/day. With respect to administration in a clinical setting, a NOAEL could be identified at 1.06 mg/kg/day based upon histopathological findings in the tracheal bifurcation, liver, and uterus at 2.85 mg/kg/day. AUC values in males and females associated with the NOAEL were 29.5 and 42.5 ng·hr/mL, respectively.

Rats

In a 13-week inhalation toxicology study (WD2007/02012/00; submitted in support of dose selection for a 2-year carcinogenicity study), rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day (see Review #01 dated June 3, 2009). Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. Histopathological findings were observed for the larynx at doses ≥38 µg/kg/day, nasal cavity/sinuses at doses ≥102 µg/kg/day, and nasopharynx for a single rat at 288 µg/kg/day. Histopathological findings in the larynx

were judged to be nonspecific irritant effects. Histopathological findings in the nasal cavity/sinuses and nasopharynx were judged to be not relevant to oral inhalation used in a clinical setting. The NOAEL with respect to clinical use was judged to be 924 µg/kg/day. AUC values in males and females associated with the NOAEL were 22.6 and 36.2 ng·hr/mL, respectively.

Dogs

The sponsor's inhalation toxicology studies with GSK573719 administered to dogs were confounded by a series of problems. The sponsor's initial 4-week inhalation toxicology study with dogs was complicated by findings of granulomatous inflammation in the lung that were attributed to inhalation of plant material from the diet (WD2005/01423/00). The sponsor conducted a second 4-week inhalation toxicology study with dogs (WD2006/03294/00) to identify a feeding regimen to reduce the possibility of inhalation of particulate matter from the diet. The sponsor made modifications to method for delivery of GSK573719 to dogs in the 13-week inhalation toxicology study that inadvertently resulted in significant reductions of delivered inhalation doses as compared to earlier studies. The sponsor conducted a 4-week inhalation toxicology study with dogs (WD2008/01504/00) to determine the source of the problem for delivery of GSK573719 in the 13-week study as well as to identify the best method for delivery of GSK573719 to dogs.

In a 28-day inhalation toxicology study with dogs (WD2005/01423/00), GSK573719 was administered by face mask fitted with an oropharyngeal tube at estimated achieved doses of 0, 16.2, 208.0, and 2757.6 µg/kg/day (pulmonary deposited doses were 4.0, 52, and 689.4 µg/kg/day, respectively). Heart rates were increased up to 1.7-fold for male and female dogs in the 208.0 and 2757.6 µg/kg/day groups during the treatment period. Histopathological findings were observed in the lung, thymus, nasal cavity/sinuses, larynx, and trachea. Findings in the nasal cavity/sinuses were judged to be irrelevant to clinical administration by oral inhalation. In the lung, granulomatous inflammation was observed for all male treatment groups and females in the high dose group; the incidence and severity did not display dose-response relationships. Slight to moderate multifocal acute and/or subacute bronchoalveolar inflammation was observed for 1 male at 16.2 µg/kg/day and 1 male and 2 females 2757.6 µg/kg/day. In the larynx, minimal to moderate acute/subacute inflammation was observed for males and females at 2757.6 µg/kg/day. Mineralization and exudate were also evident for females at 2757.6 µg/kg/day. In the trachea, slight to moderate epithelial degeneration/regeneration and minimal acute/subacute inflammation were observed for females at 2757.6 µg/kg/day. Granulomatous inflammation in the lung was judged to unrelated to the test article based upon results of a follow-up 28-day inhalation toxicology study with dogs (WD2006/03294/00) (see Summary and Evaluation). The NOAEL was identified as 208 µg/kg/day based upon histopathological findings in the lung, larynx, and trachea for the high dose group. Estimated AUC values for males and females in the 208 µg/kg/day group on day 28 were 25.9 and 29.9 ng·hr/mL, respectively (determined by extrapolation from the high dose group).

In a 13-week inhalation toxicology study, dogs received GSK573719 at estimated achieved doses of 0, 40.7, 187, and 1070 µg/kg/day. The control group was exposed to lactose containing magnesium stearate. In Week 13, at predose, 3/6 males and 4/6 females given 1070 µg/kg/day showed no respiratory sinus arrhythmia (RSA) (expressed by decreased RR interval variability) and all dogs given 1070 µg/kg/day had increased heart rates (up to 1.8X pretest individual values). Immediately following a dose of 1070 µg/kg/day, no RSA was noted in 4/6 males and 4/6 females, which was associated with further increases in heart rates (up to 2.1X predose individual values) in 2/6 males and 3/6 females. The changes in most animals were returned to pre-test values, but RSA was not observed in females during the recovery phase. On Day 1, a female given 1070 µg/kg/day had elevated serum cTnI concentrations at 3 hr (0.171µg/L), 7 hr (0.189 µg/L), and 24 hr (0.082 µg/L). By Week 13 and at the end of the recovery period, the serum cTnI concentrations were comparable to pre-dose and concurrent controls. Additionally on Day 1, a male given 1070 µg/kg/day showed minimal to mildly higher serum cTnI concentrations at 3 hr (0.044 µg/L) and 7 hr (0.101 µg/L) when compared to pre-dose and concurrent control values. By 24 hr the serum cTnI concentration was comparable to the pre-dose value. By 24 hr the serum cTnI concentration was comparable to the pre-dose value. Increased troponin I concentrations suggest the possibility of cardiac injury although histopathological examinations of hearts found no evidence of damage. No cardiac damage was evident in shorter term studies. Observed histopathological findings appeared to be background/spontaneous and unrelated to treatment. Systemic exposure to GSK573719 measured in dogs from this study was lower than that seen in dogs in the two 4-week studies when given by oropharyngeal tube inhalation exposure. It is considered possible that the lower systemic exposures seen in this study related to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in this study. The histopathological results found in the present study may not accurately reflect the local toxicity of GSK573173. The NOAEL was identified as the high dose of 1070 µg/kg/day based upon lack of treatment-related histopathological finding; however, this absolute dose might not be comparable to other studies due to problems with drug delivery. Cardiac effects appear to be monitorable in a clinical setting. Systemic exposures for males and females in the high dose group during week 13 were 30.6 and 28.6 ng·hr/mL, respectively.

Genetic toxicology:
GSK573719A was negative in the in vitro bacterial reverse mutation assay, in vitro mouse lymphoma assay, and in vivo rat micronucleus assay (Reviewed in Review #01 dated June 3, 2009).

Reproductive toxicology:
In a female fertility and early embryonic development study, rats received estimated achieved inhalation doses of 3.37, 29.1, 100, and 294 µg/kg/day (Reviewed in Review #01 dated June 3, 2009). Body weight gains were unaffected. Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day. Body weight gains for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control,

respectively. Body weight loss occurred for the high dose group (-2.9%). Dose selection in the present study appeared to be adequate. There were no effects on fertility or reproductive performance for female rats treated with inhalation doses up to 294 µg/kg/day (equivalent to a pulmonary deposited dose of 29.4 µg/kg/day).

GSK573719A was administered by nose-only inhalation for 1 hr each day from gestation days 6 to 17 to pregnant female Sprague-Dawley rats at estimated achieved doses of 0, 0.0317, 0.0969, and 0.278 mg/kg/day (Reviewed in Review #01 dated June 3, 2009). Body weight gains from gestation days 6 to 18 were unaffected. Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day. Body weight gains for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group. Dose selection in the present study appeared to be adequate. There were no external, skeletal, or visceral malformations. Increased incidences of minor skeletal and visceral abnormalities/variants were observed for treatment groups. The incidence of ventral arch of the 1st cervical vertebra was increased for all treatment groups in a dose-related manner. Incidences of metatarsals (incomplete ossification/unossified) and phalanges (none ossified) bilateral hindlimb were increased for all treatment groups although dose-response relationships were not present. Subdural hemorrhages were evident in the mid and high dose groups. Hemorrhage in the cerebellum was observed in the mid and high dose groups although a dose-response relationship was not present. Hemorrhage within cerebral hemispheres was observed in the high dose group. GSK573719 was not teratogenic in rats at inhaled doses up to 0.278 mg/kg/day (equivalent to a pulmonary deposited dose of 0.028 mg/kg/day or 27.8 µg/kg/day).

In an embryofetal development study, time-mated female New Zealand white rabbits received GSK573719 at estimated inhaled doses of 0, 0.0285, 0.0889 or 0.306 mg/kg/day from gestation days 7 to 19 (Reviewed in Review #01 dated June 3, 2009). Body weight gains from gestation days 7 to 20 for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups were reduced as compared to the control although a dose-response relationship was not evident. Food consumption for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups from gestation days 7 to 19 was reduced to 77.3, 78.15, and 73.1% of the control, respectively. Gravid uterine weights for the 0.0899 and 0.305 mg/kg/day groups were reduced to 94.2 and 92.3% of the control (0.52 kg) although the toxicological significance of these differences was not clear. Fetal body weights (male, female, and overall) were slightly reduced for all treatment groups although the significance of these differences was not clear. There were no treatment-related external, visceral, or skeletal malformations. Two fetuses in the high dose group were observed with thymus-undescended horn. The incidence of caudal vertebrae-incomplete ossification/unossified was increased for the high dose group. The incidence of sternebrae-bipartite ossified/offset alignment was increased for the high dose group and slightly exceeded the historical control incidence. GSK573719 was not teratogenic in rabbits with inhaled doses up to 0.306 mg/kg/day.

2.6.6.3 Repeat-dose toxicity

Rats

Study title: **14-Day Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat (Magnesium Stearate Bridging Study)**

Key study findings:

● The objectives of this 14-day nose-only inhalation bridging toxicology study (Study no.: WD2006/03225/00) with rats were to identify potential differences of the toxicity profile and toxicokinetics of GSK573719A caused by excipients, (b) (4) (b) (4) and magnesium stearate and to support the use of magnesium stearate in a clinical formulation of GSK573719. A comparator group received GSK573719A without either of these excipients (i.e., lactose only). The target dose of GSK573719A was 1000 µg/kg/day.

● Histopathological findings were evident in the tracheal bifurcation, trachea, lung, larynx, nasal cavity/sinuses, nasopharynx, and liver. Histopathological findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to the clinical setting of oral inhalation. Findings in the larynx were judged to be rodent-specific, nonspecific effects of inhalation administration and not relevant to humans.

● At the tracheal bifurcation, incidences of degeneration/regeneration of the epithelium were increased for males and females that received GSK573719 formulated in lactose (b) (4) (G3) or lactose/magnesium stearate (G4). The incidence was also slightly increased for females that received GSK573719 formulated in lactose only (G5).

● In the trachea, degeneration/regeneration of the epithelium was evident for 1 of 10 females that received GSK573719 formulated in lactose/magnesium stearate (G4).

●In the lung, incidences of hemorrhage were increased for males and females that received GSK573719 formulated in lactose/magnesium stearate (G4).

● Exposures (AUC and C_{max}) to GSK573719 were >2-fold higher for males and females that received GSK573719 formulated in lactose/ (b) (4) or lactose/magnesium stearate as compared to lactose only.

● The toxicity profile and toxicokinetics of GSK573719 formulated in magnesium stearate or (b) (4) were relatively comparable in rats.

Study no.: WD2006/03225/00
Volume #, and page #: Volume 10 and 11, Pages 1-538
Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: May 5, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Micronized GSK573719A from input batch number EE3939C (99.5% purity as free base) was used in this study.
GSK573719A powder was blended at a nominal concentration of 4% (w/w) with 8% (w/w) (b) (4) (batch number R159636) in lactose (batch number B143232, Lot number 051089255) for use in Group 3 (blend batch number 78903/78922-05-10-05-05).
GSK573719A powder was blended at a nominal concentration of 4% (w/w) with 1% (w/w) magnesium stearate (batch number C512923, Lot number 061114989) in lactose (batch number B143232, Lot number 051089255) for use in Group 4 (blend batch number 79190-06-05-06-02)
GSK573719A powder was blended at a nominal concentration of 4% (w/w) in lactose (batch number B143232, Lot number 051089255) for use in Group 5 (blend batch number 79190-09-05-06-03)

The vehicle comprised either lactose only (Group 1) or 1% (w/w) magnesium stearate in lactose (blend batch number 79190-06-05-06-01) (Group 2)

Methods
Doses: The objective of this study was to compare the potential toxicity and toxicokinetics of GSK573719A when blended with magnesium stearate during daily nose-only inhalation exposure (duration 60 min) to the rat for a minimum of 14 days with that observed when blended with lactose only or lactose and (b) (4)

GSK573719

Group	Average achieved aerosol chamber concentration (µg/L) [a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day) [a,b]	
			Male	Female	Male	Female	Males	Female
1 (Lactose)	0	60	0.244	0.161	0.414	0.247	0	0
							Average = 0	
2+ (Lactose/ Magnesium Stearate)	0	60	0.244	0.158	0.412	0.242	0	0
							Average = 0	
3 # GSK573719 (Lactose/ [redacted] (b) (4)	40.2	60	0.237	0.156	0.398	0.238	1436	1581
							Average = 1509	
4+ GSK573719 (Lactose/ Magnesium Stearate)	39.8	60	0.236	0.151	0.397	0.229	1420	1575
							Average = 1498	
5 GSK573719 (Lactose)	36.8	60	0.242	0.155	0.409	0.235	1306	1456
							Average = 1381	

a. Expressed in terms of GSK573719.

b. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract

D = (RMVxTxC) / (BW)

D dose (µg/kg/day)

Respiratory Minute Volume

(RMV) $0.499 \times BW(kg)^{0.809}$(L/min) [Bide, 2000]

T Duration of exposure/day (minutes)

C Aerosol GSK573719 concentration (µg/L)

BW Group average body weight for study (Kg) Days 1 to 14

The average aerosol chamber concentration and estimated achieved dose of GW857238 in Group 3 was 67.3 µg/L and 2526 µg/kg/day, respectively.

+ The average aerosol chamber concentration and estimated achieved dose of Magnesium Stearate in Group 2 was 5.2 µg/L and 195 µg/kg/day, respectively and in Group 4 was 5.5 µg/L and 207 µg/kg/day, respectively.

Reference ID: 3330354

Average achieved chamber (b) (4) aerosol concentrations and resulting estimated achieved doses (males and females combined) of (b) (4) are presented below.

Group	Average achieved aerosol chamber concentration (µg/L) [a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day) [a,b]	
			Male	Female	Male	Female	Males	Female
1	0	60	0.244	0.161	0.414	0.247	0	0
							Average = 0	
2	0	60	0.244	0.158	0.412	0.242	0	0
							Average = 0	
3	67.3	60	0.237	0.156	0.398	0.238	2405	2647
							Average = 2526	
4	0	60	0.236	0.151	0.397	0.229	0	0
							Average = 0	
5	0	60	0.242	0.155	0.409	0.235	0	0
							Average = 0	

a. Expressed in terms o (b) (4)
b. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract
D = (RMVxTxC) / (BW)
D dose (µg/kg/day)
RMV 0.499xBW(kg) $^{0.809}$(L/min) [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol (b) (4) concentration (µg/L)
BW Group average body weight for study (Kg) Days 1 to 14

Average achieved chamber Magnesium Stearate aerosol concentrations and resulting estimated doses of Magnesium Stearate are presented in the table below.

Group	Average achieved aerosol chamber concentration (µg/L) [a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day) [a,b]	
			Male	Female	Male	Female	Males	Female
1	0	60	0.244	0.161	0.414	0.247	0	0
							Average = 0	
2	5.2	60	0.244	0.158	0.412	0.242	185	204
							Average = 195	
3	0	60	0.237	0.156	0.398	0.238	0	0
							Average = 0	
4	5.5	60	0.236	0.151	0.397	0.229	196	218
							Average = 207	
5	0	60	0.242	0.155	0.409	0.235	0	0
							Average = 0	

a. Expressed in terms of Magnesium Stearate.
b. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract
D = (RMVxTxC) / (BW)
D dose (µg/kg/day)
RMV 0.499xBW(kg) $^{0.809}$(L/min) [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol Magnesium Stearate concentration (µg/L)
BW Group average body weight for study (Kg) Days 1 to 14

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolised GSK573719 are presented below.

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	MMAD (µm)	GSD
1	0	(b) (4)	
2	0		
3	40.2		
4	39.8		
5	36.8		

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolised (b) (4) are presented below.

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	MMAD (µm)	GSD
1	0	(b) (4)	
2	0		
3	67.3		
4	0		
5	0		

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolised Magnesium Stearate are presented below.

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	MMAD (µm)	GSD
1	0	(b) (4)	
2	5.2		
3	0		
4	5.5		
5	0		

Chamber aerosol concentrations: A measured volume of air was drawn from exposure chambers through an open-face glass fiber filter, fitted to a sampling holder attached to the sampling port. The lactose vehicle control group (Group 1) was exposed to lactose only and the magnesium stearate in lactose control group (Group 2) was exposed to only magnesium stearate in lactose. The concentration of lactose in the aerosol given to Group 1 was targeted to be the same as the concentration of lactose given to Group 5 and the concentration of magnesium stearate in the aerosol given to Group 2 was targeted to be the same as the concentration of magnesium stearate given to Group 4. Lactose concentrations in aerosols were determined by a gravimetric method. Magnesium stearate concentrations were determined by atomic absorption spectroscopy. GSK573719 and (b) (4) concentrations were measured by HPLC.

Particle size distribution: A measured volume of air was drawn from inhalation exposure chambers through a cascade impactor attached to a sampling port on days 1 or 8. Filters were weighed and assayed for GSK573719, (b) (4) or magnesium stearate.

Species/strain: Male and Female Crl:CD(SD) rats were obtained from (b) (4)

Number/sex/group or time point (main study): 10 rats/sex/group
Route, formulation, volume, and infusion rate: Rats were exposed by nose-only inhalation exposure. The inhalation exposure system consisted of standard nose-only inhalation chambers of modular design with 20 separate ports in each row. Forty exposure ports were available on each exposure chamber. The animals were restrained in polycarbonate restraint tubes which were inserted onto the chambers. The test atmospheres were generated into the top section of the inhalation chambers using an extended duration powder delivery system (EDPDS). Animals were exposed for 60 min/day.
Satellite groups used for toxicokinetics or recovery: 6 rats/sex/group
Age: Rats were 12 weeks old on day 1 of treatment.

Weight: Body weight ranges were 359 to 421 g for males and 214 to 260 g for females on day 1 of treatment.
Unique study design or methodology (if any): None

Observation and Times:
Clinical signs: Viability checks were conducted twice daily. Clinical signs were recorded pre- and post dose.
Body weights: Body weights were recorded twice weekly.
Food consumption: Food consumption was measured weekly.
Ophthalmoscopy: Ophthalmic examinations were performed prior to the start of dosing and prior to dosing on day 14.
EKG: Not performed.
Hematology: Blood samples for measurement of a complete panel of hematology parameters were collected at termination.
Clinical chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected at termination.
Urinalysis: Urine samples for measurement of a complete panel of urinalysis parameters were collected overnight (approximately 21 hr) at termination.
Gross pathology: Rats were sacrificed after treatment for 14 days.
Organ weights: Absolute and relative organ weights were measured for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, prostate, spleen, testes, and thymus.
Histopathology: Tissue samples were processed to paraffin wax, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. A complete battery of tissues was examined. All tissues were examined from all groups. Stage-dependent evaluation of spermatogenesis was conducted. Sections of testes from terminal main study animals were prepared and stained by with Periodic acid Schiff's-Hematoxylin (PASH). A qualitative examination of spermatogenic stages was made for normal progression of the stages of the spermatogenic cycle, cell associations, and proportions expected to be present during normal spermatogenesis for all animals. A peer review of selected microscopic tissues sections and pathology data interpretation was completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected from toxicokinetic animals on days 1 and 14 immediately postdose and at 0.5, 1, 2, 4, 8, and 23 hr after completion of the 60 min dosing period. Blood samples collected from naïve animals were used to assess for potential contamination during sample analyses. GSK573719 was measured using a HPLC/MS/MS method with a LLOQ of 0.1 ng/mL and a linear range of 0.1 to 100 ng/mL.

Results:

Mortality: One female rat receiving GSK573719 with (b) (4) in lactose (No. 3504) was found dead on day 1 after dosing in the home cage. Histopathological findings were observed in the nasal cavity/sinuses (degeneration/regeneration in the vomeronasal organ), larynx (ulceration, multifocal, with inflammation, hemorrhagic exudate, and foreign material in the oropharynx), lung (focal inflammation), nasopharynx (focal

ulceration with exudate), thymus (multifocal hemorrhage), kidneys (congestion), and liver (congestion). The relationship of the death to drug cannot be completely excluded although there were no additional deaths as dosing progressed and no deaths were occurred in groups that received GSK573719A with (b) (4) or magnesium stearate where systemic exposure to GSK573719 was >2-fold higher.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Body weight gains were reduced for males in Groups 3, 4, and 5 (24, 8.2, and 40.9% of the control BW gain, respectively) and females in Groups 3 and 5 (7.7 and 32.2% of the control BW gain, respectively). Females in Group 4 lost body weight (decrease of 1.7% of initial BW).

Food consumption: Food consumption was unaffected.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

Hematology: There were no treatment-related effects on hematology parameters.

Clinical chemistry: Differences of clinical chemistry parameters between control and treatment groups were small and appeared to have no toxicological significance.

Urinalysis: Differences of urinalysis parameters between control and treatment groups were small and appeared to have no toxicological significance.

Gross pathology: In the liver, 3 of 10 males that received GSK573719 formulated in lactose only were reported with area pale. This finding might correlate with histopathological findings of tension lipidosis. Enlargement of the tracheobronchial lymph node was reported for 2 of 10 males that received GSK573719 formulated in lactose only; however, there was no corresponding histopathological finding. A dark area in the lung was reported for 1 of 10 males in each of the treatment groups that received GSK573719 regardless of formulation; however, there was no apparent corresponding histopathological finding.

Organ weights: Thymus weights were decreased (up to approximately 25%) for males and females received GSK573719 regardless of formulation; however, there were no corresponding histopathological findings.

Histopathology: Histopathological findings were evident in the tracheal bifurcation, trachea, lung, larynx, nasal cavity/sinuses, nasopharynx, and liver. Histopathological findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to the clinical setting of oral inhalation. Findings in the larynx were judged to be rodent-specific, nonspecific effects of inhalation administration and not relevant to humans.

At the tracheal bifurcation, incidences of degeneration/regeneration of the epithelium were increased for males and females that received GSK573719 formulated in

lactose (b) (4) (G3) or lactose/magnesium stearate (G4). The incidence was also slightly increased for females that received GSK573719 formulated in lactose only (G5).

In the trachea, degeneration/regeneration of the epithelium was evident for 1 of 10 females that received GSK573719 formulated in lactose/magnesium stearate (G4).

In the lung, incidences of hemorrhage were increased for males and females that received GSK573719 formulated in lactose/magnesium stearate.

There were multiple findings in the nasal cavity/sinuses. Minimal to moderate changes related to the inhalation of GSK573719 were observed in the respiratory, transitional and olfactory epithelium of animals with magnesium stearate or (b) (4) Slight differences in the incidence and/or severity of the lesions between the blends of GSK573719 containing either (b) (4) or magnesium stearate were considered negligible and likely due to differences in individual animal exposures to the test article, GSK573719. In animals given GSK573719 with lactose only, milder changes were seen in the epithelia although the vomeronasal organ was still significantly affected.

There were multiple findings in the nasopharynx. Similar microscopic findings were seen in rats given GSK573719 with magnesium stearate or (b) (4) but less severe in those receiving GSK573719 in lactose alone.

In the liver, the incidence of tension lipidosis was increased for males receiving GSK573719 with lactose only. This finding was not evident in longer duration studies with comparable and higher doses.

Histopathological findings were generally comparable for GSK573719 formulated in (b) (4) or magnesium stearate.

Microscopic findings following a 14-day treatment period

Organ/Tissue	Males					Females				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Tracheal bifurcation										
-degeneration/regeneration epithelium, G1	0/10	1/10	8/10	7/10	1/10	0/10	0/9	7/9	5/10	2/9
Trachea										
-degeneration/regeneration epithelium, G1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Lung										
-hemorrhage, G1-2	0/10	1/10	3/10	1/10	0/10	1/10	0/10	2/10	0/10	0/10
Larynx										
-hyperplasia/squamous, metaplasia, G1-3	0/10	1/10	10/10	10/10	10/10	0/10	0/10	9/10	10/10	10/10
-inflammation, subacute, G1-3	0/10	1/10	10/10	10/10	8/10	0/10	0/10	9/10	10/10	7/10
-necrosis, ventral pouch cartilage, G1-3	0/10	0/10	10/10	10/10	9/10	0/10	0/10	9/10	10/10	9/10
-exudate, G2	0/10	0/10	1/10	0/10	0/10	0/10	0/10	2/10	2/10	0/10
-ulceration, G2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10

Organ/Tissue	Males					Females				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Nasal cavity/sinuses										
-degeneration/regeneration vomeronasal organ, G1-2	0/10	0/10	1/10	1/10	2/10	0/10	0/10	7/10	4/10	5/10
-metaplasia, squamous, transitional,epithelium,G1-3	0/10	0/10	9/10	10/10	0/10	0/10	0/10	7/10	8/10	0/10
-hyperplasia, transitional, epithelium, G1-2	0/10	0/10	10/10	10/10	1/10	1/10	0/10	8/10	9/10	2/10
-erosion, respiratory epithelium, Grade 2	0/10	0/10	1/10	5/10	0/10	0/10	0/10	1/10	1/10	0/10
-metaplasia, squamous, respiratory epithelium,G1-3	0/10	0/10	10/10	10/10	1/10	0/10	0/10	6/10	10/10	1/10
-hyperplasia, respiratory epithelium, G1-3	0/10	0/10	10/10	10/10	3/10	0/10	0/10	9/10	10/10	3/10
-erosion/ulceration, olfactory epithelium, G1-3	0/10	0/10	4/10	7/10	0/10	0/10	0/10	6/10	10/10	0/10
-metaplasia, squamous, olfactory epithelium, G2	0/10	0/10	5/10	5/10	0/10	0/10	1/10	6/10	9/10	2/10
-degeneration, olfactory epithelium, G1-3	0/10	0/10	3/10	6/10	1/10	0/10	0/10	0/10	0/10	0/10
Nasopharynx										
-metaplasia, squamous, respiratory epithelium,G1-2	0/10	0/10	1/10	4/10	0/10	1/10	0/10	1/10	2/10	0/10
-degeneration/regeneration respiratory epithelium,G1-2	0/10	0/10	3/10	9/10	0/10	0/10	0/10	6/10	10/10	4/10
-hyperplasia/hypertrophy, goblet cells, G1-2	3/10	10/10	10/10	7/10	5/10	3/10	8/10	6/10	4/10	6/10
-ulceration, G2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10
Liver										
-tension lipidosis, G1	0/10	0/10	1/10	0/10	3/10	1/10	1/10	0/10	0/10	1/10

Toxicokinetics: Exposures (AUC and C_{max}) to GSK573719 were >2-fold higher for males and females that received GSK573719 formulated in lactose (b) (4) or lactose/magnesium stearate as compared to lactose only. There were no consistent differences between males and females.

Parameter+	Day	Male			Female		
		Estimated Achieved Dose (µg/kg/day)			Estimated Achieved Dose (µg/kg/day)		
		1509[a]	1498[b]	1381[c]	1509[a]	1498[b]	1381[c]
$AUC_{(0-t)}$ (ng.h/mL)	Day 1	189	181	62.7	229	202	95.6
	Day 14	219	199	89.6	154	267	69.9
C_{max} (ng/mL)	Day 1	138	159	26.7	190	133	49.6
	Day 14	55.2	90.0	22.4	59.2	123	29.8

+ Calculated values derived by multiplying the dose-normalized data (refer to Appendix 4) by the overall estimated achieved dose.

a. GSK573719/lactose (b) (4)
b. GSK573719/lactose/Magnesium stearate
c. GSK573719/lactose

Dogs

Study title: **A 14-Day Study to Examine the Influence of Magnesium Stearate on the Toxicity and Toxicokinetics of GSK573719A by Inhalation Administration to Beagle Dogs**

Key study findings:

● The objectives of this 14-day inhalation bridging toxicology study (Study no.: WD2006/03669/00) with dogs were to identify potential differences of the toxicity profile and toxicokinetics of GSK573719A caused by excipients, (b) (4) and magnesium stearate and to support the use of magnesium stearate in a clinical formulation of GSK573719. A comparator group received GSK573719A without either of these excipients (i.e., lactose only). The target dose of GSK573719A was 1000 µg/kg/day.

● Femoral pulse rates were generally increased for male and female treatment groups on days 1, 2, and 14 at 10 min to 24 hr postdose. On day 12, at predose, no dogs receiving GSK573719A (Groups 3-5) displayed respiratory sinus arrhythmia (RSA) and heart rates were higher compared to the pretreatment period. Immediately postdose, increased heart rates and no RSA were observed in all dogs from Groups 3-5. Cardiac troponin I concentrations were increased for all male treatment groups on day 1 at 3, 7, and 24 hr postdose. Cardiac troponin I concentrations were increased for females in Groups 4 and 5 at 3, 7, and 24 hr postdose. No increases of cardiac troponin I concentrations were evident on day 14.

● Histopathological findings were evident in the gallbladder, lungs, trachea, tracheal bifurcation, trachea, and nose/turbinate. The finding in the nose/turbinate was judged to be irrelevant to the clinical setting with oral inhalation of the test article.

● Findings in the gallbladder consisted of foci of minimal to slight myofiber degeneration/regeneration associated with variable hemorrhage and inflammation present in the tunica muscularis of animals dosed with all GSK573719 formulations.

● Findings for lungs included alveolar inflammation for 1 female in GSK573719/lactose group, an increased incidence of cellular debris and mucus in the bronchiole lumen females in the GSK573719 (b) (4) /lactose group, and a microgranuloma for one female in the GSK573719A/lactose group.

● Goblet cell hyperplasia in the tracheal bifurcation was observed for 1 female in the GSK573719/ (b) (4) lactose group.

● Lymphoid follicular aggregates and inflammatory cells/cell debris were observed in the trachea for 1 female in the GSK573719/lactose group.

● The toxicity profile and toxicokinetics were generally comparable for GSK573719 formulated in (b) (4) or magnesium stearate. Histopathological findings in the gallbladder appeared to be associated with relatively high systemic exposures to the test article (i.e., AUC values on day 14 ranged from 52.732 to 102.689 ng·hr/mL).

Study no.: WD2006/03669/00
Volume #, and page #: Volume 18, Pages 1-451
Conducting laboratory and location: (b) (4)

Date of study initiation: October 24, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Micronized GSK573719A powder (from GSK input batch number R230164 purity 99.6%) was included in all test article formulations at a nominal concentration of 40% (w/w). Micronized GSK573719A was blended in lactose only (blend batch number EE116517C) or in lactose, with either micronized (b) (4) at a nominal concentration of 8% (w/w) (blend batch number EE116517D) or in lactose with magnesium stearate at a nominal concentration of 1% (w/w) (blend batch number EE116517E). (b) (4) was taken from lot number 041058716 and magnesium stearate was taken from lot number 061114989.

Vehicle control blend: Magnesium stearate powder was blended in lactose powder at a nominal concentration of 1% (w/w) (blend batch number EE16517B). Lactose (control) was taken from batch number 061118478.

Methods
Doses: Three groups of Beagle dogs (3/sex/group) were exposed to dry powder formulations of 40% (w/w) micronized GSK573719A blended in lactose only, or blended in lactose with either 1% (w/w) magnesium stearate or 8% (w/w) micronized (b) (4) (b) (4) by inhalation administration by oropharyngeal tube, 10 minutes/day for 14 days with GSK573719 at a target dose of 1000 µg/kg/day. Two control groups were similarly exposed to lactose only or vehicle (a dry powder blend of magnesium stearate blended in lactose at a nominal concentration of 1% (w/w) at the same concentration of lactose or magnesium stearate as the GSK573719/lactose or GSK573719/magnesium stearate/lactose groups, respectively.

Inhaled doses and particle sizes of GSK573719

Group Number	Treatment	GSK573719				
		Target dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day)[a,d]	Target Aerosol Concentration (µg/L)[a]	Averaged Achieved Aerosol Concentration (µg/L)[a]	MMAD ± GSD [a e]
1[b]	Lactose	0	0	0	0	(b) (4)
2[c]	Vehicle (Lactose/magnesium stearate)	0	0	0	0	
3	GSK573719A/ (b) (4) lactose	1000	1018	304	306	
4	GSK573719A/ magnesium stearate/ lactose	1000	1023	304	307	
5	GSK573719A/ lactose	1000	1103	304	333	

a. Expressed in terms of the parent compound, GSK573719.
b. Exposed to lactose only.
c. Exposed to vehicle (1% (w/w) magnesium stearate in lactose) only.
d. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
e. MMAD = Mass Median Aerodynamic Diameter µm, GSD = Geometric Standard Deviation

D = (RMVxTxC)/ (BW)
D Dose (µg/kg/day)
RMV (respired minute volume L/minute) $0.499 \times BW(kg)^{0.809}$ (Bide, 2000)
T Duration of exposure/day (minutes)
C Aerosol concentration (µg/L)
BW Group average daily body weight for the study (kg)

Inhaled doses and particle sizes of (b) (4) ((b) (4) and magnesium stearate

Group Number	Treatment	GW857238		magnesium stearate		
		Estimated Achieved Dose (µg/kg/day)[a,d]	Overall Achieved Aerosol Concentration (µg/L)[a]	Estimated Achieved Dose (µg/kg/day)[a,d]	Achieved Aerosol Concentration (µg/L)[a]	MMAD ± GSD [a e]
1[b]	Lactose	-	-	-	-	-
2[c]	Vehicle (Lactose/magnesium* stearate)	-	-	23	6.75	3.4 ±3.65
3	GSK573719A/ (b) (4) lactose	196	58.94	-	-	2.1 ±2.62
4	GSK573719A/ magnesium stearate/ lactose	-	-	31	9.35	3.8 ±2.11
5	GSK573719A/ lactose	-	-	-	-	-

a. Expressed in terms of the parent compound, GW857238 or expressed as magnesium stearate, determined by the measurement of magnesium by atomic absorption spectroscopy.
b. Exposed to lactose only.
c. Exposed to vehicle (1% w/w magnesium stearate in lactose) only.
d. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
e. MMAD = Mass Median Aerodynamic Diameter µm, GSD = Geometric Standard Deviation

D = (RMVxTxC)/ (BW)
D Dose (µg/kg/day)
RMV (respired minute volume L/minute) $0.499 \times BW(kg)^{0.809}$ (Bide, 2000)
T Duration of exposure/day (minutes)
C aerosol concentration (µg/L)
BW Group average daily body weight for the study (kg)

Aerosol concentrations of GSK573719, (b) (4) and magnesium stearate were measured with glass fiber filters fitted to an open face filter holder at a representative animal breathing point. Gravimetric and chemical analyses were conducted. GSK573719 and (b) (4) were measured by HPLC with UV detection. Magnesium stearate was measured by atomic absorption spectroscopy.

Particle size distribution was measured with a Mercer-style cascade impactor. Impactor stages were analyzed for GSK573719, (b) (4) and magnesium stearate.

Species/strain: Male and Female beagle dogs were obtained from (b) (4)

Number/sex/group or time point (main study): 3 dogs/sex/group
Route, formulation, volume, and infusion rate: Each aerosol delivery system consisted of a dust generator (Wright Dust Feed), an aerosol conditioning chamber fitted with four outlets, aerosol delivery tubing, Y-piece connections with isolating ball valve, and oropharyngeal tube attached. The aerosol exhaust system included a collapsible aerosol reservoir, further aerosol tubing, and a series of particulate filters, a flow meter with integral needle valve, and an extract pump. The oropharyngeal tubes were

constructed of silicone rubber and were inserted into the mouth of the dogs during dosing.

A separate atmosphere generation system was employed to dose each group of animals. The compressed air flow rate exiting the Wright Dust Feed (WDF) for Groups 1, 3, 4, and 5 was set to 20 L/min and an additional dilution flow of compressed air was supplied at 10 L/min to the conditioning chamber by an inlet that was adjacent to the aerosol entry point. The aerosol-laden air exiting the conditioning chamber (30 L/min, equal to 7.5 L/min/animal and each of two spare positions for sampling) was to four active outlets to potential animal exposure positions by clinical aerosol tubing with a Y-piece providing the attachment point for the ball valve and oropharyngeal tube. For Group 2, the air flow rate exiting the WDF was set to 15 L/min and the dilution flow was supplied at 12 L/min. Effective from Day 5 of treatment, the calibrated dilution flow was decreased to 12 L/min (overall system air flow of 27 L/min) in order to increase the atmosphere concentration. Two aerosol outlets were used to supply aerosol to the animals assigned to the study and the remaining outlets were used for the collection of aerosol samples. This arrangement was necessary due to the short exposure duration and the occasional requirement for the simultaneous collection of two samples (i.e., concentration and particle size distribution).

Exhaust air from the aerosol delivery system was delivered to a separate exhaust plenum incorporating a meteorological balloon pressure compensation reservoir that was exhausted at a flow of 27 L/min for Group 2. The air was passed through a filter system to remove the test article particulate before the air was discharged to atmosphere.

Satellite groups used for toxicokinetics or recovery: None
Age: Dogs were 13-14 months old at the start of treatment.
Weight: Body weight ranges were 8.2-10.3 kg for males and 6.9-9.1 kg for females.
Unique study design or methodology (if any): The objective of this study was to determine if there was any effect of magnesium stearate on the toxicity and toxicokinetics of GSK573719 in order to support the use of magnesium stearate in a clinical formulation of GSK573719. Previously, (b) (4) (b) (4) was used as an excipient in GSK573719 formulations; however, its use was discontinued due to issues of alveolar macrophage accumulation and pulmonary inflammation. The novel excipient (b) (4) (b) (4) or magnesium stearate was included in the GSK573719/1actose formulations to aid chemical stability.

Observation and Times:
Clinical signs: Animals were observed for viability and clinical signs twice daily. During the dosing period, animals were observed immediately after the dosing period, 1-2 hr postdose, and as late as possible in the day.
Body weights: Body weights were measured pretreatment and on days 1, 8, and 14.
Food consumption: Food consumption was measured daily.
Ophthalmoscopy: Ophthalmic examinations were conducted during pretreatment and at the end of the dosing period.

EKG: Electrocardiograms were recorded during pretreatment and week 2 using leads I, II, III, aVR, aVL, aVF, rV2, V2, and V10. Tracings were evaluated for abnormalities, heart rate, QRS complex, and P, PR, QRS, ST, QT, QTc, and RR intervals. Femoral pulse was measured on days 1 and 2 and during week 2 at 10 min and at 1, 2, 8, and 24 hr postdose. Blood samples for measurement of cardiac Troponin I were collected on days 1 and 14 at predose and 3, 7, and 24 hr after the start of dosing. Samples were shipped to the sponsor for analysis by immunoassay with chemoluminescent detection (assay range was 0.006 to 50 µg/L).
Hematology: Blood samples for measurement of a complete panel of hematology parameters were collected during pretreatment and on day 15.
Clinical chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected during pretreatment and on day 15.
Urinalysis: Urine samples for measurement of a complete panel of urinalysis parameters were collected overnight (approximately 16 hr) during pretreatment and week 2.
Gross pathology: Dogs were sacrificed on day 15 and submitted to gross necropsy examination.
Organ weights: Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, prostate, testes, and thymus.
Histopathology: Tissue samples were processed to paraffin wax, sectioned at 3 to 5 µm, stained with hematoxylin and eosin, and examined by light microscopy. A complete battery of tissues was examined. Tissues from all groups were examined. A peer review of selected microscopic tissues sections and pathology data interpretation was completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on days 1 and 14 immediately postdose and 0.33, .75, 1, 3, 7, and 24 hr after the start of dosing. Plasma samples were shipped to the sponsor for analysis. Naïve blood samples were available for the analysis. GSK573719 was measured using a HPLC/MS/MS method with a LLOQ of 0.1 ng/mL and a linear range of 0.1 to 100 ng/mL.

Results:

Mortality: None

Clinical signs: Dry mouth was seen at a higher incidence in all groups given GSK573719 generally immediately postdose and up to 2 hr postdose. Dry nose was seen in all groups given GSK573719, generally during dosing with a higher incidence in the group given GSK573719/ (b) (4) lactose. Swollen neck was observed primarily 2 hr postdose, starting on day 11 in all groups given GSK573719, but was seen at a higher incidence in the group given GSK573719/ (b) (4) /lactose. Excessive salivation was observed in all groups (including lactose and magnesium/lactose control groups) during dosing and immediately postdose although the response was exacerbated in animals given GSK573719. Excessive salivation was most likely due to problems with palatability of the test article and limited to time of dosing. Dry mouth occurred

somewhat later postdose following systemic absorption of the test article. Clinical signs of dry mouth, dry nose, and swollen neck were attributed to the antimuscarinic properties of the test article.

Body weights: There were no treatment-related effects on body weight gain.

Food consumption: Food consumption appeared to be lower for female treatment groups although body weight gain was unaffected.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

EKG: On Day 12, at predose, there was no respiratory sinus arrhythmia (RSA) on any ECGs taken from dogs receiving GSK573719A (Groups 3-5) and heart rates were higher compared to pretest period in most dogs (up to 1.5-fold). Immediately following GSK573719A administration, increased heart rates (up to 2.2-fold) were observed in all dogs from Groups 3, 4, and 5. Also no RSA was observed in all animals from Groups 3-5.

There were no test article-related effects on ST segment, P, PR, QRS, QT, and QTc intervals following administration of GSK573719A.

ECG Findings at Weeks 2 (Predose and Postdose)

Parameter	Time	Males					Females				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Heart rate bpm	Predose	88	104	146 (140%)	158 (152%)	133 (151%)	130	94	124 (132%)	141 (150%)	146 (112%)
	Postdose	68	83	176 (212%)	184 (222%)	140 (206%)	106	81	156 (193%)	172 (212%)	175 (165%)
RR interval msec	Predose	695	594	412 (69%)	381 (64%)	453 (65%)	485	650	486 (75%)	430 (66%)	413 (85%)
	Postdose	890	784	342 (44%)	326 (42%)	434 (49%)	598	797	391 (49%)	350 (44%)	343 (57%)

For Groups 3 and 4, values in parentheses are relative to Group 2-Control. For Group 5, values in parentheses are relative to Group 1-Control.

Femoral pulse rates were generally increased for male and female treatment groups on days 1, 2, and 14 at 10 min to 24 hr postdose. Maximum increases on day 1 were 176% for males in Group 3 at 1 hr, 192% for males in Group 5 at 10 min, 140% for females in Group 3 at 8 hr, 207% for females in Group 4 at 2 hr, and 185% for females in Group 5 at 10 min and 2 hr. Maximum increases on day 2 were 122-143% for males in Group 3 from 10 min to 24 hr, 120 to 167% for males in Group 4 from 10 min to 24 hr, 197% for males in Group 5 at 2 hr, 124% for females in Group 3 at 10 min, 132-184% for females in Group 4 from 10 min to 24 hr, and 120 to 198% for females in Group 5 from 1 to 24 hr. Maximum increases on day 14 were 153% for males in Group 3 at 8 hr, 159% for males in Group 4 at 1 hr, 163% for males in Group 5 at 1 hr, 122-149% for females in Group 3 from 1 to 24 hr, 119 to 177% for females in Group 4 at 10 min to 24 hr, and 113 to 151% for females in Group 5 at 10 min to 24 hr.

Cardiac troponin I concentrations were increased for all male treatment groups on day 1 at 3, 7, and 24 hr postdose. Cardiac troponin I concentrations were increased for females in Groups 4 and 5 at 3, 7, and 24 hr postdose. No increases of cardiac troponin I concentrations were evident on day 14.

Cardiac Troponin I Concentrations on Day 1

Cardiac Troponin I Concentrations (µg/L)

Day 1 - Males

	Predose	3hr	7hr	24hr
Group-1	0.006	0.010	0 007	0.009
Group-2	0.009	0.012	0 009	0.007
Group-3	0.007	0.073	0 064	0.037
%Control	75.269	623.932	714.444	552.239
Group-4	0.011	0.064	0 040	0.033
%Control	118.280	547.009	441.111	492.537
Group-5	0.007	0.025	0 031	0.043
%Control	111.667	254.639	438 571	490.805

Day 1 - Females

	Predose	3hr	7hr	24hr
Group-1	0.008	0.013	0.016	0.008
Group-2	0.007	0.012	0.011	0.008
Group-3	0.010	0.016	0.018	0.013
%Control	136.986	135.833	159.292	172.727
Group-4	0.009	0.035	0.036	0.016
%Control	123.288	289.167	318.584	207.792
Group-5	0.007	0.079	0.042	0.047
%Control	87.013	607.692	259.509	583.750

Cardiac Troponin I Concentrations on Day 14

Cardiac Troponin I Concentrations (µg/L)

Day 14 - Males

	Predose	3hr	7hr	24hr
Group-1	0.006	0.007	0.009	0.008
Group-2	0.006	0.009	0.007	0.006
Group-3	0.007	0.014	0.011	0.007
%Control	116.667	155.172	146.575	116.667
Group-4	0.009	0.011	0.013	0.010
%Control	150.000	122.989	178.082	166.667
Group-5	0.006	0.008	0.015	0.009
%Control	100.000	105.479	161.290	112.987

Day 14 - Females

	Predose	3hr	7hr	24hr
Group-1	0 006	0.008	0 008	0.009
Group-2	0 006	0.007	0 009	0.009
Group-3	0 009	0.009	0 010	0.011
%Control	150 000	132.857	111.494	121.505
Group-4	0 007	0.011	0 009	0.008
%Control	111 667	157.143	106 897	82.796
Group-5	0 008	0.008	0 009	0.007
%Control	122 222	100.000	112 500	74.444

Hematology: There were no treatment-related changes of hematology and coagulation parameters.

Clinical chemistry: There were no treatment-related changes of clinical chemistry parameters.

Urinalysis: There were no treatment-related changes of urinalysis parameters.

Gross pathology: Moderate to severe (bile) distension of the gallbladder was observed at necropsy in both sexes in all GSK573719 formulations. In several cases this finding was associated with abnormal bile content (black/granular bile content) or focal black serosal discoloration. Formulation had no effect on the incidence of the finding in GSK573719-treated males, but a lower incidence was seen in females given GSK573719/magnesium stearate/lactose.

Gross pathological findings in the gallbladder from dogs after treatment for 14 days

Incidence Test Article-Related Findings: Gallbladder

	Males					Females				
Group Number	1[1]	2[2]	3[3]	4[4]	5[5]	1[1]	2[2]	3[3]	4[4]	5[5]
Target dose GSK 573719 µg/kg/day	0	0	1000	1000	1000	0	0	1000	1000	1000
No. Animals Examined	3	3	3	3	3	3	3	3	3	3
Gallbladder: Distended	0	0	3	3	3	0	0	2	1	3
Abnormal bile content (black/granular bile)	0	0	1	0	0	0	0	0	0	1
Focal black serosal discoloration	0	0	0	1	0	0	0	0	0	0

1.Exposed to Lactose only
2.Exposed to magnesium stearate in lactose only.
3.Exposed to 5% (w/w) GSK573719A and 8% (w/w) GW857238X in lactose.
4.Exposed to 5% (w/w) GSK573719A and 1% (w/w) magnesium stearate in lactose.
5.Exposed to 5% (w/w) GSK573719A in lactose.

Organ weights: Prostate weights were decreased for all male treatment groups that received GSK573719; however, there were no corresponding histopathological findings.

Histopathology: Histopathological findings were evident in the gallbladder, lungs, trachea, tracheal bifurcation, trachea, and nose/turbinate. The profile of target organs with GSK573719 was unaffected by (b) (4) or magnesium stearate.

Gallbladder: Foci of minimal to slight myofiber degeneration/regeneration associated with variable hemorrhage and inflammation, were present in the tunica muscularis of animals dosed with all GSK573719 formulations. With one exception (female #4931 given GSK573719/lactose/magnesium stearate), these changes were associated macroscopically, with the observation of distension of the gallbladder with or without abnormal bile content. Although the incidence of grossly distended gallbladders was lower in the females given GSK573719/magnesium stearate/lactose, there were no meaningful differences in incidence of myofiber degeneration or gallbladder distension across formulations.

Lungs: Alveolar inflammation was observed for 1 female in GSK573719/lactose group. The incidence of cellular debris and mucus in the bronchiole lumen was increased for females in the GSK573719/ (b) (4) lactose group. A minimal focal microgranuloma was observed in the lung of one female given GSK573719A/lactose.

Tracheal bifurcation: Goblet cell hyperplasia in the tracheal bifurcation was observed for 1 female in the GSK573719/ (b) (4) lactose group.

Trachea: Lymphoid follicular aggregates and inflammatory cells/cell debris were observed for 1 female in the GSK573719/lactose group.

Nasal Turbinates: One female given GSK573719/magnesium stearate/lactose had multiple foci of minimal bilateral degeneration, ulceration and regeneration of the respiratory epithelium. These findings were judged to irrelevant to the clinical setting where the test article is administered by oral inhalation.

Histopathological findings in dogs after treatment for 14 days

Organ/Tissue	Males					Females				
	Vehicle		GSK573719			Vehicle		GSK573719		
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Gallbladder										
-muscular: myofiber degeneration/regeneration associated with hemorrhage/inflammation, Grade 1-2	0/3	0/3	2/3	2/3	1/3	0/3	0/3	0/3	1/3	1/3
Lungs										
-acute/subacute inflammation, alveolar, Grade 2	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
-bronchioles: lumen cellular debris/mucus, Grade 1	1/3	0/3	0/3	0/3	1/3	1/3	0/3	3/3	1/3	1/3
-microgranuloma, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Tracheal bifurcation										
-goblet cell hyperplasia, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
Trachea										
-lymphoid follicular aggregates, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
-lumen: inflammatory cells/cell debris, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Nose/Turbinate Section 2										
-nasal mucosa (respiratory), degeneration/regeneration, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3
Nose/Turbinate Section 3										
-nasal mucosa (respiratory), degeneration/regeneration, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3

Toxicokinetics: AUC values on day 14 were up to 4-old greater than values on day 1 suggestive of accumulation occurring during the process of achieving steady-state levels. On day 14, AUC values were generally highest for males and females receiving GSK573719 with lactose only. AUC values were generally similar for males and females on day 14. C_{max} values on days 7 and 14 were greater than values on day 1 for males receiving GSK573719 with magnesium stearate + lactose or lactose only and females receiving GSK573719 with lactose only. C_{max} values were erratic for females

Toxicokinetic parameters for GSK573719 in dogs on days 1, 7, and 14

Analyte	Parameter [1]	Day	Male			Female		
			Estimated Achieved Dose GSK573719			Estimated Achieved Dose GSK573719		
			1018 µg/kg/day	1023 µg/kg/day	1103 µg/kg/day	1018 µg/kg/day	1023 µg/kg/day	1103 µg/kg/day
Excipient			G	M	L	G	M	L
GSK573719	$AUC_{(0-t)}$ (ng.h/mL)	Day 1	48.151	37.033	38.274	10.282	33.350	26.582
		Day 14	75.332	79.589	96.843	52.732	79.590	102.689
	C_{max} (ng/mL)	Day 1	106.890	47.365	53.054	16.492	65.779	35.406
		Day 7[2]	109.944	85.932	132.360	21.582	29.053	82.725
		Day 14	71.871	78.976	99.601	36.750	75.600	88.019

[1]Calculated values, derived from dose-normalized toxicokinetic parameters (see Appendix 20, Table 2) multiplied by the average study estimated achieved dose.
[2] Samples only taken at 10 min (IAD) post dosing on Day 7.
L=GSK573719+ lactose, M = GSK573719+ magnesium stearate/lactose, G = GSK573719+GW857838/lactose

Study title: **A 28-Day Inhalation Toxicity Study of a Powder Aerosol Formulation in the Beagle Dog**

Key study findings:

● In a 28-day inhalation toxicology study with dogs (WD2005/01423/00), GSK573719 was administered by face mask fitted with an oropharyngeal tube at estimated achieved doses of 0, 16.2, 208.0, and 2757.6 µg/kg/day (pulmonary deposited doses were 4.0, 52, and 689.4 µg/kg/day, respectively).

● Heart rates were increased up to 1.7-fold for male and female dogs in the 208.0 and 2757.6 µg/kg/day groups during the treatment period.

● Histopathological findings were observed in the lung, thymus, nasal cavity/sinuses, larynx, and trachea. Findings in the nasal cavity/sinuses were judged to be irrelevant to clinical administration by oral inhalation.

● In the lung, granulomatous inflammation was observed for all male treatment groups and females in the high dose group; the incidence and severity did not display dose-response relationships. Slight to moderate multifocal acute and/or subacute bronchoalveolar inflammation was observed for 1 male at 16.2 µg/kg/day and 1 male and 2 females 2757.6 µg/kg/day.

● In the thymus, lymphoid atrophy was observed for the control and all treatment groups. An increased severity was noted for males at 208.0 µg/kg/day and males and females at 2757.6 µg/kg/day; however, the incidence in the control group was high and the relationship of these findings to treatment was considered questionable.

● In the larynx, minimal to moderate acute/subacute inflammation was observed for males and females at 2757.6 µg/kg/day. Mineralization and exudate were also evident for females at 2757.6 µg/kg/day.

● In the trachea, slight to moderate epithelial degeneration/regeneration and minimal acute/subacute inflammation were observed for females at 2757.6 µg/kg/day

● Granulomatous inflammation in the lung was judged to unrelated to the test article (see Summary and Evaluation). The NOAEL was identified as 208 µg/kg/day based upon histopathological findings in the lung, larynx, and trachea for the high dose group. Estimated AUC values for males and females in the 208 µg/kg/day group on day 28 were 25.9 and 29.9 ng·hr/mL, respectively (determined by extrapolation from the high dose group).

Study no.: WD2005/01423/00
Volume #, and page #: Volume 15, Pages 1-444
Conducting laboratory and location: (b) (4)

Date of study initiation: September 8, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Micronized GSK573719A, from input batch 70683AA001, Lot number 051093955 (83.8% purity as free base), was supplied by the sponsor. GSK573719A powder was blended with lactose powder BP/USNF (batch number B143232, Lot number 051089255) at a nominal concentration of 4% (w/w) with 8% (w/w) (b) (4), batch number R159636).

The vehicle consisted of 8% (w/w) (b) (4) in lactose from the same batches as those used for test article preparation.

Methods
Doses: GSK573719 was administered at estimated achieved doses of 0, 16.2, 208.0, and 2757.6 µg/kg/day.

Inhaled doses and particle sizes of GSK573719A

GSK573719A

Group Number	Target Dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day)[a,c]	Target Mask Aerosol Concentration (µg/L)	Achieved Mask Aerosol Concentration (µg/L)[a]	MMAD ± GSD[b]
1	0	0	0	0	(b) (4)
2	10	16.2	0.4	0.4	
3	100	208.0	4.2	4.1	
4	2000	2757.6	83.3	61.2	

a. Expressed in terms of parent compound (GSK573719)
b. MMAD = Mass Median Aerodynamic Diameter (µm), GSD = Geometric Standard Deviation
c. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/(BW)
D Dose (µg/kg/day)
RMV Measured during the pretreatment period and Week 4 using the Buxco BioSystem XA (L/min)
T Duration of exposure/day (minutes)
C GSK573719 Aerosol concentration (µg/L)
BW Group average body weight for study (kg) Days 1-28

Inhaled doses and particle sizes of (b) (4)

GW857238X

Group Number	Estimated Achieved Dose (µg /kg/day)[a,c]	Achieved Mask Aerosol Concentration (µg/L)[a]	MMAD ± GSD[b]
1	11759.3	240.4	(b) (4)
2	32.3	0.8	
3	375.3	7.4	
4	11003.2	244.2	

a. Expressed in terms of (b) (4)
b. MMAD = Mass Median Aerodynamic Diameter (µm), GSD = Geometric Standard Deviation
c. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/(BW)
D Dose (µg/kg/day)
RMV Measured during the pretreatment period and Week 4 using the Buxco BioSystem XA (L/min)
T Duration of exposure/day (minutes)
C (b) (4) Aerosol concentration (µg/L)
BW Group average body weight for study (kg) Days 1-28

Mask aerosol concentrations were measured using open-face glass fiber filters fitted to a sampling device. Filters were analyzed for GSK573719 and (b) (4) by HPLC.

Particle size distributions were determined using a cascade impactor on days 2 and 25. Aerosols were classified into a series of size ranges to determine the amount of mass aerosol collected in each size range. The filter samples collected from the stages of the cascade impactor were analyzed by chemical methods for GSK573719 and (b) (4) content using HPLC. The MMAD and GSD were calculated from gravimetric and analytical data.

Species/strain: Male and Female beagle dogs were obtained from (b) (4)

Number/sex/group or time point (main study): 3 dogs/sex/group
Route, formulation, volume, and infusion rate: Dogs received daily inhaled doses of GSK573719A or vehicle for 60 min per day. GSK573719A was administered by a face mask fitted with an oropharyngeal tube that was used to depress the tongue, consisting of a plastic cylinder fitted over the dog's muzzle in such a way that the nose was inside the cylinder and the animal was mouth breathing through the short oropharyngeal tube. An aerosol was generated from powder test article using a positive flow delivery system and introduced into a mixing chamber by predried compressed air. The resulting atmosphere was carried to the dog mask. A medical breathing bag was used to provide the dogs with a reservoir of additional air. The mixing chamber was operated under slight positive pressure monitored as a differential pressure across a constriction in the exhaust line using a Magnehelic™ gauge. Compressed air was introduced into the chamber at 30 L/min from the top with further air being introduced into the bottom of the chamber at 40 L/min with a scavenging exhaust attached. The test atmospheres were generated into the chamber air inlet using a Venturi T-section supplied with pre-dried compressed air. The various test article concentrations were achieved by altering the rate of test article introduction into the feed nozzle of the T-section.
Satellite groups used for toxicokinetics or recovery: None
Age: Dogs were 8 to 9 months old at the start of treatment
Weight: Body weight ranges were 6.4 to 8.7 kg for males and 5.6 to 8.4 kg for females.
Unique study design or methodology (if any): None

Observation and Times:
Clinical signs: Dogs were observed twice daily for moribundity/mortality. Animals were also observed immediately postdose and 1-4 hr postdose.
Body weights: Body weights were measured weekly.
Food consumption: Food consumption was recorded daily.
Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of dosing and during week 4.
EKG: Electrocardiograms were recorded once during pretreatment and during week 4 using leads I, II, III, aVR, aVL, and aVF. Traces were examined for rhythm and abnormalities. Quantitative measures of heart rate, PR, QRS, QT, and RR intervals were performed. QTc was calculated using Van de Waters equation. Heart rate was measured on days 1 and 28 before dosing, just prior to blood collection, immediately after dosing, and at 0.5, 1, 2, 4, 8, and 23 hr after dosing. Heart rates were also measured from days 2 to 7 before dosing, immediately after dosing, and 4 hr after dosing.

Respiratory minute volume: During the pretreatment period (twice) and at the end of week 4, respiratory minute volume was recorded over a 15-min period for each animal.
Hematology: Blood samples for measurement of a complete panel of hematology and coagulation parameters were collected during weeks -3 and 4.
Clinical chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected during weeks -3 and 4. Cardiac troponin I was measured by ELISA (b) (4) on days 1 and 28 before dosing and at 4, 8, and 23 hr postdose.
Urinalysis: Urine samples for measurement of a complete panel of urinalysis parameters were collected overnight during weeks -3 and 4.
Gross pathology: Dogs were sacrificed on day 29 and submitted to necropsy examinations.
Organ weights: Absolute and relative organ weights were measured for the adrenal glands, brain, heart, kidneys, liver, lung, ovaries, prostate, testes, and thymus.
Histopathology: Tissues were processed to paraffin wax, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. A complete battery of tissues was examined. Tissues from all groups were examined. Stage-dependent evaluation of spermatogenesis was conducted on sections of testes from control and high dose animals. Sections were prepared and stained with Periodic Acid Schiff's-Hematoxylin. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on days 1 and 28 prior to dosing and at 0.5, 1, 2, 4, 8, and 23 hr postdose. GSK573719 was measured using a HPLC/MS/MS method with a LLOQ of 0.1 ng/mL and a linear range of 0.1 to 100 ng/mL.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Body weight gains were reduced for males and females in the mid dose group (1.4 and 2.1% increases of initial body weights, respectively) as compared to controls (6.6 and 6.1% increases of initial body weights, respectively). Body weights for males and females in the high dose group were decreased by 2.3 and 1.9%, respectively.

Food consumption: Food consumption for males in the low, mid, and high dose groups were decreased to 87.7, 79.2, and 78% of the control, respectively.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

EKG: Heart rates were increased up to 1.7-fold primarily for males and females in the high dose group. Increased heart rates were also observed for males and females in the

mid dose group although the magnitude and incidence of increased heart rates were less than observed for the high dose group. These increases appeared to occur throughout the dosing period. Troponin I concentrations on days 1 and 28 were below the LLOQ (value not provided in the study report) for all male and female control and treatment groups. There were no significant changes of PR, QT and QTc intervals as well as QRS complex duration.

Heart rate and ECG parameters during week 4

Parameter	Time	Males				Females			
		Control	LD	MD	HD	Control	LD	MD	HD
Heart rate bpm	Predose	120	130	100	147	113	90	143	153
	Immediately Postdose	100	130	133	173 (173%)	120	127	133	180 (150%)
RR Interval msec	Predose	519	494	640	411	565	667	422	392
	Immediately Postdose	617	462	453	350	506	492	454	334 (152%)

Heart rate measurements

Day	Time	Males				Females			
		Control	LD	MD	HD	Control	LD	MD	HD
1	Immediately Postdose	108	110	85	138 (128%)	114	96	101	159 (139%)
	0.5 hr	102	104	107	136 (133%)	103	110	98	122
	1 hr	108	100	109	164 (152%)	101	88	109	151 (150%)
	2 hr	100	102	112	166 (166%)	104	102	106	156 (150%)
	4 hr	112	99	119	152 (136%)	103	102	102	138 (134%)
	8 hr	127	125	124	142 (112%)	99	115	121	135 (136%)
	23 hr	127	110	122	141 (111%)	107	97	119	133 (124%)
2	Predose	127	110	122	141 (111%)	107	97	119	133 (124%)
	Immediately Postdose	115	112	115	153 (133%)	105	89	148 (141%)	168 (160%)
	4 hr	103	108	132 (128%)	143 (139%)	98	98	115	147 (150%)
3	Predose	132	92	121	133	107	91	131	118
	Immediately Postdose	119	105	120	169 (142%)	95	99	154 (162%)	197 (207%)
	4 hr	116	103	130	158 (136%)	100	115	139 (139%)	147 (147%)
4	Predose	133	91	135	150	103	107	142	148
	Immediately Postdose	106	117	152 (143%)	170 (160%)	119	99	159 (134%)	211 (177%)
	4 hr	105	105	130 (124%)	158 (150%)	108	105	130 (120%)	159 (147%)

5	Predose	115	104	137 (119%)	140 (122%)	111	92	137 (123%)	132 (119%)
	Immediately Postdose	108	115	142 (131%)	194 (180%)	92	91	160 (174%)	187 (203%)
	4 hr	112	120	149 (133%)	141 (126%)	110	104	130 (118%)	139 (126%)
6	Predose	128	103	128	159 (124%)	117	101	127	149 (127%)
	Immediately Postdose	138	96	120	186 (135%)	116	90	160 (138%)	187 (161%)
	4 hr	114	96	117	148 (130%)	101	102	121	145 (144%)
7	Predose	133	115	123	132	121	101	119	155 (128%)
	Immediately Postdose	109	119	140	182 (167%)	105	97	140 (133%)	179 (170%)
	4 hr	113	98	132	163 (144%)	109	103	125	165 (151%)
28	Predose	117	94	115	141 (121%)	120	101	112	155 (129%)
	Immediately Postdose	123	107	119	139 (113%)	118	109	125	160 (136%)
	0.5 hr	115	103	119	150 (130%)	116	102	132	150 (129%)
	1 hr	118	107	128	147 (125%)	121	108	137	158 (131%)
	2 hr	133	112	136	146 (110%)	121	108	137	158 (131%)
	4 hr	122	106	132	142 (116%)	119	107	133	137 (115%)
	8 hr	136	112	124	138	123	115	127	138 (112%)
	23 hr	119	103	103	124	95	91	113	125 (132%)

Hematology: There were no toxicologically significant changes of hematology parameters.

Clinical chemistry: There were no toxicologically significant changes of clinical chemistry parameters.

Urinalysis: There were no toxicologically significant changes of urinalysis parameters.

Gross pathology: Gross necropsy findings were evident for males and female in the high dose group that might correlate to histopathological findings. For females in the high dose group, necropsy findings were observed in the lung (area depressed [1/3], area raised [2/3], discoloration dark [1/3], firm [1/3]), thymus (small [1/3]), and tracheobronchial LN (area dark [1/3], enlargement [1/3]) that might correlate with histopathological findings. For males in the high dose group, necropsy findings were observed in the lung (pale foci [1/3]) and tracheobronchial LN (enlargement [1/3]).

Organ weights: Differences of absolute and relative organ weights were evident between control and treatment groups for the thymus, lung, prostate, and ovary.

Absolute and relative lung weights were increased for females in the HD group that might correlate with histopathological findings of granulomatous and bronchoalveolar inflammation.

Absolute and relative thymus weights were decreased for all male and female treatment groups that appeared to correlate with findings of increased severity of lymphoid atrophy.

Absolute and relative prostate weights were increased for males in the high dose group although there were no corresponding histopathological findings.

Absolute and relative ovaries weights were decreased for all female treatment groups although there were no corresponding histopathological findings.

Organ weights

Organ	Males				Females			
	Control	LD	MD	HD	Control	LD	MD	HD
Lung g	NC	NC	NC	NC	56.8540	58.6633	53.3727	80.4960 (142%)
Lung %BW	NC	NC	NC	NC	0.82802	0.93800	0.85727	1.21742 (147%)
Thymus g	5.2627	4.3770 (83%)	3.9707 (75%)	2.6147 (50%)	5.8040	3.1653 (55%)	3.1753 (55%)	4.0560 (70%)
Thymus %BW	0.06796	0.5392 (79%)	0.5306 (78%)	0.03687 (54%)	0.08764	0.04988 (57%)	0.05166 (59%)	0.05861 (67%)
Prostate g	3.5817	4.0740	2.7697	4.8170 (135%)				
Prostate %BW	0.04770	0.04965	0.03698	0.06705 (141%)				
Ovary g					1.5773	0.7653 (49%)	0.8407 (53%)	0.8713 (55%)
Ovary %BW					0.02290	0.01210 (53%)	0.01345 (59%)	0.01367 (60%)

Histopathology: Histopathological changes were observed in the lung, thymus, nasal cavity/sinuses, larynx, and trachea.

In the lung, there were several histopathological findings as follows:

- Granulomatous inflammation was observed for all male treatment groups and females in the high dose group. In the areas with granulomatous inflammation, macrophages were numerous and giant cell formation was a prominent feature. On occasion, foreign material compatible with plant particles could be clearly identified within the granulomas. The incidence and severity of granulomatous inflammation for male treatment groups displayed no dose-response relationship. Based upon results of a second 28-day

inhalation toxicology study with dogs (WD2006/03294/00), granulomatous inflammation in the lung was attributed to aspiration of feed and unrelated to GSK573719.

- Eosinophilic material and interstitial inflammation were observed for all male treatment groups; however, these findings were judged to be spontaneous and unrelated to treatment. Interstitial inflammation is a common finding for young beagle dogs with a background incidence of 22-28%. Eosinophilic material was a background finding in other studies.

- Slight to moderate multifocal acute and/or subacute bronchoalveolar inflammation was observed for 1 male at 16.2 µg/kg/day and 1 male and 2 females 2757.6 µg/kg/day; a dose-response relationship was not evident for male treatment groups.

- Minimal multifocal mineralization was observed for 1 female in the high dose group.

In the thymus, lymphoid atrophy was observed for the control and all treatment groups. An increased severity was noted for males at 208.0 µg/kg/day and males and females at 2757.6 µg/kg/day. The incidence in the male and female control groups was high and the relationship of these findings to treatment was considered questionable.

Several histopathological changes were evident in the nasal cavity/sinuses. In the respiratory epithelium, minimal to moderate squamous metaplasia with or without keratinization at 2757.6 µg/kg/day and minimal to marked degeneration/regeneration at ≥16.2 µg/kg/day were noted in the septum and/or turbinates at levels 2 and 3, respectively. Changes observed in the squamous epithelium consisted of slight to moderate hyperplasia at level 1 of the nasal cavity at 2757.6 µg/kg/day. In the olfactory epithelium, minimal to slight degeneration/regeneration was noted at levels 3 and 4 at ≥208.0 µg/kg/day and at level 4 at 2757.6 µg/kg/day. Acute/subacute inflammation, ulceration of the squamous and/or respiratory epithelium (males only), and goblet cell hyperplasia (one female) was also noted at 2757.6 µg/kg/day. Dilatation and atrophy of Bowman's glands were observed for all male and female control and treatment groups although the incidence and severity was increased at 2757.6 µg/kg/day. Hyperplasia of Bowman's gland was observed for 1 male at 2757.6 µg/kg/day. An increase in PAS/AB staining of Bowman's glands indicated an increase of mucin secretion by the glands for males and females at 2757.6 µg/kg/day. These findings in the nasal cavities/sinuses were judged to be irrelevant to the clinical setting with administration by oral inhalation.

In the larynx, minimal to moderate acute/subacute inflammation was observed for males and females at 2757.6 µg/kg/day. Mineralization and exudate was also evident for females at 2757.6 µg/kg/day.

In the trachea, slight to moderate epithelial degeneration/regeneration and minimal acute/subacute inflammation were observed for females at 2757.6 µg/kg/day.

For males in the control and high dose groups, seminiferous tubules were evaluated with respect to their stage in the spermatogenic cycle and the integrity of the various cell

types present within the different stages. No cell- or stage-specific abnormalities were noted.

Histopathological findings in dogs following a 28-day treatment period

Organ/Tissue	Males				Females			
	0	16.2	208	2757.6	0	16.2	208	2757.6
Lung								
-inflammation, granulomatous	0/3	1/3	1/3	1/3	0/3	0/3	0/3	2/3
Grade 1	0	0	1	0	0	0	0	1
Grade 2	0	0	0	1	0	0	0	1
Grade 3	0	1	0	0	0	0	0	0
Lung								
-material, eosinophilic	0/3	1/3	1/3	1/3				
Grade 1								
Lung								
-inflammation, interstitial,	0/3	1/3	1/3	1/3	0/3	0/3	0/3	1/3
Grade 2-3								
Lung								
-inflammation, bronchoalveolar	0/3	1/3	0/3	1/3	0/3	0/3	0/3	2/3
Grade 2	0	1	0	1	0	0	0	1
Grade 3	0	0	0	0	0	0	0	1
Lung								
-mineralization, Grade 2	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Thymus								
-atrophy, lymphoid	2/3	2/3	3/3	3/3	2/3	2/3	3/3	2/3
Grade 1	0	1	0	0	1	1	1	1
Grade 2	2	1	2	1	1	1	2	0
Grade 3	0	0	1	2	0	0	0	1
Nasal Cavity/Sinuses								
-degeneration/regeneration, respiratory epithelium	0/3	1/3	2/3	3/3	0/3	0/3	1/3	3/3
Grade 1	0	1	1	0	0	0	1	0
Grade 2	0	0	1	2	0	0	0	0
Grade 3	0	0	0	1	0	0	0	2
Grade 4	0	0	0	0	0	0	0	1
Nasal Cavity/Sinuses								
-degeneration/regeneration, olfactory epithelium	0/3	0/3	0/3	2/3	0/3	0/3	1/3	2/3
Grade 1	0	0	0	1	0	0	1	1
Grade 2	0	0	0	1	0	0	0	1
Nasal Cavity/Sinuses								
-hyperplasia, squamous epithelium	0/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3
Grade 2	0	0	0	2	0	0	0	2
Grade 3	0	0	0	1	0	0	0	1
Nasal Cavity/Sinuses								
-metaplasia, squamous, respiratory epithelium	0/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3
Grade 1	0	0	0	1	0	0	0	1
Grade 2	0	0	0	1	0	0	0	2
Grade 3	0	0	0	1	0	0	0	0
Nasal Cavity/Sinuses								
-ulceration, respiratory and	0/3	0/3	0/3	2/3	0/3	0/3	0/3	0/3

Organ/Tissue	Males				Females			
	0	16.2	208	2757.6	0	16.2	208	2757.6
squamous epithelium Grade 1 Grade 2	 0 0	 0 0	 0 0	 1 1	 0 0	 0 0	 0 0	 0 0
Nasal Cavity/Sinuses -inflammation, acute and subacute, Grade 1	0/3	0/3	0/3	1/3	0/3	0/3	0/3	2/3
Nasal Cavity/Sinuses -dilatation and atrophy, Bowman's glands Grade 1 Grade 2 Grade 3	1/3 1 0 0	1/3 1 0 0	2/3 2 0 0	2/3 0 1 1	2/3 2 0 0	3/3 3 0 0	1/3 1 0 0	3/3 1 2 0
Nasal Cavity/Sinuses -hyperplasia, Bowman's gland Grade 1	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
Nasal Cavity/Sinuses -increased Periodic Acid Schiff/Alcian blue staining Grade 1	0/3	0/3	0/3	2/3	0/3	0/3	0/3	3/3
Nasal Cavity/Sinuses -hyperplasia and hypertrophy, goblet cells, Grade 2	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Larynx -inflammation, acute and subacute Grade 1 Grade 3	0/3 0 0	0/3 0 0	0/3 0 0	1/3 0 1	0/3 0 0	0/3 0 0	0/3 0 0	3/3 2 1
Larynx -mineralization, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Larynx -exudate, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Trachea -inflammation, acute and subacute, Grade 2	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Trachea -degeneration/regeneration, epithelium Grade 2 Grade 3	0/3 0 0	0/3 0 0	0/3 0 0	0/3 0/3 0/3	0/3 0 0	0/3 0 0	0/3 0 0	2/3 1/3 1/3

Toxicokinetics: The sponsor reported that toxicokinetic data collected for dogs in the 16.2 and 208.0 µg/kg/day groups were highly variable. T_{max} data were highly variable (in some instances at the 9 or 24 hour time points), there were a number of anomalously high values, and it was not possible to distinguish a recognizable plasma concentration profile from the data. Based upon these problems, the sponsor did not report data for the 16.2 and 208.0 µg/kg/day groups.

There were no differences in systemic exposure between males and females in the 2757.6 µg/kg/day group. With repeat dosing, AUC_{0-t} and C_{max} values on day 28 were 3- to 4-fold greater than values on day 1. Estimated AUC values for males and females in

the 208 µg/kg/day group on day 28 were 25.9 and 29.9 ng·hr/mL, respectively (determined by extrapolation from the high dose group).

Plasma samples from vehicle control animals, collected at the same time points and occasions as the GSK573719A treated animals, were analyzed for GSK573719. A total of 17 samples (out of 84) contained quantifiable levels of GSK573719 above the lower limit of quantification (0.1 ng/mL). The range was 0.110 to 0.391 ng/mL with one animal having a plasma concentration of 1.26 ng/mL at one time point. Naïve samples showed detectable levels of drug in five of six samples (0.112-1.4 ng/mL). The quantifiable levels of GSK573719 in control samples were considered to be a result of external contamination because of the relatively low levels of quantifiable GSK573719, lack of any corresponding toxicokinetic profile, and evidence of similar concentrations of GSK573719 in naïve samples. In addition, GSK573719 was not detected in the control exposure mask aerosol on days 1 or 28.

Table 1 Summary of the Toxicokinetic Parameters for GSK573719 Derived from Serial Plasma Concentration-Time Results from Male and Female Dogs Following Inhalation of GSK573719A for 28 Days.

Day	Estimated Achieved Dose Male/Female[1] (µg/kg/day)	AUC_{0-t} (ng.h/mL)		C_{max} (ng/mL)		T_{max}[2] (h)	
		Male	Female	Male	Female	Male	Female
Day 1	18.2/13.2	NR	NR	NR	NR	NR	NR
	223/211	NR	NR	NR	NR	NR	NR
	1810/2660	94.7	82.8	44.0	45.6	1.02	1.03
Day 28	28.4/19.8	NR	NR	NR	NR	NR	NR
	86.9/172	NR	NR	NR	NR	NR	NR
	2300/6140	344	396	154	172	1.02	1.02

1. Estimated achieved dose on the relevant toxicokinetic sampling day
2. T_{max} values were derived from averaged actual sampling time
n = 3 males and 3 females per timepoint per dose group
NR: Not Reported.

Study title: **<u>A 28-Day Investigative Toxicity of a Powder Aerosol Formulation in the Beagle Dog</u>**

Key study findings:

● The objective of this 28-day inhalation toxicology study with dogs (WD2006/03294/00) was to investigate the effect of a modification to feeding methods, designed to reduce the possibility of inhalation of particulate matter from the diet, on the formation of granulomas in the lung seen in a previous 28-day study (WD2005/01423/00). Foreign material compatible with plant material found in the diet was identified within granulomas.

● Group 1, 2, 3, and 4 received GSK573719 at estimated achieve doses of 0, 22, 2254, and 1835 µg/kg/day, respectively. The feeding regimen for dogs in Groups 1, 2, and 3

was modified to provide pelleted diet mixed with water 3 hr after completion of dosing for a period of 2 hr (identified as new feeding regimen, NFR). Dogs in Group 4 were given dry pelleted diet prior to dosing for approximately 6 hr (commencing in the morning), and removed generally during dosing or up to 40 min following the completion of dosing (identified as old feeding regimen (OFR) used in the previous study).

● Dry eyes/decreased tear production, attributed to the pharmacological action of GSK573719, was evident in all treatment groups. Ophthalmic findings, attributed to dry eyes/decreased tear production, were evident for 1 dog in each of the high dose/NFR and high dose/OFR groups.

● Increased heart rates (up to 1.6-fold) were evident for male and female dogs in the high dose/NFR and high dose/OFR groups. Increases were more pronounced on day 1 as compared to days 25 or 27.

● Histopathological examinations of tissues were limited to gross lesions, bronchi, larynx, lungs, tracheobronchial LN, nasal cavity, pharynx, trachea, and tracheal bifurcation. Findings were observed in the lung, larynx, trachea, tracheal bifurcation, bronchus, and nasal cavity/sinuses. Findings in the nasal cavity/sinuses were judged to be not relevant to the clinical setting with oral inhalation of the drug product.

● Microscopic findings considered to be related to the old feeding regimen (OFR) were seen in the lungs. Dogs given the high dose/OFR (Group 4) had minimal to slight granulomatous inflammation in the lungs (1/3 males and 2/3 females), which was not seen in dogs given the high dose/new feeding regimen (Group 3). The male dog with granulomatous inflammation in the lungs also had suppurative bronchoalveolar inflammation and both of these inflammatory foci contained foreign material that was consistent in appearance with food/plant material. Increased incidences of interstitial inflammation were observed for all male treatment groups. An increased incidence of accumulation of foamy alveolar macrophages was evident for male dogs given the high dose/OFR (Group 4).

● For the larynx, acute inflammation and degeneration/necrosis of the epithelium was observed for all male treatment groups and females in the high dose/NFR (Group 3) and high dose/old feeding regimen (Group 4) groups. Exudate was also evident for all male treatment groups and females in the high dose/NFR (Group 3) and high dose/OFR (Group 4) groups.

● For the trachea, acute inflammation was observed for all male treatment groups and females in the low dose/NFR and high dose/NFR groups. Degeneration/regeneration of the epithelium was observed for male and female dogs in the high dose/NFR (Group 3) and high dose/OFR (Group 4) groups. Single cell necrosis was observed for 1 male in the high dose/NFR (Group 3) group. Exudate in the lumen and epithelial necrosis were each observed for 1 male in the high dose/OFR (Group 4) and 1 female in the high dose/NFR (Group 3) group.

● For the tracheal bifurcation, acute inflammation and degeneration/regeneration of the epithelium were each observed for one male and one female in the high dose/NFR (Group 3) group.

● For the bronchus, acute inflammation and degeneration/regeneration of the epithelium were observed for males in the low dose and high dose/NFR (Groups 2 and 3) groups and females in the high dose/NFR (Group 3) group.

● Results of this study suggest that granulomatous inflammation observed in the previous 28-day toxicology study was associated with aspiration of feed and not a direct test article effect. The pharmacological action of the test material on mucous secretion in the respiratory tract and upper digestive system (dryness/lack of normal secretions) could have played a major role in this accidental inhalation of the exogenous material. A NOAEL was not established in the present study based upon histopathological findings in the bronchus, trachea, larynx, and lung. Many of the histopathological findings (e.g., accumulation of foamy macrophages in the lung and acute inflammation and degeneration/regeneration of the epithelium in the trachea and bronchus) were not consistent with the other two 28-day inhalation toxicology studies. The new feeding regimen appeared to minimize the potential for inhalation of particulate matter from the diet.

Study no.: WD2006/03294/00
Volume #, and page #: Volume 16, Pages 1-504
Conducting laboratory and location: (b) (4)

Date of study initiation: June 6, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Micronized GSK573719A (from batch number EE73761A, Lot number 061118857, 83.8% purity as free base) was blended in lactose (batch number B143232, Lot number 051089255) at 2 nominal concentrations of 0.4% and 4% w/w with a nominal 8% w/w micronized (b) (4) (batch number R168290). Control animals received the vehicle alone, 8% w/w (b) (4) blended in lactose (batch number R168290 and B143232, respectively).

Methods
Doses: Group 1, 2, 3, and 4 received GSK573719 at estimated achieve doses of 0, 22, 2254, and 1835 µg/kg/day, respectively. The feeding regimen for dogs in Groups 1, 2, and 3 was modified to provide pelleted diet mixed with water 3 hr after completion of dosing for a period of 2 hr (identified as new feeding regimen). Dogs in Group 4 were given dry pelleted diet prior to dosing for approximately 6 hr (commencing in the morning), and removed generally during dosing or up to 40 min following the completion of dosing (identified as old feeding regimen).

Inhalation doses of GSK573719A

Group	Average achieved aerosol mask concentration (µg /L)[a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day)[a, b]	
			Male	Female	Male	Female	Males	Females
1	0	60	9.25	7.36	11.05	8.54	0	0
							Average = 0	
2	0.56	60	7.44	5.54	10.69	8.87	23	21
							Average = 22	
3	58.7	60	5.77	6.57	11.17	8.61	1819	2688
							Average = 2254	
4	64.1	60	4.76	4.09	10.64	8.07	1721	1949
							Average = 1835	

a. Expressed in terms of parent compound, GSK573719.
b. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/(BW)
D dose (µg/kg/day)
RMV Respiratory minute volume; Measured during the pretreatment period and Week 4 using the Buxco BioSystem XA (L/min)
T Duration of exposure/day (minutes)
C Aerosol GSK573719 concentration (µg/L)
BW Group average body weight for study (kg) Days 1 to 27.

Inhalation doses of [redacted] (b) (4)

Average mas[redacted] (b) (4) aerosol concentrations and resulting estimated doses (males and females combined) o[redacted] (b) (4) were as follows:

Group	Average achieved aerosol mask concentration (µg /L)[a]	Time of exposure (mins)	RMV (L/min)		Body weight (kg)		Estimated achieved dose (µg/kg/day)[a, b]	
			Male	Female	Male	Female	Males	Females
1	177	60	9.25	7.36	11.05	8.54	8890	9153
							Average = 9022	
2	8.4	60	7.44	5.54	10.69	8.87	351	315
							Average = 333	
3	174	60	5.77	6.57	11.17	8.61	5393	7966
							Average = 6680	
4	179	60	4.76	4.09	10.64	8.07	4805	5443
							Average = 5124	

a. Expressed in terms o[redacted] (b) (4)
b. Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/(BW)
D dose (µg/kg/day)
RMV Respiratory minute volume; Measured during the pretreatment period and Week 4 using the Buxco BioSystem XA (L/min)
T Duration of exposure/day (minutes)
C Aerosol [redacted] (b) (4) concentration (µg/L)
BW Group average body weight for study (kg) Days 1 to 27.

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolised GSK573719 are presented below.

Group number	Average achieved mask aerosol concentration (µg/L)	MMAD (µm)	GSD
1	0	(b) (4)	
2	0.56		
3	58.7		
4	64.1		

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) data for aerosolised (b) (4) are presented below.

Group number	Average achieved mask aerosol concentration (µg/L)	MMAD (µm)	GSD
1	177	(b) (4)	
2	8.4		
3	174		
4	179		

Mask aerosol concentrations were measured using open-face glass fiber filters fitted to a sampling device. Filters were analyzed for GSK573719 and (b) (4) by HPLC.

Particle size distributions were determined using a cascade impactor on days 2 and 25. Aerosols were classified into a series of size ranges to determine the amount of mass aerosol collected in each size range. The filter samples collected from the stages of the cascade impactor were analyzed by chemical methods for GSK573719 and (b) (4) content using HPLC. The MMAD and GSD were calculated from gravimetric and analytical data.

Species/strain: Male and Female beagle dogs were obtained from (b) (4)

Number/sex/group or time point (main study): 3 dogs/sex/group
Route, formulation, volume, and infusion rate: Dogs received GSK573719 or the vehicle once daily for 60 minutes by oropharyngeal inhalation by face mask fitted with a short

tube (tongue depressor) that was used to depress the tongue, consisting of a plastic cylinder fitted over the dog's muzzle in such a way that the nose was inside the cylinder and the animal was mouth breathing through the short tube. An aerosol was generated from powder control or test article using a positive flow delivery system and introduced into a mixing chamber by pre-dried compressed air. The resulting atmosphere was carried to the dog mask. A medical breathing bag was used to provide the dogs with a reservoir of additional air. The mixing chamber was operated under slight positive pressure monitored as a differential pressure across a constriction into the exhaust line using a Magnehelic™ gauge. Compressed air was introduced into the chamber at 30 L/min from the top with further air being introduced into the bottom of the chamber at 40 L/min with a scavenging exhaust attached. The test and vehicle control atmospheres were generated into the chamber air inlet using a Venturi T-section supplied with pre-dried compressed air. The various aerosol concentrations were achieved by altering the rate of control or test article introduction into the feed nozzle of the T-section.
Satellite groups used for toxicokinetics or recovery: None
Age: On day 1, animals were approximately 12 months old.
Weight: Body weight ranges were 9.6 to 11.6 kg for males and 7.6 to 9.2 kg for females.
Unique study design or methodology (if any): The objective of this study was to investigate the effect of a modification to feeding methods, designed to reduce the possibility of inhalation of particulate matter, on the formation of granulomas in the lung seen previously in a 28-day oropharyngeal inhalation toxicity study in the dog (WD2005/0I423/00J over a 28-day repeat dose period (60 minutes daily). Foreign material compatible with plant material found in the diet was identified within granulomas.

Observation and Times:
Clinical signs: Animals were observed twice daily for moribundity/mortality. Animals were observed for clinical signs post dosing upon return to the home cage, 1 to 4 hr postdose, and at the end of the working day.
Body weights: Body weights were measured weekly.
Food consumption: Daily food consumption was measured semi-quantitatively.
Ophthalmoscopy: Ophthalmic examinations were conducted during pretreatment and week 4. Shirmer tear test strips were applied to both eyes of each animal for 1 min during the pretreatment period and weeks 1 and 4 (2-4 hr postdose) to evaluate moisture content.
EKG: Electrocardiographic recordings using leads I, II, III, aVR, aVL, and aVF leads were conducted during the pretreatment period and week 4. During the treatment period, recordings were made predose and near T_{max} (immediately postdose). Quantitative measurements of heart rate, PR, QRS, QT, and RR intervals were performed. Recordings were evaluated for rhythm and abnormalities. Heart rates were measured on days 1 and 25 immediately after dosing and at 0.5, 1, 2, 4, 8, and 23 hr postdose.
Respiratory minute volume: Respiratory minute volumes were measured over a 15 min period during the pretreatment period and once predose during week 4 (day 23).
Hematology: Blood samples for measurement of a complete panel of hematology parameters were collected during weeks -2 and 4.

Clinical chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected during weeks -2 and 4.
Urinalysis: Urine samples for measurement of complete panel of urinalysis parameters were collected overnight during weeks -2 and 4.
Gross pathology: Dogs were sacrificed on day 29 and submitted to gross necropsy examinations.
Organ weights: Absolute and relative organ weights were measured for the adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, testes, and thymus.
Histopathology: Histopathological examinations of tissues were limited to the bronchi, gross lesions, larynx, lungs, tracheobronchial LN, nasal cavity, pharynx, trachea, and tracheal bifurcation. Tissues were processed to paraffin wax, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. A peer review of selected microscopic tissues sections and pathology data interpretation was completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on days 1 and 25 immediately after dosing and at 0.5, 1, 2, 4, 8, and 23 hr after dosing. Frozen plasma samples were shipped to GSK Drug Metabolism and Pharmacokinetics for analysis. GSK573719 was measured using a HPLC/MS/MS method with a LLOQ of 0.1 ng/mL and a linear range of 0.1 to 100 ng/mL.

Results:

Mortality: None.

Clinical signs: Clinical signs for treatment groups included decreased activity, abnormal breathing sounds, dry eyes, dry gums, and tremors.

Clinical signs	Males				Females			
	New Feeding Regimen			OFR	New Feeding Regimen			OFR
	0	22	2254	1835	0	22	2254	1835
N =	3	3	3	3	3	3	3	3
Activity decreased	0	0	2	1	0	0	3	0
Breathing sounds, abnormal	0	0	1	1	0	0	1	1
Eyeball dry/L	0	3	3	3	0	3	3	3
Eyeball dry/R	0	3	3	3	0	3	3	3
Gums dry	0	3	3	3	3	2	3	3
Tremors	0	0	3	1	0	0	3	3

OFR = Old Feeding Regimen

Body weights: Males and females in Group 4 (1835 µg/kg/day, Old feeding regimen) lost 5.4 and 3.3% of initial body weights during the treatment period.

Food consumption: Food consumption was reduced for females in Group 3 and males and females in Group 4. A concurrent control group for Group 4 was not included in the study.

Food consumption, sum total of weeks 1 to 4

Group	Males	Females
Group 1: NFR/0	4356.33	4117.63
Group 2: NFR/22	4288.54	4547.37
Group 3: NFR/2254	4110.93	3539.60 (86%)
Group 4: OFR/1835	907.40	895.13

NFR=New Feeding Regimen (Feed was moistened with water); OFR=Old Feeding Regimen

Ophthalmoscopy: Unilateral conjunctival hyperemia and mucoid discharge were noted in one animal (male No. 303) of the 2254 µg/kg/day/new feeding regimen group and one animal (female No. 451) of the 1835 µg/kg/day/old feeding regimen groups. For the female, there were additional findings of slight corneal edema and vascularization in one eye. These findings were attributed pharmacological actions of GSK573719 (i.e., dry eyes, decreased tear production).

The Schirmer Tear Test revealed that moisture content of the eyes of dogs given 2254 µg/kg/day/new feeding regimen and dogs given 1835 µg/kg/day/old feeding regimen was lower in Weeks 1 and 4 when compared to control values. Moisture content was also slightly reduced for dogs given 22 µg/kg/day/new feeding regimen.

Schirmer Tear Test, test strip reading (mm)

Group/Dose	Week 1, 2-4 hr PD		Week 4, 2-4 hr PD	
	Males	Females	Males	Females
G1/ Control	21.3	22.7	19.3	16.8
G2/22 µg/kg/day	16.2	17.0	15.2	16.3
G3/2254 µg/kg/day	1.8	1.7	3.0	5.5
G4/1835 µg/kg/day	1.3	3.3	2.7	2.3

EKG: Increased heart rates (up to 1.6-fold) were evident for male and female dogs in the high dose/NFR and high dose/OFR groups. Increases were more pronounced on day 1 as compared to days 25 or 27. There were no significant changes of PR, QRS, QT, and RR intervals.

Heart rate and ECG parameters on day 27 predose and immediately postdose

Parameter	Time	Males				Females			
		New Feeding Regimen			OFR	New Feeding Regimen			OFR
		0	22	2254	1835	0	22	2254	1835
Heart rate bpm	Predose	113	103	120	120	127	97	153	130
	Immediately postdose	120	113	170 (142%)	173 (144%)	120	123	190 (158%)	157 (131%)
RR Interval msec	Predose	536	586	502	502	528	632	397 (75%)	468
	Immediately postdose	502	547	354 (71%)	348 (69%)	529	497	316 (60%)	386 (73%)

Heart rate measurements

Day	Time	Males				Females			
		New Feeding Regimen			OFR	New Feeding Regimen			OFR
		0	22	2254	1835	0	22	2254	1835
1	Predose	101	100	91	91	87	96	86	83
	Immediately Postdose	81	78	157 (194%)	212 (262%)	98	87	197 (201%)	244 (249%)
	0.5 hr	107	93	208 (194%)	199 (186%)	95	85	229 (241%)	165 (174%)
	1 hr	99	84	220 (222%)	199 (201%)	87	83	225 (259%)	234 (269%)
	2 hr	92	101	210 (228%)	196 (213%)	86	86	211 (245%)	212 (247%)
	4 hr	85	113	190 (224%)	196 (231%)	107	95	201 (188%)	164 (153%)
	8 hr	119	98	136	142	107	102	148 (138%)	127 (119%)
	23 hr	108	94	124 (114%)	134 (124%)	102	93	126 (124%)	124 (122%)
25	Predose	108	113	126 (112%)	131 (121%)	91	102	126 (138%)	115 (126%)
	Immediately Postdose	99	88	141 (142%)	127 (128%)	105	101	130 (124%)	129 (123%)
	0.5 hr	98	102	129 (132%)	120 (122%)	96	111	143 (149%)	110
	1 hr	102	120 (118%)	129 (126%)	119 (117%)	88	106 (120%)	142 (161%)	103 (117%)
	2 hr	88	92	135 (153%)	134 (152%)	86	106 (123%)	116 (135%)	121 (141%)
	4 hr	110	107	123	117	113	113	144 (127%)	128 (113%)
	8 hr	110	108	119	121	108	124 (114%)	130 (111%)	107
	23 hr	134	96	120	125	92	105 (114%)	118 (128%)	132 (143%)

Hematology: There were no toxicologically significant changes of hematology parameters.

Clinical chemistry: Triglyceride levels for males in the high dose/NFR (Group 3) group were increased to 0.720 mmol/L as compared to a concentration of 0.387 mmol/L for the male control group.

Urinalysis: There were no treatment-related changes of urinalysis parameters.

Gross pathology: A dark area in the lung was observed for 1 of 3 males in Group 4 (Old Feeding Regimen). A depressed area in the lung was observed for 1 of 3 females in Group 4 (Old Feeding Regimen). These findings may correlate with observed histopathological findings in the lung.

Organ weights: Absolute and relative lung weights were increased for female treatment groups, although observed histopathological findings in the lung do not appear to be responsible for increased weights. Thymus weights were decreased for male and female treatment groups; however, histopathological examinations of the thymus were not conducted for all dogs. Ovary weights were increased for females in the mid and high dose groups; however, histopathological examinations of the ovaries were not conducted.

Organ weights

Organ	Males				Females			
	New Feeding Regimen			Old FR	New Feeding Regimen			Old FR
	0	22	2254	1835	0	22	2254	1835
Lung g	NC	NC	NC	NC	69.8563	79.0750 (113%)	81.5030 (117%)	79.6087 (114%)
Lung %BW	NC	NC	NC	NC	0.80790	0.88717 (110%)	096209 (119%)	1.01064 (125%)
Thymus g	11.1430	7.1267 (64%)	9.4463 (85%)	6.9650 (63%)	8.9693	6.1083 (68%)	6.2707 (70%)	4.7220 (53%)
Thymus %BW	0.10032	0.06687 (67%)	0.08391 (84%)	0.06720 (67%)	0.10289	0.06784 (66%)	0.07351 (71%)	0.06038 (59%)
Ovary g					0.8847	0.9237 (104%)	1.2197 (138%)	1.1113 (126%)
Ovary %BW					0.01018	0.01018	0.01429 (140%)	0.01377 (135%)

Histopathology: Histopathological examinations of tissues were limited to gross lesions, bronchi, larynx, lungs, tracheobronchial LN, nasal cavity, pharynx, trachea, and tracheal bifurcation for all groups. Findings were observed in the lung, larynx, trachea, tracheal bifurcation, bronchus, and nasal cavity/sinuses. Findings in the nasal cavity/sinuses were judged to be not relevant to the clinical setting with oral inhalation of the drug product.

Microscopic findings considered to be related to the old feeding regimen (OFR) were seen in the lungs. Dogs given the high dose/OFR (Group 4) had minimal to slight granulomatous inflammation in the lungs (1/3 males and 2/3 females), which was not seen in dogs given the high dose/new feeding regimen (Group 3). The male dog with granulomatous inflammation in the lungs also had suppurative bronchoalveolar inflammation and both of these inflammatory foci contained foreign material that was consistent in appearance with food/plant material. Increased incidences of interstitial inflammation were observed for all male treatment groups; however, this finding was evident at an incidence of 1 of 3 dogs for the male and female control groups and all female treatment groups. Interstitial inflammation is a common finding for young beagle dogs with a background incidence of 22-28%. An increased incidence of accumulation of foamy alveolar macrophages was evident for male dogs given the high dose/OFR (Group 4).

For the larynx, acute inflammation and degeneration/necrosis of the epithelium were observed for all male treatment groups and females in the high dose/new feeding

regimen (NFR) (Group 3) and high dose/old feeding regimen (Group 4) groups. Exudate was also evident for all male treatment groups and females in the high dose/NFR (Group 3) and high dose/OFR (Group 4) groups.

For the trachea, acute inflammation was observed for all male treatment groups and females in the low dose/NFR and high dose/NFR groups. Degeneration/regeneration of the epithelium was observed for male and female dogs in the high dose/NFR (Group 3) and high dose/OFR (Group 4) groups. Single cell necrosis was observed for 1 male in the high dose/NFR (Group 3) group. Exudate in the lumen and epithelial necrosis were each observed for 1 male in the high dose/OFR (Group 4) and 1 female in the high dose/NFR (Group 3) group.

For the tracheal bifurcation, acute inflammation and degeneration/regeneration of the epithelium were each observed for one male and one female in the high dose/NFR (Group 3) group.

For the bronchus, acute inflammation and degeneration/regeneration of the epithelium were observed for males in the low dose and high dose/NFR (Groups 2 and 3) groups and females in the high dose/NFR (Group 3) group.

There were multiple findings for the nasal cavities/sinuses that included degeneration/necrosis, ulceration/erosion, hyperplasia, and/or metaplasia of the squamous, respiratory and/or olfactory epithelium; acute/subacute inflammation; exudate; and hyperplasia/hypertrophy of goblet cells. One or more of these findings occurred in all animals given GSK573719A (Groups 2 to 4) and ranged from minimal to severe in grade. In general, the dogs given the low dose/new feeding regimen (Group 2) had a lower severity of lesions compared to the Group 3 and 4 dogs, and also did not have epithelial ulceration/erosion or exudate. A dose response was evident between dogs given the low dose/NFR (Group 2) and those given the high dose/NFR (Group 3). In general, the severity and/or incidence of these findings were similar between dogs given the high dose/OFR (Group 4) and the dogs given the high dose/NFR (Group 3).

Histopathological findings in dogs following a 28-day treatment period

Organ/Tissue	Males				Females			
	New Feeding Regimen			Old FR	New Feeding Regimen			Old FR
	0	22	2254	1835	0	22	2254	1835
Lung -inflammation, granulomatous, Grade 1-2	0/3	0/3	0/3	1/3	0/3	0/3	0/3	2/3
Lung -inflammation, interstitial, Grade 1 Grade 2	1/3 1 0	2/3 2 0	2/3 2 0	3/3 3 0	1/3 1 0	1/3 0 1	1/3 1 0	1/3 1 0
Lung -foamy macrophage accumulation Grade 1	1/3 1	0/3 0	1/3 1	2/3 1	0/3 0	1/3 1	0/3 0	1/3 0

Organ/Tissue	Males				Females			
	New Feeding Regimen			Old FR	New Feeding Regimen			Old FR
	0	22	2254	1835	0	22	2254	1835
Grade 2	0	0	0	1	0	0	0	1
Lung								
-inflammation, bronchoalveolar, Grade 1	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
Larynx								
-degeneration/necrosis epithelium	0/3	1/3	2/3	3/3	0/3	0/3	3/3	2/3
Grade 1	0	1	0	1	0	0	1	1
Grade 2	0	0	1	2	0	0	0	0
Grade 3	0	0	0	0	0	0	0	1
Grade 4	0	0	1	0	0	0	2	0
Larynx								
-inflammation, acute	0/3	1/3	3/3	3/3	0/3	0/3	2/3	2/3
Grade 1	0	1	1	1	0	0	0	1
Grade 2	0	0	1	2	0	0	0	0
Grade 3	0	0	0	0	0	0	2	1
Grade 4	0	0	1	0	0	0	0	0
Larynx								
-exudate	0/3	1/3	1/3	2/3	0/3	0/3	0/3	1/3
Grade 1	0	1	0	2	0	0	0	1
Grade 2	0	0	1	0	0	0	0	0
Trachea								
-inflammation, acute	0/3	1/3	3/3	2/3	0/3	1/3	2/3	0/3
Grade 1	0	1	3	1	0	1	1	0
Grade 3	0	0	0	1	0	0	1	0
Trachea								
-degeneration/regeneration, epithelium	0/3	0/3	2/3	1/3	0/3	0/3	1/3	1/3
Grade 1	0	0	1	0	0	0	0	1
Grade 2	0	0	1	0	0	0	0	0
Grade 3	0	0	0	0	0	0	1	0
Grade 4	0	0	0	1	0	0	0	0
Trachea								
-necrosis, single cell, Grade 1	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
Trachea								
-exudate, lumen, Grade 2	0/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3
Trachea								
-necrosis, epithelium, Grade 2-4	0/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3
Tracheal bifurcation								
-inflammation, acute, Grade 1	0/3	0/3	1/3	0/3	0/3	0/3	1/3	0/3
Tracheal bifurcation								
-degeneration/regeneration, epithelium, Grade 1	0/3	0/3	1/3	0/3	0/3	0/3	1/3	0/3
Bronchus								
-inflammation, acute, Grade 1	0/3	1/3	2/3	0/3	0/3	0/3	1/3	0/3
Bronchus								
-degeneration/regeneration, epithelium, Grade 1	0/3	1/3	1/3	0/3	0/3	0/3	1/3	0/3
Nasal Cavity/Sinuses								
-degeneration/necrosis,	0/3	3/3	3/3	3/3	0/3	2/3	3/3	3/3

Organ/Tissue	Males				Females			
	New Feeding Regimen			Old FR	New Feeding Regimen			Old FR
	0	22	2254	1835	0	22	2254	1835
squamous epithelium								
Grade 1	0	3	0	0	0	2	0	0
Grade 3	0	0	3	2	0	0	2	1
Grade 4	0	0	0	1	0	0	1	2
Nasal Cavity/Sinuses								
-degeneration/necrosis, respiratory epithelium	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3
Grade 1	0	3	0	0	0	3	0	0
Grade 2	0	0	3	2	0	0	0	1
Grade 3	0	0	0	1	0	0	1	2
Grade 4	0	0	0	0	0	0	2	0
Nasal Cavity/Sinuses								
-degeneration/necrosis, olfactory epithelium	0/3	1/3	3/3	2/3	0/3	0/3	2/3	3/3
Grade 1	0	1	2	2	0	0	2	3
Grade 2	0	0	1	0	0	0	0	0
Nasal Cavity/Sinuses								
-ulceration/erosion, respiratory epithelium	0/3	0/3	2/3	3/3	0/3	0/3	3/3	3/3
Grade 2	0	0	2	1	0	0	0	1
Grade 3	0	0	0	2	0	0	1	2
Grade 4	0	0	0	0	0	0	1	0
Grade 5	0	0	0	0	0	0	1	0
Nasal Cavity/Sinuses								
-ulceration/erosion, squamous epithelium	0/3	0/3	3/3	3/3	0/3	0/3	3/3	3/3
Grade 1	0	0	0	1	0	0	2	0
Grade 2	0	0	3	0	0	0	0	2
Grade 3	0	0	0	2	0	0	0	1
Grade 4	0	0	0	0	0	0	1	0
Nasal Cavity/Sinuses								
-metaplasia, squamous, respiratory epithelium	0/3	0/3	3/3	3/3	0/3	3/3	3/3	3/3
Grade 1	0	0	1	0	0	3	0	2
Grade 2	0	0	2	0	0	0	3	1
Grade 3	0	0	0	2	0	0	0	0
Grade 4	0	0	0	1	0	0	0	0
Nasal Cavity/Sinuses								
-hyperplasia, squamous epithelium	0/3	0/3	3/3	3/3	0/3	2/3	3/3	3/3
Grade 1	0	0	1	3	0	2	0	2
Grade 2	0	0	2	0	0	0	3	1
Nasal Cavity/Sinuses								
-hyperplasia/hypertrophy, goblet cells, Grade 1	0/3	1/3	2/3	3/3	0/3	2/3	2/3	2/3
Nasal Cavity/Sinuses								
-inflammation, acute/subacute	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3
Grade 1	0	3	0	0	0	3	0	0
Grade 2	0	0	3	3	0	0	1	2
Grade 3	0	0	0	0	0	0	2	1
Nasal Cavity/Sinuses								

Organ/Tissue	Males				Females			
	New Feeding Regimen			Old FR	New Feeding Regimen			Old FR
	0	22	2254	1835	0	22	2254	1835
-exudate, Grade 1-2	0/3	0/3	2/3	3/3	0/3	0/3	3/3	3/3

Toxicokinetics: GSK573719 was quantifiable in the plasma of all dogs dosed with GSK573719; however, plasma concentration data for male and female dogs in the low dose/NFR (Group 2) group were highly variable and toxicokinetic parameters were not reported. Plasma C_{max} and AUC values on day 1 were slightly higher for males and females in the high dose/NFR group as compared to the high dose/OFR group. On day 25, plasma C_{max} and AUC values for males and females in the high dose/NFR group were slightly lower as compared to day 1; however, this was not observed for the high dose/OFR group. Plasma C_{max} and AUC values were relatively comparable for male and female dogs of the same treatment groups.

GSK573719 was sporadically detectable in plasma samples (10 of 84 samples) from male and female controls on days 1 and 25 at levels ranging from 0.147 to 1.36 ng/mL. GSK573719 was also detectable in 2 of 6 naïve samples.

Parameter+	Day	Male			Female		
		New Feeding Regimen		Old Feeding Regimen	New Feeding Regimen		Old Feeding Regimen
		22 µg/kg/day	2254 µg/kg/day	1835 µg/kg/day	22 µg/kg/day	2254 µg/kg/day	1835 µg/kg/day
$AUC_{(0-t)}$ (ng.h/mL)	Day 1	NR	390	284	NR	358	275
	Day 25	NR	185	295	NR	179	283
C_{max} (ng/mL)	Day 1	NR	264	195	NR	289	255
	Day 25	NR	45.1	91.4	NR	62.7	110

+ Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.
NR Not reported since toxicokinetic parameters could not be calculated or were not reported due to variability of the data.

Study title: **Toxicity Study by Inhalation Administration to Beagle Dogs for 13 Weeks Followed by a 4-Week Recovery Period**

Key study findings:

● In a 13-week inhalation toxicology study (WD2007/01512/00), dogs received GSK573719 at estimated achieved doses of 0, 40.7, 187, and 1070 µg/kg/day. The control group was exposed to lactose containing magnesium stearate.

● In Week 13, at predose, 3/6 males and 4/6 females given 1070 µg/kg/day showed no respiratory sinus arrhythmia (RSA) (expressed by decreased RR interval variability) and all dogs given 1070 µg/kg/day had increased heart rates (up to 1.8X pretest individual values). Immediately following a dose of 1070 µg/kg/day, no RSA was noted in 4/6 males and 4/6 females, which was associated with further increases in heart rates (up

to 2.1X predose individual values) in 2/6 males and 3/6 females. The changes in most animals were returned to pre-test values, but RSA was not observed in females during the recovery phase.

● On Day 1, a female given 1070 µg/kg/day had elevated serum cTnI concentrations at 3 hr (0.171µg/L), 7 hr (0.189 µg/L), and 24 hr (0.082 µg/L). By Week 13 and at the end of the recovery period, the serum cTnI concentrations were comparable to pre-dose and concurrent controls. Additionally on Day 1, a male given 1070 µg/kg/day showed minimal to mildly higher serum cTnI concentrations at 3 hr (0.044 µg/L) and 7 hr (0.101 µg/L) when compared to pre-dose and concurrent control values. By 24 hr the serum cTnI concentration was comparable to the pre-dose value. By 24 hr the serum cTnI concentration was comparable to the pre-dose value. Increased troponin I concentrations suggest the possibility of cardiac injury although histopathological examinations of hearts found no evidence of damage. No cardiac damage was evident in shorter term studies.

● Observed histopathological findings appeared to be background/spontaneous and unrelated to treatment. Systemic exposure to GSK573719 measured in dogs from this study was lower than that seen in dogs in the two 4-week studies when given by oropharyngeal tube inhalation exposure. It is considered possible that the lower systemic exposures seen in this study related to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in this study. The modifications were intended to improve the stability of the concentration of GSK573719 in aerosols generated in the dosing system. The histopathological results found in the present study may not accurately reflect the local toxicity of GSK573173.

● The NOAEL was identified as the high dose of 1070 µg/kg/day based upon lack of treatment-related histopathological finding; however, this absolute dose might not be comparable to other studies due to problems with drug delivery. Cardiac effects appear to be monitorable in a clinical setting. Systemic exposures for males and females in the high dose group during week 13 were 30.6 and 28.6 ng·hr/mL, respectively.

Study no.: WD2007/01512/00
Volume #, and page #: Volumes 19, 20, and 21, Pages 1-1021
Conducting laboratory and location: (b) (4)

Date of study initiation: December 19, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Micronized GSK573719A (from GSK input batch number R230166) was formulated as a dry powder blend in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate (input batch number 061114989). The GSK573719 blend was designated as GSK blend batch number EE132023.

Magnesium stearate powder from (input batch number 061114989

Methods

Doses: GSK573719 was administered at estimated achieved doses of 0, 40.7, 187, and 1070 µg/kg/day (using aerosol concentrations of 12.1, 55.6, and 318 µg/L, respectively) for 10 min/day for 13 weeks. The control group was exposed to lactose containing magnesium stearate.

Overall Average Aerosol Concentration, Estimated Achieved Doses and Particle Size Distribution of GSK573719

Study Design					
Group Number	Target Dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day)[a,e]	Target Aerosol Concentration (µg/L)[a]	Achieved Aerosol Concentration (µg/L)[a]	MMAD ± GSD[b]
1[c]	0	0	0	0	(b) (4)
2[d]	35	40.7	11	12.1	
3[d]	187	187	57	55.6	
4[d]	1000	1070	304	318	

a. Expressed in terms of parent compound (GSK573719)
b. MMAD = Mass Median Aerodynamic Diameter µm, GSD = Geometric Standard Deviation
c. Exposed to lactose with a nominal 1% (w/w) magnesium stearate.
d. Exposed to GSK573719A blended in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate.
e. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/ (BW)
D Dose (µg/kg/day)
RMV (respired minute volume) (L/min) $0.499 \times BW(kg)^{0.809}$ (Bide, 2000)
T Duration of exposure/day (minutes)
C GSK573719 aerosol concentration (µg/L)
BW Group average body weight for the study (kg)
\- Not determined

Overall Average Aerosol Concentration, Estimated Achieved Doses and Particle Size Distribution of Magnesium Stearate

Study Design			
Group Number	Estimated Achieved Dose (µg/kg/day)[a,e]	Achieved Aerosol Concentration (µg/L)[a]	MMAD ± GSD[b]
1[c]	28.5	8.49	(b) (4)
2[d]	1.01	0.30	
3[d]	4.16	1.24	
4[d]	25.1	7.48	

a. Expressed as magnesium stearate, determined by the measurement of magnesium by atomic absorption spectroscopy.
b. MMAD = Mass Median Aerodynamic Diameter µm, GSD = Geometric Standard Deviation
c. Exposed to lactose with a nominal 1% (w/w) with magnesium stearate.
d. Exposed to GSK573719A blended in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate
e. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/ (BW)
D Dose (µg/kg/day)
RMV (respired minute volume) (L/min) $0.499 \times BW(kg)^{0.809}$ (Bide, 2000)
T Duration of exposure/day (minutes)
C Magnesium Stearate aerosol concentration (µg/L)
BW Group average body weight for the study (kg)

Species/strain: Male and Female beagle dogs where obtained from (b) (4) Artificial tears were administered to all animals postdose daily throughout the course of the study with the exception of days for Schirmer tear tests assessments.
Number/sex/group or time point (main study): 4 dogs/sex/group
Route, formulation, volume, and infusion rate: Each aerosol delivery system consisted of a dust generator (Wright Dust Feed), an aerosol conditioning chamber fitted with two outlets, an acrylic sub-chamber fitted with four outlets, aerosol delivery tubing, Y -piece connections with isolating ball valve, and oropharyngeal tube attached. The aerosol exhaust system included a scavenger valve system, further aerosol tubing and a series of particulate filters, a flow meter with integral needle valve, and an extract pump. The oropharyngeal tubes were constructed of silicone rubber and were inserted into the mouth of the dogs. The aerosol was delivered to the dog via the oropharyngeal tube for 10 min.

Separate atmosphere generation systems were used to dose the control and test article groups of animals. The compressed air flow rate exiting the Wright Dust Feed (WDF) for the control group was set to 15 L/min and for the test article groups was set to 20 L/min. An additional dilution flow of compressed air was supplied at 12 L/min for the control group and 10 L/min for the test article groups to the conditioning chamber by an inlet that was adjacent to the aerosol entry point. The aerosol-laden air exiting the conditioning chamber (27 L/min, equal to 6.75 L/min/animal for the control group and 30 L/min, equal to 7.5 L/min/animal for the treated groups and each of two spare positions for sampling) was delivered to each end of an acrylic sub-chamber. Each acrylic sub-chamber had four active outlets to potential animal exposure positions by clinical aerosol tubing with a Y -piece providing the attachment point for the ball valve and oropharyngeal tube. Two aerosol outlets were used to supply aerosol to the animals assigned to the study and the remaining outlets were used for the collection of aerosol samples. This arrangement was necessary due to the short exposure duration and the occasional requirement for the simultaneous collection of two samples (i.e., concentration and particle size distribution).

Exhaust air from the aerosol delivery system was delivered to a separate exhaust plenum, incorporating an in-line scavenger valve system that operated at a flow that was approximately 5 L/min greater than the aerosol production flow of 32 L/min for the control group and 35 L/min for the test article groups. During dosing the excess air demand of the scavenger valve system was drawn directly from the room environment through an in-line flow meter and a pre-determined opening pressure valve that served to prevent the flow of aerosol laden air into the exposure laboratory. The air was passed through a filter system to remove the test article particulate before the air was discharged to atmosphere.

Aerosol concentrations of GSK573719 and magnesium stearate were measured with glass fiber filters located at the point of attachment of a representative oropharyngeal tube and the volume of air sampled was measured. Concentrations of GSK573719 were measured by HPLC with UV detection. Concentrations of magnesium stearate were measured by atomic absorption spectroscopy.

Particle size distributions of GSK573719 and magnesium stearate were measured from the inhalation exposure system using cascade impactors located at the point of attachment of a representative oropharyngeal tube. Samples were collected during weeks 1, 4, 8, and 13. Impactor stages were analyzed chemically for GSK573719 using a HPLC with UV detection and magnesium stearate by atomic absorption spectroscopy.

Satellite groups used for toxicokinetics or recovery: An additional 2 dogs/sex/group were included in the control and high dose groups for a 4-week recovery period.
Age: Dogs were 11 to 12 months old on the first day of dosing.
Weight: Body weight ranges were 6.8 to 9.4 kg for males and 6.1 to 8.5 kg for females.
Unique study design or methodology (if any): Systemic exposure to GSK573719 measured in dogs from this study was lower than that seen in dogs in the two 4-week

studies when given by oropharyngeal tube inhalation exposure. It is considered possible that the lower systemic exposures seen in this study related to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in this study. The modifications were intended to improve the stability of the concentration of GSK573719 in aerosols generated in the dosing system.

Observation and Times:
Clinical signs: Animals were observed for moribundity/mortality and clinical signs twice daily.
Body weights: Body weights were measured weekly.
Food consumption: Food consumption was measured daily.
Ophthalmoscopy: Ophthalmic examinations were conducted during the pretreatment period and at the end of week 13. Tear production in both eyes was assessed with the Schirmer tear test during the pretreatment period, during week 1, 4, and 13 (predose, 2-4 hr after the start of dosing), and at the end of the recovery period.
EKG: Electrocardiograms were recorded using leads I, II, III, aVR, aVL, aVF, rV2, V2, and V10 during the pretreatment period, week 13, and at the end of the recovery period. Tracings were evaluated for abnormalities and heart rate. P, PR, QRS, ST, QT, QTc (Van dewater), and RR were calculated. Femoral pulse was measured on day 1, during week 4, and week 13 (predose, immediately postdose, and at 0.5, 1, 3, 7, and 24 hr postdose). Blood samples for measurement of Troponin I were collected during weeks 1 and 13 at predose and at 1, 3, 7, and 24 hr postdose. Samples were shipped to the sponsor for analysis by immunoassay with chemoluminescent detection (assay range was 0.006 to 50 µg/L).
Hematology: Blood samples for measurement of a complete panel of hematology parameters were collected during weeks 4 and 13.
Clinical chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected during weeks 4, 6, 12, and 13 and at the end of the recovery period. Blood samples for measurement of cholecystokinin and somatostatin were collected during weeks 6 and 12 at predose and at 2 hr after completion of dosing, 1 hr after removal of food (nominally 5 hr after completion of dosing), and 24 hr after completion of dosing. Blood samples were also obtained at the end of the recovery period. Samples for measurement of cholecystokinin and somatostatin were shipped to GSK for analysis.
Urinalysis: Urine samples for the measurement of a complete panel of urinalysis parameters were collected overnight (~16 hr) during the pretreatment period, weeks 4 and 13, and recovery week 4.
Gross pathology: After the end of the 13-week treatment and 4-week recovery periods, 4 and 2 dogs/sex/group, respectively, were sacrificed and submitted to necropsy examination.
Organ weights: Absolute and relative organ weights were measured for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, prostate, testes, and thymus.
Histopathology: Tissue samples were processed to paraffin wax, sectioned (3-5 µm), stained with hematoxylin and eosin, and examined by light microscopy. A complete panel of tissues was examined and tissues from all groups were examined. A peer review of selected microscopic tissues sections and pathology data interpretation was

completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were measured on day 1 and during weeks 4, 9, and 13. Blood samples were collected at immediately postdose and at 0.33, 0.5, 0.75, 1, 3, 7, and 24 hr postdose. Blood samples collected from naïve dogs were also included in analyses. Plasma samples were analyzed for GSK573719 using a HPLC/MS/MS method. The assay had a LLOQ of 0.1 ng/mL and a linear range of 0.1 to 100 ng/mL.

Results:

Mortality: None

Clinical signs: Treatment-related clinical signs included dry mouth, dry nose, swollen neck, and excessive salivation. Incidences of the clinical signs in the report were provided on an individual animal basis and summary tables were not provided. Dry mouth was evident throughout the dosing period with a dose-related incidence for all male and female treatment groups. Swollen neck was observed for males in the mid and high dose groups sporadically from days 86 to 92. Swollen neck was observed for all female treatment groups beginning on day 68 and continuing to the end of the dosing period. Excessive salivation was observed immediately postdose for the mid and high dose groups and attributed to palatability problems with the test article. Dry mouth, dry nose, and swollen neck were attributed to the antimuscarinic properties of the test article.

Food consumption: Food consumption was unaffected.

Ophthalmoscopy: There were no treatment-related ophthalmic findings; however, treatment-related decreases of tear production were observed.

In week 1, treatment-related decreases of tear production were observed for all male treatment groups and females in the high dose group (0.2 to 0.9X pretest mean values). In weeks 4 and 13 (pre and post dose), decreased tear production was noted in animals given 1070 µg/kg/day (0.2 to 0.7X pretest mean values), and in Week 13 (pre and post dose), decreased tear production was also noted in animals given 187 µg/kg/day (0.6 to 0.8 X pretest mean values).

At the end of the recovery phase tear production was comparable to pretest values for animals given 1070 µg/kg/day.

EKG: In Week 13, at predose, 3/6 males and 4/6 females given 1070 µg/kg/day showed no respiratory sinus arrhythmia (RSA) (expressed by decreased RR interval variability) and all dogs given 1070 µg/kg/day had increased heart rates (up to 1.8X pretest individual values). Immediately following a dose of 1070 µg/kg/day, no RSA was noted in 4/6 males and 4/6 females, which was associated with further increases in heart rates

(up to 2.1X predose individual values) in 2/6 males and 3/6 females. The changes in most animals were returned to pre-test values, but RSA was not observed in females during the recovery phase.

There were no test article-related effects on ST segment, P, PR, QRS, QT, and QTc intervals following administration of GSK573719A.

On Day 1, a female given 1070 µg/kg/day had elevated serum cTnI concentrations at 3 hr (0.171µg/L), 7 hr (0.189 µg/L), and 24 hr (0.082 µg/L). By Week 13 and at the end of the recovery period, the serum cTnI concentrations were comparable to pre-dose and concurrent controls.

Additionally on Day 1, a male given 1070 µg/kg/day showed minimal to mildly higher serum cTnI concentrations at 3 hr (0.044 µg/L) and 7 hr (0.101 µg/L) when compared to pre-dose and concurrent control values. By 24 hr the serum cTnI concentration was comparable to the pre-dose value.

ECG evaluations during week 13 (predose and postdose) and at the end of the recovery period

Parameter	Time	Males				Females			
		Control	LD	MD	HD	Control	LD	MD	HD
Heart rate bpm	Predose	101	94	117 (116%)	122 (121%)	88	90	98	106 (121%)
	Postdose	95	86	108 (110%)	129 (136%)	96	92	78	136 (142%)
	Recovery	86	-	-	125 (145%)	91	-	-	126 (139%)
RR msec	Predose	599	650	519 (87%)	515 (86%)	704	681	626 (89%)	578 (82%)
	Postdose	642	706	588 (92%)	511 (80%)	675	663	797	490 (73%)
	Recovery	702	-	-	491 (70%)	661	-	-	478 (72%)

Femoral pulse pressures were increased during week 4 for males in the mid dose group and males and females in the high dose group as compared to controls; however, these differences were not evident on day 1 or during week 13.

Femoral pulse measurements during weeks on day 1 and during weeks 4 and 13 (values in parentheses are percent of concurrent control)

Day/Wk	Time	Males				Females			
		Control	LD	MD	HD	Control	LD	MD	HD
Wk 4	Predose	82.0	NC	65.5	103.3 (126%)	71.7	NC	NC	98.3 (137%)
	10m	98.7	NC	117.0 (119%)	114.0 (116%)	94.0	NC	NC	122.3 (130%)
	30m	82.7	NC	81.3	105.3 (127%)	82.0	NC	NC	113.3 (138%)
	1hr	80.7	NC	96.0	105.3 (130%)	88.7	NC	NC	111.3 (125%)

	3hr	78.7	NC	105.0	110.0 (140%)	77.3	NC	NC	109.3 (141%)
	7hr	90.0	NC	92.0	110.0 (122%)	88.0	NC	NC	109.3 (124%)
	24hr	99.3	NC	85.0	92.7	78.7	NC	NC	87.3

NC= No change

Hematology: Changes of hematology parameters were generally small and appeared to have little or no toxicological significance. Eosinophil counts for females in the high dose group during weeks 4 and 13 were increased to 159 and 139% of the control (0.27 and 0.33 x 10^3/µL, respectively.) Monocyte counts for females in the low, mid, and high dose groups during week 13 were increased to 138, 129, and 191% of the control (0.34 x 10^3/µL), respectively.

Clinical chemistry:
Urea concentration was increased in both males and females at 187 and 1070 µg/kg/day in Week 4 (1.3 to 1.5X control mean values), with recovery by Week 13 (1.1 to 1.3X control mean values). There were no treatment-related histopathological findings in the kidneys.

Triglyceride levels were elevated for males in the high dose group during weeks 4 and 13. Alkaline phosphatase activities were increased for female treatment groups during week 13; however, observed increases were not toxicologically significant.

Due to non-specific interference in the cholecystokinin assay it was not possible to report any plasma cholecystokinin measurements from Weeks 6 or 12.

Due to technical issues with the extraction of somatostatin from plasma samples and insufficient sample remaining to perform repeat measurements there were no data to report for Week 6.

Clinical chemistry parameters at predose and weeks 4 and 13

Parameters	Time	Males				Females			
		0	41	187	1070	0	41	187	1070
BUN mg/dL	Wk4	17	18	24 (141%)	26 (153%)	NC	NC	NC	NC
Triglyceride mg/dL	Wk4	38	39	44	52 (137%)	NC	NC	NC	NC
	Wk13	36	39	39	46 (128%)	NC	NC	NC	NC
Alkaline phosphatase U/L	Predose	NC	NC	NC	NC	63	63	84 (133%)	75 (119%)
	Wk13	NC	NC	NC	NC	58	79 (136%)	98 (169%)	88 (152%)

NC= No change

Urinalysis: There were no treatment-related changes of urinalysis parameters.

Gross pathology: The gallbladder of 1 male given 1070 µg/kg/day contained moderate amounts of black granular material at the end of the treatment phase. There were no corresponding histopathological findings.

The heart nodule corresponded to a hematocyst.

Gross pathological findings at the end of the treatment and recovery periods

Organ/Tissue	Sex	End of the treatment period				End of the recovery period	
		0	41	187	1070	0	1070
Gallbladder							
-abnormal contents	M	0/4	0/4	0/4	1/4	0/2	0/2
	F	0/4	0/4	0/4	0/4	0/2	0/2
Heart							
-nodule(s)	M	0/4	0/4	0/4	1/4	0/2	0/2
	F	0/4	0/4	0/4	0/4	0/2	0/2

Organ weights: At the end of the dosing period, absolute and relative prostate weights were increased (up to 1.3-fold) for male treatment groups and absolute and relative thymus weights were decreased (up to 35%) for female treatment groups; however, there were no corresponding histopathological findings.

At the end of the recovery period, absolute and relative thymus weights were decreased (up to 29%) for males in the high dose group, absolute and relative prostate weights were increased (up to 1.65-fold) for males in the high dose group, and absolute and relative thymus (up to 1.78-fold), ovaries (up to 1.85-fold), and liver weights (up 1.39-fold) were increased for females in the high dose group; however, there were no corresponding histopathological findings.

Histopathology: Observed histopathological findings appeared to be background/spontaneous and unrelated to treatment. Achieved systemic exposures in this study were significantly lower as compared to the two earlier 4 week studies. The sponsor's analysis indicated problems with drug delivery in this study. These histopathological results found in the present study may not accurately reflect the local toxicity of GSK573173.

Toxicokinetics: Toxicokinetic data was highly variable for the low dose group. Systemic exposures were particularly low for males and females in the low dose group on day 1 and females in the low dose group during weeks 9 and 13. AUC and C_{max} values increased with elevating dose. Dose proportionality was evident for males during weeks 9 and 13. However, increases were greater than dose proportional for females (e.g., systemic exposure increased almost 2-fold above that expected based upon dose proportionally).

Systemic exposure to GSK573719 measured in dogs from this study was lower than that seen in dogs in the two 4-week studies when given by oropharyngeal tube inhalation exposure. It is considered possible that the lower systemic exposures seen in this study related to modifications made to the air flow balancing system of the

oropharyngeal tube dosing system used in this study. The modifications were intended to improve the stability of the concentration of GSK573719 in aerosols generated in the dosing system.

Table 1 Mean Toxicokinetic Parameters for GSK573719 from Male and Female Dogs Following Inhalation via Oropharyngeal Tube of GSK573719A for 13 Weeks

Sex	Day/Week	Target Dose Group (µg/kg/day)	Estimated Achieved Dose[1] (µg/kg/day)	AUC_{24}[2] (ng.h/mL)	C_{max}[2] (ng/mL)	T_{max}[3] (h)
Male	Day 1	35	56.1	0.168	0.777	0.167
		187	204	3.74	2.12	0.167
		1000	1210	9.57	12.3	0.167
	Week 9	35	33.4	1.87	1.10	0.167
		187	168	5.08	3.19	0.167
		1000	1080	27.1	16.0	0.167-0.333
	Week 13	35	43.9	1.59	1.59	0.167
		187	209	6.58	4.60	0.167
		1000	1230	30.6	12.9	0.167
Female	Day 1	35	56.4	0.0738	0.267	0.167
		187	209	1.22	2.80	0.167
		1000	1240	13.1	17.8	0.167
	Week 9	35	33.6	0.0655	0.271	0.167
		187	173	4.42	2.50	0.167
		1000	1120	39.1	24.1	0.167
	Week 13	35	44.9	0.101	0.432	0.167
		187	216	3.17	1.51	0.167
		1000	1260	28.6	12.9	0.167

1. Estimated achieved dose of GSK573719 on the relevant toxicokinetic sampling day
2. AUC_{24} and C_{max} are the mean of n=4 dogs except 1000 µg/kg/day group which n=6
3. T_{max} values are the median of n=4 except 1000 µg/kg/day group which n=6

Study title: **<u>Dose Range Finding Study by Inhalation Administration to Beagle Dogs for 4 Weeks</u> (Non-GLP Study)**

Key study findings:

● The current study was designed to identify an optimal inhalation exposure system and characterize the toxicity of GSK573719 administered to dogs using this system for 4 weeks to aid in dose selection for the future 9-month inhalation toxicology study with dogs. Systemic exposure to GSK573719 seen in an earlier 13 week inhalation toxicity study in dogs (WD2007/01512/00) was lower than that seen in the dog in previous studies when given by oropharyngeal tube inhalation exposure (WD2006/03228/00 and WD2006/03669/00). It is considered possible that the lower systemic exposures seen in

the 13 week study relate to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in that study.

● An exposure system equipped with the passive scavenger was selected for use. Dogs were exposed to GSK573719 at 1130 and 2390 µg/kg/day using this system for 4 weeks; however, the 1130 µg/kg/day group was only composed of 3 male dogs. A concurrent control group was not included.

● On day 1, there were dose-related increases of pulse rates (up to 2.3-fold). Increases were also evident on day 7 and during week 4 although not as pronounced as day 1.

● Histopathological examinations of tissues were limited to gross lesions, gallbladder, larynx, lungs, nasal turbinates, trachea, tracheal bifurcation, and tracheobronchial LN. Microscopic changes associated with administration of GSK573719 were present in the lungs, trachea, tracheal bifurcation and larynx at 1130 and/or 2390 µg/kg/day.

● A NOAEL was not identified based upon histopathological findings in the lung, trachea, tracheal bifurcation, and larynx.

Study no.: WD2008/01504/00
Volume #, and page #: Volume 22, Pages 1-120
Conducting laboratory and location: (b) (4)

Date of study initiation: Not provided (Conducted after the 13-week inhalation toxicology study)
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, and % purity: GSK573719A (input batch no.R289410) was administered as a dry powder formulation blended in Lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate.

Methods
Doses: This study was conducted in two phases. Control groups were not included in either phase.

The objectives of this study were to:
a) select the most appropriate oropharyngeal tube inhalation exposure system that will provide acceptable systemic exposure to GSK573719 in male beagle dogs (Phase 1).
b) determine the tolerability and toxicokinetics of GSK573719 in male and female beagle dogs when given for 4 weeks, using the oropharyngeal tube inhalation exposure system selected in Phase 1 of the study, to aid dose selection for a future 9 month study and combination toxicity studies with GSK573719 (Phase 2).

Phase 1: GSK573719 was given to 4 male dogs (Group 1) by 10 minute oropharyngeal tube inhalation exposures in a series of four 2-day cycles at estimated achieved average doses of 806, 799, 621, or 647 µg/kg/day (aerosol concentrations of 242, 240,190, or 198 µg/L) for each of the two day cycles respectively. Two-day cycles were separated by a 1 or 2-day washout period where no dose was administered.

Summary of Average Achieved Aerosol Concentrations and Estimated Achieved Doses of GSK573719

Group Number [1]	Target aerosol concentration (µg/L) [2, 3]	Average achieved aerosol concentration (µg/L) [2]	% Change from target aerosol concentration	Estimated achieved dose (µg/kg/day) [2]
				Males
1 (Cycle 1)	300	242	-19	806
1 (Cycle 2)	300	240	-20	799
1 (Cycle 3)	300	190	-37	621
1 (Cycle 4)	300	198	-34	647

1. Group 1: exposed to GSK573719A blended in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate.
   Cycle 1: Meteorological Balloon (reference) exposure system used over two days.
   Cycle 2: Scavenger exposure system used (with a PDOP valve and a flowmeter) over two days.
   Cycle 3: Passive scavenger exposure system used over two days.
   Cycle 4: Meteorological Balloon (reference) exposure system used over two days.
   Each cycle was separated by 1 or 2 day washout periods.
2. Expressed in terms of GSK573719 (the parent compound).
3. All exposures performed using common dust feed conditions selected during aerosol generation trials. No attempt was made to correct the generation settings to achieve the target aerosol concentration.

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/ BW

D Estimated Dose (µg/kg/day)

RMV Respired minute volume (L/min) $0.499 \times BW(kg)^{0.809}$ (Bide et al, 2000)

T Duration of exposure/day (minutes)

C Aerosol concentration of GSK573719 (µg/L) in inhaled air

BW Most recent available group average body weights (kg) on these occasions (Study Days 1 and 8 as appropriate).

Summary of Group Particle Size Distribution Results for GSK573719 Aerosols (Phase 1)

Group number [3]	Average achieved aerosol concentration (µg/L) [4]	MMAD (µm) [1]	GSD [2]
1 (Cycle 1)	242	(b) (4)	
1 (Cycle 2)	240		
1 (Cycle 3)	190		
1 (Cycle 4)	198		

1 Mass Median Aerodynamic Diameter.
2 Geometric Standard Deviation.
3 Group 1: exposed to GSK573719A blended in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate.
Cycle 1: Meteorological Balloon (reference) exposure system used over two days.
Cycle 2: Scavenger exposure system used (with a PDOPvalve and a flowmeter) over two days.
Cycle 3: Passive scavenger exposure system used over two days.
Cycle 4: Meteorological Balloon (reference) exposure system used over two days.
4 Expressed in terms of GSK573719 (the parent compound).

Cycle 1 (Days 1 and 2) and Cycle 4 (Days 11 and 12) using a Meteorological Balloon (reference) System: The aerosol exhaust system comprised a combination of clinical aerosol tubing, rigid, smooth bore PYC piping, in-line aerosol filters and a meteorological balloon. During dosing, once partially inflated, the meteorological balloon acted to provide a partial buffer between the rates of aerosol production, exhaust flow and the respiratory requirements of the dogs. Also, the reservoir served as a sensitive visual indicator of the balance of air flow into and out of the inhalation system. The extract air flow rate was set at 30 L/min for this phase of treatment. This system was used to dose the animals in the dose range finding and 14-Day inhalation toxicity study with GSK573719 (WD2006/03228/00 and WD2006/03669/00).

Cycle 2 (Days 5 and 6) using a Scavenger system equipped with a flow meter and a predetermined opening pressure valve (PDOP valve): This exposure system was identical to the balloon system except that a scavenger system comprising a variable area flow meter and pediatric PDOP valve (2.5cm H_2O) replaced the meteorological balloon downstream of the rigid PYC exhaust plenum. The extract air flow rate was calibrated to a flow of 35 L/min with the balance airflow drawn passively into the exposure system from the room environment through the scavenger system. This system was used to dose the animals in a 13 week inhalation toxicity study with GSK573719 (WD2007/01512/00).

Cycle 3 (Days 8 and 9) using a Passive Scavenger system: For this exhaust system the meteorological balloon and rigid PYC piping exhaust manifold was replaced by a plastic chamber of identical dimensions to the sub-chamber used in the delivery system. The extract air flow rate through the exhaust system was calibrated to a flow of 40 L/min and the difference between the total inlet flow (30 L/min) and exhaust flow was drawn from the room air through an unrestricted port into the chamber. This system has not been used previously to administer GSK573719 to dogs.

The washout days were Days 3, 4, 7 and 10.

Phase 2
GSK573719 was given to 3 male dogs (Group 2) and 3 male and 3 female dogs (Group 3) at estimated achieved doses of 1130 or 2390 µg/kg/day (aerosol concentrations of 344 or 713 µg/L) respectively once daily for 4 weeks by oropharyngeal tube inhalation exposure using the passive scavenger system as described in Cycle 3 of Phase 1.

Summary of Average Achieved Aerosol Concentrations and Estimated Achieved Doses of GSK573719

Group Number [1]	Target aerosol concentration (µg/L) [2]	Average achieved aerosol concentration (µg/L) [2]	% Change from target aerosol concentration	Estimated achieved dose (µg/kg/day) [2]	
				Males	Females
2	300	344	+15	1130	N/A
				Average = 1130	
3	600	713	+19	2340	2430
				Average = 2390	

N/A: Not Applicable.
1. Group 2 and 3: exposed to GSK573719A blended in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate.
2. Expressed in terms of GSK573719 (the parent compound).

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/ BW

D Estimated Dose (µg/kg/day)

RMV Respired minute volume (L/min) $0.499 \times BW(kg)^{0.809}$ (Bide et al, 2000)

T Duration of exposure/day (minutes)

C Aerosol concentration of GSK573719 (µg/L) in inhaled air

BW The average of the group mean body weight (kg) on Days 1, 8, 15 and 22.

Summary of Group Particle Size Distribution Results for GSK573719 Aerosols (Phase 2)

Group number [3]	Average achieved aerosol concentration (µg/L) [4]	MMAD (µm) [1]	GSD [2]
2	344	(b) (4)	
3	713		

1 Mass Median Aerodynamic Diameter.
2 Geometric Standard Deviation.
3 Group 2 and 3: exposed to GSK573719A blended in lactose at a nominal concentration of 40% (w/w) with a nominal 1% (w/w) magnesium stearate using a passive scavenger exposure system.
4 Expressed in terms of GSK573719 (the parent compound).

Aerosol concentrations of GSK573719 were measured using glass fiber filter, fitted to an open faced filter holder at a representative breathing point. Gravimetric and chemical analyses were conducted. The glass fiber filters were analyzed for GSK573719 content using HPLC.

Particle Size Distributions were determined using a Marple Cascade Impactor. Samples were collected once for each cycle of the Group 1 exposures and during Weeks 1 and 4 of Phase 2 of the study for gravimetric and chemical analysis (all groups). The material collected on each impaction stage was analyzed chemically for GSK573719A content using HPLC.

Species/strain: Beagle dogs (source was not provided).
Number/sex/group or time point (main study): Groups 1: 4 male dogs, Group 2: 3 male dogs, and Group 3: 3 dogs/sex/group
Route, formulation, volume, and infusion rate: Inhalation
Satellite groups used for toxicokinetics or recovery: None
Age: Not provided.

Weight: Mean body weights at the start of treatment were 8.3 kg for male dogs in Group 1, 9.0 kg for male dogs in Group 2, and 8.9 kg for males dogs and 7.5 kg for female dogs in Group 3.
Unique study design or methodology (if any): Systemic exposure to GSK573719 seen in an earlier 13 week inhalation toxicity study in dogs (WD2007/01512/00) was lower than that seen in the dog in previous studies when given by oropharyngeal tube inhalation exposure (WD2006/03228/00 and WD2006/03669/00). It is considered possible that the lower systemic exposures seen in the 13 week study relate to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in that study. The current study was designed to identify an optimal inhalation exposure system and characterize the toxicity of GSK573719 in dogs using this system to aid in dose selection for the future 9-month inhalation toxicology study with dogs.

Observation and Times:
Clinical signs: Animals were observed daily for viability and clinical signs.
Body weights: Body weights were measured weekly for Phase 2 animals.
Food consumption: Food consumption was measured for Phase 1 and 2 animals
Ophthalmoscopy: Schirmer tear test was performed on days -3, 1, 14, and 25 for Phase 2 animals only.
EKG: Femoral pulse was measured for Phase 2 animals on days 1 and 7 and during week 4.
Hematology: Not performed.
Clinical chemistry: Not performed.
Urinalysis: Not performed.
Gross pathology: Necropsy examinations of tissues were conducted for Phase 2 animals although a list of tissues was not provided.
Organ weights: Not performed.
Histopathology: Histopathological examinations of tissues were conducted for Phase 2 animals. Examinations of tissues were limited to gross lesions, gallbladder, larynx, lungs, nasal turbinates, trachea, tracheal bifurcation, tracheobronchial LN.
Toxicokinetics: Toxicokinetic evaluation was performed on each day of treatment in Phase 1 and days 1, 7, 14, and 27 in Phase 2. Plasma samples were analyzed for GSK573719 using a HPLC/MS/MS method. The assay had a LLOQ of 0.1 ng/mL and a linear range of 0.1 to 100 ng/mL.

Results:

Mortality: There were no deaths in either Phase 1 or 2.

PHASE 1:

Toxicokinetics: Toxicokinetic (TK) results following completion of Phase I indicated that the achieved exposure, in terms of C_{max}, in animals given GSK573719 using the exposure system equipped with a flow meter and PDOP valve scavenger (Cycle 2, Phase 1) was lower than that seen in animals given the test article using the other two

exposure systems. In terms of C_{max}, cycle 2 was approximately 2-fold lower than cycle 3 and cycle 3 was almost equivalent to cycle 1. Cycle 2 (the valve and flow meter equipped system) was judged to be unsuitable for use in Phase 2 of this study.

The TK results for the passive scavenger (Cycle 3, Phase 1) and balloon equipped exhaust systems (Cycle 1, Phase 1) were sufficiently similar for the methods to be considered comparable in terms of test article delivery and systemic exposure. In addition, the passive scavenger system was judged to offer greater operational simplicity, with two fewer observation points (balloon and exhaust flow) for the operating technicians to monitor, and a lower potential risk of accidental contamination of the external environment.

The exposure system equipped with the passive scavenger was selected for the Phase 2 administrations.

	Day	Male			
Parameter+	Estimated Achieved Dose				
Cycles	1		2	3	4
	806 µg/kg/day		799 µg/kg/day	621 µg/kg/day	647 µg/kg/day
C_{max} (ng/mL)	1	105	56.3	92.9	ND
	2	81.7	53.5	87.3	ND

+ Mean estimated achieved dose over two days; ND – No Data, samples not analysed
Cycle 1: Meteorological Balloon (reference) exposure system used over two days.
Cycle 2: Scavenger exposure system used (with a PDOPvalve and a flowmeter) over two days.
Cycle 3: Passive scavenger exposure system used over two days.
Cycle 4: Meteorological Balloon (reference) exposure system used over two days.

PHASE 2:

Clinical signs: Ocular mydriasis was noted beginning Day 2 for the duration of the study in animals given 2390 µg/kg/day (both sexes), while in animals given 1130 µg/kg/day, the incidence was lower and onset was later (Day 10). Dry eyes, nose, mouth and vomit were noted occasionally at 1130 µg/kg/day (males) and at 2390 µg/kg/day (both sexes). Swelling of the neck was recorded immediately post dose on several days in animals given 1130 and 2390 µg/kg/day. Incidences of the clinical signs in the report were provided on an individual animal basis and summary tables were not provided. Clinical signs of mydriasis, dry eyes, dry nose, and swelling of the neck were attributed to the anticholinergic properties of the test article.

Body weights: A concurrent control group was not included for comparison.

Food consumption: A concurrent control group was not included for comparison.

Ophthalmoscopy: On Days 1, 14 and 25, minimal to no tear production (relative to pretest) was noted 2 to 4 hr postdose in males given 1130 µg/kg/day and males and females given 2390 µg/kg/day. On Days 1, 14 and 25, tear production prior to dose administration in males given 1130 µg/kg/day and males and females given 2390 µg/kg/day GSK573719 was 0.7X pretest and 0.4 to 0.9X pretest, respectively. Decreased tear production was attributed to the anticholinergic properties of the test article.

Schirmer Tear Test (mm/min)

	Day 1				Day 14				Day 25			
	Predose		2-4 hr PD		Predose		2-4 hr PD		Predose		2-4 hr PD	
	L	R	L	R	L	R	L	R	L	R	L	R
G2M	18	20	2	1	15	11	5	3	11	14	8	7
G3M	17	17	1	2	9	8	0	0	7	11	3	3
G3F	15	15	0	1	6	4	0	0	9	7	0	0

EKG: On Day 1, there was a dose-related increase in pulse rates between 10 min and 3 hr (up to 1.7X predose) in males given 1130 µg/kg/day and between 10 min and 7 hr (up to 2.3X predose) in males and females given 2390 µg/kg/day. Pulse rates generally returned to predose values between 7 and 24 hr after the start of dosing.

On day 7, males given 1130 µg/kg/day had an increase of pulse rate at 10 min, males given 2390 µg/kg/day had increases of pulse rate (up to 1.3X pre dose) between 10 and 30 min, and no changes were noted for females given 2390 µg/kg/day. During week 4, males given 1130 µg/kg/day had no increase of pulse rate, males given 2390 µg/kg/day had an increase of pulse rate (up to 1.5X predose) between 10 min and 3 hr, and females given 2390 µg/kg/day had an increase at 10 min. Increased heart rate was attributed to the anticholinergic properties of the test article.

Femoral pulse pressure (values in parentheses represent percent of predose)

Day/Week	Time	Males		Females
		1130 µg/kg	2390 µg/kg	2390 µg/kg
Day 1	Predose	77	91	79
	10 min	127 (165%)	191 (210%)	174 (220%)
	30 min	111 (144%)	176 (193%)	177 (224%)
	1 hr	111 (144%)	146 (160%)	179 (227%)
	3 hr	101 (131%)	131 (144%)	149 (189%)
	7 hr	101 (131%)	140 (154%)	150 (190%)
	24 hr	95 (123%)	108	113 (143%)
Day 7	Predose	100	101	NC
	10 min	162 (162%)	131 (130%)	NC
	30 min	NC	121 (120%)	NC
	1 hr	NC	101	NC
	3 hr	NC	99	NC
	7 hr	NC	79	NC
	24 hr	NC	97	NC
Week 4	Predose	NC	91	108
	10 min	NC	133 (146%)	133 (123%)
	30 min	NC	123 (135%)	NC

	1 hr	NC	112 (123%)	NC
	3 hr	NC	121 (133%)	NC
	7 hr	NC	96	NC
	24 hr	NC	100	NC

Gross pathology: The gallbladder was distended for 1 male in Group 3; however, there were no corresponding histopathological findings.

Histopathology: Microscopic changes associated with administration of GSK573719 were present in the lungs, trachea, tracheal bifurcation and larynx at 1130 and/or 2390 µg/kg/day.

Lungs: Degeneration/regeneration of the bronchiolar epithelium was present in 2 males and 1 female given 2390 µg/kg/day. Subacute/chronic inflammation was evident for all dogs at 1130 and 2390 µg/kg/day. Cellular debris and mucus in the bronchiole lumen were observed for dogs at 1130 and 2390 µg/kg/day.

Trachea/Tracheal Bifurcation: Minimal epithelial degeneration and regeneration was present at the tracheal bifurcation and in the upper trachea of 1 female given 2390 µg/kg/day, which also exhibited pulmonary epithelial changes. At the top of the trachea, mixed inflammatory cells in the mucosa were evident for males at 1130 µg/kg/day and males and females at 2390 µg/kg/day. At the bottom of the trachea, mixed inflammatory cells in the mucosa were evident for males and females at 2390 µg/kg/day.

Larynx: A focus of minimal ulceration, associated with slight acute/subacute inflammation, was found in 1 male given 2390 µg/kg/day. An area of slight acute/subacute inflammation, without ulceration, also occurred in 1 female at the same dose. Foci of minimal epithelial degeneration/regeneration, without ulceration or inflammation, were present in 1 male and 1 female given 2390 µg/kg/day. Mixed inflammatory cells in the mucosa were evident for all dogs at 1130 and 2390 µg/kg/day.

Histopathological findings for dogs at 1130 and 2390 µg/kg/day of Phase 2

Organ/Tissue	Males		Females
	1130	2390	2390
Lungs			
-bronchiolar epithelium, degeneration/regeneration, Grade 1	0/3	2/3	1/3
-subacute/chronic inflammation, perivascular, peribronchiolar, subcapsular, Grade 1-2	3/3	3/3	3/3
-increased alveolar/intraalveolar macrophages, Grade 1-2	2/3	0/3	0/3
-interstitial subacute/chronic inflammation, Grade 1	1/3	0/3	0/3
-bronchioles: lumen cell	2/3	2/3	1/3

Organ/Tissue	Males		Females
	1130	2390	2390
debris/mucus, Grade 1			
Trachea (bottom) -mucosa: mixed inflammatory cells, Grade 1	0/3	1/3	1/3
Trachea (middle) -mucosa: mixed inflammatory cells, Grade 1	2/3	0/3	0/3
Trachea (top) -mucosa : epithelium, degeneration/regeneration, Grade 1 -mucosa: mixed inflammatory cells, Grade 1	0/3 2/3	0/3 1/3	1/3 1/3
Tracheal bifurcation -mucosa: epithelium, degeneration/regeneration, Grade 1 -mucosa: mixed inflammatory cells, Grade 1	0/3 1/3	0/3 1/3	1/3 1/3
Larynx, Level 1 -mucosa: acute/subacute inflammation, Grade 2 -mucosa: mixed inflammatory cells, Grade 1	0/3 3/3	0/3 3/3	1/3 3/3
Larynx Level 2 -mucosa: epithelium – ulcer(s), Grade 1 -mucosa: acute/subacute inflammation, Grade 2 -epithelium: degeneration/regeneration, Grade 1	0/3 0/3 0/3	1/3 1/3 1/3	0/3 0/3 1/3

Toxicokinetics: C_{max} values increased in a dose proportional manner on days 14 and 27. The C_{max} value for males at 1130 µg/kg/day on day 1 was greater than value for males at 2390 µg/kg/day. C_{max} values for males at 1130 and 2390 µg/kg/day on day 7 were not proportional.

Parameter+	Day	Male		Female
		Estimated Achieved Dose		Estimated Achieved Dose
		1130 µg/kg/day	2390 µg/kg/day	2390 µg/kg/day
C_{max} (ng/mL)	1	167	147	246
	7	113	157	217
	14	165	282	404
	27	173	378	344

+Calculated values derived by multiplying the dose-normalized data (see Appendix 12, Table 1) by the overall estimated achieved dose.

Histopathology inventory (optional)

Study	GSK573719					
	14-days WD2006/032 25/00	28-days WD2005/014 23	28-days WD2006/032 94/00	13-weeks WD2007/015 12/00	28-days WD2008/015 04/00	14-days WD2006/036 69/00
Species	Rat	Dog	Dog	Dog	Dog	Dog
Adrenals	X*	X*	*	X*		X*
Aorta	X	X		X		X
Bone Marrow smear						
Bone (femur)	X	X		X		X
Brain	X*	X*	*	X*		X*
Bronchi	w/Lungs	X	X	w/Lungs	w/Lungs	w/Lungs
Cecum	X	X		X		X
Cervix	X	X		X		X
Colon	X	X		X		X
Duodenum	X	X		X		X
Epididymis	X	X		X		X
Esophagus	X	X		X		X
Eye	X	X		X		X
Fallopian tube						
Gallbladder		X		X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland	X					
Heart	X*	X*	*	X*		X*
Ileum	X	X		X		X
Injection site						
Jejunum	X	X		X		X
Kidneys	X*	X*	*	X*		X*
Lachrymal gland						
Larynx	X	X	X	X	X	X
Liver	X*	X*	*	X*		X*
Lungs	X*	X*	X*	X*	X	X*
Lymph nodes, cervical				X		X
Lymph nodes mandibular	X	X				
Lymph nodes, mesenteric	X	X		X		X
Lymph nodes, popliteal		X		X		X
Lymph nodes, tracheobronchial	X	X	X	X	X	X
Mammary Gland	X	X		X		X
Nasal cavity	X	X	X	X	X	X
Optic nerves	X	X		X		X
Ovaries	X*	X*	*	X*		X*
Pancreas	X	X		X		X
Parathyroid	X (w/Thy)	X (w/Thy)		X (w/Thy)		X (w/Thy)
Peripheral nerve						
Pharynx	X	X	X	X		X
Pituitary	X	X		X		X
Prostate	X*	X*	*	X*		X*
Rectum		X		X		X
Salivary gland, submandibular	X	X		X		X
Salivary gland, sublingual	X					
Salivary gland, parotid	X	X		X		X

Sciatic nerve	X	X		X		X
Seminal vesicles	X					
Skeletal muscle	X	X		X		X
Skin	X	X		X		X
Spinal cord	X	X		X		X
Spleen	X*	X		X		X
Sternum	X	X (BM)		X (BM)		X (BM)
Stomach	X	X		X		X
Testes	X*	X*	*	X*		X*
Thymus	X*	X*	*	X*		X*
Thyroid	X (w/PT)	X (w/PT)		X (w/PT)		X (w/PT)
Tongue	X	X		X		X
Tonsils		X		X		X
Trachea	X	X	X	X	X	X
Tracheal bifurcation	X	X	X	X	X	X
Urinary bladder	X	X		X		X
Uterus	X	X		X		X
Vagina	X	X		X		X
Zymbal gland						

X, histopathology performed
*, organ weight obtained

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **Inhaled Female Fertility and Early Embryonic Development Study in Rats**

Key study findings:

● In a female fertility and early embryonic development study, rats received estimated achieved inhalation doses of 3.37, 29.1, 100, and 294 µg/kg/day.

● Body weight gains were unaffected. Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day. Body weight gains in this previous study for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group. Dose selection in the present study appeared to be adequate.

● There were no effects on fertility or reproductive performance for female rats treated with inhalation doses up to 294 µg/kg/day (equivalent to a pulmonary deposited dose of 29.4 µg/kg/day).

Study no.: WD2007/00763/00
Volume #, and page #: Volume 22, Pages 1 to 193

Conducting laboratory and location: (b) (4)

Date of study initiation: January 30, 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: GSK573719A [Input batch R230166, Lot 061120527] was micronized and blended with lactose powder BP/USNF (Batch number 612435, Lot 061121863) at a nominal concentration of 0.5% (w/w) (as base) with magnesium stearate [input batch C512923] at a nominal concentration of 1% (w/w) [blended batch number BVR/H/1009/07, content uniformity of 95.1%].

The vehicle, magnesium stearate [input batch C512923, Lot 06114989] was blended with lactose powder BP/USNF (Batch number 612435, Lot 061121863) at a nominal concentration of 1% (w/w) [blended batch number BVR/H/1008/07].

Methods
Doses: Groups of 25 females were treated by inhalation with lactose containing 1 % (w/w) magnesium stearate or GSK573719A (0.5% (w/w) with 1% (w/w) magnesium stearate in lactose) at target doses of 3, 30, 100, and 300 µg/kg/day for 60 min per day. Estimated achieved doses were 3.37, 29.1, 100, and 294 µg/kg/day. Females were treated daily for 2 weeks before pairing, throughout pairing, and until Day 7 after mating.

Average Chamber Aerosol Concentration and Estimated Achieved Doses of GSK573719.

Group Number	Target chamber aerosol concentration (μg/L)[1]	Average achieved chamber aerosol concentration (μg/L)[1]	% change from target chamber aerosol concentration	Estimated achieved dose (μg/kg/day)[1]
2	0.0800	0.0872	+9.0	3.37
3	0.800	0.750	-6.3	29.1
4	2.70	2.59	-4.2	100
5	8.00	7.63	-4.6	294

1. Doses and concentrations are expressed in terms of parent compound, GSK573719.
Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)

D dose (μg/kg)
RMV Respired minute volume (L) $0.499 \times BW(kg)^{0.809}$ [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol GSK573719 concentration (μg/L)
BW Group average body weight for study (kg)

Average Chamber Aerosol Concentration and Estimated Achieved Doses of Magnesium Stearate

Group Number	Average achieved chamber aerosol concentration (μg/L)[1]	Estimated achieved dose (μg/kg/day)
1	9.79	380
2	0.0894	3.46
3	0.773	30.0
4	3.42	132
5	10.5	403

1. Concentrations are expressed in terms of Magnesium Stearate.
Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)

D dose (μg/kg)
RMV Respired minute volume (L) $0.499 \times BW(kg)^{0.809}$ [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol Magnesium Stearate concentration (μg/L)
BW Group average body weight for study (kg)

The GSK573719 concentration was targeted in preference to the Magnesium Stearate concentration (Groups 2 to 4). The estimated achieved doses for all groups were close to the target.

Particle Size Distribution Results of GSK573719

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
2	0.0872	(b) (4)	
3	0.750		
4	2.59		
5	7.63		

1 Concentrations are expressed in terms of parent compound, GSK573719
MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Particle Size Distribution Results of Magnesium Stearate

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
1	9.79	(b) (4)	
2	0.0894		
3	0.773		
4	3.42		
5	10.5		

1 Concentrations are expressed in terms of Magnesium Stearate
2 Bimodal particle distribution observed
MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Aerosol concentrations: A measured volume of air was drawn from exposure chambers through a microfiber filter, fitted to an open-faced filter holder. The glass fiber filters were analyzed for GSK573719 using a UPLC method with UV detection. Magnesium stearate concentration was determined by analyzing the quartz filters for magnesium using atomic absorption spectroscopy.

Particle size distribution: A measured volume of air was drawn through a cascade impactor. Samples were collected during weeks 1 and 3 for gravimetric and chemical analyses. Samples for chemical analysis of GSK573719 and for magnesium stearate were collected separately.

Species/strain: Female CRL:CD®(SD)IGS BR rats were obtained from (b) (4) A small number of male rats (10 weeks old) were obtained from the same source for mating purposes only. Females were 9-12 weeks old with a body weight range of 210-264 g at the start of estrous cycle evaluation.
Number/sex/group: 25 female rats/group.
Route, formulation, volume, and infusion rate: Female rats received vehicle or drug by nose-only inhalation once daily for 60 min. Aerosols were generated using a Wright

Dust Feed mechanism by suspending material scraped from the surface of the a reservoir of compressed test article powder blend in a stream of dry compressed air. Aerosols were conducted into nose-only inhalation exposure chambers. The inhalation exposure system consisted of an ADG nose-only inhalation exposure chamber, restraining tubes, a dust generator (to produce an aerosol from the powder supplied), air supply, and extract lines, which attached to the top and bottom of the chamber, respectively, and an air supply to the dust generator. A filtration system was incorporated in the extract line. The nose-only inhalation chamber was a modular apparatus of aluminum alloy construction consisting of a base unit, a variable number of animal exposure sections, and a top section incorporating a central aerosol inlet surrounded by a tangential air inlet. The WDF mechanism was mounted on the top of the aerosol conditioning pre-chamber and the aerosol passed through it into the top of the exposure chamber. Sixty exposure ports were available on each exposure chamber (20 ports/exposure section). Five individual exposure chambers were used in the study.
Satellite groups used for toxicokinetics:
Study design: Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day. Body weight gains for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group. Treatment-related histopathological findings were observed in the nasal cavity/sinuses, larynx, nasopharynx, lung, tracheobronchial bifurcation, and prostate.

Female rats were treated with estimated achieved doses of 3.37, 29.1, 100, and 294 µg/kg/day for 2 weeks before pairing, throughout pairing, and until day 7 after mating. During the treatment period, animals were observed prior to exposure, during exposure, 1-2 hr after completion of dosing of all groups, and as late as possible in the day. Body weights were measured on gestation days 0, 7, 10, 14, 17, and 20. Food consumption was measured weekly during the 2-week pretreatment period and the treatment period before mating, and daily on days 0-6, 7-13, and 14-19. Vaginal smears were collected for 14 days prior to the start of dosing and during the treatment period until paired. After 2 weeks of treatment, females were paired on a 1 to 1 basis with stock males for a period of up to 2 weeks. Females were sacrificed on gestation day 20 and submitted to necropsy examinations. The ovaries were removed and the corpora lutea were counted. The uterus was weighed and its contents were examined. Implantation sites, resorptions, live and dead fetuses were counted, and their relative positions were recorded. A gross examination of each placenta from live fetuses was conducted. Live fetuses were weighted, euthanized, and examined externally.
Parameters and endpoints evaluated: Fertility and early embryonic development were assessed following administration by nose-only inhalation to female Sprague-Dawley rats.

Results

Mortality: There were three deaths prior to mating. None of the deaths were judged to be treatment-related. Female #57 administered 29.1 µg/kg/day collapsed following smearing on day 15 of the treatment phase and died. Necropsy findings were evident in

the lung (congested), liver (pale subcapsular area), uterus (fluid distension), and stomach (white nodule near the limiting ridge). Female #96 administered 100 µg/kg/day was found dead having turned in the restraint tube during exposure on day 14. Necropsy findings were evident in the lung (complete collapse). Female #104 administered 294 µg/kg/day was found dead having turned in the restraint tube during exposure on Day 5. There were no necropsy findings for this animal.

Clinical signs: There were no treatment-related clinical signs. Estrous cycle length and pre-coital interval were unaffected.

Body weight: Body weight gains were unaffected from days 1 to 15 prior to mating and gestation days 0 to 7.

Food consumption: Food consumption was unaffected the two weeks prior to mating and gestation days 0 to 6.

Necropsy: Gravid uterine weights were unaffected by treatment.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Mating and fertility indexes were unaffected. Numbers of corpora lutea, implantation sites, resorptions (early, late, and total), and live young (male, female, and total) were unaffected. Sex ratios, pre- and post-implantation losses, litter weight, litter size, and fetal body weight (male, female, and overall) were unaffected.

Mating Performance and Fertility - group values

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1F	25	25	25	100	100	100
2F	25	25	22	100	88	88
3F	24	24	24	100	100	100
4F	24	24	24	100	100	100
5F	24	24	24	100	100	100

Embryofetal development

Study title: **Inhaled Embryo-Fetal Development Study in Rats**

Key study findings:

● GSK573719A was administered by nose-only inhalation for 1 hr each day from gestation days 6 to 17 to pregnant Sprague-Dawley rats at estimated achieved doses of 0, 0.0317, 0.0969, and 0.278 mg/kg/day.

● Body weight gains from gestation days 6 to 18 were unaffected. Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day. Body weight gains for females from the dose selection study in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group. Dose selection in the present study appeared to be adequate.

● There were no external, skeletal, or visceral malformations. Increased incidences of minor skeletal and visceral abnormalities/variants were observed for treatment groups.

● The incidence of ventral arch of the 1st cervical vertebra was increased for all treatment groups in a dose-related manner. Incidences of metatarsals (incomplete ossification/unossified) and phalanges (none ossified) bilateral hindlimb were increased for all treatment groups although dose-response relationships were not present.

● Subdural hemorrhages were evident in the mid and high dose groups. Hemorrhage in the cerebellum was observed in the mid and high dose groups although a dose-response relationship was not present. Hemorrhage within cerebral hemispheres was observed in the high dose group.

● GSK573719 was not teratogenic in rats at inhaled doses up to 0.278 mg/kg/day (equivalent to a pulmonary deposited dose of 0.028 mg/kg/day or 27.8 µg/kg/day).

Study no.: WD2007/00764/00
Volume #, and page #: Volume 23, Pages 1-145
Conducting laboratory and location: (b) (4)

Date of study initiation: January 30, 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: GSK573719A [Input batch R230166, Lot 061120527] was micronized and blended with lactose powder BP/USNF (Batch number 612435, Lot 061121863) at a nominal concentration of 0.5% (w/w) (as base) with magnesium stearate [Input batch C512923, Lot 061114989] at a nominal concentration of 1% (w/w) [blended batch number BVR/H/1009/07, content uniformity of 95.1%].

The vehicle, magnesium stearate [Input batch C512923] was blended with lactose powder BP/USNF (Batch number 612435, Lot 061121863) at a nominal concentration of 1% (w/w) [blended batch number BVR/H/1008/07].

Methods

Doses: GSK573719A was administered by nose-only inhalation for 1 hr each day from gestation days 6 to 17 to pregnant Sprague-Dawley rats at estimated achieved doses of 0, 0.0317, 0.0969, and 0.278 mg/kg/day (aerosol concentrations of 0.831, 2.55, and 7.30 µg/L, respectively).

GSK573719

Group	Average achieved aerosol chamber concentration (µg/L)[1]	Time of exposure (mins)	Body weight[2] (g)	Estimated achieved dose (mg/kg/day)[1]
1	-	60	295	0
2	0.831	60	281	0.0317
3	2.55	60	287	0.0969
4	7.30	60	284	0.278

1. Expressed in terms of GSK573719 (parent compound)
2. Average of the group mean daily body weight results.

Magnesium Stearate

Group	Average achieved aerosol chamber concentration (µg/L)[1]	Time of exposure (mins)	Body weight[2] (g)	Estimated achieved dose (mg/kg/day)[1]
1	9.76	60	295	0.369
2	1.01	60	281	0.0386
3	3.00	60	287	0.114
4	9.68	60	284	0.369

1. Expressed in terms of Magnesium Stearate
2. Average of the group mean body daily body weight results.

Summary of Group Particle Size Distribution Results for GSK573719

Group number	Average achieved chamber aerosol concentration (µg/L)	MMAD (µm)	GSD
2	0.831	(b) (4)	
3	2.55		
4	7.30		

MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Summary of Group Particle Size Distribution Results for Magnesium Stearate

Group number	Average achieved chamber aerosol concentration (µg/L)	MMAD (µm)	GSD
1	9.76	(b) (4)	
2	1.01		
3	3.00		
4	9.68		

MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Chamber aerosol concentrations and particle size distributions were measured as described in reviews of other studies.

Species/strain: Time-mated female Crl:CD(SD)IGS BR rats were obtained from (b) (4) At the start of treatment (gestation day 6), rats were approximately 10-12 weeks old and the body weight range was 229-302 g.
Number/sex/group: 22 females/group
Route, formulation, volume, and infusion rate: Females rats received vehicle or drug by nose-only inhalation once daily for 60 min. Aerosols were generated using a Wright Dust Feed mechanism by suspending material scraped from the surface of the a reservoir of compressed test article powder blend in a stream of dry compressed air. Aerosols were conducted into nose-only inhalation exposure chambers. See the fertility and early embryonic development study (WD2007/00763/00) for additional details on the inhalation exposure system.
Satellite groups used for toxicokinetics: None
Study design: Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day. Body weight gains for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group. Treatment-related histopathological findings were observed in the nasal cavity/sinuses, larynx, nasopharynx, lung, tracheobronchial bifurcation, and prostate.

GSK573719A was administered by nose-only inhalation for 1 hr each day from gestation days 6 to 17 to pregnant Sprague-Dawley rats at estimated achieved doses of

0, 0.0317, 0.0969, and 0.278 mg/kg/day. During the treatment period, animals were observed prior to exposure, during exposure, 0.5-2 hr after completion of dosing, and as late as possible in the day. Body weights were measured on gestation days 3, 6-18, and 21. Food consumption was measured on gestation days 3-5, 6-8, 9-11, 12-14, 15-17, and 18-20. Female rats were sacrificed on gestation day 21 and submitted to necropsy examinations. The number of corpora lutea on each ovary was counted. The uterus with ovaries was removed and weighed and its contents were examined. Implantation sites, resorptions, and live and dead fetuses were counted and relative positions were recorded. A gross examination of each placenta from live and dead fetuses was conduced. Live fetuses were weighed and euthanized. Approximately half of the fetuses in each litter were subjected to gross internal examination of the organs of the neck, thorax, and abdominal cavities by microdissection. These fetuses were eviscerated and skeletons were processed, stained with Alizarin Red, and examined. The heads from remaining fetuses examined for visceral abnormalities.
Parameters and endpoints evaluated: The objective of this study was to determine the influence of GSK573719A on pregnancy and embryo-fetal survival and development when administered by nose-only inhalation to female Sprague-Dawley rats.

Results

Mortality (dams): There were no deaths. Two dams spontaneously delivered on gestation day 21 (Female #24 in the low dose group and Female #82 in the high dose group).

Clinical signs (dams): There were no treatment-related clinical signs.

Body weight (dams): Body weight gains from gestation days 6 to 18 were unaffected.

Food consumption (dams): Food consumption from gestation days 6 to 18 was unaffected.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): Gravid uterine weights were unaffected. Numbers of corpora lutea, implantation sites, resorptions (early, late, and total), and live young (male, female, and total). Sex ratios, pre- and post-implantation losses, litter weight, litter size, and fetal body weights (male, female, and total) were unaffected.

Offspring (malformations, variations, etc.): There were no external, skeletal, or visceral malformations. Increased incidences of minor skeletal and visceral abnormalities/variants were observed for treatment groups. The incidence of ventral arch of the 1st cervical vertebra was increased for all treatment groups in a dose-related manner. Incidences of metatarsals (incomplete ossification/unossified) and phalanges (none ossified) bilateral hindlimb were increased for all treatment groups although dose-response relationships were not present. Subdural hemorrhages were evident in the mid and high dose groups. Hemorrhage in the cerebellum was observed in the mid and

high dose groups although a dose-response relationship was not present. Hemorrhage within cerebral hemispheres was observed in the high dose group.

Minor skeletal abnormalities/variants

Parameter	0	0.0317	0.0969	0.278
Fetuses/Litters examined	136/22	120/20	133/22	115/19
Ventral arch of 1st cervical vertebra	5/4	9/6	10/7	19/9
Metatarsals on the 1st digit (incomplete ossification/ unossified)	3/2	25/11	19/7	8/7
Phalanges (none ossified) bilateral hindlimb	19/10	36/13	33/12	26/12

Minor visceral abnormalities

Parameter	0	0.0317	0.0969	0.278
Fetuses/Litters examined	136/22	123/20	135/22	113/19
Hemorrhage, cerebellum	0/0	0/0	4/4	3/3
Hemorrhage within cerebral hemisphere	0/0	0/0	0/0	2/2

Note: Maternal toxicity was not evident in the present study although maternal toxicity (i.e., decreased body weight gain) was demonstrated in the study used for dose selection.

Study title: **Inhaled Embryo-Fetal Development Study in Rabbits**

Key study findings:

● In an embryofetal development study, time-mated female New Zealand white rabbits received GSK573719 at estimated inhaled doses of 0, 0.0285, 0.0889 or 0.306 mg/kg/day from gestation days 7 to 19.

● Body weight gains from gestation days 7 to 20 for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups were reduced (i.e., body weights on day 20 were changed by -0.77, +0.28, and -0.28% as compared to body weights on day 7, respectively; control was +1.2%) as compared to the control although a dose-response relationship was not evident. Food consumption for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups from gestation days 7 to 19 was reduced to 77.3, 78.15, and 73.1% of the control, respectively.

● Gravid uterine weights for the 0.0899 and 0.305 mg/kg/day groups were reduced to 94.2 and 92.3% of the control (0.52 kg) although the toxicological significance of these differences was not clear. Fetal body weights (male, female, and overall) were slightly reduced (up to 9.3%) for all treatment groups although the significance of these differences was not clear.

● There were no treatment-related external, visceral, or skeletal malformations. Two fetuses in the high dose group were observed with thymus-undescended horn. The incidence of caudal vertebrae-incomplete ossification/unossified was increased for the high dose group. The incidence of sternebrae-bipartite ossified/offset alignment was increased for the high dose group and slightly exceeded the historical control incidence.

● GSK573719 was not teratogenic in rabbits with inhaled doses up to 0.306 mg/kg/day. The sponsor is conducting a second embryofetal development study with GSK573719 in rabbits using the intravenous route of administration (see Review of Amendment #030 dated May 10, 2010).

Study no.: WD2007/00762/00
Volume #, and page #: Volume 23, Pages 1-199
Conducting laboratory and location: (b) (4)

GSK
Park Road
Ware
Hertfordshire
SG12 0DP
England

Date of study initiation: March 27, 2007
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:
GSK573719A (input batch R230166, Lot 061120527) was micronized and blended with lactose powder BP/USNF (Batch Number 612435, Lot Number 061121863) at a nominal concentration of 2% (w/w) (as base), with Magnesium Stearate (Input batch C512923, Lot 061114989) at a nominal concentration of 1 % (w/w) (blended batch number BVR/H/I 0 12/07, content uniformity 99.4%).

The vehicle, Magnesium Stearate (Input batch C512923, Lot 061114989) was blended with lactose powder BP/USNF (Batch Number 612435, Lot Number 061121863) at a nominal concentration of 1% (w/w) (blended batch number BVR/H/I011/07).

Methods

Doses: Time-mated female New Zealand white rabbits received GSK573719 at estimated inhaled doses of 0, 0.0285, 0.0889 or 0.306 mg/kg/day (achieved chamber concentrations of 0, 1.23, 3.77 and 13.0 µg/L, respectively) from gestation days 7 to 19.

Groups of 22 mated females received vehicle or drug formulations by snout-only inhalation once daily on Days 7 to 19 pc at the doses shown in the dose table.

Group Number	Target Dose[1] (mg/kg/day)	Estimated Achieved Dose[1,2] (mg/kg/day)	Target Chamber Aerosol Concentration[1] (µg/L)	Achieved Chamber Aerosol Concentration[1,2] (µg/L)	Number of Females
1	0	0	0	0	22
2	0.03	0.0285	1.27	1.23	22
3	0.1	0.0889	4.2	3.77	22
4	0.3	0.306	12.7	13.0	22

1. Doses and concentrations are expressed in terms of parent compound
2. As determined by chemical analysis

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/(BWx1000)

D	dose (mg/kg)
RMV (Respiratory minute volume L/min)	$0.499 \times BW(kg)^{0.809}$ [Bide et al, 2000]
T	Duration of exposure/day (minutes)
C	Aerosol GSK573719 concentration (µg/L)
BW	Group average body weight for study (kg)

Magnesium Stearate

Group Number	Estimated Achieved Dose[1,2] (mg/kg/day)	Achieved Chamber Aerosol Concentration[1,2] (µg/L)	Number of Females
1	0.121	5.29	22
2	0.0119	0.513	22
3	0.0360	1.53	22
4	0.128	5.44	22

1 Doses and concentrations are expressed in terms of Magnesium Stearate
2 As determined by chemical analysis

Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/(BWx1000)

D	dose (mg/kg)
RMV (Respiratory minute volume L/min)	$0.499 \times BW(kg)^{0.809}$ (Bide et al, 2000)
T	Duration of exposure/day (minutes)
C	Aerosol Magnesium Stearate concentration (µg/L)
BW	Group average body weight for study (kg)

Particle Size Distribution Results of GSK573719

Group number	Average achieved chamber aerosol concentration (µg/L)[1]	MMAD (µm)	GSD
2	1.23	(b) (4)	
3	3.77		
4	13.0		

1. Concentrations are expressed in terms of parent compound
MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Reference ID: 3330354

Particle Size Distribution Results of Magnesium Stearate

Group number	Average achieved chamber aerosol concentration (μg/L)[1]	MMAD (μm)	GSD
1	5.29	(b) (4)	
2	0.513		
3	1.53		
4	5.58		

1. Concentrations are expressed in terms of Magnesium Stearate
2. The particle size distribution was bimodal suggesting 2 distinct subpopulations of particles. The MMAD for each subpopulation is presented

MMAD - Mass median aerodynamic diameter (μm)
GSD - Geometric standard deviation

Chamber aerosol concentrations: A measured volume of air was drawn from exposure chambers through a microfiber filter, for GSK573719 or magnesium stearate, mounted in an open face filter holder. Samples were collected daily from each dose group for gravimetric analysis and chemical analysis. The microfiber filters were analyzed for GSK573719 content using UPLC with UV. The microfiber filters were analyzed for magnesium stearate content using AA.

Particle size determinations: A measured volume of air was drawn from inhalation exposure chambers containing GSK573719 and Magnesium Stearate through a cascade impactor Samples were collected during Week 1 and 2 and subjected to gravimetric analysis prior to chemical analysis. The samples were analyzed for GSK573719 and magnesium stearate content.

Species/strain: Timed-mated female New Zealand white rabbits were obtained for this study from (b) (4) At arrival, the rabbits were day 1 post coitum (day of mating was designated gestation day 0) and were approximately 20 to 23 weeks of age. At the initiation of treatment on gestation day 7, the body weight range was 3.15 kg and 3.76 kg.
Number/sex/group: 22 time-mated female rabbits/group
Route, formulation, volume, and infusion rate: During the 13 day treatment period rabbits received daily nose-only inhaled doses of GSK513719 or vehicle for 60 min/day. The inhalation exposure system comprised an ADG snout-only inhalation exposure chamber, restraining tubes, a Wright Dust Feed dust generator (to produce an aerosol from the powder supplied), air supply, and extract lines, which attached to the top and bottom of the chamber respectively and an air supply to the dust generator. The ADG nose-only inhalation chamber was a modular apparatus of aluminum alloy construction consisting of a base unit, a variable number of animal exposure sections, and a top section incorporating a central aerosol inlet surrounded by a tangential air inlet. During dosing each chamber was housed in an enclosed ventilated cabinet. The WDF mechanism was mounted on the top of the aerosol conditioning pre-chamber and the

aerosol passed through it into the top of the exposure chamber. Twenty-four exposure ports were available on each exposure chamber (6 ports/exposure section). Four individual exposure chambers were used in the study.
Satellite groups used for toxicokinetics: None
Study design: Dose selection was based upon two dose range finding studies. In the first dose range finding study (WD2006/03186/00), unmated female rabbits (4/group) received GSK573719 by nose-only inhalation at mean achieved doses of 0.128, 0.257, or 1.82 mg/kg/day for 13 days. Following exposure to 0.128, 0.257, or 1.82 mg/kg/day, body weights were reduced by -2.9, -2.6, and -5.1% of initial body weights on day 1, respectively. Food consumption was reduced at all doses as compared to pretreatment levels. In the second dose range finding study, mated female rabbits (5/group) received GSK573719 by nose-only inhalation at mean achieved doses of 0, 0.0428, or 0.305 mg/kg/day from gestation days 7 to 19. Body weights from gestation days 7 to 20 with doses of 0, 0.0428, or 0.305 mg/kg/day were increased by 5.25, 1.64, and 1.56%, respectively. Food consumption and gravid uterine weight at 0.305 mg/kg/day were reduced to 75.5 and 72% of the control, respectively. Litter weight and overall fetal body weight at 0.305 mg/kg/day were reduced to 75.4 and 86.7% of the control, respectively.

Time-mated female New Zealand white rabbits received GSK573719 at estimated inhaled doses of 0, 0.0285, 0.0889 or 0.306 mg/kg/day (achieved chamber concentrations of 0, 1.23, 3.77 and 13.0 µg/L, respectively) from gestation days 7 to 19. During the treatment period, animals were observed immediately after dosing, during exposure, between 0.5 and 2 hr after completion of dosing, and as late as possible during the work day. Body weights were measured on gestation days 1, 2, 4, 7 to 20, 25, and 29. Food consumption was measured daily from gestation days 4 to 29. Females were sacrificed on gestation day 29 and submitted to necropsy examinations. The number of corpora lutea on each ovary was counted. The uterus was weighed and its contents examined. Implantation sites, resorptions (early and late), and live and dead fetuses were counted and their relative positions were recorded. A gross examination was performed on each placenta from live and dead fetuses. Live fetuses were weighed and euthanized. Live fetuses were sexed and abdominal and thoracic viscera of all fetuses were examined by microdissection. The heads of approximately one-half of the fetuses were sectioned and examined by the Wilson's technique (Wilson 1965) for abnormalities. The torsos of these fetuses and the other half of the fetuses (intact fetuses) were processed for skeletal examination. All skeletons, except fetal heads fixed in Bouin's solution, were stained with Alizarin Red Sand subsequently examined for skeletal development.

On day 5 of dosing (gestation day 11), blood samples for measurement of plasma drug concentrations were collected from 6 rabbits per group immediately after dosing and at 0.25, 0.5,1, 5, and 23 hours after completion of dosing. Naïve samples were processed alongside the study samples (immediately after dosing and 5 and 23 hr after dosing). Samples were transferred to GlaxoSmithKline for analysis. Plasma samples were analyzed for GSK573719 using a validated analytical method based on protein precipitation, followed by HPLC-MS/MS analysis. The lower limit of quantification (LLQ)

for GSK573719 was 0.084 ng/mL using a 50 µL aliquot of rabbit plasma with a higher limit of quantification (HLQ) of 42.16 ng/mL.

Parameters and endpoints evaluated: The objective of this study was to determine the effects of GSK573 719 on pregnancy and embryo-fetal development when administered by snout-only inhalation to female New Zealand white rabbits.

Results

Mortality (dams): There were three deaths.

One female (#77) in the 0.306 mg/kg/day group aborted her litter on gestation day 19.

One female (#66) in the 0.0889 mg/kg/day group was sacrificed on gestation day 28 due to clinical signs indicating a possible abortion, although there was no evidence at necropsy and uterine examination of abortion. A small amount of clotted blood was surrounding the placenta and fetus L3.

One female (#63) in the 0.0889 mg/kg/day group was sacrificed on gestation day 28 due to poor condition (i.e., thin appearance, watery discharge from the eyes, brown stained fur and tail, and a period of reduced/negligible food intake). Post mortem examination confirmed thin appearance and gastrointestinal disturbances (i.e., abnormal contents in GI tract, cecum contents dark and fluid, stomach distended with fluid, liver-all lobes pale, and gallbladder-distended). This was not considered to be test article-related as similar effects were not observed in other animals given 0.0889 mg/kg/day or at higher doses.

Clinical signs (dams): There were no treatment-related clinical signs.

Body weight (dams): Body weight gains from gestation days 7 to 20 for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups were reduced as compared to the control although a dose-response relationship was not evident.

Body weight gains from gestation days 7 to 19

Parameter	0 mg/kg/day	0.0285 mg/kg/day	0.089 mg/kg/day	0.308 mg/kg/day
BW (kg), Day 7	4.16	3.88	3.51	3.57
BW (kg), Day 20	4.21	3.85	3.52	3.56
Δ (kg), D20-D7	0.05	-0.03	0.01	-0.01
% of Initial BW (D7)	1.2%	-0.77%	0.28%	-0.28%

Food consumption (dams): Food consumption for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups from gestation days 7 to 19 was reduced to 77.3, 78.15, and 73.1% of the control, respectively.

Toxicokinetics: AUC and C_{max} values increased with elevating dose although increases were greater than dose proportional from the low to mid dose and dose proportional from the mid to high dose.

Parameter[1]	Day pc	Dose (mg/kg/day)		
		0.0285	0.0889	0.306
Mean AUC $_{(0-t)}$ (ng/h.mL)	11	0.217[2]	2.02[3]	10.9[4]
Mean C_{max} (ng/mL)	11	0.158	1.03	1.78

1 Calculated values derived by multiplying the dose normalised data by the overall estimated achieved dose
2 AUC calculated up to 0 - 2 hours
3 AUC calculated up to 0 - 6 hours
4 AUC calculated up to 0 - 24 hours

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): A total of 1, 1, 3 and 4 females were non pregnant, 0, 0, 2, and 1 females were killed prior to study termination and 0, 1, 0, and 0 had total litter loss at 0, 0.0285, 0.0889, and 0.306 mg/kg/day. respectively. This left a total of 21, 20, 17, and 17 females with live litters at 0, 0.0285, 0.0889, and 0.306 mg/kg/day groups, respectively, which were available for assessment of embryo-fetal development on gestation day 29.

Numbers of corpora lutea, implantation sites, resorptions (early, late, and total), and live young (male, female, and total) were unaffected. Sex ratios, pre-implantation loss, post-implantation loss, litter weight, and litter size were unaffected. Gravid uterine weights for the 0.0899 and 0.305 mg/kg/day groups were reduced to 94.2 and 92.3% of the control (0.52 kg) although the toxicological significance of these differences was not clear. Fetal body weights (male, female, and overall) were slightly reduced for all treatment groups although the significance of these differences was not clear.

Fetal body weights

Group	Male Fetal BW, g	Female Fetal BW, g	Overall BW, g
1	45.45	44.06	45.15
2	42.22 (92.9%)	41.63 (94.5%)	44.02* (93.1%)
3	41.21 (90.7%)	41.06 (93.2%)	41.10* (91%)
4	43.12 (94.9%)	41.20 (93.5%)	42.30* (93.7%)

Offspring (malformations, variations, etc.): There were no treatment-related external, visceral, or skeletal malformations. Two fetuses in the high dose group were observed with thymus-undescended horn. The incidence of caudal vertebrae-incomplete ossification/unossified was increased for the high dose group. The incidence of sternebrae-bipartite ossified/offset alignment was increased for the high dose group and slightly exceeded the historical control incidence (Highest incidence of 3/3 for 151 fetuses/18 litters). The incidence of metacarpals/phalanges-incomplete ossification/unossified was increased for the high dose group although the incidence appeared to be within the historical control (Highest incidence of 22/7 for 175 fetuses/20 litters).

The number of fetuses with 12/13 or 13/13 ribs or 20 thoracolumbar vertebrae was reduced for the high dose group although these findings were not regarded as adverse. Incidences of 5th sternebrae or other sternebrae incompletely ossified/unossified were decreased for all treatment groups although these findings were not regarded as adverse.

Minor visceral abnormalities

Parameter	0 mg/kg/day	0.0285 mg/kg/day	0.089 mg/kg/day	0.308 mg/kg/day
Fetuses/Litters examined	162/21	177/20	142/17	137/17
Thymus-undescended horn	0/0	0/0	0/0	2/1

Minor skeletal abnormalities/variants

Parameter	0 mg/kg/day	0.0285 mg/kg/day	0.089 mg/kg/day	0.308 mg/kg/day
Fetuses/Litters examined	162/21	177/20	142/17	137/17
Sternebrae -bipartite ossified/offset alignment	1/1	1/1	0/0	4/2
Metacarpals/phalanges -incomplete ossification/unossified	7/5	8/4	8/7	12/7
Caudal vertebrae -incomplete ossification/unossified	0/0	0/0	0/0	2/2
Number with 12/13 or 13/13 ribs	82/18	80/20	79/16	56/15
20 thoracolumbar vertebrae	25/10	27/9	23/10	13/7
5th Sternebrae – incomplete ossification/unossified	33/10	23/9	14/6	7/5
Other Sternebrae – incomplete ossification/unossified	11/4	12/7	9/5	6/4
Total	39/11	33/13	22/10	11/8

2.6.6.9 Discussion and Conclusions

GSK573719 is a muscarinic acetylcholine receptor (mAChR) antagonist under development for the treatment of COPD. Inhalation toxicology studies with exposure duration up to 13 weeks were conducted in mice, rats, and dogs. Clinical signs of mydriasis, dry mouth, dry nose, and swollen neck as well as increased femoral pulse pressure observed in dogs could be attributed to the antimuscarinic properties of GSK573719. Increased heart rates (up to 2-fold) were observed in dogs that could be attributed to the antimuscarinic properties of the test article. Elevations of troponin I were observed in dogs on day 1, but not after several weeks of repeat dose administration. The elevations of troponin I suggested the potential for cardiac injury; however, histopathological examination of the heart found no evidence of injury. Heart rate is monitorable in a clinical setting. Target organs/tissues of toxicity in mice, rats,

and dogs, were primarily confined to respiratory tract that consisted of findings in the nasal cavities/sinuses, nasopharynx, larynx, trachea, tracheal bifurcation, bronchus, and lung and were judged to be indicative of local irritation. In the 13-week mouse study, there were additional findings in the liver (focal inflammation) and uterus (luminal dilatation). In a 14-day inhalation bridging toxicology studies with dogs, toxic effects were identified in the gallbladder (i.e., foci of minimal to slight myofiber degeneration/regeneration associated with variable hemorrhage and inflammation present in the tunica muscularis); however, longer duration studies with lower exposure levels did not replicate these findings suggesting the finding was associated with high dose exposure. Differences of target organs/tissues between species are shown in the table below.

2.6.6.10 Tables and Figures

Histopathological findings in 13-week inhalation toxicology studies with mice and rats and the 4-week inhalation toxicology study with dogs (WD2005/01423/00) are shown in the tables below. Granulomatous inflammation in the lungs of dogs was determined to be unrelated to treatment.

Target organs in mice and rats following inhalation exposure for 13 weeks and dogs following inhalation exposure for 4 weeks (WD2005/01423/00)

Target Organs/Tissues of Toxicity		
Mice/13 weeks (WD2007/01600/00)	**Rats/13 weeks** (WD2007/02012/00)	**Dogs/4 weeks** (WD2005/01423/00)
		Heart -increased heart rate -elevations of Troponin I -no histopathological findings
		Lung -inflammation, granulomatous (?) -material, eosinophilic -inflammation, interstitial -inflammation, bronchoalveolar -mineralization
		Trachea -inflammation, acute and subacute -degeneration/regeneration, epithelium
Tracheal bifurcation -epithelial hyperplasia at bifurcation, minimal -loss of cilia at bifurcation -loss of cilia at bronchial bifurcation, minimal		
Larynx -arytenoid, squamous hyperplasia, minimal-slight -ventral cartilage **necrosis**, minimal-marked	**Larynx** -metaplasia, squamous, Grade 1-2 -squamous, metaplasia, submucosal glands, Grade 1-2	**Larynx** -inflammation, acute and subacute -mineralization -exudate

Target Organs/Tissues of Toxicity		
Mice/13 weeks (WD2007/01600/00)	**Rats/13 weeks** (WD2007/02012/00)	**Dogs/4 weeks** (WD2005/01423/00)
-ventral epithelium, keratinization, minimal-moderate -ventral epithelium, squamous hyperplasia/metaplasia, minimal-marked -ventral gland, ductal dilatation, minimal-moderate -ventral gland, mucous cell hypertrophy, minimal-moderate -ventral pouch epithelium, squamous hyperplasia, minimal-marked -ventral region, subacute/chronic inflammation, minimal -ventrolateral/lateral epithelial squamous hyperplasia/ metaplasia, minimal-moderate	-inflammation, Grade 1-2 -**necrosis, Grade 1-2**	
Nasal turbinates -Bowman's glands, atrophy, slight -Bowman's glands, epithelial hyperplasia, minimal-slight -Bowman's glands, hypertrophy and eosinophilic inclusions, minimal-slight -Bowman's glands, inflammation, minimal-slight -exudate in nasal passages -maxillary sinus, respiratory epithelium, eosinophilic inclusions, minimal-moderate -olfactory epithelium, atrophy, minimal-marked -olfactory epithelium, degeneration/regeneration, minimal-moderate -olfactory epithelium, eosinophilic inclusions, minimal-marked -olfactory epithelium, **erosion**, minimal -olfactory epithelium, **ulceration**, slight -olfactory nerve fibers, atrophy, slight-marked -olfactory/respiratory junction, dilated gland(s), minimal-moderate -respiratory epithelium, degeneration/regeneration, minimal-moderate -respiratory epithelium,	**Nasal cavity/sinuses** -hyperplasia, transitional epithelium, Grade 1-2 -degeneration/regeneration, respiratory epithelium, Grade 1-3 -degeneration/regeneration, olfactory epithelium, Grade 1-2 -degeneration/regeneration, vomeronasal organ, Grade 1 -hyperplasia/hypertrophy, goblet cells, Grade 1 -inflammation, Grade 1-2 -exudate, Grade 1	**Nasal Cavity/Sinuses** -degeneration/regeneration, respiratory epithelium -degeneration/regeneration, olfactory epithelium -hyperplasia, squamous epithelium -metaplasia, squamous, respiratory epithelium -metaplasia, squamous, respiratory epithelium -ulceration, respiratory and squamous epithelium -inflammation, acute and subacute -dilatation and atrophy, Bowman's glands -hyperplasia, Bowman's gland -increased Periodic Acid Schiff/Alcian blue staining -hyperplasia and hypertrophy, goblet cells -inflammation, acute and subacute

Target Organs/Tissues of Toxicity		
Mice/13 weeks (WD2007/01600/00)	**Rats/13 weeks** (WD2007/02012/00)	**Dogs/4 weeks** (WD2005/01423/00)
eosinophilic inclusions, minimal-moderate -respiratory epithelium, inflammation in lamina propria, minimal-slight -respiratory epithelium, squamous metaplasia, minimal-moderate -Steno's gland, epithelial hyperplasia, slight -transitional epithelium, degeneration/regeneration, minimal-slight -transitional epithelium, eosinophilic inclusions, minimal-moderate -transitional epithelium, inflammation, minimal-slight -vomeronasal organ, degeneration/regeneration/ inflammatory cells, minimal		
Nasopharynx -epithelial degeneration/ regeneration, minimal-slight -epithelial eosinophilic inclusions, minimal-slight	**Nasopharynx** -metaplasia, squamous, nasopharynx, Grade 1	
Liver (?) -inflammation, focal, minimal		
Uterus (?) -luminal dilatation		
		Thymus (?) -atrophy, lymphoid
		Gallbladder* - foci of minimal to slight myofiber degeneration/regeneration associated with variable hemorrhage and inflammation present in the tunica muscularis

(?) Questionable relationship to treatment

* Observed in a 14-day inhalation bridging toxicology study (Study no.: WD2006/03669/00) with dogs. Histopathological findings in the gallbladder appeared to be associated with relatively high systemic exposures to the test article (i.e., AUC values on day 14 ranged from 52.732 to 102.689 ng·hr/mL and were not observed in 4- and 13-week inhalation toxicology studies with dogs.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

GSK573719 is a pan active muscarinic acetylcholine receptor (mAChR) antagonist that is under development for the treatment of chronic obstructive pulmonary disease (COPD).

Pharmacology: GSK573719A is a high affinity, pan active muscarinic antagonist. The compound competes with [^{3}H]-N-methyl-scopolamine binding for human recombinant receptor membranes from CHO cells stably expressing the M1, M2, M3, M4 and M5 receptors with K_i values of 0.16, 0.15, 0.06, 0.05, 0.13 nM, respectively. Intranasal administration of GSK573719A to mice resulted in a time and dose-related inhibition of methacholine-induced bronchoconstriction. The ED50 at 5-hr post-administration for GSK573719A was 0.02 µg/mouse. GSK573719A had a long, dose-related duration of bronchoprotection. A single intranasal dose of 0.05 µg/mouse inhibited methacholine-induced bronchoconstriction for up to 7 days. GSK573719A dose-dependently inhibited acetylcholine-induced bronchoconstriction and exhibited a dose-related duration of action. At doses of ≥2.5 µg/guinea pig, GSK573179A, caused inhibition ≥50% for more than 48 hr. The duration of inhibition for GSK573719A and tiotropium was comparable.

Safety Pharmacology: Effects of GSK573719 on CNS, cardiovascular, and respiratory functions were assessed. Effects of GSK573719 on neurobehavior effects, body temperature, and motor activity were assessed in male rats following single doses of 36, 322, or 1994 µg/kg administered by nose only inhalation. Moderately dilated pupil was observed for rats at 322 and 1994 µg/kg, which is an expected pharmacological effect, but no other changes in CNS were observed. GSK573719A inhibited the hERG tail current in a concentration-dependent manner with a nominal IC50 value of 9.41 µM. Effects of single intravenous doses of GSK573719A at 0.3, 3, and 10 µg/kg on arterial pressures, heart rate, and ECG parameters were evaluated in dogs. At 10 µg/kg GSK573719A, there was an increase in heart rate with a maximum change of 49 bpm, from predose average of 65 bpm, which returned towards the predose average approximately 30 min after infusion. In agreement with increased heart rate, RR interval decreased to 0.545 sec from a predose average of 0.974 sec following infusion of 10 µg/kg GSK573719A. RR interval returned to approximate predose values by 30 min after the end of dosing. The changes in RR interval are associated with the tachycardia seen following dosing with GSK573719A at 10 µg/kg. Following dosing with GSK573719A at 10 µg/kg, incidences of second degree atrioventricular (AV) block were observed in 3 of 4 animals. Observed effects of increased heart rate and corresponding decreased RR interval as well as AV block can be attributed to the antimuscarinic effects of GSK573719. Effects of GSK573719 on respiratory function were assessed following single inhaled doses to rats of 36, 215, or 2660 µg/kg by nose only inhalation. Single achieved inhaled doses of 215 or 2260 µg/kg GSK573719 produced increases in respiratory rate (18 to 45%) and decreases in tidal volume (3 to 17%).

ADME: The ADME of GSK573719 was examined in rats, dogs, and humans. Half-lives of GSK573719 in rats and dogs following intravenous administration were 1.42 and 11.6 hr, respectively. The terminal elimination half-lives (t½) of GSK573719 in humans calculated following 14-day repeat dose inhalation administration of 750 and 1000

µg/day were estimated to be 26.3 and 27.7 hr, respectively. GSK573719 was a substrate for p-glycoprotein.

Plasma protein binding in mouse, rat, rabbit, dog, and human ranged from 74.7 to 88.8% and was concentration-independent. Red blood cell associations of GSK573719 in dog, human, rabbit, rat, and mouse were generally low. Studies in rats and dogs suggested extensive distribution into tissues and rapid clearance suggestive of a metabolic clearance. GSK573719 was a substrate for p-glycoprotein. Oral bioavailability of GSK573719 was minimal in both rats and dogs. Following intravenous administration in rats, the highest levels of radioactivity were present in the kidney cortex, kidney medulla, liver, pituitary gland, small intestine wall, thyroid, and pancreas.

In vitro metabolism of GSK573719 was examined with hepatocytes from mouse, rat, rabbit, dog, and human. Studies with human hepatocytes and microsomes found that the main routes of metabolism of GSK573719 were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The major routes of metabolism in humans were also present in rat and dog. O-dealkylation (as observed in human) was a major pathway in the mouse, but only a minor pathway in the rabbit. Hydroxylation (at the position observed in M33 in human) was also a major pathway in the mouse (M21) but a minor pathway in rabbit hepatocytes (M22). Metabolism of GSK573719 in human liver microsomes was mediated primarily by CYP2D6, with some contribution from CYP3A4. GSK573719 is also metabolized by the CYP1A1, which is known to be expressed extrahepatically. GSK573719A was a marked direct inhibitor of CYP2D6 activity (IC50 = 0.1 µM) and CYP3A4 activities (IC50 = 1.0 µM for DEF and 8.0 for 7BQ).

Following repeat inhalation of GSK573719A at a dose of 1 mg/day for 7 days to healthy male subjects and to a healthy male population of CYP2D6 poor metabolizers, GSK573719 and GSK339067 (M14, product of O-dealkylation) were the only GSK573719-related components detected in 5 min, 1 hr, and 0-24 hr pooled human plasma extracts and in 0-4 hr pooled human urine. The proportion of GSK339067 (relative to the parent drug, GSK573719) in CYP2D6 poor metabolizers compared to normal metabolizers was similar in the 5 min pooled plasma samples, but decreased over 3-fold in the 1 hr pooled plasma samples. In the 0-24 hr pooled plasma samples, the proportion of GSK339067 was approximately 18% in 2D6 poor metabolizers and 35% in healthy subjects. In 0-4 hr pooled urine samples, the proportion of GSK339067 relative to the parent drug, GSK573719, was comparable in both populations at 6 to 7%. CYP2D6 appears to play a significant role in the metabolism of GSK573719 in humans.

Following intravenous administration of 1 mg/kg GSK573719 to rats, 65.3 and 16.9% of the radioactive dose were found in the feces and urine, respectively. Following intravenous administration of GSK573719 to dogs, the major route of elimination of drug-related material was by the feces (61.8% of the dose). Urinary excretion accounted for 11.9% of the dose. The primary route of elimination of GSK573719 in dogs was biliary excretion.

Supporting Toxicology Studies: In support of the proposed clinical trial, the sponsor conducted inhalation toxicology studies in mice, rats, and dogs with duration up to 13 weeks, a standard battery of genetic toxicology tests, a fertility and reproductive performance study in rats, and embryofetal development studies in rats and rabbits.

Inhalation Toxicology Studies: The sponsor has conducted inhalation toxicology studies with GSK573719A administered to mice, rats, and dogs for exposure periods up to 13 weeks. Early toxicology studies up to 4 weeks in duration were conducted using excipients, lactose and [redacted] (b) (4) However, safety concerns of alveolar macrophage accumulation were identified regarding the use of [redacted] (b) (4) (See IND [redacted] (b) (4) Type A Meeting Minutes dated September 28, 2005). The sponsor discontinued the use of [redacted] (b) (4) It is noted that magnesium stearate is an approved excipient for use by the inhalation route (e.g., Foradil® Certihaler™). Bridging studies with duration of 14 days were conducted in rats and dogs to compare the toxicity profile and toxicokinetics of GSK573719 formulated with [redacted] (b) (4) or magnesium stearate. Thirteen (13)-week inhalation toxicology studies with GSK573719A administered to mice, rats, and dogs were conducted using excipients, lactose and magnesium stearate. The sponsor's studies with dogs were confounded by several problems that are discussed below.

14-Day bridging inhalation toxicology studies with rats and dogs to compare the toxicity profile and toxicokinetics of GSK573719 formulated in [redacted] (b) (4) or magnesium stearate: These 14-day inhalation bridging toxicology studies in rats and dogs found no significant differences in toxicity profile and toxicokinetics of GSK573719 formulated in [redacted] (b) (4) or magnesium stearate. There were some differences compared to lactose alone.

The objectives of this 14-day nose-only inhalation bridging toxicology study (Study no.: WD2006/03225/00) with rats were to identify potential differences of the toxicity profile and toxicokinetics of GSK573719A caused by excipients, [redacted] (b) (4) and magnesium stearate and to support the use of magnesium stearate in a clinical formulation of GSK573719. A comparator group received GSK573719A without either of these excipients (i.e., lactose only). The target dose of GSK573719A was 1000 µg/kg/day. Histopathological findings were evident in the tracheal bifurcation, trachea, lung, larynx, nasal cavity/sinuses, nasopharynx, and liver. Histopathological findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to the clinical setting of oral inhalation. Findings in the larynx were judged to be rodent-specific, nonspecific effects of inhalation administration and not relevant to humans. At the tracheal bifurcation, incidences of degeneration/regeneration of the epithelium were increased for males and females that received GSK573719 formulated in lactose/ [redacted] (b) (4) (G3) or lactose/magnesium stearate (G4). The incidence was also slightly increased for females that received GSK573719 formulated in lactose only (G5). In the trachea, degeneration/regeneration of the epithelium was evident for 1 of 10 females that received GSK573719 formulated in lactose/magnesium stearate (G4). In the lung, incidences of hemorrhage were increased for males and females that received GSK573719 formulated in lactose/magnesium stearate (G4). Exposures (AUC and C_{max}) to GSK573719 were >2-fold higher for males and females that received

GSK573719 formulated in lactose/ (b) (4) or lactose/magnesium stearate as compared to lactose only. The toxicity profile and toxicokinetics of GSK573719 formulated in magnesium stearate or (b) (4) were relatively comparable in rats.

The objectives of this 14-day inhalation bridging toxicology study (Study no.: WD2006/03669/00) with dogs were to identify potential differences of the toxicity profile and toxicokinetics of GSK573719A caused by excipients, (b) (4) and magnesium stearate and to support the use of magnesium stearate in a clinical formulation of GSK573719. A comparator group received GSK573719A without either of these excipients (i.e., lactose only). The target dose of GSK573719A was 1000 µg/kg/day. Femoral pulse rates were generally increased for male and female treatment groups on days 1, 2, and 14 at 10 min to 24 hr postdose. On day 12, at predose, no dogs receiving GSK573719A (Groups 3-5) displayed respiratory sinus arrhythmia (RSA) and heart rates were higher compared to the pretreatment period. Immediately postdose, increased heart rates and no RSA were observed in all dogs from Groups 3-5. Cardiac troponin I concentrations were increased for all male treatment groups on day 1 at 3, 7, and 24 hr postdose. Cardiac troponin I concentrations were increased for females in Groups 4 and 5 at 3, 7, and 24 hr postdose. No increases of cardiac troponin I concentrations were evident on day 14. There were no histopathological findings in the heart suggestive of injury. Histopathological findings were evident in the gallbladder, lungs, trachea, tracheal bifurcation, trachea, and nose/turbinate. The finding in the nose/turbinate was judged to be irrelevant to the clinical setting with oral inhalation of the test article. Findings in the gallbladder consisted of foci of minimal to slight myofiber degeneration/regeneration associated with variable hemorrhage and inflammation present in the tunica muscularis of animals dosed with all GSK573719 formulations. Findings for lungs included alveolar inflammation for 1 female in GSK573719/lactose group, an increased incidence of cellular debris and mucus in the bronchiole lumen females in the GSK573719 (b) (4) /lactose group, and a microgranuloma for one female in the GSK573719A/lactose group. Goblet cell hyperplasia in the tracheal bifurcation was observed for 1 female in the GSK573719/ (b) (4) lactose group. Lymphoid follicular aggregates and inflammatory cells/cell debris were observed in the trachea for 1 female in the GSK573719/lactose group. The toxicity profile and toxicokinetics were generally comparable for GSK573719 formulated in (b) (4) or magnesium stearate. Histopathological findings in the gallbladder appeared to be associated with relatively high systemic exposures to the test article (i.e., AUC values on day 14 ranged from 52.732 to 102.689 ng·hr/mL) and were not observed in 4- and 13-week inhalation toxicology studies with dogs discussed below.

13-Week inhalation toxicology study with mice: In a 13-week inhalation toxicology study (WD2007/01600/00; submitted in support of dose selection for a 2-year carcinogenicity study), mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day (see Review #01 dated June 3, 2009). Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Additional findings were evident in the liver and uterus. Histopathological findings were observed in the

larynx at all doses, nasal turbinates at doses ≥0.287 mg/kg/day, and nasopharynx at doses of 1.06 and 2.85 mg/kg/day; however, findings in the larynx were judged to be nonspecific irritant effects with no relevance to humans and findings in the nasal turbinates and nasopharynx were judged to be not relevant to oral inhalation used in a clinical setting. Histopathological findings in the tracheal bifurcation (epithelial hyperplasia and loss of cilia), liver (focal inflammation), and uterus (luminal dilatation) were observed at 2.85 mg/kg/day. These findings in the liver and uterus were of questionable relationship to treatment. With respect to administration in a clinical setting, a NOAEL could be identified at 1.06 mg/kg/day based upon histopathological findings in the tracheal bifurcation, liver, and uterus at 2.85 mg/kg/day. AUC values in males and females associated with the NOAEL were 29.5 and 42.5 ng·hr/mL, respectively.

13-Week inhalation toxicology study with rats: In a 13-week inhalation toxicology study (WD2007/02012/00; submitted in support of dose selection for a 2-year carcinogenicity study), rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day (see Review #01 dated June 3, 2009). Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. Histopathological findings were observed for the larynx at doses ≥38 µg/kg/day, nasal cavity/sinuses at doses ≥102 µg/kg/day, and nasopharynx for a single rat at 288 µg/kg/day. Histopathological findings in the larynx were judged to be nonspecific irritant effects. Histopathological findings in the nasal cavity/sinuses and nasopharynx were judged to be not relevant to oral inhalation used in a clinical setting. The NOAEL with respect to clinical use was judged to be 924 µg/kg/day. AUC values in males and females associated with the NOAEL were 22.6 and 36.2 ng·hr/mL, respectively.

4- and 13-Week Inhalation toxicology studies with dogs: The sponsor's inhalation toxicology studies with GSK573719 administered to dogs were confounded by a series of problems. The sponsor's initial 4-week inhalation toxicology study with dogs (WD2005/01423/00) was complicated by findings of granulomatous inflammation in the lung that were attributed to inhalation of plant material from the diet. The sponsor conducted a second 4-week inhalation toxicology study with dogs (WD2006/03294/00) to identify a feeding regimen that reduced the possibility of inhalation of particulate matter from the diet. The sponsor made modifications to method for delivery of GSK573719 to dogs in the 13-week inhalation toxicology study (WD2007/01512/00) that inadvertently resulted in significant reductions of delivered inhalation doses as compared to earlier studies. The sponsor conducted a 4-week inhalation toxicology study with dogs (WD2008/01504/00) to determine the source of the problem for delivery of GSK573719 in the 13-week study as well as to identify the best method for delivery of GSK573719 to dogs.

In a 28-day inhalation toxicology study with dogs (WD2005/01423/00), GSK573719 was administered by face mask fitted with an oropharyngeal tube at estimated achieved doses of 0, 16.2, 208.0, and 2757.6 µg/kg/day (pulmonary deposited doses were 4.0,

52, and 689.4 µg/kg/day, respectively). Heart rates were increased up to 1.7-fold for male and female dogs in the 208.0 and 2757.6 µg/kg/day groups during the treatment period. Histopathological findings were observed in the lung, thymus, nasal cavity/sinuses, larynx, and trachea. Findings in the nasal cavity/sinuses were judged to be irrelevant to clinical administration by oral inhalation. In the lung, granulomatous inflammation was observed for all male treatment groups and females in the high dose group; the incidence and severity did not display dose-response relationships. Slight to moderate multifocal acute and/or subacute bronchoalveolar inflammation was observed for 1 male at 16.2 µg/kg/day and 1 male and 2 females 2757.6 µg/kg/day. In the thymus, lymphoid atrophy was observed for the control and all treatment groups. An increased severity was noted for males at 208.0 µg/kg/day and males and females at 2757.6 µg/kg/day; however, the incidence in the control group was high and the relationship of these findings to treatment was considered questionable. In the larynx, minimal to moderate acute/subacute inflammation was observed for males and females at 2757.6 µg/kg/day. Mineralization and exudate were also evident for females at 2757.6 µg/kg/day. In the trachea, slight to moderate epithelial degeneration/regeneration and minimal acute/subacute inflammation were observed for females at 2757.6 µg/kg/day. Granulomatous inflammation in the lung was judged to unrelated to the test article (see 28-day follow-up study; WD2006/03294/00). The NOAEL was identified as 208 µg/kg/day based upon histopathological findings in the lung, larynx, and trachea for the high dose group. Estimated AUC values for males and females in the 208 µg/kg/day group on day 28 were 25.9 and 29.9 ng·hr/mL, respectively (determined by extrapolation from the high dose group).

The objective of this 28-day inhalation toxicology study with dogs (WD2006/03294/00) was to investigate the effect of a modification to feeding methods, designed to reduce the possibility of inhalation of particulate matter from the diet, on the formation of granulomas in the lung seen in a previous 28-day study (WD2005/01423/00). Foreign material compatible with plant material found in the diet was identified within granulomas. Group 1, 2, 3, and 4 received GSK573719 at estimated achieve doses of 0, 22, 2254, and 1835 µg/kg/day, respectively. The feeding regimen for dogs in Groups 1, 2, and 3 was modified to provide pelleted diet mixed with water 3 hr after completion of dosing for a period of 2 hr (identified as new feeding regimen, NFR). Dogs in Group 4 were given dry pelleted diet prior to dosing for approximately 6 hr (commencing in the morning), and removed generally during dosing or up to 40 min following the completion of dosing (identified as old feeding regimen (OFR) used in the previous study). Dry eyes/decreased tear production, attributed to the pharmacological action of GSK573719, was evident in all treatment groups. Ophthalmic findings, attributed to dry eyes/decreased tear production, were evident for 1 dog in each of the high dose/NFR and high dose/OFR groups. Increased heart rates (up to 1.6-fold) were evident for male and female dogs in the high dose/NFR and high dose/OFR groups. Increases were more pronounced on day 1 as compared to days 25 or 27. Histopathological examinations of tissues were limited to gross lesions, bronchi, larynx, lungs, tracheobronchial LN, nasal cavity, pharynx, trachea, and tracheal bifurcation. Findings were observed in the lung, larynx, trachea, tracheal bifurcation, bronchus, and nasal cavity/sinuses. Findings in the nasal cavity/sinuses were judged to be not relevant to the

clinical setting with oral inhalation of the drug product. Microscopic findings considered to be related to the old feeding regimen (OFR) were seen in the lungs. Dogs given the high dose/OFR (Group 4) had minimal to slight granulomatous inflammation in the lungs (1/3 males and 2/3 females), which was not seen in dogs given the high dose/new feeding regimen (Group 3). The male dog with granulomatous inflammation in the lungs also had suppurative bronchoalveolar inflammation and both of these inflammatory foci contained foreign material that was consistent in appearance with food/plant material. Increased incidences of interstitial inflammation were observed for all male treatment groups. An increased incidence of accumulation of foamy alveolar macrophages was evident for male dogs given the high dose/OFR (Group 4). For the larynx, acute inflammation and degeneration/necrosis of the epithelium was observed for all male treatment groups and females in the high dose/NFR (Group 3) and high dose/old feeding regimen (Group 4) groups. Exudate was also evident for all male treatment groups and females in the high dose/NFR (Group 3) and high dose/OFR (Group 4) groups. For the trachea, acute inflammation was observed for all male treatment groups and females in the low dose/NFR and high dose/NFR groups. Degeneration/regeneration of the epithelium was observed for male and female dogs in the high dose/NFR (Group 3) and high dose/OFR (Group 4) groups. Single cell necrosis was observed for 1 male in the high dose/NFR (Group 3) group. Exudate in the lumen and epithelial necrosis were each observed for 1 male in the high dose/OFR (Group 4) and 1 female in the high dose/NFR (Group 3) group. For the tracheal bifurcation, acute inflammation and degeneration/regeneration of the epithelium were each observed for one male and one female in the high dose/NFR (Group 3) group. For the bronchus, acute inflammation and degeneration/regeneration of the epithelium were observed for males in the low dose and high dose/NFR (Groups 2 and 3) groups and females in the high dose/NFR (Group 3) group. Results of this study suggest that granulomatous inflammation observed in the previous 28-day toxicology study (WD2005/01423/00) was associated with aspiration of feed and not a direct test article effect. The pharmacological action of the test material on mucous secretion in the respiratory tract and upper digestive system (dryness/lack of normal secretions) could have played a major role in this accidental inhalation of the exogenous material. A NOAEL was not established in the present study based upon histopathological findings in the bronchus, trachea, larynx, and lung. Many of the histopathological findings (e.g., accumulation of foamy macrophages in the lung and acute inflammation and degeneration/regeneration of the epithelium in the trachea and bronchus) were not consistent with the other two 28-day inhalation toxicology studies. The new feeding regimen appeared to minimize the potential for inhalation of particulate matter from the diet.

In a 13-week inhalation toxicology study (WD2007/01512/00), dogs received GSK573719 at estimated achieved doses of 0, 40.7, 187, and 1070 µg/kg/day. The control group was exposed to lactose containing magnesium stearate. In Week 13, at predose, 3/6 males and 4/6 females given 1070 µg/kg/day showed no respiratory sinus arrhythmia (RSA) (expressed by decreased RR interval variability) and all dogs given 1070 µg/kg/day had increased heart rates (up to 1.8X pretest individual values). Immediately following a dose of 1070 µg/kg/day, no RSA was noted in 4/6 males and 4/6 females, which was associated with further increases in heart rates (up to 2.1X

predose individual values) in 2/6 males and 3/6 females. The changes in most animals were returned to pre-test values, but RSA was not observed in females during the recovery phase. On Day 1, a female given 1070 µg/kg/day had elevated serum cTnI concentrations at 3 hr (0.171µg/L), 7 hr (0.189 µg/L), and 24 hr (0.082 µg/L). By Week 13 and at the end of the recovery period, the serum cTnI concentrations were comparable to pre-dose and concurrent controls. Additionally on Day 1, a male given 1070 µg/kg/day showed minimal to mildly higher serum cTnI concentrations at 3 hr (0.044 µg/L) and 7 hr (0.101 µg/L) when compared to pre-dose and concurrent control values. By 24 hr the serum cTnI concentration was comparable to the pre-dose value. By 24 hr the serum cTnI concentration was comparable to the pre-dose value. Increased troponin I concentrations suggest the possibility of cardiac injury although histopathological examinations of hearts found no evidence of damage. No cardiac damage was evident in shorter term studies. Observed histopathological findings appeared to be background/spontaneous and unrelated to treatment. Systemic exposure to GSK573719 measured in dogs from this study was lower than that seen in dogs in the two 4-week studies when given by oropharyngeal tube inhalation exposure. It is considered possible that the lower systemic exposures seen in this study related to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in this study. The modifications were intended to improve the stability of the concentration of GSK573719 in aerosols generated in the dosing system. The histopathological results found in the present study may not accurately reflect the local toxicity of GSK573173. The NOAEL was identified as the high dose of 1070 µg/kg/day based upon lack of treatment-related histopathological finding; however, this absolute dose might not be comparable to other studies due to problems with drug delivery. Cardiac effects appear to be monitorable in a clinical setting. Systemic exposures for males and females in the high dose group during week 13 were 30.6 and 28.6 ng·hr/mL, respectively.

The sponsor conducted a study (WD2008/01504/00) designed to identify an optimal inhalation exposure system and characterize the toxicity of GSK573719 administered to dogs using this system for 4 weeks to aid in dose selection for the future 9-month inhalation toxicology study with dogs. Systemic exposure to GSK573719 seen in an earlier 13 week inhalation toxicity study in dogs (WD2007/01512/00) was lower than that seen in the dog in previous 4-week studies when given by oropharyngeal tube inhalation exposure (WD2006/03228/00 and WD2006/03669/00). It is considered possible that the lower systemic exposures seen in the 13 week study relate to modifications made to the air flow balancing system of the oropharyngeal tube dosing system used in that study. An exposure system equipped with the passive scavenger was selected for use. Dogs were exposed to GSK573719 at 1130 and 2390 µg/kg/day using this system for 4 weeks; however, the 1130 µg/kg/day group was only composed of 3 male dogs. A concurrent control group was not included. On day 1, there were dose-related increases of pulse rates (up to 2.3-fold). Increases were also evident on day 7 and during week 4 although not as pronounced as day 1. Histopathological examinations of tissues were limited to gross lesions, gallbladder, larynx, lungs, nasal turbinates, trachea, tracheal bifurcation, and tracheobronchial LN. Microscopic changes associated with administration of GSK573719 were present in the lungs, trachea,

tracheal bifurcation and larynx at 1130 and/or 2390 µg/kg/day. A NOAEL was not identified based upon histopathological findings in the lung, trachea, tracheal bifurcation, and larynx.

Safety margins for the highest proposed clinical dose of 1000 µg/day are shown in the table below. Given that observed toxicities in the 13-week inhalation toxicology study with mice and rats and 4-week inhalation toxicology study with dogs were confined to the respiratory tract, NOAELs were re-expressed on a µg/g lung weight/day basis. Safety margins on a µg/g lung weight/day basis were >10 for rats and approximately 5 to 6 for dogs, which were judged to be acceptable. Systemic exposures at the NOAELs of the 13-week toxicology studies with mice, rats, and dogs and 4-week toxicology study with dogs provide safety margins >1, which are acceptable. In the 14-day bridging inhalation toxicology study with dogs, histopathological findings were evident in the gallbladder that appeared to be associated with relatively high systemic exposures to the test article (i.e., AUC values on day 14 ranged from 52.732 to 102.689 ng·hr/mL) and were not observed in 4- and 13-week inhalation toxicology studies with dogs.

Safety margins for the highest proposed clinical dose of 1000 µg/day

Species/Study	NOAEL			Safety margins for a clinical dose of 1000 µg/day		
	µg/kg/day (Deposited)	µg/g LW/day	AUC ng·hr/mL	20 µg/kg/day	1 µg/g LW/day	AUC = 8.64 ng·hr/mL[a]
Mouse, 13 weeks (WD2007/01600/00)	100	16	29.5/42.5	5	16	3.4/4.9
Rat, 13 weeks (WD2007/02012/00)	92.4	23.6	22.6/36.2	4.6	23.6	2.6/4.2
Dog, 4 weeks (WD2005/01423/00)	52	5.12/6.47	25.9[b]/29.9[b]	2.6	5.12/6.47	3.0/3.5
Dog, 13 weeks (WD2007/01512/00)	268[c]	-	30.6/28.6	-	-	3.5/3.3

a. The steady-state AUC_T achieved following an inhaled dose of 1000 µg/day was 8.64 ng·hr/mL (estimated based on day 14 in healthy subjects).
b. Estimated AUC value from the high dose in the study.
c. Not comparable to other studies due to lower than expected delivery of the test article by inhalation.

Genetic Toxicology: GSK573719A was negative in the in vitro bacterial reverse mutation assay, in vitro mouse lymphoma assay, and in vivo rat micronucleus assay.

Reproductive Toxicology Studies: The sponsor conducted reproductive toxicology studies with GSK573719 consisting of a fertility and reproductive performance study in rats and embryofetal development studies in rats and rabbits. In a female fertility and early embryonic development study, rats received estimated achieved inhalation doses

of 3.37, 29.1, 100, and 294 µg/kg/day. Body weight gains were unaffected. There were no effects on fertility or reproductive performance. In an embryofetal development study, rats received estimated achieved inhalation doses of 0, 0.0317, 0.0969, and 0.278 mg/kg/day. Body weight gain and food consumption were unaffected. Some potentially treatment-related minor skeletal and visceral abnormalities were observed. In an embryofetal development study, rabbits received estimated achieved inhalation doses of 0, 0.0285, 0.0889, and 0.306 mg/kg/day. Body weight gain and food consumption was decreased at all doses. Overall fetal body weights were decreased. There were some potentially treatment-related minor visceral and skeletal abnormalities. GSK573719 did not appear to be teratogenic in rats and rabbits. The sponsor is conducting a second embryofetal development study with GSK573719 in rabbits using the intravenous route of administration (see Review of Amendment #030 dated May 10, 2010).

Use of the Excipient, Magnesium Stearate: Magnesium stearate is being used as an excipient in the drug product. The sponsor initially used [redacted] (b) (4) as an excipient; however, issues of macrophage accumulation and pulmonary inflammation in inhalation toxicology studies with rats led to the discontinuation of its use. The maximum clinical dose of magnesium stearate in the proposed clinical trial is 150 µg/day (3 µg/kg/day for a 50 kg subject). The approved dose of magnesium stearate in the approved Foradil® Certihaler™ product is 56 µg/day. Pulmonary deposited doses of magnesium stearate in vehicle-control groups from the 13-week inhalation toxicology studies with mice, rats, and dogs were 6.9, 2.3, and 7.1 µg/kg/day, respectively, which were not associated with adverse effects. Further, magnesium stearate does not appear to alter the toxicity or toxicokinetic of GSK573719. However, these pulmonary deposited doses do not provide adequate safety margins for the maximum clinical dose of magnesium stearate. The sponsor has rights of reference to a 6-month inhalation toxicology study with magnesium stearate in rats. The NOAEL (pulmonary deposited dose) was identified as 180 µg/kg/day, which supports a maximum clinical dose of 18 µg/kg/day.

Genotoxic Impurity: [redacted] (b) (4) The chemist, Dr. Craig Bertha, indicated that this compound possesses [redacted] (b) (4) This was a theoretical impurity. Its presence in the drug substance has not been confirmed. Thus, levels in the drug substance, if any, were not known. An evaluation of this compound by the Computational Toxicology Service was requested. The computational toxicology evaluation of this compound predicted that it possessed a high genotoxic potential based upon results of MC4PC and MDL-QSAR analyses. A published Ames test with this compound was reported to be negative [redacted] (b) (4) however, this result was judged to be questionable based upon the structure of the compound (i.e., [redacted] (b) (4)) and the results of the computational toxicology analysis. Levels of [redacted] (b) (4) should be controlled as described in Draft Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (December

Reference ID: 3330354

2008). The sponsor is monitoring levels of this impurity (Amendment #014 dated September 17, 2009).

Communication to sponsor: The following comment was conveyed to the sponsor in a facsimile dated August 24, 2009.

Non-Clinical

1. [redacted] (b) (4) [redacted] (b) (4) A computational toxicology evaluation of this compound predicted a high genotoxic potential based upon results of MC4PC and MDLQSAR analyses. The negative Ames test result with this compound as reported by [redacted] (b) (4) [redacted] (b) (4) was judged to be questionable based upon the structure of the compound [redacted] (b) (4) and results of the computational toxicology evaluation. Levels of [redacted] (b) (4) should be controlled as described in *"Draft Guidance for Industry- Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (December 2008)."*

In a submission dated September 17, 2009, the sponsor agreed to this comment.

Internal Comments:
An assessment of stage-dependent spermatogenesis was conducted in the 4-week inhalation toxicology study with rats (WD2005/01422/00) and there were no treatment-related effects (Confirmed in Document #010 dated July 15, 2009).

In review of the 13-week inhalation toxicology study with mice (WD2007/01600/00), it was noted that incidences of slight or moderate retinal folds were increased for male and female treatment groups as compared to the concurrent control. The sponsor provided information as follows: "The toxicology study report states that "retinal folds were reported on some animals in both sexes dosed at 0.287, 1.06 and 2.85 mg/kg/day. These are, however, considered to be developmental abnormalities and an incidental finding in younger mice." (Report WD2007/01600/00). In the individual animal data (Appendix 1), the examining ophthalmologist recorded synonymous use of the terms retinal folds and retinal rosettes. The literature supports the interpretation of retinal folds and rosettes as a manifestation of retinal dysplasia, a developmental defect (Noden, 1985; Cook, 2007; Grahn, 2007). This information appears to resolve this question.

Recommendation: The proposed clinical trial appears reasonably safe and should be allowed to proceed.

External Comment: None

Reviewer signature: _____

Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Molly E. Shea, Ph.D.

cc: list:
IND104,479, HFD-570
RaggioM, HFD-570
SheaM, HFD-570
RobisonT, HFD-570

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-104479	ORIG-1	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	GSK573719 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.

/s/

TIMOTHY W ROBISON
05/25/2010

MOLLY E SHEA
05/25/2010

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:	104,479
Supporting document/s:	#058
Sponsor's letter date:	February 14, 2011
CDER stamp date:	February 14, 2011
Product:	GSK573719 (Muscarinic receptor antagonist)
Indication:	COPD
Sponsor:	GlaxoSmithKline P.O. Box 13398 Five Moore Drive Research Triangle Park North Carolina 27709-3398
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Timothy W. Robison, Ph.D., D.A.B.T.
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

GSK573719, a M3 muscarinic antagonist, is under development alone (IND 104,479) and in combination (IND 106,616) with GW642444, a β_2 adrenergic agonist, for the treatment of COPD. Two-year rat and mouse carcinogenicity studies with GSK573719 are currently ongoing. In the present submission, the sponsor has requested to lower doses used in these ongoing carcinogenicity studies based upon observed decreases of body weight gains.

1.2 Brief Discussion of Nonclinical Findings

The sponsor has conducted chronic toxicology studies with rats up to 6 months and dogs up to 9 months.

Two year carcinogenicity studies with GSK573719 in mice and rats are in progress.

1.3 Recommendations

Internal comments: In the present submission, the sponsor has requested to lower doses used in these ongoing carcinogenicity studies based upon observed decreases of body weight gains.

<u>2-year carcinogenicity study with rats</u>: The sponsor has proposed dose reduction for male and female treatment groups from target doses of 30, 100 and 300µg/kg/day to target doses of 15, 50 and 150 µg/kg/day, respectively.

Treatment of rats with GSK573719 at doses up to 300 µg/kg/day for 70 weeks had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains. The reduced doses would still provide large multiples over systemic exposure achieved with the MRHD. The ECAC, in emails dated February 18, 2010, agreed with the sponsor's proposed dose reduction for the 2-year carcinogenicity study with rats.

<u>2-year carcinogenicity study with mice</u>: The sponsor has proposed dose reduction for male treatment groups from target doses of 60, 200, and 600 µg/kg/day to target doses of 30, 100, and 300 µg/kg/day, respectively.

Treatment of mice with GSK573719 at doses up to 600 µg/kg/day in males and 200 µg/kg/day in females up to week 70 had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains for male treatment groups. The reduced doses would still provide large multiples over systemic exposure achieved with the MRHD. The ECAC, in emails dated February 18, 2010, agreed with the sponsor's proposed dose reduction for males in the 2-year carcinogenicity study with mice.

External comments: The sponsor's proposed dose reductions for male and female rats and male mice in ongoing 2-year carcinogenicity studies are acceptable based upon ECAC concurrence. Miranda Raggio communicated this information to the sponsor by telephone.

2 Drug Information

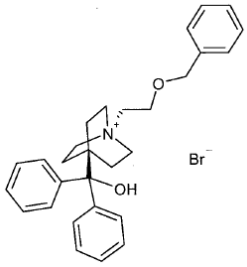
2.1 Drug

Code name: GSK573719

Chemical name: 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide

Molecular formula/molecular weight: $C_{29}H_{34}NO_2 \cdot Br$
508.5 (GSK573719A as quaternary ammonium bromide compound)
428.6 (as free cation, GSK573719)

Structure:



Pharmacologic class: GSK573719 is a quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist.

2.2 Relevant INDs, NDAs, and DMFs

IND 106,616 (GSK, GSK573719 + GW642444)

2.8 Regulatory Background

Dose selection for the 2-year carcinogenicity studies with GSK573719 in mice and rats were reviewed by the ECAC on June 3, 2009.

3 Studies Submitted

3.1 Studies Reviewed

The sponsor submitted a proposal to reduce doses for male and female rats and male mice in the 2-year carcinogenicity studies based upon observe decreased of body weight gain.

3.3 Previous Reviews Referenced

Pharmacology and Toxicology Review of IND 104,479 dated June 3, 2009
ECAC recommendations and conclusions for 2-year carcinogenicity studies with mice and rats dated June 3, 2011

11 Integrated Summary and Safety Evaluation

GSK573719, a M3 muscarinic antagonist, is under development alone (IND 104,479) and in combination (IND 106,616) with GW642444, a β_2 adrenergic agonist, for the treatment of COPD. Two-year rat and mouse carcinogenicity studies with GSK573719 are currently ongoing. In the present submission, the sponsor has requested to lower doses used in these ongoing carcinogenicity studies based upon observed decreases of body weight gains.

2-Year inhalation carcinogenicity study with rats

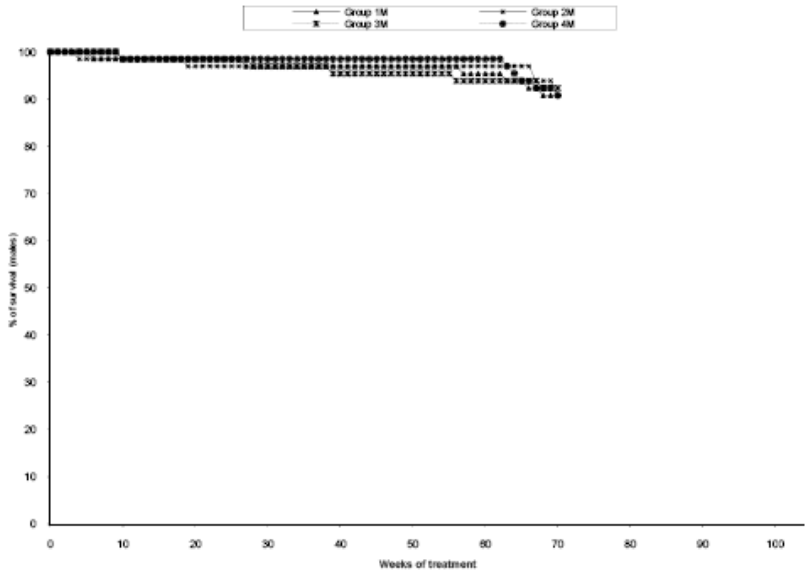
The target doses of GSK573719 for the carcinogenicity study with rats, as agreed with the ECAC in June 2009, are 30, 100 and 300 µg/kg/day. Dose selection was based on MTD (body weight effects). Dosing in this study has completed Week 70. Survival was unaffected for both male and female treatment groups.

For female treatment groups, there were non dose-related reductions of percent body weight gain compared to controls (21, 16 and 24% reduction in body weight gain for the low, mid, and high doses, respectively, compared to the concurrent control). This was accompanied by a minimal, dose related reduction of percent food consumption (range 7 10%). Absolute body weights for females in the low, mid, and high dose groups were decreased to approximately 88.5, 91, and 87.2% of the control, respectively.

In male treatment groups, there were small dose related reductions of percent body weight gain (5, 12 and 14% reduction in body weight gain for the low, mid, and high doses, respectively, compared to the concurrent control). This was accompanied by minimal, dose related reductions of food consumption (range 1 8%). Absolute body weights for males in the low, mid, and high dose groups were decreased to approximately 97, 92, and 89% of the control, respectively.

Figure 1 Survival in male and female rats through week 70

Graph 1: Current survival curve for male rats (main study rats, weeks 1-70)



Graph 2: Current survival curve for female rats (main study rats, weeks 1-70)

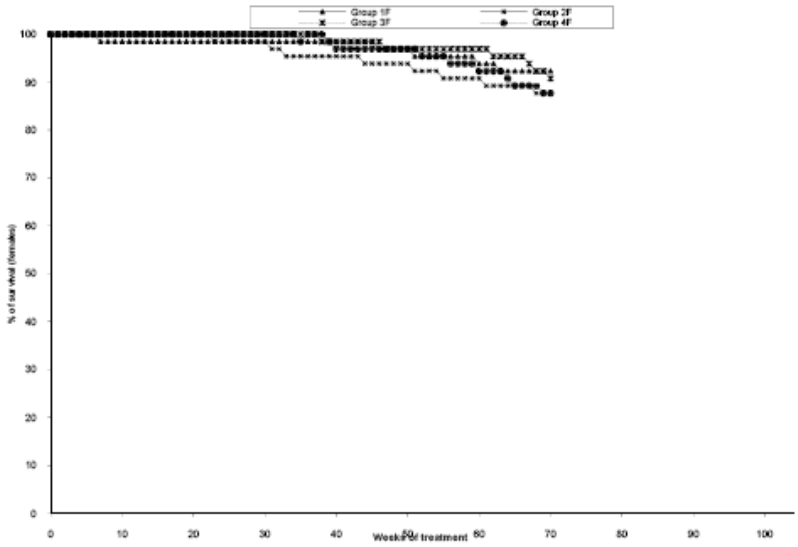
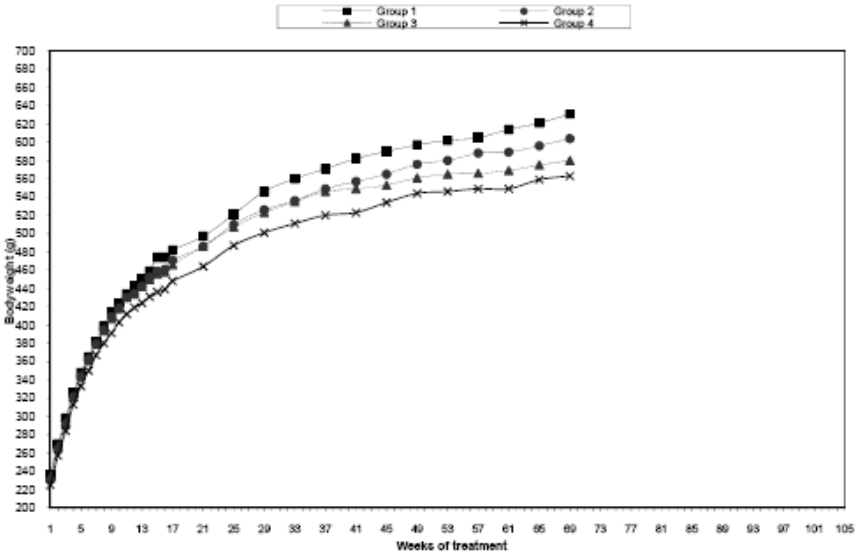


Figure 2 Body weights for male and female rats through week 68

Graph 3: Current bodyweight curve for male rats (main study rats. Weeks 1-68)



Graph 4: Current bodyweight curve for female rats (main study rats, weeks 1-68)

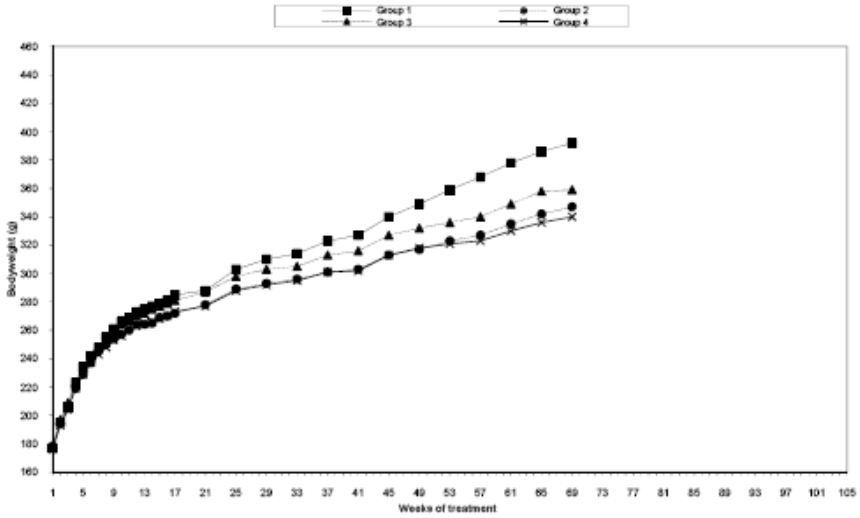


Table 1 Body weight gain, food consumption, and survival for male and female rats through week 70

Current Data on Body weight gain, food consumption and survival:

Group Number	MALE				FEMALE			
	1	2	3	4	1	2	3	4
Target dose (µg/kg/day) GSK573719	0	30	100	300	0	30	100	300
Mean body weight gain (g) Day 1 to Week 68	395	374	347	338	215	169	180	164
% of control	-	95	88	86	-	79	84	76
Mean food consumption at Week 68(g/animal/wk)	177	176	167	163	137	127	124	123
% of control	-	99	94	92	-	93	91	90
% survival at Week Number 70	91	91	92	91	92	88	91	88

Plasma C_{max} and AUC values for GSK573719 increased in an approximate dose proportional manner. There were no notable differences in systemic exposure to GSK573719 between the sexes within any of the dose groups and the data is consistent with previous data in the rat. Estimated systemic overage compared to the maximum recommended human dose (MRHD) of 125mcg is given below.

Table 2 Exposure multiples in male and female rats as compared to the MRHD

Estimated systemic overage compared to the MRHD of 125mcg.

Target Dose (µg/kg/day)	Estimated AUC exposure overage compared to the maximum clinical dose (125mcg)		Estimated Cmax exposure overage compared to the maximum clinical dose (125mcg)	
	Males	Females	Males	Females
30	NC	6	1.7	3.1
100	32	50	4.7	9.5
300	174	182	18.1	22.3
Exposure overages calculated using 1) human exposure data for 125µg/day based on repeat dose data (14 days) in COPD patients in study AC4113073 (Day 14 AUC_{0-t}- 0.07ng.h/mL and Cmax = 0.1ng/ml (geometric mean) and 2) Week 26 toxicokinetic exposure data from the ongoing carcinogenicity study (Table 2, Appendix 1)				

Sponsor's Proposal: The sponsor proposes to implement an immediate dose reduction in both male and female rats by reducing the exposure time on the inhalation chambers from 60 to 30 min. The sponsor considers this change the most efficient way to implement a dose reduction at this stage given the current stage of the study (week 71) and will result in a dose reduction from target doses of 30, 100 and 300µg/kg/day to target doses of 15, 50 and 150 µg/kg/day. This will allow immediate exploration of the sensitive part of the dose response curve based on multiple of the MHRD for the remainder of the study period. GSK573719 is expected to achieve approximately 3, 20 and 90 fold margins over human AUC at the MRHD for the proposed new target doses of 15, 50 and 150µg/kg/day, respectively. To implement any lower doses would require preparation and calibration of new blend strengths, which would take approximately 8 weeks to implement (i.e. to prepare, analyze the new blends and calibrate new aerosol concentrations on the inhalation chambers). Additional satellite animals will be introduced to the study to monitor the systemic exposure at the new target doses. Work is ongoing to validate a lower level of quantification at 20 pg/mL for GSK573719 in rat plasma to assist in better defining the plasma concentration profile of GSK573719 at lower inhaled doses (e.g. 15 µg/kg/day).

Evaluation: Treatment of rats with GSK573719 at doses up to 300 µg/kg/day up to week 70 had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains for male and female treatment groups. The reduced doses would still provide large multiples over systemic exposure

achieved with the MRHD. It is noted that the LOQ for the analytical method to measure GSK573719 might be inadequate to detect lower concentrations of GSK573719, possibly at ≤50 µg/kg/day. Exposure margins would decrease 2-fold with respect to local toxicity although this is not a typical parameter for dose selection in a carcinogenicity study and there is still a relatively large exposure margin at the high dose. The ECAC, in emails dated February 18, 2011, agreed with the sponsor's proposed dose reduction for male and female rats in the 2-year carcinogenicity study.

Table 3 Exposure margins for pulmonary deposited dose (Rat/Human)

Proposed doses µg/kg/day	PDD µg/kg/day	Exposure margins for clinical dose of 125 µg (2.1 µg/kg/day)
15	1.5	0.7
50	5	2.4
150	15	7.1

2-Year inhalation carcinogenicity study with GSK573719 in mice

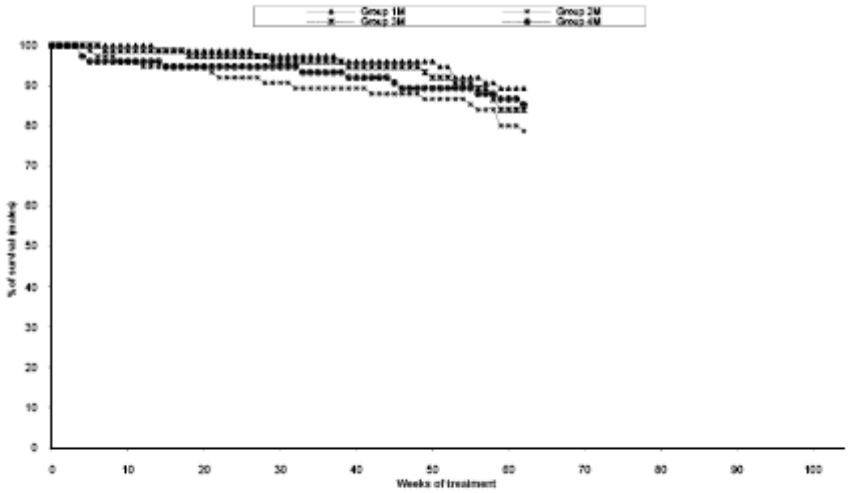
The target doses of GSK573719 for the carcinogenicity study with mice, as agreed with the ECAC in June 2009, are 60, 200 and 600µg/kg/day for males and 20, 60 and 200 µg/kg/day for females. Dose selection was based on MTD (effects in the larynx). Dosing in this study has completed Week 63. Survival appears to be >80% in all male and female treatment groups.

In males, there was a non dose related reduction in bodyweight gain compared to controls (8, 6, and 26% in the low, mid, and high doses, respectively, compared to the concurrent control). This was accompanied by a minimal reduction in food consumption (range 7 10%). Absolute body weight for males in the high dose was reduced to 90.5% of the control.

There was no effect on body weight gain in females at the low dose and only minor reductions at the mid and high doses (6 and 5%, respectively), accompanied by only minor reductions of food consumption at the mid and high doses (3%) compared to the concurrent control.

Figure 3 Survival for male and female mice through week 62

Graph 7: Current survival curve for male mice (main study mice, weeks 1-62)



Graph 8: Current survival curve for female mice (main study mice, weeks 1-62)

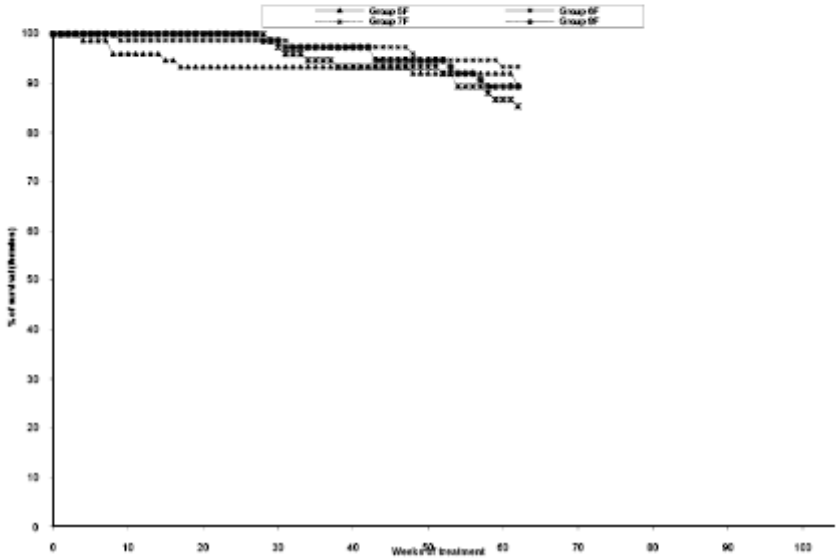
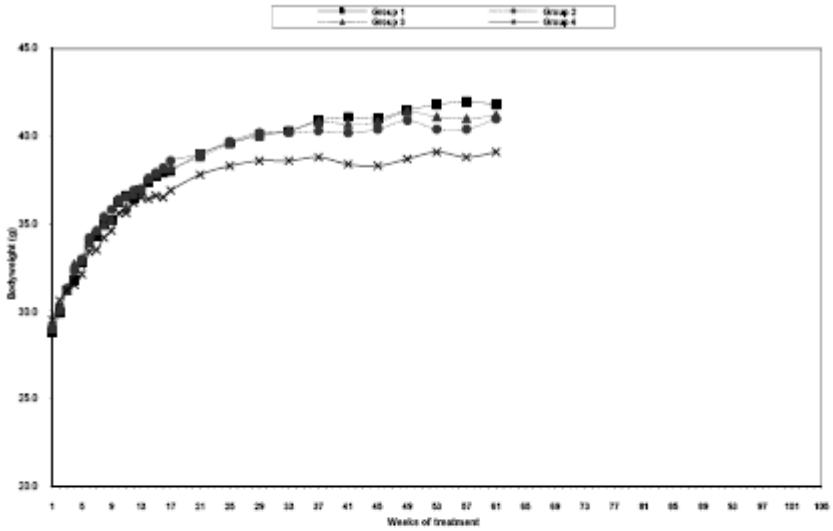


Figure 4 Body weights for male and female mice through week 60

Graph 9: Current bodyweight curve for male mice (main study mice, weeks 1-60)



Graph 10: Current bodyweight curve for female mice (main study mice, weeks 1-56)

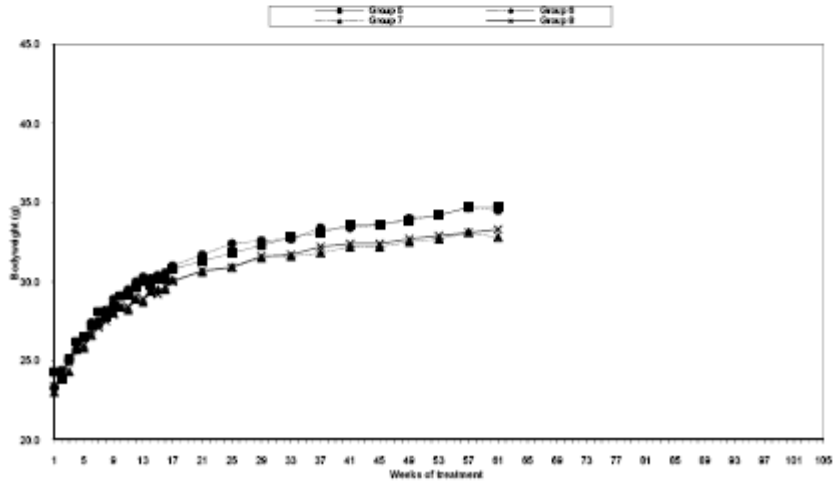


Table 4 Body weight gains, food consumption, and survival for male and female mice

Current Data on Body weight gain, food consumption and survival:

Group Number	MALE				FEMALE			
	1	2	3	4	5	6	7	8
Target dose (µg/kg/day) GSK573719	-	60	200	600	-	20	60	200
Mean body weight gain (g) Day 1 to Week 60	13.0	11.9	12.2	9.6	10.4	11.2	9.8	9.9
% of control	-	92	94	74	-	108	94	95
Mean food consumption at Week 60 (g/animal/wk)	41	38	40	37	35	37	34	34
% of control	-	93	98	90	-	106	97	97
% survival at start of Week 63	89	79	84	85	89	95	85	89

AUC and C_{max} values for GSK573719 generally increased in an approximate dose proportional manner. Exposures appeared to be relatively comparable between males and females. Estimated systemic overage compared to the MRHD of 125mcg is given below.

Table 5 Exposure multiples in male and female mice as compared to the MRHD

Estimated systemic overage compared to the MRHD of 125mcg.

Males:		
Target Dose (µg/kg/day)	Estimated AUC exposure overage compared to the maximum clinical dose (125mcg)	Estimated Cmax exposure overage compared to the maximum clinical dose (125mcg)
60	NC	4.4
200	26	11.5
600	121	27.5
Females:		
Target Dose (µg/kg/day)	Estimated AUC exposure overage compared to the maximum clinical dose (125mcg)	Estimated Cmax exposure overage compared to the maximum clinical dose (125mcg)
20	NC	2.5
60	21	9.2
200	96	36
Exposure overages calculated using 1) human exposure data for 125µg/day based on repeat dose data (14 days) in COPD patients in study AC4113073 (Day 14 AUC_{0-t}- 0.07ng.h/mL and Cmax = 0.1ng/ml (geometric mean) and 2) Week 26 toxicokinetic exposure data from the ongoing carcinogenicity study (Table 4, Appendix 2)		

Proposal: The sponsor proposes to implement an immediate dose reduction in male mice. The percent reduction in body weight gain only exceeded 10% for high dose males; however, the reduction in body weight gain for low dose males is currently 8%. The sponsor considers it prudent to implement a dose reduction across all male groups. The sponsor proposes to implement an immediate dose reduction for male treatment groups by reducing the exposure time on the inhalation chambers from 60 to 30 min. This is considered the most efficient way to implement the dose reduction at this stage given the current stage of the study (week 63) and will result in a dose reduction for male treatment groups from target doses of 60, 200, and 600 µg/kg/day to target doses of 30, 100, and 300 µg/kg/day, respectively. This will allow immediate exploration of the sensitive part of the dose response curve in males based on multiples of the MHRD for the remainder of the study period. GSK573719 is expected to achieve approximately 3.5, 3, and 60 fold exposure margins over the human AUC at the MRHD for the proposed new target doses of 30, 100 and 300 µg/kg/day, respectively. To implement any lower doses would require preparation and calibration of new blend strengths, which would take approximately 8 weeks to implement (i.e. to prepare, analyze the new blends and calibrate new aerosol concentrations on the inhalation chambers). Additional satellite animals will be introduced to the study to monitor the systemic exposure at the

new target doses. As for the rat study, work is ongoing to validate a lower level of quantification at 20 pg/mL for GSK573719 in mouse plasma to assist in better defining the plasma concentration profile of GSK573719 at lower inhaled doses.

Evaluation: Treatment of mice with GSK573719 at doses up to 600 µg/kg/day in males and 200 µg/kg/day in females up to week 70 had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains for male treatment groups. The reduced doses would still provide large multiples over systemic exposure achieved with the MRHD. It is noted that the LOQ for the analytical method to measure GSK573719 might be inadequate to detect lower concentrations of GSK573719 (≤60 µg/kg/day). Exposure margins would decrease 2-fold with respect to local toxicity although this is not a typical parameter for dose selection in a carcinogenicity study and there is still a relatively high exposure margin at the high dose. The ECAC, in emails dated February 18, 2011, agreed with the sponsor's proposed dose reduction for male mice in the 2-year carcinogenicity study.

Table 6 Exposure margins for pulmonary deposited dose (Male Mouse/Human)

Proposed doses µg/kg/day	PDD µg/kg/day	Exposure margins for clinical dose of 125 µg (2.1 µg/kg/day)
30	3	1.4
100	10	4.8
300	30	14.3

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

/s/

TIMOTHY W ROBISON
02/18/2011

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

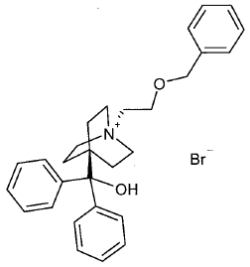
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 106,616
Review number: #01
Sequence number/date/type of submission: #001/November 13, 2009/Initial Submission
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: GlaxoSmithKline
P.O. Box 13398
Five Moore Drive
Research Triangle Park
North Carolina 27709-3398
Manufacturer for drug substance: Same as above.

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Products
HFD #: 570
Review completion date: February 10, 2010

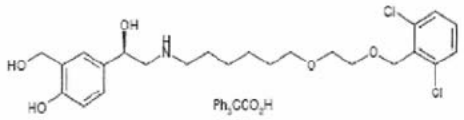
Drug:

Code name: GSK573719
Chemical name: 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide
Molecular formula/molecular weight: $C_{29}H_{34}NO_2 \cdot Br$
508.5 (GSK573719A as quaternary ammonium bromide compound)
428.6 (as free cation, GSK573719)
Structure:



Code name: GW642444
Chemical name: Triphenylacetic acid-4-{(1R)-2-[(6-2{2-[2,6-dichlorobenzyl)oxy] ethoxy{hexyl)amino]-1-hydroxyethyl{-2-(hydroxymethyl)phenol (1:1)
Molecular formula/molecular weight: $C_{24}H_{33}Cl_2NO_5 \cdot C_{20}H_{16}O_2$ (as triphenyl acetate salt) / 774.78 g/mole (as the triphenylacetate salt), 486.43 g/mole (as the free base)
Structure:

Reference ID: 3330354



Relevant INDs/NDAs/DMFs:
IND 74,696 (GSK, GW642444 Inhalation Powder, Long-acting β_2 agonist)
IND [redacted] (b) (4)
IND 77,855 (GSK, GW642444 + Fluticasone)
IND [redacted] (b) (4)
IND 104,479 (GSK, GSK573719)

Drug class: GSK573719 is a quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist.

GW642444 is a long-acting β_2-adrenergic agonist.

Intended clinical population: Chronic obstructive pulmonary disease (COPD)

Clinical formulation: GSK573719/GW642444 Inhalation Powder in the Novel Dry Powder Inhaler for oral inhalation is a [redacted] (b) (4) in molded plastic, with a dose counter. Each inhaler contains two foil blister strips, with each strip containing 30 regularly distributed blisters. One strip contains 500 µg/blister of GSK573719, as the quaternary ammonium bromide salt GSK573719A (micronized), [redacted] (b) (4) magnesium stearate and lactose monohydrate. The second strip contains 25 µg/blister of GW642444, as the triphenylacetate salt GW642444M (micronized), [redacted] (b) (4) magnesium stearate and lactose monohydrate. Magnesium stearate is added to the powder formulation [redacted] (b) (4)

Table 1 Composition of the GSK573719 Blister Strip for GSK573719/GW642444 Inhalation Powder, 500/25 Microgram

Component	Quantity per Blister[1]	Function	Reference to Standard
GSK573719A (micronised [redacted] (b) (4)	593 microgram	Active	GlaxoSmithKline
Magnesium Stearate	75 microgram	[redacted] (b) (4)	NF or Ph.Eur.
Lactose Monohydrate	to 12.5 mg		NF or Ph.Eur.

Note:
1. [redacted] (b) (4)
2.

Table 2 **Composition of the GW642444 Blister Strip for GSK573719/GW642444 Inhalation Powder, 500/25 Microgram**

Component	Quantity per Blister[1]	Function	Reference to Standard
GW642444M (micronised)[2]	40 microgram	Active	GlaxoSmithKline
Magnesium Stearate	125 microgram	(b) (4)	NF or Ph.Eur.
Lactose Monohydrate	to 12.5 mg		NF or Ph.Eur.

Note:
1. (b) (4)
2.

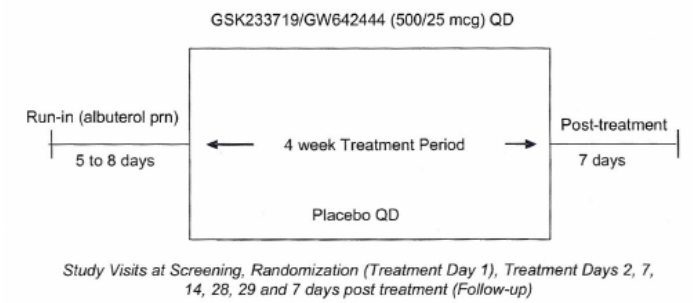
Route of administration: Oral Inhalation

Proposed clinical protocol:
Safety, tolerability, pharmacokinetics, and pharmacodynamics of the combination of GSK573719 and GW642444 in subjects with COPD (Clinical Study Identifier DB2113120)

This is a multicenter, randomized, double-blind, placebo-controlled, parallel group study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of the combination of GSK573719 (500 µg) Inhalation Powder and GW642444 (25 µg) Inhalation Powder administered once-daily by a novel dry powder inhaler (Novel DPI) over 28 days in subjects with COPD. Approximately 50 subjects (male or female ≥40 years old) with a diagnosis of COPD will be randomized in order to ensure at least 45 subjects complete the 28-day treatment. Subjects will be randomized using a 4:1 ratio to 500 µg GSK573719 / 25 µg GW642444 or placebo. The primary endpoint is change from baseline in weighted mean heart rate over 0-6 hr postdose at day 28.

A female is eligible to enter and participate in this study if she is of non-childbearing potential (i.e., physiologically incapable of becoming pregnant), including any female who is post-menopausal. If indicated, menopause can be confirmed by serum follicle stimulating hormone (FSH) levels >40 mIU/mL and estradiol <40pg/mL (<140pmol/L).

Figure 1 Study Schematic Diagram



Previous clinical experience: To date, 15 clinical studies to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single and repeat dose administration have been completed with the individual components of the combination product. These comprise of five clinical studies for GSK573719 Inhalation Powder in a total of 91 healthy subjects and 62 subjects with COPD and ten clinical studies for GW642444 Inhalation Powder in a total of 56 healthy subjects, 726 subjects with COPD, and 690 subjects with asthma.

Table 1 Summary of Completed GSK573719 Clinical Studies

Study Number	Design	Number of Subjects	Treatments	Formulation and Device
Studies in Healthy Volunteers				
AC4105209	R, DB, PC, XO Single Dose Ascending	19	GSK573719 - 10, 20, 60, 100, 250 & 350 mcg, tiotropium 18 mcg, or placebo	Lactose (b) (4) via DISKUS
AC4106889	R, DB, PC, PG, 14-Day Repeat Dose Ascending	36	GSK573719 - 250 and 750, 1000 mcg QD or placebo	Lactose (b) (4) via DISKUS
AC4110106	R, DB, PC Dose Ascending, Single and 7-day Repeat dose	36	Part 1: Normal CYP2D6 subjects: GSK573719 – 100, 500, and 1000 mcg or placebo single dose then 500 or 1000 µg or placebo QD 7-days Part 2: Poor CYP2D6 activity subjects: GSK573719 – Cohort 1) 100 mcg or placebo single dose then100 mcg or placebo QD 7-days, then 500 mcg or placebo single dose then 500 mcg or placebo QD 7 days. Cohort 2) 500 mcg or placebo single dose, then 500 mcg or placebo QD 7 days, then 1000 mcg or placebo single dose, then 1000 mcg or placebo QD 7 days	Lactose/MgSt via Novel DPI
Studies in COPD subjects				
AC4108123	R, DB, PC, XO, Single Dose Ascending	24	GSK573719 - 250, 500 & 1000 mcg, tiotropium 18 mcg, or placebo	Lactose (b) (4) via DISKUS
AC4105211	R, DB, PC, PG,7-Day, Repeat Dose Ascending	38	GSK573719 – 250mcg QD, 1000mcg QD, or placebo	Lactose/MgSt via Novel DPI

R=randomised, DB=double-blind, PC=placebo-controlled, PG=parallel group, XO=crossover, QD=once daily, (b) (4) MgSt=magnesium stearate, CDY2P6= cytochrome P450 Isoenzyme 2D6; DPI=dry powder inhaler

Reference ID: 3330354

Table 2 Summary of Completed GW642444 Clinical Studies

Study Number	Design	Number of Subjects	Treatments	Formulation and Device
Studies in Healthy Volunteers				
B2C10001	R, DB, PC single dose ascending	20	GW642444H 12.5, 50, 100, 200, 400, and 600mcg	Lactose via DISKUS
B2C108784	R, DB, PG, PC 14-day repeat dose ascending	36	GW642444M 25, 50 and 100mcg	Lactose/MgSt via DISKUS
Studies in Asthma Subjects				
B2C101762	R, DB, XO, PC 7-day repeat dose ascending	28	GW642444H 50, 100 and 200mcg	Lactose via DISKUS
B2C106093	R, DB, PC, XO 14-day	55	GW642444H 25, 100 and 400mcg, salmeterol 50mcg BID	Lactose via DISKUS
B2C104604	R, DB, XO, PC single dose ascending	14	GW642444M 25, 100 and 200mcg, GW642444H 100mcg	Lactose (b) (4) via DISKUS
B2C106996	R, DB, PC, XO 1-day dose-ascending	22	GW642444M 25, 50 and 100mcg, GW642444H 100mcg	Lactose/MgSt via DISKUS
B2C109575	R, DB, PC, PG, 28-day, dose ranging	607	GW642444M 3, 6.25, 12.5, 25, 50 mcg QD and placebo	Lactose/MgSt via Novel DPI
Studies in COPD Subjects				
B2C108562	R, DB, PC, PG 15-day	68	GW642444H 100 and 400mcg QD, salmeterol 50mcg BID	Lactose via DISKUS
B2C110165	R, DB, PC, XO, IB 1-day dose ascending	20	GW642444M 25, 50, and 100mcg QD, GW642444H 100mcg QD	Lactose/MgSt via DISKUS
B2C111045	R, DB, PC, PG, 28-day, dose ranging	602	GW642444M 3, 6.25, 12.5, 25, 50 mcg QD and placebo	Lactose/MgSt via Novel DPI

R=randomized, DB=double-blind, PC=placebo-controlled, PG=parallel group, XO=crossover, IB=incomplete block, BID=twice daily, QD=once daily, (b) (4) MgSt=magnesium stearate, Novel DPI=Novel Dry Powder Inhaler, M= triphenylacetate salt, H= α-phenylcinnamate salt.

One phase I study with co-administration of GSK573719 Inhalation Powder and GW642444 Inhalation Powder has recently been completed. This study (DB2113208) was a randomized, double-blind, placebo-controlled, four-way crossover study that evaluated the safety, tolerability, pharmacodynamics, and pharmacokinetics of a single inhaled dose of GSK573719 Inhalation Powder (500 µg) and GW642444 Inhalation Powder (50 µg) delivered individually by the Novel DPI and in combination from separate Novel DPIs in 16 healthy volunteers of Japanese decent. The sponsor's summary indicated that single inhaled doses of GSK573719 Inhalation Powder, GW642444 Inhalation Powder, and the combination of GSKS73719 and GW642444 Inhalation Powders were found to be well tolerated. There were no significant changes in vital signs, hematology, or biochemistry assessments, which were attributed to study drug and no significant 12- lead ECG or 24-hr Holter abnormalities during the study.

Human Pharmacokinetics:

Pharmacokinetic of single inhaled doses of GSK573719 and GW642444 delivered individually by the Novel DPI and in combination from separate Novel DPIs (DB2113208): A single study was completed in a total of 16 healthy male or female Japanese subjects to investigate the safety, tolerability, pharmacokinetics and

pharmacodynamics of single inhaled doses of GSK573719 (500 µg) and GW642444 (50 µg) delivered individually by the Novel DPI and in combination from separate Novel DPIs. The bioanalytical method used to detect plasma GSK573719 concentrations [lower limit of quantification (LLQ = 10 pg/mL)] was not sensitive enough to fully characterize the PK profile of GSK573719 following single dose administration as concentrations in plasma samples collected at time points later than 6 hr were generally not quantifiable.

Following single dose administration of either GSK573719 alone or the GSK573719 and GW642444 combination, GSK573719 was rapidly absorbed with most of the C_{max} values occurring at 5 min followed by rapid declines of plasma concentrations. The terminal phase half life (t½) was estimated to be 1-3 hr. A comparison of $AUC_{0-\infty}$ values for GSK573719+GW642444 versus GSK573719 alone suggested a large increase in exposure (617.322 pg·hr/mL vs. 368.322 pg·hr/mL, respectively).

The bioanalytical method used to detect plasma GW642444 concentrations [lower limit of quantification (LLQ) = 30 pg/mL] was not sensitive enough to fully characterize the PK profile of GW642444 following single dose administration of GW642444 either alone or in combination. Following a single dose administration of 50 µg GW642444 alone, plasma concentrations of GW642444 were measurable up to 1 hr, but not quantifiable (NQ) after 4 hr postdose. Following single dose administration of 50 µg GW642444 with GSK573719, plasma concentrations of GW642444 were measurable up to 2 hr; however, all were NQ by 5 hr postdose.

Following single dose administration of GW642444 alone or the combination of GSK573719 and GW642444, GW642444 was rapidly absorbed with most of the C_{max} values occurring at 5 min followed by rapid declines of plasma concentrations. The large amount of NQ data at later time points suggested rapid metabolism, distribution and elimination of drug from systemic circulation. A comparison of $AUC_{0-\infty}$ values for GSK573719+GW642444 versus GW642444 alone suggested a potential increase in exposure (315.562 pg·hr/mL vs. 233.768 pg·hr/mL, respectively).

Table 7 Summary Statistics of GSK573719 PK Parameters

Parameter	Treatment	N	n	Geometric Mean	95% CI	CVb(%)
AUC(0-0.25) (h•pg/mL)	719 500μg	15	15	126.519	(82.969, 192.928)	88.7
	719 500μg/444 50μg	15	15	179.603	(140.326, 229.873)	46.9
AUC(0-2) (h•pg/mL)	719 500μg	15	15	312.130	(194.090, 501.959)	104.3
	719 500μg/444 50μg	15	15	413.471	(315.589, 541.712)	51.8
AUC(0-4) (h•pg/mL)	719 500μg	15	15	377.787	(226.444, 630.280)	116.2
	719 500μg/444 50μg	15	15	513.611	(380.274, 693.702)	58.5
AUC(0-t) (h•pg/mL)	719 500μg	15	15	374.225	(213.422, 656.186)	134.0
	719 500μg/444 50μg	15	15	537.640	(380.159, 760.359)	69.2
AUC(0-∞) (h•pg/mL)	719 500μg	15	15	368.704	(196.798, 690.770)	161.7
	719 500μg/444 50μg	15	15	617.322	(434.415, 877.242)	70.4
C_{max} (pg/mL)	719 500μg	15	15	578.301	(224.078, 1492.478)	421.3
	719 500μg/444 50μg	15	15	1289.384	(991.659, 1676.496)	50.2
$t_{1/2}$ (h)	719 500μg	15	12	1.563	(1.288, 1.897)	31.2
	719 500μg/444 50μg	15	15	1.776	(1.166, 2.703)	88.2
t_{max} (h)*	719 500μg	15	13	0.080	(0.08, 0.13)	NA
	719 500μg/444 50μg	15	15	0.080	(0.08, 0.08)	NA
t_{last} (h)*	719 500μg	15	13	4.000	(1.00, 8.00)	NA
	719 500μg/444 50μg	15	15	5.000	(1.00, 16.00)	NA

*presented as Median and range;
NA : not applicable;
AUC(0-t) = area under concentration-time curve from time 0 to time of last quantifiable concentration; C_{max} = maximum observed plasma concentration; t_{max} = time of maximum observed plasma concentration; t_{last}= last time point where the concentration is above the limit of quantification, CI = confidence interval.

Table 9 Summary Statistics of GW642444 PK Parameters

Parameter	Treatment	N	n	Geometric Mean	95% CI	CV(%)
AUC(0-1) (h•pg/mL)	444 50□g	16	16	207.995	(171.502, 252.254)	37.4
	719 500□g/444 50□g	15	15	225.337	(177.127, 286.669)	45.6
AUC(0-t) (h•pg/mL)	444 50□g	16	16	234.342	(183.621, 299.074)	48.3
	719 500□g/444 50□g	15	15	271.343	(204.657, 359.759)	54.4
AUC(0-∞) (h•pg/mL)	444 50□g	16	16	233.768	(176.956, 308.819)	56.0
	719 500□g/444 50□g	15	15	315.562	(237.212, 419.791)	55.2
Cmax (pg/mL)	444 50□g	16	16	495.929	(396.693, 619.989)	43.8
	719 500□g/444 50□g	15	15	499.298	(330.787, 753.651)	85.9
$t_{1/2}$ (h)	444 50□g	16	15	0.422	(0.361, 0.493)	28.9
	719 500□g/444 50□g	15	14	0.707	(0.515, 0.971)	59.3
t_{max} (h)*	444 50□g	16	16	0.080	(0.08, 0.10)	NA
	719 500□g/444 50□g	15	15	0.080	(0.08, 0.08)	NA
t_{last} (h)*	444 50□g	16	16	1.500	(1.00, 4.00)	NA
	719 500□g/444 50□g	15	15	2.000	(0.08, 5.00)	NA

*presented as Median and range;
NA : not applicable;
AUC (0-t) = area under concentration-time curve from time 0 to time of last quantifiable concentration; Cmax = maximum observed plasma concentration; tmax = time of maximum observed plasma concentration; tlast= last time point where the concentration is above the limit of quantification, CI = confidence interval.

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

Studies reviewed within this submission:

STUDY:	**STUDY NUMBER**:
SAFETY PHARMACOLOGY:	
Cardiovascular effects:	
GW642444M and GSK573719A: The Effect of Single Intravenous Administration on Cardiovascular Function in the Conscious Beagle Dog	FD2008/00365/00
TOXICOLOGY:	
GW642444M and GSK573719A: Toxicity Study by Inhalation Administration to Rats for 4 Weeks	FD2009/00392/00
GSK573719A and GW642444M: A 4-Week Combination Inhalation Toxicity Study in Dogs	FD2009/00391/00

Studies <u>not</u> reviewed within this submission: None

Drug History: During the Pre-IND meeting for IND104,479 (GSK573719 monoproduct), comments were conveyed to the sponsor regarding the combination of GSK573719 and GW642444.

3. We noted in your briefing package that your proposed future clinical studies include dosing with a combination of your product in this IND (GSK573719) and one from another IND (GW642444). To support clinical dosing with the combination of GSK573719 and GW642444, a bridging study with the combination in the most appropriate species should be conducted. The duration should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies for a combination of two NMEs (three months). Short-term studies with the combination in both a rodent and non-rodent may be needed to identify the most appropriate species. We recommend that the combination be tested at doses that produce exposure ratios that are relevant to the intended clinical use. Prior to clinical dosing with the combination exceeding three months, toxicology studies with each NME should be complete (i.e., six months in a rodent and nine months in a nonrodent). Further, a fertility and reproductive performance study in rats and embryofetal studies in rats and rabbits for each NME should be completed. We recommend an embryofetal development study with the combination in the most appropriate species prior to enrollment of women of childbearing potential.

Best Available Copy

In the initial submission of IND 104,479, the sponsor submitted draft protocols for a 13-week inhalation toxicology study with the combination in dogs and an embryofetal study with the combination administered by the subcutaneous route in rabbits. The dog was identified as the most appropriate species for further study based upon 4-week inhalation toxicology studies with the combination in rats and dogs. The rabbit was

identified as the most appropriate species based upon embryofetal development studies conducted in rats and rabbits with monoproducts alone.

The following comments were conveyed to the sponsor in a facsimile dated September 1, 2009 (Typographical errors regarding Group numbers have been corrected).

1. We agree that the dog is most appropriate species for the 13-week inhalation toxicology study to assess the potential toxic effects of the combination, GSK573719 + GW642444. The general design of the 13-week study appears acceptable given that ratios of doses are based on the maximum and minimum predicted clinical ratios of GSK573719 with GW642444 (i.e. 1:1 or 200:1 for GSK573719:GW642444).

a. The dose of GSK573719 in Group 3 (20 µg/kg/day) appears to be low with respect to the clinical pulmonary deposited dose and systemic exposure although the dose ratio with respect to GW642444 is acceptable. Further, the dose of GSK573719 in Group 4 (60 µg/kg/day) appears to be low with respect to the clinical pulmonary deposited dose although the dose ratio with respect to GW642444 is acceptable. The doses of GSK573719 in Groups 3 and 4 do not appear to support the maximum proposed clinical dose of GSK573719 at 1000 µg/day.

b. It is recommended that the pre-study adaptation phase proposed for dogs in Groups 4, 5, and 8 should not be conducted as this procedure may potentially reduce the observed β-adrenergic toxicity for these treatment groups (i.e., pre-exposure to lower doses may offer protection against subsequent exposure to higher doses, see Toxicology and Applied Pharmacology 69: 199-205, 1983).

2. The choice of the rabbit to assess the effects of the combination of GSK573719+GW642444 on embryofetal development appears acceptable. The subcutaneous route to maximize systemic exposure and proposed ratios of doses also appear to be acceptable.

The Division's position that a pre-adaptation phase should not be used was reiterated in a facsimile dated October 9, 2009.

Your response to Division Comment 1a is generally acceptable although this will be a review issue at the time the 13-week dog study with the combination is submitted to determine if adequate safety margin are present for proposed clinical doses.

With regard to your response to Division Comment 1b, it is the Division's general position that a pre-adaptation phase should not be used in dog studies with β_2 agonists as this may lead to cardio-protection against subsequent exposure to larger doses. Thus, the pre-adaptation phase might potentially prevent a full characterization of the toxicity of the drug. Further, such procedures are not used in a clinical setting. We recommend that the pre-adaptation phase not be used in the proposed 13-week dog study with the combination.

It is noted that the sponsor ultimately elected to use the pre-adaptation phase in the 13-week inhalation toxicology study with dogs.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

GSK573719A is a high affinity, pan active muscarinic antagonist and GW642444 is a long-acting β_2 agonist. The combination is under development for the treatment of COPD. GSK573719 competes with [^{3}H]-N-methyl-scopolamine binding for human recombinant receptor membranes from CHO cells stably expressing the M1, M2, M3, M4 and M5 receptors with K_i values of 0.16, 0.15, 0.06, 0.05, 0.13 nM, respectively. The potency of GW642444 agonist activity in human adrenoreceptors was similar to that of isoprenaline and R,R-formoterol. Intrinsic activity of GW642444 was lower than isoprenaline and R,R-formoterol, but higher than salmeterol (1.53 versus 2.67, 2.17 and 1.0, sequentially). Both compounds inhibited bronchoconstriction in various animal models.

Effects of GW642444 and GSK573719 administered by the intravenous route alone or in combination, on arterial pressure, heart rate, and ECG parameters were assessed in dogs. Increased heart rate was observed following infusion of 0.3 µg/kg GW642444 alone, 0.3 µg/kg GSK573719 alone, and 0.3 µg/kg GW642444 with 0.3 µg/kg GSK573719. There were also small reductions of RR intervals following infusion concomitant to the increase in heart rates. It is noted that these doses are low as compared to the proposed therapeutic dose (inhalation dose of 10 µg/kg GSK573719 and 0.5 µg/kg GW642444) although the route is different.

2.6.2.4 Safety pharmacology

Cardiovascular effects:

GW642444M and GSK573719A: The Effect of Single Intravenous Administration on Cardiovascular Function in the Conscious Beagle Dog (FD2008/00365/00)

Methods: Effects of GW642444 and GSK573719, administered by the intravenous route over 1 min alone or in combination, on arterial pressure, heart rate, and ECG parameters were assessed in conscious, restrained, telemetered male beagle dogs. Using a Latin square design, each dog received GW642444 vehicle (20% (v/v) Polyethylene Glycol 400 in aqueous 8% (w/v) 2-Hydroxypropyl Beta Cyclodextrin), GSK573719 vehicle (0.9% (w/v) Aqueous Sodium Chloride), GW642444 (Batch Number, 071137023, K064213, 99.4% pure, 62.4% pure as GW642444) at 0.3 µg/kg or GSK573719 (Batch Number R360972, 99.8% pure, 83.8% pure as GSK573719) at 0.3 µg/kg, alone or in combination. There were at least 6 days between each treatment.

Results: A transient increase in heart rate was observed following infusion of 0.3 µg/kg GW642444 with GSK573719 vehicle and 0.3 µg/kg GW642444 with 0.3 µg/kg GSK573719, resulting in peak increases to 111 bpm (from a mean predose of 78 bpm) and 109 bpm (from a mean predose of 75 bpm), respectively, occurring 6 min from the start of infusion. Heart rate was elevated following 0.3 µg/kg GW642444 with

GSK573719 vehicle (between 6 to 71 min, and 86 to 111 min), and GW642444 vehicle with 0.3 µg/kg GSK573719 (21 to 51 min, and 96 to 121 min), compared to combined vehicle treatment.

There was a small reduction in RR interval following infusion of 0.3 µg/kg GW642444 with GSK573719 vehicle, GW642444 vehicle with 0.3 µg/kg GSK573719, and 0.3 µg/kg GW642444 with 0.3 µg/kg GSK573719, concomitant to the increase in heart rate. A small reduction in QT interval (10-20%) associated with the change in heart rate was also observed after dosing 0.3 µg/kg GW642444 with GSK573719 vehicle, compared to combined vehicle treatment. No test article-related changes in PR or QRS intervals were noted following any dosing regime.

Infusion of 0.3 µg/kg GW642444 with 0.3 µg/kg GSK573719, resulted in a small non-statistically significant prolongation in rate corrected QT interval from 16 min after the start of infusion, with a mean peak vehicle corrected change from predose of 9 msec occurring at 31 minutes. This increase may have been mediated by one dog (7337 AK).

Infusion of 0.3 µg/kg GW642444 with 0.3 µg/kg GSK573719 increased mean systolic and diastolic blood between 126 min and the end of recording at 3 hr. Peak increases were less than 10%.

Following intravenous administration of GW642444 or GSK573719 either alone or in combination at a nominal dose of 0.3 µg/kg to male dogs, GW642444 was quantifiable for up to 30 min after dosing while GSK573719 was only quantifiable to between 1 and 5 min after dosing.

It is noted that the doses used in this study were low as compared to the proposed therapeutic dose (inhalation dose of 10 µg/kg GSK573719 and 0.5 µg/kg GW642444) although the route is different.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Rats

Study title: **GW642444M and GSK573719A: Toxicity Study by Inhalation Administration to Rats for 4 Weeks**

Key study findings:

● In a 4-week toxicology study, GSK573719A and GW642444M, in combination and alone, were given as dry powder formulations in lactose to rats by nose only inhalation administration once daily for 60 min/day. Selection of the doses used in this study was based on the minimum and maximum combination ratios of GSK573719 or GW642444

to be given to humans in clinical studies (200:1 and 1:1 for GSK573719:GW642444). Target doses of 0/0, 1000/5, 1000/50, 1000/1000, 1000/0, and 0/1000 µg/kg/day (GSK573719/ GW642444) were selected for this study.

● Histopathological findings were observed in the lungs + bronchi, tracheal bifurcation, liver, mandibular LN, larynx, nasopharynx, and nasal turbinates. Findings in the larynx were judged to be rat-specific irritant effects commonly associated with inhalation toxicology studies. Findings in the nasopharynx and nasal turbinates were associated with nose-only inhalation and judged to be irrelevant for oral inhalation in the clinical setting. Findings for the mandibular LN occurred with GSK573719 alone.

● In the lungs + bronchi, findings of alveolar hemoglobin crystals with associated inflammatory cell infiltrate were observed in groups treated with combination of GSK573173 + GW642444. There was no evidence of a dose-response relationship. These findings were also observed at a lower incidence in groups treated with GSK573719 alone. The sponsor indicated that this finding has been seen recently at a similar incidence in control Sprague-Dawley rats from the testing laboratory.

● In the tracheal bifurcation, inflammatory cell infiltration was observed at increased incidences for males treated with the low dose of the combination or with either monoproduct alone and for females treated with the two lower doses of the combination or GW642444 alone. Loss of cilia, basophilia, and cell flattening at the bifurcation were observed at increased incidences for males treated with the low and high doses of the combination or GSK573719 alone and for females treated with the three doses of the combination or GSK573719 alone.

● The incidence of focal hepatocyte necrosis was increased for females treated with the high dose of the combination.

● AUC and C_{max} values for GSK573719 were generally comparable alone and in combination with GW642444. However, AUC and C_{max} values for GW642444 tended to be lower in combination with GSK573719 as compared to alone.

● There was no evidence of additive or synergistic toxic effects with the combination. Toxic effects observed with the combination products were generally consistent with those observed with monoproducts alone. Many findings appeared to be consistent with general inflammatory or irritation effects and could not be ascribed as class effects.

Study no.: FD2009/00392/00
Volume #, and page #: Volumes 24 and 25, Pages 1-582
Conducting laboratory and location: (b) (4)

(b) (4)

[redacted]

GlaxoSmithKline
PCD DMPK
GSK R&D
Park Road
Ware
Hertfordshire
SG12 0DP
England

Date of study initiation: September 5, 2008
GLP compliance: No. Unaudited Draft Toxicology Report
QA report: yes ( ) no (X) Unaudited Draft Toxicology Report
Drug, lot #, and % purity: GSK573719A and GW642444M (premicronized; Lot Nos. 081168732 and 071148413, respectively) were administered as a dry powder formulation blended in Lactose (Lot No. 071138760) with Magnesium Stearate (Lot No. 061127066). See table below for further details.

Material	Input Material Lot numbers	Input Material Purity	Blend Batch Number	Blend concentration (nominal)	Content uniformity	Blend Expiry	Blend Storage
Group 1: Lactose Magnesium Stearate	071138760 061127066	N/A	BVR/H/1009/08	Lactose with a nominal 1% (w/w) magnesium stearate	N/A	18 May 2009	At ambient temperature, protected from light
Group 2: GSK573719A and GW642444M Magnesium Stearate	081168732 071148413 061127066	99.8% 99.2%	BVR/H/1013/08	Nominal 40% (w/w) GSK573719 with nominal 0.2% (w/w) GW642444 with nominal 1% (w/w) magnesium stearate blended in lactose	100.4% GSK573719, 89.8% GW642444	TBC	2-8°C
Group 3: GSK573719A and GW642444M Magnesium Stearate	081168732 071148413 061127066	99.8% 99.2%	BVR/H/1014/08	Nominal 40% (w/w) GSK573719 with nominal 2% (w/w) GW642444 with nominal 1% (w/w) magnesium stearate blended in lactose	99.9% GSK573719, 98.1% GW642444	TBC	2-8°C
Group 4: GSK573719A and GW642444M Magnesium Stearate	081168732 071148413 061127066	99.8% 99.2%	BVR/H/1011/08	Nominal 6% (w/w) GSK573719 with nominal 6% (w/w) GW642444 with nominal 1% (w/w) magnesium stearate blended in lactose	98.6% GSK573719, 98.3% GW642444	TBC	2-8°C
Group 5: GSK573719A Magnesium Stearate	0081168732 061127066	99.8%	BVR/H/1012/08	Nominal 6% (w/w) GSK573719 with nominal 1% (w/w) magnesium stearate blended in lactose	97.8%	21 May 2009	At ambient temperature, protected from light
Group 6: GW642444M Magnesium Stearate	071148413 061127066	99.2%	BVR/H/1010/08	Nominal 6% (w/w) GW642444 with nominal 1% (w/w) magnesium stearate blended in lactose	97.1%	TBC	2-8°C

Methods

Doses: GSK573719A and GW642444M, in combination and alone, were given as dry powder formulations in lactose to rats by nose only inhalation administration once daily for 60 min/day for 4 weeks. Selection of the doses used in this study was based on the minimum and maximum combination ratios of GSK573719 or GW642444 to be given to humans in clinical studies (200:1 and 1:1 for GSK573719:GW642444). Target doses of 0/0, 1000/5, 1000/50, 1000/1000, 1000/0, and 0/1000 µg/kg/day (GSK573719/ GW642444) were selected for this study.

GSK573719

Group Number	Target Dose[1] (µg/kg/day)	Estimated Achieved Dose[1,3] (µg/kg/day)	Target Chamber Aerosol Concentration[1] (µg/L)	Average Achieved Chamber Aerosol Concentration[1,2] (µg/L)	Number/Sex
Toxicology Animals					
1[4]	0	0	0	0	10
2	1000	817	27	21.9	10
3	1000	1200	27	32.4	10
4	1000	1060	27	28.8	10
5	1000	757	27	20.6	10
6	0	0	0	0	10
Toxicokinetic Animals					
1[4]	0	0	0	0	12
2	1000	816	27	22.0	12
3	1000	1190	27	32.1	12
4	1000	1055	27	28.7	12
5	1000	755	27	20.5	12
6	0	0	0	0	12

1. Doses and concentrations are expressed in terms of the parent compound, GSK573719
2. As determined by chemical analysis
3. Estimated achieved inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/ (BW)

D	Dose (µg/kg)
RMV(L/min)	Respired minute volume (L/min) calculated from the formula $0.499 \times BW(kg)^{0.809}$ [Bide, 2000]
T	Duration of exposure/day (minutes)
C	GSK573719 aerosol concentration (µg/L)
BW	Group average body weight for study (kg)

4. Control received vehicle (1% w/w Magnesium Stearate in lactose) at a feed rate that targeted the lactose concentration to be similar to that of Group 4

GSK573719: Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day

Sex	Dose µg/kg/day	Deposited Dose[1] µg/kg/day	BW G	Total Dose µg/day	LW g	Deposited Dose µg/g LW/day
Males	817	81.7	422	34.5	1.440	23.9
	1200	120.0	437	52.4	1.528	34.3
	1060	106.0	452	47.9	1.487	32.2
	757	75.7	442	33.5	1.484	22.5
Females	817	81.7	243	19.85	1.147	17.3
	1200	120.0	251	30.1	1.215	24.8
	1060	106.0	266	28.2	1.265	22.3

	757	75.7	260	19.7	1.221	16.1

1. A deposition factor of 0.10 was used in calculations.
2. Mean terminal body weights at the end of the treatment period.
3. Absolute lung weights from the sponsor's data.

GW642444:

Group Number	Target Dose[1] (µg/kg/day)	Estimated Achieved Dose[1,3] (µg/kg/day)	Target Chamber Aerosol Concentration[1] (µg/L)	Average Achieved Chamber Aerosol Concentration[1,2] (µg/L)	Number/Sex
Toxicology Animals					
1[4]	0	0	0	0	10
2	5	4.37	0.13	0.118	10
3	50	60.7	1.30	1.64	10
4	1000	1040	27	28.2	10
5	0	0	0	0	10
6	1000	869	27	23.9	10
Toxicokinetic Animals					
1[4]	0	0	0	0	12
2	5	4.36	0.13	0.117	12
3	50	60.7	1.30	1.64	12
4	1000	1040	27	28.4	12
5	0	0	0	0	12
6	1000	851	27	23.5	12

1. Doses and concentrations are expressed in terms of the parent compound, GW642444
2. As determined by chemical analysis
3. Estimated achieved inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

D = (RMVxTxC)/ (BW)

D	Dose (µg/kg)
RMV(L/min)	Respired minute volume (L/min) calculated from the formula $0.499 \times BW(kg)^{0.809}$ [Bide, 2000]
T	Duration of exposure/day (minutes)
C	GW642444 aerosol concentration (µg/L)
BW	Group average body weight for study (kg)

4. Control received vehicle (1% w/w Magnesium Stearate in lactose) at a feed rate that targeted the lactose concentration to be similar to that of Group 4

GW642444: Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day

Sex	Dose µg/kg/day	Deposited Dose[1] µg/kg/day	BW G	Total Dose µg/day	LW g	Deposited Dose µg/g LW/day
Males	4.36	0.44	422	0.186	1.440	0.13
	60.7	6.1	437	2.67	1.528	1.74
	1040	104.0	452	47.0	1.487	31.6
	851	85.1	485	41.3	1.541	26.8
Females	4.36	0.44	243	0.107	1.147	0.12
	60.7	6.1	251	1.53	1.215	1.26
	1040	104.0	266	27.7	1.265	21.9
	851	85.1	280	23.8	1.237	19.3

1. A deposition factor of 0.10 was used in calculations.
2. Mean terminal body weights at the end of the treatment period.
3. Absolute lung weights from the sponsor's data.

Magnesium stearate

Group Number	Estimated Achieved Dose[1,3] (µg/kg/day)	Achieved Chamber Aerosol Concentration[1,2] (µg/L)	Number/ Sex
Toxicology Animals			
1[4]	177	4.78	10
2	18.2	0.488	10
3	27.2	0.733	10
4	166	4.50	10
5	144	3.91	10
6	143	3.92	10
Toxicokinetic Animals			
1[4]	177	4.80	12
2	18.5	0.499	12
3	27.9	0.754	12
4	169	4.59	12
5	140	3.80	12
6	137	3.79	12

1. Doses and concentrations are expressed in terms of magnesium stearate
2. As determined by chemical analysis
3. Estimated achieved inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:

$D = (RMVxTxC)/(BW)$

D	Dose (µg/kg)
RMV(L/min)	Respired minute volume (L/min) calculated from the formula $0.499xBW(kg)^{0.809}$ [Bide, 2000]
T	Duration of exposure/day (minutes)
C	magnesium stearate aerosol concentration (µg/L)
BW	Group average body weight for study (kg)

4. Control received vehicle (1% w/w Magnesium Stearate in lactose) at a feed rate that targeted the lactose concentration to be similar to that of Group 4

Particle Size Distribution Results of GSK573719

Group Number	Average Achieved Chamber Aerosol Concentration [1,2] (µg/L)	MMAD (µm)	GSD
2	21.9	(b) (4)	
3	32.4		
4	28.8		
5	20.6		

1. Concentrations are expressed in terms of the parent compound, GSK573719
2. As determined by chemical analysis

MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Particle Size Distribution Results of GW642444

Group Number	Average Achieved Chamber Aerosol Concentration [1,2] (µg/L)	MMAD (µm)	GSD
2	0.118	(b) (4)	
3	1.64		
4	28.2		
6	23.9		

1. Concentrations are expressed in terms of the parent compound, GW642444
2. As determined by chemical analysis

MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

Particle Size Distribution Results of Magnesium Stearate

Group Number	Average Achieved Chamber Aerosol Concentration [1,2] (µg/L)	MMAD (µm)	GSD
1	4.78	(b) (4)	
2	0.488		
3	0.733		
4	4.50		
5	3.91		
6	3.92		

1. Concentrations are expressed in terms of magnesium stearate
2. As determined by chemical analysis

MMAD - Mass median aerodynamic diameter (µm)
GSD - Geometric standard deviation

A measured volume of air was drawn from exposure chambers at a rate of 5 L/min through a microfiber filter, mounted in an open face filter holder. Samples were collected daily from each dose group for gravimetric analysis. Samples were collected daily for weeks 1 and 2, and three times per week for the remainder of the study from each dose group for chemical analysis. GSK573719 and GW642444 content were measured by UPLC with UV detection. Magnesium stearate content was measured using atomic absorption spectroscopy.

A measured volume of air was drawn from chamber level 2 through a cascade impactor. Samples were collected during Weeks 1 and 4 for GSK573719 and GW642444 analysis. Samples for Magnesium Stearate were collected during either preliminary

trials or other exposure sessions with no animals present due to impracticalities of collection of particle size samples during the dosing sessions.

Species/strain: Male and Female Sprague-Dawley (Crl:CD(SD)) rats were obtained from (b) (4)
Number/sex/group or time point (main study): 10 rats/sex/group
Route, formulation, volume, and infusion rate: Rats received daily nose-only inhaled doses of GSK573719/GW642444 in combination or alone or vehicle for 60 min/day. The vertical position of animals on the exposure chamber during dosing was changed on each week of the study to minimize any bias resulting from variations in test material concentration at the 3 exposure levels of the chamber.

Aerosols were generated using a Wright Dust Feed (WDF) mechanism by suspending the test formulation scraped from the surface of compressed powder blend in a stream of oil free compressed air. Aerosols were conducted into ADG inhalation exposure chambers configured as follows: The inhalation exposure system comprised an ADG nose-only inhalation exposure chamber, restraining tubes, a dust generator (to produce an aerosol from the powder supplied), air supply and extract lines, which attached to the top and bottom of the chamber, respectively, and an air supply to the dust generator. A filtration system was incorporated in the extract line.

The ADG nose-only inhalation chamber was a modular apparatus of aluminum alloy construction comprising a base unit, a variable number of animal exposure sections, and a top section incorporating a central aerosol inlet surrounded by a tangential air inlet. During dosing each chamber was housed in an enclosed ventilated cabinet. The WDF mechanism was mounted on the top of the aerosol conditioning pre-chamber and the aerosol passed through it into the top of the exposure chamber. Sixty exposure ports were available on each exposure chamber (20 ports/exposure section). Six individual exposure chambers were used in the study.

Satellite groups used for toxicokinetics or recovery: 12 rats/sex/group were included for toxicokinetic assessments.
Age: Rats were 11 to 12 weeks old at the start of treatment.
Weight: Body weight ranges were 337 to 442 g for males and 190 to 287 g for females.
Unique study design or methodology (if any): Selection of the doses used in this study was based on the minimum and maximum combination ratios of GSK573719 or GW642444 to be given to humans in clinical studies (200:1 and 1:1 for GSK573719:GW642444).

Observation and Times:
Clinical signs: Viability checks were performed twice daily. During the treatment period, animals were observed for clinical signs immediately before dosing, during exposure, immediately after dosing, 1-2 hr postdose, and late in the day.
Body weights: Body weights were measured twice weekly.
Food consumption: Food consumption was recorded weekly.

Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of dosing and during week 4.
EKG: Not performed.
Hematology: Blood samples for measurement of a complete set of hematology parameters were collected during week 4.
Clinical chemistry: Blood samples for measurement of a complete set of clinical chemistry parameters were collected during week 4.
Urinalysis: Urine samples were collected overnight (approximately 16 hr) during week 4 for assessment of a complete panel of urinalysis parameters.
Gross pathology: Surviving toxicology animals were sacrificed after 4 weeks of treatment and submitted to gross necropsy examinations.
Organ weights: Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes, and thymus.
Histopathology: Tissue samples were processed to paraffin wax, sectioned, stained with hematoxylin and eosin, and examined microscopically for all Group 1 and 4 animals killed at the end of the dosing period, all unscheduled deaths (toxicology animals), and macroscopic abnormalities from all toxicology animals. Respiratory tract tissues with treatment-related findings or suspected treatment related findings prior to peer review in the high dose combination group (larynx, lungs, nasal cavity, nasopharynx, trachea, tracheal bifurcation) were examined for all Group 2 and 3 animals and in the groups given each test article alone (Groups 5 and 6). Sections of testes from controls, and Groups 2, 3 and 4 terminal necropsy animals were prepared and stained with Periodic Acid Schiff's-Hematoxylin (PASH). A qualitative examination of spermatogenic stages was made for normal progression of the stages of the spermatogenic cycle, cell associations, and proportions expected to be present during normal spermatogenesis. A peer review of selected microscopic tissue sections and pathology data interpretation was completed. An additional peer review was performed by the sponsor. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on days 1, 13, and 28 immediately after dosing and at 0.5, 1, 2, 4, 6, 8, and 23 hr postdose. Plasma samples prepared from naïve animals were available for assessment of potential contamination during processing of plasma samples obtained from drug-treated animals. Plasma samples were shipped to the sponsor for measurement of GSK573719 and GW642444 concentrations. Concentrations of GSK573719 and GW642444 were measured by HPLC-MS/MS. The LLOQ for GSK573719 and GW642444 was 0.1 ng/mL.
Results:

Mortality: One male (# 119) given GW642444 alone at 869 µg/kg/day was found dead in the restraint tube during dosing on Day 5 in Week 1. No clinical signs had been noted in this animal. Histopathological findings were observed in larynx (squamous metaplasia), nasal turbinates (mineralization of the olfactory epithelium), and kidneys (unilateral papillary tubular dilatation). This death was probably unrelated to GW642444 as there were no furthers deaths as treatment progressed.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Body weight gains were decreased for male and female rats in Groups 2, 3, and 5, which might be attributed to GSK573719. Body weight gains were increased for males and females in Groups 4 and 6, which can be attributed to the exaggerated pharmacological action of GW642444.

Body weight gains from days 1 to 29 expressed as a percent of the control

Sex	Control	Group 2	Group 3	Group 4	Group 5	Group 6
Males	100.0%	66.9%	88.2%	113.2%	43.2%	137.7%
Females	100.0%	56.7%	94.6%	141.3%	49.4%	125.0%

Food consumption: Food consumption was unaffected for male and female treatment groups.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

Hematology: Changes of white and red blood cell parameters were evident between control and treatment groups; however, the significance of these changes was unclear.

White blood cell (WBC) counts were slightly increased for males in the mid and high dose combination groups and females in all combination groups. These increases were primarily attributed to elevations of lymphocyte counts. Neutrophil counts were decreased for females in all combination groups and females in the GW642444 only group. Eosinophils counts were increased for males in the high dose combination group and both monoproduct only groups. Basophil counts were increased for males in all combination groups and females in the mid and high dose combination groups. Monocyte counts were increased for males and females in the mid and high dose combination groups and females in the monoproduct only groups. Large unstained cells were increased for males in the high dose combination group and females in all combination groups. APPT values were decreased for females in the GSK573719 only group and males and females in the GW642444 only groups.

Hematocrit, hemoglobin concentrations, and red blood cell counts were slightly increased (<10%) for all female treatment groups. MCH, MCHC, and MCV values were slightly decreased (<5%) for males and females in the GSK573719 and GW642444 only groups. The significance of these changes was unclear.

Hematology parameters, week 4

Parameter	Males						Females					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
WBC x 10^9/L	10.68	10.76	13.39* (125%)	13.25* (124%)	10.93	9.14	6.56	8.15 (124%)	8.44 (129%)	9.22* (141%)	7.57 (115%)	7.08 (108%)
Neutrophils x 10^9/L	NC	NC	NC	NC	NC	NC	1.72	0.97 (56%)	0.64* (37%)	1.32* (77%)	1.52	0.68 (40%)
Lymphocytes x 10^9/L	8.89	9.05	11.44* (129%)	11.03 (124%)	8.79	7.41	4.58	6.84* (149%)	7.43* (162%)	7.49* (164%)	5.72 (125%)	6.12 (134%)
Eosinophils x 10^9/L	0.08	0.07	0.10	0.14 (175%)	0.11* (138%)	0.14* (175%)						
Basophils x 10^9/L	0.04	0.08 (200%)	0.09* (225%)	0.06* (150%)	0.03	0.02	0.02	0.03	0.05* (250%)	0.04* (200%)	0.02	0.02
Monocytes	0.18	0.19	0.22	0.24	0.19	0.17	0.11	0.13	0.16	0.17	0.15	0.22

x 10^9/L			(122%)	(133%)					(145%)	(155%)	(136%)	(200%)
LUC x 10^9/L	0.08	0.11	0.10	0.13* (163%)	0.07	0.08	0.05	0.07 (140%)	0.08 (160%)	0.09* (180%)	0.05	0.05
APTT sec	20.7	19.8	21.7	19.1	21.4*	17.1* (83%)	22.55	24.3	19.8*	19.6*	16.1* (71%)	17.5* (78%)

Clinical chemistry: Changes of clinical chemistry parameters were evident between control and treatment groups although the significance of most changes was unclear. There were no treatment-related histopathological findings in the liver or kidneys that appear to correlate with these changes.

Glyceraldehyde dehydrogenase (GLDH) activities were increased for males in GS573719 and GW642444 only groups. Potassium concentrations were increased for male and female treatment groups; this is a known pharmacological effect of β_2 agonists. Urea concentrations were slightly increased for males and females in all combination groups and males in the GW642444 only group. Creatinine concentrations were slightly increased for males and females in the high dose combination group and GSK573719 and GW642444 only groups. Glucose levels were slightly increased for males and females in the GSK573719 only group. Calcium levels were slightly decreased for males and females in the GSK573719 and GW642444 only groups. Triglyceride levels were slightly decreased for males in the high dose combination group. Cholesterol levels were slightly increased for females in the GSK573719 only group. ALT and AST values were slightly increased for males and females in the high dose combination group and the GW642444 only group.

Clinical chemistry parameters, week 4

Parameter	Males						Females					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
GLDH U/L	8	7	6	6	24* (300%)	11* (138%)	NC	NC	NC	NC	NC	NC
K+ mmol/L	3.8	4.3* (113%)	4.5* (118%)	4.7* (124%)	4.7* (124%)	4 8* (126%)	3.5	3.9* (111%)	4.2* (120%)	4.3* (123%)	4.1* (117%)	4.4* (126%)
Urea mmol/L	4.76	5.65* (119%)	5.83* (123%)	6.91* (145%)	4.81	5.46 (115%)	5.55	6.21 (112%)	6.06 (109%)	6.87* (124%)	4.72	5.66
Creatinine µmol/L	29	31	28	35* (121%)	32* (110%)	36* (124%)	33	32	31	36* (109%)	35 (106%)	39* (118%)
Glucose mmol/L	6.52	7.27	7.22	7.30	8.04 (123%)	6 39	5.90	6.57	5.76	5.79	7.90* (134%)	6.09
Ca2+ mmol/L	2.70	2.64	2.69	2.66	2.61* (97%)	2 54* (94%)	2.66	2.65	2.68	2.65	2.54* (96%)	2.52 (95%)
Triglyceride mmol/L	0.46	0.41	0.41	0.39 (85%)	0.51	0 34 (74%)	NC	NC	NC	NC	NC	NC
Cholesterol mmol/L	NC	NC	NC	NC	NC	NC	1.74	1.77	1.62	1.68	2.13 (122%)	1.82
ALT U/L	43	43	41	54* (126%)	47	49	34	32	33	43* (126%)	39	43 (126%)
AST U/L	75	80	74	88* (117%)	93	100* (133%)	81	81	81	90	93	108* (133%)

* Statistical significance was achieved, $p \leq 0.05$
NC = no change

Urinalysis: Urine volumes were increased for males and females in all combination groups and the GW642444 only group. Urinary creatinine concentrations were decreased for males and females in all combination groups and the GW642444 only group. Total creatinine output (TCRR) was increased for males and females in the GW642444 only group. Total glucose output (TGLR) was decreased for males in the high dose combination group and GSK573719 only group. The significance of these

differences between control and treatment groups appeared to be minimal as there were no treatment-related histopathological findings in the kidneys.

Urinalysis parameters, week 4

Parameter	Males						Females					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
Volume mL	8.1	9.4	13.0* (160%)	12.5* (154%)	6.9	12.9* (159%)	4.1	6.0 (146%)	5.9 (144%)	6.7* (163%)	4.7	6.5* (159%)
UCreatinine µmol/L	9888	8748 (89%)	6852* (69%)	7363* (75%)	9708	8205* (83%)	9277	6220* (67%)	5925* (64%)	6659* (72%)	8289	8871
TCRR µmol	75.995	NC	NC	NC	NC	103.05* (136%)	34.155	NC	NC	NC	NC	51.854* (152%)
TGLR µmol	6.407	6.372	6 249	3.279 (51%)	4.377 (68%)	8.018	NC	NC	NC	NC	NC	NC

NC = No Change

Gross pathology: Gross pathological findings were evident in the lungs + bronchi that consisted of congestion for 2 of 10 females that received the high dose of the combination and 1 of 10 females that received GSK573719 alone and pale area(s) for 4 of 10 females in each of the GSK573710 and GW642444 alone groups.

Organ weights: Absolute and relative organ weights were unaffected.

Histopathology: Histopathological findings were observed in the lungs + bronchi, tracheal bifurcation, liver, mandibular LN, larynx, nasopharynx, and nasal turbinates. Findings in the larynx were judged to be rat-specific irritant effects commonly associated with inhalation toxicology studies. Findings in the nasopharynx and nasal turbinates were associated with nose-only inhalation and judged to be irrelevant for oral inhalation in the clinical setting.

In the lungs + bronchi, findings of alveolar hemoglobin crystals with associated inflammatory cell infiltrate were observed in groups treated with combination of GSK573173 + GW642444. All findings were characterized as focal and minimal. There was no evidence of a dose-response relationship given that the dose of GSK573719 was relatively constant across all treatment groups and dose of GW642444 was increased, but the findings did not parallel the increased dose of GW642444. These findings appeared to be attributable to GSK573719 alone although the incidence was somewhat lower in this group. The sponsor indicated that this finding has been seen recently at a similar incidence in control Sprague-Dawley rats from the testing laboratory.

In the tracheal bifurcation, inflammatory cell infiltration was observed at increased incidences for males treated with the low dose of the combination or with either monoproduct alone and for females treated with the two lower doses of the combination or GW642444 alone. Loss of cilia, basophilia, and cell flattening at the bifurcation were observed at increased incidences for males treated with the low and high doses of the combination or GSK573719 alone and for females treated with the three doses of the combination or GSK573719 alone.

The incidence of focal hepatocyte necrosis was increased for females treated with the high dose of the combination.

For the mandibular LN, incidences of apoptosis of plasma cells, plasmacytosis, and prominent numbers of Russell bodies were increased for males treated with GSK573719 alone.

There multiple histopathological findings in the larynx. Minimal epithelial keratinization of the arytenoids was observed for males given the high dose of the combination. Epithelial keratinization of the ventral and lateral walls was observed for males and females give the high dose of the combination and females given GSK573719 alone. Erosion of the epithelium of the ventral and lateral walls was observed for males given the low dose of the combination. Inflammatory cell infiltrate in the lamina propria and epithelium of the ventral and lateral walls was observed for males and females given the low dose of the combination. Necrosis of the ventral cartilage was observed for males and females at all doses of the combination as well as GSK573719 alone. Squamous epithelial hyperplasia of the arytenoids was observed at all doses of the combination and both monoproducts alone. Squamous metaplasia of the ventral and lateral walls was observed at all doses of the combination as well as GSK573719 alone although the severity was increased with the combination.

Findings in the nasopharynx included goblet cell hyperplasia at the low and mid doses of the combination, inflammatory cell infiltration in the lamina propria for males and females the received the low dose of the combination, and degeneration/regeneration of the respiratory epithelium for male that received the low dose of the combination and males and females that received the high dose of the combination.

There were multiple findings in the nasal turbinates as detailed in the table below. Major findings included inflammatory cell infiltrate in the lamina propria of the nasolacrimal duct for males and females that received the low and mid dose of the combination or GSK573719 alone, atrophy and disorganization of the dorsal aspect and septum of the olfactory epithelium for males and females at all doses of the combination, degeneration/regeneration of the respiratory epithelium for males and females that received all doses of the combination or GSK573719 alone, goblet cell hyperplasia for males that received the low dose of the combination, mineralization of the respiratory epithelium for males that received all doses of the combination or GSK573719 alone, inflammatory cell infiltrate in the lamina propria of the respiratory epithelium for males and females that received the low dose of the combination, mineralization of the squamous epithelium for males that received the mid dose of the combination, inflammatory cell infiltrate in the lamina propria of the squamous epithelium for males and females that received the dose of the combination, degeneration/regeneration of vomeronasal organ at all doses of the combination or GSK573719 alone, and squamous metaplasia of the respiratory and olfactory epithelium of the vomeronasal organ for females receiving the high dose of the combination.

In the testes, seminiferous tubules were evaluated with respect to their stage in the spermatogenic cycle and the integrity of the various cell types present within the different stages. No cell or stage specific abnormalities considered to be treatment-related were identified.

Histopathological findings in the 28-day inhalation toxicology study with rats (GSK573719/GW642444)

Organ/Tissue	Males						Females					
	0/0	817/4.4	1200/61	1060/1040	757/0	0/869	0/0	817/4.4	1200/61	1060/1040	757/0	0/869
Lungs + Bronchi												
-alveolar hemoglobin crystals with associated inflammatory cell infiltrate	0/10	3/10	3/10	2/10	1/10	0/10	0/10	1/10	2/10	3/10	1/10	0/10
Tracheal bifurcation												
-inflammatory cell infiltration in lamina propria	1/10	4/10	0/10	0/10	3/10	3/9	1/10	2/10	2/10	0/10	1/10	3/10
-loss of cilia/basophilia/cell flattening at bifurcation	1/10	5/10	1/10	5/10	6/10	1/9	1/10	3/10	3/10	5/10	2/10	1/10
Liver												
-hepatocyte necrosis, focal	0/10	-	-	0/10	-	1/1	0/10	-	-	3/10	-	0/2
Mandibular LN												
-apoptosis (lymphocytolysis) plasma cells	0/10	0/4	0/3	0/10	5/8	0/1	0/10	0/1	-	0/10	0/4	-
-plasmacytosis	4/10	3/4	1/3	1/10	7/8	1/1	2/10	0/1	-	1/10	1/4	-
-prominent numbers of Russell bodies	0/10	1/4	0/3	1/10	3/8	0/1	0/10	0/1	-	0/10	0/4	-
Larynx												
-epithelial keratinization-arytenoids	0/10	0/10	0/9	2/10	0/10	0/9	0/10	0/10	1/10	0/10	0/10	0/10
-epithelial keratinization-ventral/lateral wall	0/10	1/10	1/9	5/10	0/10	0/9	0/10	1/10	2/10	5/10	3/10	0/10
-erosion-ventral/lateral wall epithelium	0/10	2/10	0/9	0/10	0/10	0/9	0/10	1/10	0/10	0/10	0/10	0/10
Larynx-Pouch												
-inflammatory cell infiltrate in lamina propria	0/10	5/10	0/10	0/10	0/10	1/9	0/10	2/10	0/10	0/10	0/10	0/10
Larynx-ventral/lateral epithelium												
-inflammatory cell infiltrate, ventral/lateral epithelium	0/10	4/10	0/10	0/10	0/10	0/9	0/10	1/10	0/10	0/10	0/10	0/10
-necrosis of ventral cartilage	0/10	1/10	8/10	10/10	9/10	0/9	0/10	10/10	10/10	10/10	10/10	0/10
-squamous epithelial hyperplasia-arytenoids	0/10	4/10	2/10	2/10	2/10	1/9	0/10	2/10	1/10	2/10	3/10	1/10
-squamous metaplasia-lateral wall	0/10	8/10	5/10	6/10	5/10	0/9	0/10	4/10	3/10	6/10	1/10	0/10
-squamous metaplasia-ventral	1/10	10/10	9/10	9/10	8/10	0/9	0/10	9/10	10/10	10/10	9/10	0/10
Nasopharynx												
-goblet cell hyperplasia	0/10	1/10	4/10	0/10	0/10	2/9	0/10	3/10	1/10	0/10	0/10	0/10
-lamina propria-inflammatory cell infiltration	0/10	1/10	0/10	1/10	0/10	0/9	1/10	2/10	0/10	0/10	0/10	0/10
-respiratory epithelium-degeneration/regeneration	0/10	3/10	0/10	6/10	0/10	0/9	0/10	0/10	0/10	1/10	0/10	0/10
Nasal turbinates												
-inflammatory cell exudate in airways	0/10	1/10	0/10	0/10	0/10	0/9	0/10	0/10	0/10	1/10	0/10	0/10
-inflammatory cell infiltrate, hard palate	0/10	0/10	0/10	0/10	0/10	0/9	0/10	0/10	0/10	0/10	2/10	0/10
-nasolacrimal duct, lamina propria-inflammatory cell infiltrate	0/10	3/10	2/10	0/10	6/10	1/9	0/10	6/10	4/10	0/10	2/10	0/10
-olfactory epithelium (dorsal aspect/septum)-	0/10	4/10	3/10	3/10	0/10	0/9	0/10	2/10	3/10	4/10	0/10	0/10

Organ/Tissue	Males						Females					
	0/0	817/4.4	1200/61	1060/1040	757/0	0/869	0/0	817/4.4	1200/61	1060/1040	757/0	0/869
atrophy/disorganization												
-olfactory epithelium-eosinophilic inclusions	0/10	1/10	0/10	0/10	0/10	0/9	0/10	0/10	0/10	0/10	0/10	0/10
-olfactory epithelium-glandular dilatation	0/10	0/10	0/10	0/10	0/10	1/9	0/10	0/10	0/10	0/10	1/10	0/10
-olfactory epithelium-mineralization	1/10	0/10	1/10	2/10	2/10	2/9	3/10	2/10	1/10	0/10	0/10	4/10
-olfactory epithelium, lamina propria-inflammatory cell infiltrate	1/10	3/10	0/10	1/10	1/10	1/9	2/10	3/10	1/10	1/10	0/10	0/10
-olfactory epithelium, turbinates-atrophy/disorganization	0/10	0/10	0/10	0/10	0/10	0/9	3/10	0/10	1/10	0/10	1/10	0/10
-olfactory nerve f bers-atrophy	0/10	2/10	1/10	1/10	0/10	0/9	0/10	1/10	0/10	1/10	0/10	0/10
-respiratory epithelium-degeneration/regeneration	0/10	6/10	8/10	8/10	4/10	0/9	0/10	3/10	2/10	6/10	2/10	0/10
-respiratory epithelium-goblet cell hyperplasia	2/10	6/10	1/10	1/10	1/10	1/9	0/10	1/10	0/10	1/10	0/10	0/10
-respiratory epithelium-mineralization	1/10	3/10	6/10	2/10	3/10	1/9	0/10	0/10	1/10	0/10	0/10	4/10
-respiratory epithelium-squamous metaplasia	0/10	0/10	0/10	1/10	1/10	0/9	0/10	0/10	0/10	1/10	0/10	0/10
-respiratory epithelium, lamina propria-inflammatory cell infiltrate	0/10	6/10	0/10	2/10	0/10	0/9	0/10	5/10	1/10	1/10	0/10	0/10
-ruptured keratin cyst(s), hard palate	0/10	0/10	0/10	0/10	0/10	0/9	0/10	1/10	0/10	0/10	0/10	0/10
-squamous epithelium-erosion	0/10	0/10	1/10	1/10	0/10	0/9	0/10	0/10	0/10	0/10	0/10	0/10
-squamous epithelium-mineralization	0/10	0/10	3/10	0/10	0/10	0/9	0/10	0/10	0/10	0/10	0/10	0/10
-squamous epithelium, lamina propria-inflammatory cell infiltrate	0/10	2/10	0/10	0/10	0/10	0/9	0/10	3/10	0/10	0/10	0/10	0/10
-vomeronasal organ-degeneration/regeneration	0/10	0/10	3/10	4/10	1/10	0/9	0/10	2/10	1/10	7/10	2/10	0/10
-vomeronasal organ-squamous metaplasia of respiratory and olfactory epithelium	0/10	0/10	0/10	0/10	0/10	0/9	0/10	0/10	0/10	3/10	1/10	0/10

Toxicokinetics: AUC and C_{max} values for GSK573719 were generally comparable alone and in combination with GW642444. However, AUC and C_{max} values for GW642444 tended to be lower in combination with GSK573719 as compared to alone.

GW642444 was not quantifiable in the low dose group. AUC and C_{max} values for GW642444 increased in an approximate dose proportional manner from the mid dose combination group to the high dose combination group.

GSK573719

Parameter+	Day	Male				Female			
		Estimated Achieved Dose GSK573719/GW642444				Estimated Achieved Dose GSK573719/GW642444			
		817/ 4.37	1200/ 60.7	1060/ 1040	757/0	817/ 4.37	1200/ 60.7	1060/ 1040	757/0
$AUC_{(0-t)}$ (ng.h/mL)	Day 1	26.1	22.8	60.4	22.7	24.5	26.4	41.3	23.5
	Day 14	34.3	39.6	35.0	37.1	30.2	38.4	26.5	19.7
	Day 28	28.6	27.6	30.7	28.0	18.0	22.8	17.0	28.8
C_{max} (ng/mL)	Day 1	9.80	7.20	29.7	8.33	5.72	7.20	19.1	6.81
	Day 14	7.35	10.8	9.54	22.0	4.90	18.0	12.7	3.79
	Day 28	6.54	4.80	9.54	5.30	4.09	3.60	6.36	6.06

+Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.

GW642444

Parameter+	Day	Male				Female			
		Estimated Achieved Dose GSK573719/GW642444				Estimated Achieved Dose GSK573719/GW642444			
		817/ 4.37	1200/ 60.7	1060/ 1040	0/869	817/ 4.37	1200/ 60.7	1060/ 1040	0/869
$AUC_{(0-t)}$ (ng.h/mL)	Day 1	NC	2.37	124	152	NC	6.13	112	141
	Day 14	NC	5.16	66.6	92.1	0.476	4.01	51.0	91.2
	Day 28	NC	2.97	70.7	124	NC	2.19	35.4	92.1
C_{max} (ng/mL)	Day 1	NC	0.971	33.3	30.4	NC	1.15	22.9	23.5
	Day 14	NC	1.52	16.6	28.7	NC	1.82	15.6	29.5
	Day 28	NC	1.34	13.5	29.5	NC	1.58	9.36	21.7

+Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.
NC Not calculated

All control samples were below the limit of quantification (0.1 ng/mL) for GW642444. GSK573719 was quantifiable in 11/143 samples from control rats (7 and 4 samples in Week 2 and 4 respectively). There was no obvious pattern to the quantifiable concentrations of GSK573719 in these samples and hence it is considered the source of the contamination to have occurred ex vivo, although no naïve plasma sample had any quantifiable concentrations of GSK573719 or GW642444.

In the groups given GSK573719 or GW642444 alone, a small proportion of the samples had quantifiable concentrations of the analyte that was not administered (17/144 samples for GSK573719 and 10/139 samples for GW642444), but these were randomly scattered between the assessment occasions and time points and concentrations were low (up to 2.93 ng/mL). This is considered to represent cross contamination of samples rather than animals but has not affected the interpretation of the toxicokinetic data.

Dogs

Study title: **GSK573719A and GW642444M: A 4-Week Combination Inhalation Toxicity Study in Dogs**

Key study findings:

● In a 4-week toxicology study, GSK573719A and GW642444M, in combination and alone, were given as dry powder formulations in lactose to dogs by oropharyngeal tube. Selection of the doses used in this study was based on the minimum and maximum combination ratios of GSK573719 or GW642444 to be given to humans in clinical studies (200:1 and 1:1 for GSK573719:GW642444). Estimated achieved doses were 0/0, 996/6.46, 190/205, 997/0, and 0/174 µg/kg/day (GSK573719/GW642444). A 3-day pre-adaptation phase was used for the 190/205 and 0/174 µg/kg/day groups.

● During the pre-adaptation phase for Groups 3 and 5, increased femoral pulse rates (up to 2-fold of baseline) were seen immediately postdose. During the treatment phase, increased femoral pulse rates (up to 2.2-fold of baseline) were seen immediately postdose in dogs given GSK573719 or GW642444 alone or in combination. The effect on pulse rates diminished over the duration of the study. ECG measurements taken immediately postdose during Week 4, confirmed increased heart rates, in males given GSK573719/GW642444 at 996/6.46 µg/kg/day and both sexes given GSK573719/GW642444 at 190/205 µg/kg/day, GSK573719 alone at 997 µg/kg/day, or GW642444 alone at 174 µg/kg/day. All dogs showed respiratory sinus arrhythmia (RSA) at pretest. RSA was observed during Week 4 (predose and postdose) in dogs that received GW642444 at 174 µg/kg/day alone or in combination with GSK573719 (Groups 3 and 5). RSA was not observed pre or post dose during week 4 in dogs given GSK573719 at a target dose of 997 µg/kg/day alone or in combination with GW642444 (Groups 2 and 4).

● On the first day of dosing during the dose escalation phase and treatment phase, increases of serum cardiac troponin (up to 142-fold of predose baseline) were observed for individual dogs that received GSK573719 or GW642444 alone or in combination. On day 23 of the treatment, serum cardiac troponin concentrations remained at baseline levels at all time points.

● Histopathological findings were observed in the heart, thymus, larynx, nose/turbinates, liver, spleen, and testes. Findings in the nasal turbinates might be irrelevant to the clinical setting where the combination drug product will be administered by oral inhalation.

● Papillary muscle fibrosis in the heart was observed for 1 female in the combination group receiving 190/205 µg/kg/day. This heart finding is a known effect of β_2 agonists. It is possible that the combined interaction of GSK573719 and GW642444 produced an enhanced cardiotoxicity given that this effect was not observed for GW642444 alone.

● The severity of thymic involution/atrophy was generally increased for treatment groups as compared to the air-control group.

● There was some evidence of irritant effects in the larynx and nose/turbinates for the 996/6.46 µg/kg/day and/or GSK573719 alone groups.

● AUC and C_{max} values for GSK573719 were unaffected by presence of GW642444. AUC and C_{max} values for GW642444 for males appeared to be generally higher in the presence of GSK573719 as compared to GW642444 alone. However, AUC values for GW642444 for females appeared to be generally higher for GW642444 alone as compared to in the presence of GSK573719.

● There was no evidence of additive or synergistic toxic effects with the combination. Toxic effects observed with the combination products were generally consistent with those observed with monoproducts alone. Many findings appeared to be consistent with general inflammatory or irritation effects and could not be ascribed as class effects.

Study no.: FD2009/00391/00
Volume #, and page #: Volumes 26 and 27, Pages 1-490
Conducting laboratory and location: (b) (4)
Date of study initiation: September 10, 2008
GLP compliance: No. Unaudited Draft Toxicology Study
QA report: yes ( ) no (X) Unaudited Draft Toxicology Study
Drug, lot #, and % purity: Micronized GSK573719A and GW642444M were blended at (b) (4). Test article blends were prepared at (b) (4). Each blend was prepared in the required proportions of test articles in vehicle (Lactose [Batch No. 071148193] with a nominal 1 % (w/w) magnesium stearate [Batch No. 071140952]). See the table below.

Material	Input Material Batch number	Input Material Purity	Blend Batch Number	Blend test article concentration (nominal)	Content uniformity	Blend Expiry/ Retest	Blend Storage
GSK573719A GW642444M (Group 2)	R360972 K021652	99.8% 99.2%	BVR/P/1022/08	30% w/w 0.15% w/w	97.2% 93.6%	28-May-09	2 to 8°C, protect from light
GSK573719A (Group 3)	R360972 K021652	99.8% 99.2%	BVR/P/1020/08	10% w/w 10% w/w	97.2% 97.2%	29-May-09	2 to 8°C, protect from light
(Group4)	R360972	99.8%	BVR/P/1021/08	30% w/w	95.8%	8-Jun-09	30 °C, protect from light
GW642444M (Group 5)	K021652	99.2%	BVR/P/1019/08	10% w/w	94.7%	28-May-09	2 to 8 °C, protect from light

Methods
Doses:

Phase 1 (Tolerance Phase, Days -3 to -1; Groups 3 and 5 only)
The sponsor stated that "Tachycardia is a known and expected pharmacological effect following administration of β2-agonists, the magnitude and duration of which diminishes upon repeat dosing." To induce tachyphylaxis to the expected cardiovascular effects of GW642444 (a β2-agonist) at a target dose of 160 µg/kg/day prior to the 4-week dosing period, dogs (3/sex/group) in Groups 3 and 5 were given a lower dose for 3 consecutive days (Phase 1) immediately prior to Phase 2 as follows:

Group	Blend	Target aerosol concentration	Average achieved aerosol concentration	Target dose	Estimated achieved dose
		(µg/L)[1]	(µg/L)[1, 2, 5]	(µg/kg/day)[1, 3]	(µg/kg/day)[1, 4, 5]
3	GSK573719/ GW642444	56/12	12.4/14.4	187/40	42.0/48.7
5	GW642444	12	14.1	40	47.4

1. Expressed in terms of GSK573719 or GW642444 (the parent compound), as appropriate.
2. Based on chemical analysis.
3. The dosage is calculated as the total inhaled body dose assuming an exposure duration of 10 minutes and a body weight of 8.5 kg (inhalable fraction F not included in calculation).

$$\text{Dose (µg/kg/day)} = \frac{\text{C (µg/L) x RMV (L/min) x D (min)}}{\text{BW (kg)}}$$

Where C = Concentration (µg/L) in air inhaled of GSK573719 or GW642444
RMV = Respiratory minute volume (L/minute).
The RMV is calculated from the formula
RMV (L/minute) = 0.499 x BW (kg) $^{0.809}$ (Bide, 2000)
D = Duration of exposure in minutes
BW = Bodyweight (kg)

4. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation above; the body weights used were group averages for Phase 1.
5. Results for GSK573719 were lower than that given as target as a result of the principal target during Phase 1 being GW642444M.

Phase 2 (4-Week Treatment Period, Days 1 to 29; all groups)

Selection of target doses used in this study was based on the likely minimum and maximum combination ratios (1:1 and 200:1 for GSK573719:GW642444) of GSK573719 with GW642444 to be given to humans in clinical studies. Target doses of 0/0, 1000/5, 187/160, 1000/0, and 0/160 µg/kg/day (GSK573719/GW642444) were selected. Beginning on day 1 and throughout the 4-week dosing period, animals in Groups 1 to 5 (3/sex/group) were administered doses as detailed in the following table:

Group	Blend	Target aerosol concentration	Average achieved aerosol concentration	Target dose	Estimated achieved dose	MMAD	GSD
		(µg/L)[1]	(µg/L)[1, 2]	(µg/kg/day)[1, 3]	(µg/kg/day)[1, 4]	(vm)	
1	Lactose/1% Magnesium Stearate Control	0	0	0	0	[redacted]	(b) (4)
2	GSK573719 /GW642444	300/1.5	298/1.93	1000/5	996/6.46		
3	GSK573719 /GW642444	56/48	56.8/61.5	187/160	190/205		
4	GSK573719	300	297	1000	997		
5	GW642444	48	52.1	160	174		

1. Expressed in terms of GSK573719 or GW642444 (the parent compound), as appropriate.
2. Based on chemical analysis.
3. The dosage is calculated as the total inhaled body dose assuming an exposure duration of 10 minutes and a body weight of 8.5 kg (inhalable fraction F not included in calculation).

$$\text{Dose (µg/kg/day)} = \frac{\text{C (µg/L) x RMV (L/min) x D (min)}}{\text{BW (kg)}}$$

Where C = Concentration (µg/L) in air inhaled of GSK573719 or GW642444
RMV = Respiratory minute volume (L/minute).
The RMV is calculated from the formula
RMV (L/minute) = 0.499 x BW (kg) $^{0.809}$ (Bide, 2000)
D = Duration of exposure in minutes
BW = Bodyweight (kg)

4. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation above; however, the body weights used were group averages for the study.

NA = Not applicable.

GSK573719: Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day

Sex	Dose µg/kg/day	Deposited Dose[1] µg/kg/day	BW G	Total Dose µg/day	LW g	Deposited Dose µg/g LW/day
Males	996	249	10.5	2614.5	92.907	28.14
	190	47.5	10.8	513.0	87.448	5.87
	997	249	9.9	2465	88.400	27.89
Females	996	249	6.8	1693.2	66.950	25.3
	190	47.5	7.1	337.25	67.678	4.98
	997	249	7.2	1792.8	67.711	26.48

1. A deposition factor of 0.25 was used in calculations.
2. Mean terminal body weights at the end of the treatment period.
3. Absolute lung weights from the sponsor's data.

GW642444: Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day

Sex	Dose µg/kg/day	Deposited Dose[1] µg/kg/day	BW G	Total Dose µg/day	LW g	Deposited Dose µg/g LW/day
Males	6.46	1.62	10.5	17.01	92.907	0.183
	205	51.3	10.8	554.0	87.448	6.34
	174	43.5	10.8	469.8	93.753	5.01
Females	6.46	1.62	6.8	11.01	66.950	0.1645
	205	51.3	7.1	364.2	67.678	5.38
	174	43.5	7.2	313.2	65.566	4.78

1. A deposition factor of 0.25 was used in calculations.
2. Mean terminal body weights at the end of the treatment period.
3. Absolute lung weights from the sponsor's data.

Overall Average Tube Aerosol Concentration and Estimated Doses of Magnesium Stearate

Study Design			
Group Number	Estimated Achieved Dose (µg/kg/day) [a, b]	Achieved Tube Aerosol Concentration (µg/L)	Number/Sex
1	34.8	10.4	3
2	27.2	8.13	3
3	16.3	4.86	3
4	25.4	7.57	3
5	13.2	3.97	3

a. Expressed in terms of Magnesium Stearate, as determined by atomic absorption spectroscopy.
b. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)
D Dose (µg]/kg/day)
RMV 0.499xBW(kg)$^{0.809}$ (L/min) [Bide, 2000].
T Duration of exposure/day (minutes)
C Tube aerosol concentration (µg/L)
b. BW Group average body weight for study (kg)

Aerosol concentrations: The concentration of magnesium stearate in the aerosol given to Group 1 was targeted to be the same as the highest concentration of magnesium stearate, which was found in Group 2. A minimum of 2 samples per group were collected on each day of exposure. A measured volume of air was drawn from exposure chambers through a microfiber filter, fitted to an open-faced filter holder attached to a spare animal breathing point. On each day and from all groups, the first sample taken from the exposure system was designated for analysis of GSK573719 and/or GW642444, as appropriate. The second sample was then taken and designated for analysis of magnesium content. The glass fiber filters analyzed for GSK573719 and for GW642444 content utilized a single HPLC method with UV detection. Magnesium stearate concentration was determined by analyzing the quartz filters for magnesium using atomic absorption spectroscopy.

Particle size distribution: A measured volume of air was drawn through a cascade impactor. Samples were collected during weeks 1 and 4 for gravimetric (where possible) and chemical analyses. Samples for chemical analysis of GSK573719 (Groups 2, 3 and 4) and GW642444 (Groups 2, 3 and 5), and for magnesium stearate (all groups) were collected separately.

Species/strain: Male and Female beagle dogs were obtained from (b) (4)

Number/sex/group or time point (main study): 3 dogs/sex/group
Route, formulation, volume, and infusion rate: Exposures consisted of 10 min/day administered using an oropharyngeal tube. Each aerosol delivery system consisted of a dust generator (Wright Dust Feed), an aerosol conditioning chamber fitted with 4 outlets, aerosol delivery tubing, Y-piece connections with isolating ball valve, and oropharyngeal tube attached. The aerosol exhaust system included a scavenger exhaust system consisting of a chamber of identical dimensions to the sub-chamber used in the delivery system, further aerosol tubing, and a series of particulate filters, a flow meter with integral needle valve, and an extract pump. A separate atmosphere generation system was employed to dose each group of animals.

The compressed air flow rate exiting the Wright Dust Feed (WDF) for Groups 1, 2, 4 and 5, was set to 20 L/min and an additional dilution flow of compressed air was supplied at 10 L/min to the conditioning chamber, by an inlet that was adjacent to the aerosol entry point. The aerosol-laden air exiting the conditioning chamber (30 L/min, equal to 7.5 L/min/animal and each of 2 spare positions for sampling) was to 4 active outlets to potential animal exposure positions by clinical aerosol tubing with a Y-piece providing the attachment point for the ball valve and oropharyngeal tube. For Group 3, the air flow rate exiting the WDF was initially set to 15 L/min and the dilution flow was supplied at 15 L/min. Two aerosol outlets were used to supply aerosol to the animals assigned to the study and the remaining outlets were used for the collection of aerosol samples.
Satellite groups used for toxicokinetics or recovery: None
Age: Dogs were 10 to 11 months old on day 1.
Weight: Body weight ranges for males at start of Phases 1 and 2 were 8.8-10.9 kg and 8.9-11.4 kg, respectively. Body weight ranges for females at start of Phases 1 and 2 were 5.7-7.2 kg and 5.7-8.0 kg, respectively.
Unique study design or methodology (if any): As described above, a pre-adaptation phase was used to induce tachyphylaxis to the cardiovascular effects of the β2 agonist, GW642444.

Observation and Times:
Clinical signs: Viability checks were performed twice daily. Clinical observations during the treatment period were conducted during and immediately after dosing, 0.5 and 2 hr after completion of dosing, and as late as possible during the day.
Body weights: Body weights were measured weekly.
Food consumption: Food consumption was measured daily.

Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of treatment and during week 4. Rate of tear production was measured using the Schirmer tear test pretest and during Phase 2 (weeks 1 and 4) predose and within 10 min after completion of dosing.
EKG: Electrocardiograms were recorded from unanesthetized animals during the pretreatment period and during week 4 using leads I, II, III, aVR, aVL, aVF, CV_5RL (rV2), CV_6LL (V2), CV_6LU (V4) and V10. Tracing were examined for abnormalities and heart rate. Femoral pulse was measured during Phase 1 for all animals in Groups 3 and 5 daily. Measurements were made predose, within 10 min after completion of dosing and at 0.5, 1, 2, 4, 6, 8 and 23 hr 50 min after the completion of dosing. Femoral pulse was measured during Phase 2 for all animals on days 1, 2, 3, and 7, and during week 4. On day 1 and during week 4, measurements were made predose, within 10 min after completion of dosing, and at 0.5, 1, 2, 4, 6, 8 and 23 hours 50 minutes. On days 2, 3, and 7, measurements were made predose and within 10 min (immediately after dosing). Blood samples for measurement of cardiac troponin I were collected from all animals in Groups 3 and 5 on day -3 of Phase 1, and from all animals on day 1 of Phase 2 and during Week 4 pre-dose and 4, 8 and 23 hours 50 minutes after the completion of dosing. TnI was measured using a 3-site sandwich immunoassay using direct chemiluminometric technology qualified for use with dogs. The LLOQ was 0.006 µg/L. Timing of measurements appeared to be adequate to assess effects when C_{max} values were achieved.
Hematology: Blood samples for measurement of a complete set of hematology parameters were collected during weeks -2 and 4.
Clinical chemistry: Blood samples for measurement of a complete set of clinical chemistry parameters were collected during weeks -2 and 4.
Urinalysis: Urine samples were collected overnight (~16 hr) during weeks -2 and 4 for a complete assessment of urinalysis parameters.
Gross pathology: After the 4-week treatment period, dogs were sacrificed and submitted to gross necropsy examinations.
Organ weights: Absolute and relative organ weights were measured for the adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, testes, and thymus.
Histopathology: Samples were processed to paraffin, sectioned (4-5 µm), stained with hematoxylin and eosin, and examined by light microscopy. Histopathological examinations of organs and tissues appeared to be complete for the control and combination groups; however, examinations were limited for groups receiving monoproducts alone. A peer review of selected microscopic tissue sections and pathology data interpretation was completed. An additional peer review was performed by the sponsor. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.
Toxicokinetics: In Phase 2 on day 1 and during weeks 2 and 4, blood samples for measurement of plasma drug concentrations were collected immediately after the completion of dosing (within 10 min) and at 0.5, 1, 2, 4, 8, and 23 hr 50 min after the completion of dosing. Plasma samples collected from naïve dogs were available to assess potential contamination during processing of samples from drug-treated dogs. Samples were shipped to the sponsor for analysis. Concentrations of GSK573719 and GW642444 in plasma from all animals (including controls) were determined by HPLC-

MS/MS by GSK PCD-DMPK (Ware).The LLOQ for GSK573719 and GW642444 was 0.1 ng/mL.

Results:

Mortality: None.

Clinical signs: Swelling of the neck, observed immediately postdose, and dry mouth were recorded in all groups given GSK573719 or GW642444, alone or in combination. The findings were observed at a greater frequency in dogs that received GSK573719 alone at an achieved dose of 997 µg/kg/day as compared to dogs that received GSK573719 in combination with GW642444. There clinical signs are generally characteristic of muscarinic antagonists.

Body weights: Body weights gains for males and females in Group 3 and male and females in Group 5 were increased, which was an expected effect of treatment with a β_2-agonist.

Body weight gain (% increase or decrease of initial body weight at week 1)

	Group 1	Group 2	Group 3	Group 4	Group 5
Males	-2.0%	1.9%	9.3%	-2.9%	8.0%
Females	-2.9%	3.0%	9.2%	3.0%	9.1%

Food consumption: Food consumption for females in Group 2 and males and females in Group 4 were reduced to 85, 83.5, and 87.8% of controls, respectively. Food consumption for males and females in Group 5 were increased to 119.9 and 107.9% of controls, respectively, which was an expected effect of treatment with a β_2-agonist.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

Decreased tear production (Schirmer tear test) was noted in animals given GSK573719 alone or in combination with GW642444.

EKG:
Femoral Pulse Rate/ECG Measurements: During the dose escalation phase for Groups 3 and 5, increased femoral pulse rates (up to 1.96X predose) were seen immediately postdose and returned to baseline from 8 to 24 hr postdose. During the treatment phase, increased femoral pulse rates (up to 2.22X predose) were seen immediately postdose in dogs given GSK573719 or GW642444 alone or in combination. The effect on pulse rates diminished (tachyphylaxis) over the duration of the study. ECG measurements taken immediately postdose during Week 4, confirmed increased heart rates (decreased RR), in males given GSK573719/GW642444 at 996/6.46 µg/kg/day (1.67X predose) and both sexes given GSK573719/GW642444 at 190/205 µg/kg/day (males: 1.77X; females: 2.07X predose), GSK573719 alone at 997 µg/kg/day (males: 1.23X; females: 1.30X predose), or GW642444 alone at 174 µg/kg/day (males: 1.77X; females: 1.67X predose). All dogs showed respiratory sinus arrhythmia (RSA) at

pretest. RSA was observed during Week 4 (predose and postdose) in dogs that received GW642444 at 174 µg/kg/day alone or in combination with GSK573719 (Groups 3 and 5). RSA was not observed pre or post dose during week 4 in dogs given GSK573719 at a target dose of 997 µg/kg/day alone or in combination with GW642444 (Groups 2 and 4).

Troponin I (cTnI) Measurements: During the dose escalation phase (Phase 1, Day -3), cTnI levels for females receiving GW642444 at 40 µg/kg/day were increased 14-fold at 4 hr, 13-fold at 8 hr, and 3.4-fold at 23 hr 50 min as compared to predose levels. cTnI concentrations reached a maximum of 0.2123 µg/L for these females. No changes were seen for males receiving GW642444 at 40 µg/kg/day (observed levels were within the biological variation of the baseline). For males receiving GSK573719/GW642444 at 187/40 µg/kg/day, cTnI levels were increased up 53-fold at 4 hr, 58-fold at 8 hr, and 17-fold at 23 hr 50 min as compared to predose levels. cTnI levels at 8 hr reached a maximum of 1.0417 µg/L. Female #3800 that received GSK573719/GW642444 at 187/40 µg/kg/day had cTnI levels that were increased 127-fold at 4 hr and 142-fold at 8 hr. Levels were recovering by 23 hr 50 min after the completion of dosing.

During the treatment phase (Phase 2, Day 1) on day 1 for males that received GSK573719 at 1000 µg/kg/day, cTnI levels were increased up 7.6-fold at 4 hr and 5.7-fold at 8 hr. cTnI reached a maximum level of 0.2253 µg/L. For female #4802 that received GSK573719 at 1000 µg/kg/day, cTnI levels were increased up to 5.1-fold at 4 and 8 hr and 3.1-fold at 23 hr 50 min. For dogs that received GSK573719/GW642444 at 1000/5 µg/kg/day, only male #2301 (up to 0.159 µg/L, 6.9-fold of its baseline), female #2801 (up to 0.304 µg/L, 25-fold of its baseline), and female #2802 (up to 4-fold of its baseline) demonstrated increases of cTnI at 4 and 8 hr. Male #2301 had recovered by 23 hr 50 min, but female #2801 still had elevated levels (0.232 µg/L, 19-fold). For dogs that received GSK573719/GW642444 at 187/160 µg/kg/day, female #3800 showed increases up to 0.603 µg/L or 5.6-fold of baseline at 4 and 8 hr. Levels had returned to baseline by 23 hr 50 min. Minimal changes were evident for male dogs. For dogs that received GW642444 at 160 µg/kg/day, cTnI levels for males were increased up to 23-fold at 4 hr and 18-fold at 8 hr. Female #5800 showed increases of cTnI up to 0.499 µg/L or 33-fold of baseline at 4 and 8 hr. Levels were returning toward baseline at 23 hr 50 min.

During the treatment phase (Phase 2, Day 23) on day 23, cTnI levels were at baseline at all time points in the previously affected animals.

The tables of troponin I concentrations provided by the sponsor were not clearly legible.

Findings of increased heart rate and increased troponin I concentrations may correlate with histopathological findings of papillary muscle fibrosis and mineralization for 1 female receiving GSK573719/GW642444 at 190/205 µg/kg/day.

Week 4: HR change from predose and presence/absence of RSA

Group 2: GSK573719/GW642444 1000/5 µg/kg/day

Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose	Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose
2300m	↑↑ (65)	+	–	–	2800f	↑ (15)	+	–	–
2301m	↑↑ (55)	+	–	–	2801f	(-20)	+	–	–
2302m	↑↑ (85)	+	–	–	2802f	(0)	+	–	–

Group 3: GSK573719/GW642444 187/160 µg/kg/day

Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose	Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose
3300m	↑↑ (70)	+	–	–	3800f	↑↑ (60)	+	+	–
					3800f*	(-15)		+	+
3301m	↑↑ (30)	+	+	–	3801f	↑↑ (110)	+	+	–
3302m	↑↑ (130)	+	+	–	3802f	↑↑ (135)	+	+	–

*Due to outside of the acceptable time variance of original, ECG collection was repeated.

Group 4: GSK573719 1000 µg/kg/day

Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose	Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose
4300m	↑↑ (37)	+	–	–	4800f	↑ (17)	+	–	–
4301m	↑↑ (30)	+	–	–	4801f	↑↑ (42)	+	–	–
4302m	↑(18)	+	–	–	4802f	↑↑ (55)	+	–	–

Group 5: GW642444 160 µg/kg/day

Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose	Animal No.	HR Change	RSA Pretest	RSA Week 4 Predose	RSA Week 4 Postdose
5300m	↑↑ (67)	+	+	–	5800f	↑↑(70)	+	+	+
5301m	↑↑ (40)	+	+	+	5801f	↑↑(30)	+	+	+
5302m	↑↑ (80)	+	+	–	5802f	↑↑ (60)	+	+	+

RSA : respiratory sinus arrhythmia
M : male,
F : female
+ : presence of RSA
- :absence of RSA
↑ : presence of increased HR compared to predose
↑↑ : presence of obvious increased HR compared to predose
( ) : values of difference between pre and postdose

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Hematology: During week 4, there were slight decreases (up to 16%) of hemoglobin concentration, hematocrit, and RBC counts for male treatment groups as compared to the control. These parameters were also decreased for female treatment groups although differences from the control were not as marked as compared to males. It was not possible to attribute these effects to the combination or monoproducts alone.

Clinical chemistry: Alkaline phosphatase (ALKP) activities during week 4 were increased 1.8-fold for males that received GW642444 alone at 174 µg/kg/day, 1.4 to 1.5-fold for male and female dogs that received GSK573719 + GW642444 at 190/205 µg/kg/day, and 1.4-fold for male dogs that received GSK573719 + GW642444 at 996/6.46 µg/kg/day. AST activity was slightly increased 1.3-fold for females that received GW642444 alone. Glyceraldehyde dehydrogenase (GLDH) activities were increased 1.31- to 1.55-fold for males and females in Groups 2 and 4, which was attributed to the effects of GSK573719. BUN concentrations during week 4 were increased 1.35- to 1.53-fold for males in Groups 2 and 3 and 1.4-fold for females in Groups 2, 3, and 4. Triglyceride levels were elevated 1.8- to 2.3-fold for males in

Groups 2 and 3 and 1.4-fold for females in Groups 3 and 5. Cholesterol levels were slightly increased up to approximately 1.18-fold for males in Groups 3 and 5.

Clinical chemistry parameters, week 4

Parameter	Males					Females				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
ALKP U/L	57	77 (135%)	77 (135%)	58	101 (177%)	89	70	133 (149%)	74	97
AST U/L	NC	NC	NC	NC	NC	33	26	32	36	44 (133%)
GLDH U/L	4.9	6.4 (131%)	4.6	7.6 (155%)	4.9	6.0	8.2 (137%)	5.8	9.0 (150%)	7.4
BUN mg/dL	17	23 (135%)	26 (153%)	17	19	16	22 (138%)	22 (138%)	22 (138%)	19
Triglyceride mg/dL	36	63 (175%)	84 (233%)	40	45	45	54	61 (136%)	53	61 (136%)
Cholesterol mg/dL	146	158	166 (114%)	135	172 (118%)	NC	NC	NC	NC	NC

NC = No Change

Urinalysis: Increases of creatinine concentration, creatinine output, and protein output were observed for male and female treatment groups; however, the significance of these differences was unclear given the lack of histopathological findings in the kidneys. These effects could not be attributed to the combination or monoproducts alone. Creatinine concentrations were increased for males in Groups 2-5 (187-214.5% of the control) and females in Groups 2-5 (136.7-159.8%). Creatinine output was increased for males in Groups 2-5 (139-222.1% of the control) and females in Groups 3-5 (113-227.3%). Protein output was increased for males in Groups 2-5 (143-200% of the control) and females in Groups 3-5 (130-200%).

Gross pathology: Gross pathological findings were observed in the gallbladder and thymus that were potentially related to treatment. There were no corresponding histopathological findings in the gallbladder although this finding was observed in other dog studies with GSK573719 and was judged to be a treatment-related effect (this finding appears to be a potential class effect of muscarinic antagonists although it is not well documented). Findings in the thymus corresponded with histopathological findings of involution/atrophy.

Gross pathological findings

Organ/Tissue	Males					Females				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Gallbladder -abnormal contents	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
Thymus -small	0/3	1/3	2/3	1/3	0/3	0/3	0/3	1/3	0/3	0/3

Organ weights: Absolute and relative thymus weights were decreased for males in Groups 2, 3, and 4 and females in Group 3, which may correspond to histopathological findings of involution/atrophy. Absolute and relative testes weights were decreased for males in Group 2, which may correspond to histopathological findings of segmental hypoplasia. Additional findings included decreased prostate weights for males in Groups 3, 4, and 5, decreased heart weights for females in Group 2, decreased kidney weights for females in Group 4, and decreased ovaries weights for females in Groups 2, 3, and 4; however, there were no corresponding histopathological changes.

Organ weights, week 4

Parameter	Males					Females				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Thymus g	6.569	4.668 (71%)	3.698 (56%)	5.359 (82%)	7.429	4.617	4.425	2.997 (65%)	5.907	5.972
Thymus %BW	0.067	0.043 (64%)	0.034 (51%)	0.053 (79%)	0.068	0.068	0.067	0.043 (63%)	0.090	0.085
Thymus %BrW	8.693	6.446 (74%)	5.036 (58%)	7.322	9.672	6.810	6.636	4.491 (66%)	8.797	9.145
Testes g	12.073	10.807 (90%)	NC	NC	NC					
Testes %BW	0.123	0.102 (82%)	NC	NC	NC					
Testes %BrW	16.105	14.547 (90%)	NC	NC	NC					

NC = No Change

Histopathology: Histopathological findings were observed in the heart, thymus, larynx, nose/turbinates, liver, spleen, and testes. Findings in the nasal turbinates might be irrelevant to the clinical setting where the combination drug product will be administered by oral inhalation.

Papillary muscle fibrosis in the heart was observed for 1 female in the combination group receiving 190/205 µg/kg/day. This heart finding is a known effect of β_2 agonists. It is possible that the combined interaction of GSK573719 and GW642444 produced an enhanced cardiotoxicity given that this effect was not observed for GW642444 alone.

The severity of thymic involution/atrophy was generally increased for treatment groups as compared to the air-control group.

There was some evidence of irritant effects in the larynx and nose/turbinates for the 996/6.46 µg/kg/day and/or GSK573719 alone groups.

There were additional findings in the liver, spleen, and testes that might potentially be related to treatment with combination of GSK573719 and GW642444.

Histopathological findings in the 28-day inhalation toxicology study with dogs (GSK573719/GW642444)

Organ/Tissue	Males					Females				
	0/ 0	996/ 6.46	190/ 205	997/ 0	0/ 174	0/ 0	996/ 6.46	190/ 205	997/ 0	0/ 174
Heart -papillary muscle: fibrosis with mineralization, Grade 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
Thymus -involution/atrophy (total) Grade 2 Grade 3 Grade 4	3/3 2 1 0	3/3 0 2 1	3/3 0 2 1	3/3 0 1 2	3/3 1 1 1	3/3 2 1 0	3/3 1 2 0	3/3 2 0 1	3/3 1 0 2	3/3 1 2 0
Larynx -erosion/ulcer, Grade 1-3 -acute/subacute inflammation, Grade 1-2 -epithelial, degeneration/ regeneration, Grade 2 -squamous epithelial hyperplasia, Grade 1	0/3 0/3 0/3 0/3	1/3 1/3 1/3 0/3	0/3 0/3 0/3 0/3	1/3 2/3 0/3 1/3	0/3 0/3 0/3 0/3	0/3 0/3 0/3 0/3	0/3 0/3 0/3 0/3	0/3 0/3 0/3 0/3	0/3 0/3 0/3 0/3	0/3 0/3 0/3 0/3
Nose/Turbinates -nasal mucosa (olfactory): epithelium degeneration/ atrophy, Grade 1 -paranasal sinuses: maxillary gland(s)/ducts(s) dilated -nasolacrimal duct(s): mucosa-mixed inflammatory cells (primarily lymphoid cells admixed with a small and variable number of neutrophils and/or eosinophils	0/3 0/3 0/3	0/3 0/3 1/3	0/3 0/3 0/3	- - -	- - -	0/3 0/3 0/3	1/3 1/3 0/3	0/3 0/3 0/3	- - -	- - -
Liver -decreased hepatocyte rarefaction, Grade 2	0/3	0/3	0/3	-	-	0/3	0/3	1/2	-	-
Spleen -increased extramedullary hematopoiesis	0/3	1/3	1/3	-	0/1	0/3	0/3	0/3	-	-
Testes -segmental hypoplasia, Grade 1-2	0/3	1/3	1/3	-	-					

Note: Histopathological examinations of organs and tissues appeared to be completed for the control and combination groups; however, examinations were limited for groups receiving monoproducts. Dashes indicate that the group was not examined.

Toxicokinetics: AUC and C_{max} values for GSK573719 were unaffected by presence of GW642444. AUC and C_{max} values for GSK573719 appeared to be higher in males as compared to females.

AUC and C_{max} values for GW642444 for males appeared to be generally higher in the presence of GSK573719 as compared to GW642444 alone. However, AUC values for GW642444 for females appeared to be generally higher for GW642444 alone as compared to in the presence of GSK573719. C_{max} values for GW642444 in females were generally comparable for GW642444 alone or in the presence of GSK573719. AUC values for GW642444 in the presence of GSK573719 appeared to be higher in males as compared to females; however, AUC values for GW642444 were relatively comparable between males and females.

A summary of the toxicokinetic parameters for GSK573719 and GW642444 corrected for the overall estimated achieved doses in male and female beagle dogs is presented below:

Toxicokinetic Parameter+	Phase 2	Male					Female				
		Overall Estimated Achieved Dose GSK573719					Overall Estimated Achieved Dose GSK573719				
		0 µg/kg/day	996 µg/kg/day	190 µg/kg/day	997 µg/kg/day	0 µg/kg/day	0 µg/kg/day	996 µg/kg/day	190 µg/kg/day	997 µg/kg/day	0 µg/kg/day
$AUC_{(0-t)}$ (ng.h/mL)	Day 1	NC	78.7	5.7	59.8	NC	NC	45.8	4.18	33.9	NC
	Day 12	NC	112	14.6	113	NC	NC	55.8	8.93	48.9	NC
	Day 27	NC	97.6	15.6	101	NC	NC	55.8	10.1	65.8	NC
C_{max} (ng/mL)	Day 1	NC	137	9.88	65.8	NC	NC	54.8	4.94	44.9	NC
	Day 12	NC	113	12.9	105	NC	NC	38.8	5.70	38.9	NC
	Day 27	NC	98.6	14.3	122	NC	NC	39.8	9.12	92.7	NC

+Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.
NC Not calculated due to plasma results being below the limit of quantification (LOQ).

Toxicokinetic Parameter+	Phase 2	Male					Female				
		Overall Estimated Achieved Dose GW642444					Overall Estimated Achieved Dose GW642444				
		0 µg/kg/day	6.46 µg/kg/day	205 µg/kg/day	0 µg/kg/day	174 µg/kg/day	0 µg/kg/day	6.46 µg/kg/day	205 µg/kg/day	0 µg/kg/day	174 µg/kg/day
$AUC_{(0-t)}$ (ng.h/mL)	Day 1	NC	13.6	221	NC	139	NC	14.4	83.6	NC	157
	Day 12	NC	17.2	373	NC	212	NC	9.88	76.5	NC	231
	Day 27	NC	11.1	252	NC	165	NC	8.08	146	NC	180
C_{max} (ng/mL)	Day 1	NC	12.5	75.0	NC	43.0	NC	9.95	40.2	NC	45.4
	Day 12	NC	10.5	67.4	NC	109	NC	6.31	42.0	NC	90.8
	Day 27	NC	6.65	149	NC	60.7	NC	4.41	65.8	NC	63.2

+Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.
NC Not calculated due to plasma results being below the LOQ.

Histopathology inventory (optional)

Study	GSK573719+GW642444	
	28-days FD2009/00392/00	28-days FD2009/00391/00
Species	Rat	Dog
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear		
Bone (femur)	X	X
Brain	X*	X*
Bronchi	w/Lungs	w/Lungs
Cecum	X	X
Cervix		X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gal 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		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Lymph nodes, popliteal		X
Lymph nodes, tracheobronchial	X	X
Mammary Gland	X	X
Nasal cavity	X	X
Optic nerves	X	
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx	X	X
Pituitary	X	X
Prostate	X*	X*
Rectum		
Salivary gland, submandibular	X	X
Salivary gland, sublingual	X	
Salivary gland, parotid	X	X
Sciatic nerve	X	X
Seminal vesicles	X	
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X

Spleen	X*	X
Sternum	X (BM)	X (BM)
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X	X
Tongue	X	X
Tonsils		X
Trachea	X	X
Tracheal bifurcation	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

2.6.6.9 Discussion and Conclusions

The sponsor has conducted 28-day inhalation toxicology studies with GSK573719 and GW642444 in rats and dogs. For rats, the primary histopathological findings were observed in the larynx, nasopharynx, and nasal turbinates that were judged to be generally irrelevant to human receiving the combination drug product by oral inhalation. Additional findings in the lung, tracheal bifurcation, liver, and mandibular LN were of uncertain relationship to treatment with the combination of GSK573719 and GW642444. The dog was sensitive to the cardiovascular effects of the combination of GSK573719 and GW642444 whereas the rat appeared to be insensitive. The dog was observed with some effects of local irritation in the larynx and nasal turbinates that were also evident in the rat. It appears that the dog would be the most relevant species for a 13 week study to assess the potential toxic effects of the combination of GSK573719 and GW642444. The dog was judged to be the most relevant species in studies with other combinations of long acting muscarinic antagonist and long acting β_2 agonists. Under IND (b) (4) the Agency concurred with the sponsor that the dog was the most relevant species.

Toxic effects observed with the combination product in rats and dogs were generally consistent with those observed with monoproducts alone. Cardiac effects observed in dogs could be attributed to the pharmacological effects of both agents. Findings of general inflammatory or irritant effects observed in various parts of the respiratory tract cannot be easily ascribed as class effects of one agent or the other.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

The sponsor has proposed to develop the combination of GSK573719 and GW642444 for the treatment of COPD. In the present submission, the sponsor submitted a clinical protocol proposing to treat COPD patients with this combination for 28 days to assess potential effects on heart rate. In support of the proposed clinical protocol, the sponsor has conducted nonclinical inhalation toxicology studies with GSK573719 alone up to 13

weeks in mice, rats, and dogs, GW642444 alone up to 26 weeks in rats and 39 weeks in dogs, and the combination of GSK573719 + GW642444 up to 4 weeks in rats and dogs.

GSK573719 alone: For inhalation toxicology studies conducted with GSK573719 alone administered to mice, rats, and dogs, toxic effects were primarily confined to respiratory tract including the nasal cavities/sinuses, nasopharynx, larynx, trachea, tracheal bifurcation, bronchus, and lung. In a 14-day inhalation bridging toxicology studies with dogs, toxic effects were identified in the gallbladder (i.e., histopathological changes); however, longer duration studies with comparable and higher exposure levels did not replicate these findings. Increased heart rates were observed in toxicology studies with dogs; however, there were no corresponding histopathological findings in the heart and this finding is monitorable in a clinical setting.

Safety margins for the highest proposed clinical dose of 500 µg/day are shown in the table below. Given that observed toxicities in the 13-week inhalation toxicology study with and rats and 4-week inhalation toxicology study with dogs were confined to the respiratory tract, NOAELs were re-expressed on a µg/g lung weight/day basis. Safety margins on a µg/g lung weight/day basis were >10 for rats and dogs, which were judged to be acceptable. Systemic exposures at the NOAELs of the 13-week toxicology studies with mice, rats, and dogs and 4-week toxicology study with dogs provide safety margins >1, which are acceptable.

Safety margins for the clinical dose of GSK573719 at 500 µg/day

Species/Study	NOAEL			Safety margins for a clinical dose of 500 µg/day		
	µg/kg/day (Deposited)	µg/g LW/day	AUC ng·hr/mL	10 µg/kg/day	0.5 µg/g LW/day	AUC = 4.32 ng·hr/mL[a]
Mouse, 13 weeks	100	16	29.5/42.5	10	32	6.8/9.8
Rat, 13 weeks	92.4	23.6	22.6/36.2	9.2	47.2	5.2/8.4
Dog, 4 weeks	52	5.12/6.47	25.9[b]/29.9[b]	5.2	10.2/12.9	6.0/7.0
Dog, 13 weeks	268[c]	-	30.6/28.6	-	-	7.0/6.6

a. The steady-state AUC_T achieved following an inhaled dose of 1000 µg/day was 8.64 ng·hr/mL (estimated based on day 14 in healthy subjects). The AUC at 500 µg/day was obtained by dividing 8.64 ng·hr/mL by 2. The clinical trial with the combination of 500 µg GSK573719 and 50 µg GW642444 indicated an AUC for GSK573719 of 617.32 pg·hr/mL although the method was considered insensitive to adequately assess PK parameters.
b. Estimated AUC value from the high dose in the study.
c. Not comparable to other studies due to lower than expected delivery of the test article by inhalation.

GW642444 alone:

In clinical trials with GW642444 alone, the sponsor has used doses up to 50 µg/day. The anticipated maximum recommended human dose for marketing is 6.25 µg/day.

Rats received GW642444M by inhalation for 13 weeks (delivered doses of 56, 658, 10400, and 38845 µg/kg; pulmonary deposited doses of 5.6, 65.8, 1040 and 3884.5 µg/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 µg/kg for local toxicities (tracheal epithelial degeneration/regeneration was seen at 658 mcg/kg) and 38800 mcg/kg (AUC_{0-t}=1485 ng·hr/mL) for systemic toxicities.

Dogs given GW642444 (delivered doses of 9.3, 66, 120/501 µg/kg) exhibited systemic toxicities in the liver (hepatocyte rarefaction, glycogen deposition, increased in periportal region and decreased in centrilobular region) and heart (left ventricle papillary muscle mineralization and/or fibrosis). Plasma troponin I assessed in week 4 of the 13-week 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 8.92 ng·h/mL) based on a minimal concern for the single incidence (1/4 male) of liver finding. Reviewer comment: Liver findings are not considered to be dose-limiting and the cardiac findings are judged to be monitorable in a clinical setting. Escalation to the high dose exposure with clinical monitoring of heart rate would appear to be acceptable.

The 13-week inhalation toxicology studies with GW642444 alone in rats and dogs provide adequate safety margins for the clinical dose of GW642444 at 25 µg/day.

Safety margins for the clinical dose of GW642444 at 25 µg/day

Species/Study	NOAEL			Safety margins for a clinical dose of 25 µg/day		
	µg/kg/day (Deposited)	µg/g LW/day	AUC pg·hr/mL	0.5 µg/kg/day	0.025 µg/g LW/day	AUC = 157.8 pg·hr/mL
Rat, 13 weeks	5.6	0.93	-	11.2	37.4	-
Dog, 13 weeks	LD = 2.32 (HD = 30/125)	-	8920			56.6

Combination of GSK573719 and GW642444: Inhalation toxicology studies up to 4 weeks have been conducted with the combination in rats and dogs.

In a 4-week toxicology study, GSK573719A and GW642444M, in combination and alone, were given as dry powder formulations in lactose to rats by nose only inhalation administration once daily for 60 min/day. Selection of the doses used in this study was based on the minimum and maximum combination ratios of GSK573719 or GW642444 to be given to humans in clinical studies (200:1 and 1:1 for GSK573719:GW642444).

Target doses of 0/0, 1000/5, 1000/50, 1000/1000, 1000/0, and 0/1000 µg/kg/day (GSK573719/ GW642444) were selected for this study.

Histopathological findings were observed in the lungs + bronchi, tracheal bifurcation, liver, mandibular LN, larynx, nasopharynx, and nasal turbinates. Findings in the larynx were judged to be rat-specific irritant effects commonly associated with inhalation toxicology studies. Findings in the nasopharynx and nasal turbinates were associated with nose-only inhalation and judged to be irrelevant for oral inhalation in the clinical setting. Findings for the mandibular LN occurred with GSK573719 alone. In the lungs + bronchi, findings of alveolar hemoglobin crystals with associated inflammatory cell infiltrate were observed in groups treated with combination of GSK573173 + GW642444. There was no evidence of a dose-response relationship. These findings were also observed at a lower incidence in groups treated with GSK573719 alone. The sponsor indicated that this finding has been seen recently at a similar incidence in control Sprague-Dawley rats from the testing laboratory. In the tracheal bifurcation, inflammatory cell infiltration was observed at increased incidences for males treated with the low dose of the combination or with either monoproduct alone and for females treated with the two lower doses of the combination or GW642444 alone. Loss of cilia, basophilia, and cell flattening at the bifurcation were observed at increased incidences for males treated with the low and high doses of the combination or GSK573719 alone and for females treated with the three doses of the combination or GSK573719 alone. The incidence of focal hepatocyte necrosis was increased for females treated with the high dose of the combination.

AUC and C_{max} values for GSK573719 were generally comparable alone and in combination with GW642444. However, AUC and C_{max} values for GW642444 tended to be lower in combination with GSK573719 as compared to alone.

There were no additive or synergistic findings with combination of GSK573719 and GW642444 in rats. The mid dose of the combination might be identified as the NOAEL.

In a 4-week toxicology study, GSK573719A and GW642444M, in combination and alone, were given as dry powder formulations in lactose to dogs by oropharyngeal tube. Selection of the doses used in this study was based on the minimum and maximum combination ratios of GSK573719 or GW642444 to be given to humans in clinical studies (200:1 and 1:1 for GSK573719:GW642444). Estimated achieved doses were 0/0, 996/6.46, 190/205, 997/0, and 0/174 µg/kg/day (GSK573719/GW642444) were selected. A 3-day pre-adaptation phase was used for the 190/205 and 0/174 µg/kg/day groups. During the pre-adaptation phase for Groups 3 and 5, increased femoral pulse rates were seen immediately postdose.

During the treatment phase, increased femoral pulse rates were seen immediately postdose in dogs given GSK573719 or GW642444 alone or in combination. The effect on pulse rates diminished over the duration of the study. ECG measurements taken immediately postdose during Week 4, confirmed increased heart rates, in males given GSK573719/GW642444 at 996/6.46 µg/kg/day and both sexes given

GSK573719/GW642444 at 190/205 µg/kg/day, GSK573719 alone at 997 µg/kg/day, or GW642444 alone at 174 µg/kg/day. All dogs showed respiratory sinus arrhythmia (RSA) at pretest. RSA was observed during Week 4 (predose and postdose) in dogs that received GW642444 at 174 µg/kg/day alone or in combination with GSK573719 (Groups 3 and 5). RSA was not observed pre or post dose during week 4 in dogs given GSK573719 at a target dose of 997 µg/kg/day alone or in combination with GW642444 (Groups 2 and 4). Minimal to mild increases in serum cardiac troponin (cTnI) were observed on the first day of dosing during the dose escalation phase and treatment phase in all groups given GSK573719 or GW642444 alone or in combination.

Histopathological findings were observed in the heart, thymus, larynx, nose/turbinates, liver, spleen, and testes. Findings in the nasal turbinates might be irrelevant to the clinical setting where the combination drug product will be administered by oral inhalation. Papillary muscle fibrosis in the heart was observed for 1 female in the combination group receiving 190/205 µg/kg/day. This heart finding is a known effect of β_2 agonists. It is possible that the combined interaction of GSK573719 and GW642444 produced an enhanced cardiotoxicity given that this effect was not observed for GW642444 alone. The severity of thymic involution/atrophy was generally increased for treatment groups as compared to the air-control group. There was some evidence of irritant effects in the larynx and nose/turbinates for the 996/6.46 µg/kg/day and/or GSK573719 alone groups.

AUC and C_{max} values for GSK573719 were unaffected by presence of GW642444. AUC and C_{max} values for GW642444 for males appeared to be generally higher in the presence of GSK573719 as compared to GW642444 alone. However, AUC values for GW642444 for females appeared to be generally higher for GW642444 alone as compared to in the presence of GSK573719.

There were no additive or synergistic findings with combination of GSK573719 and GW642444 in dog. The high dose of the combination might be identified as the NOAEL; however, a pre-adaptation phase was used for high dose of the combination. The may necessitate using the low dose of the combination for safety assessments.

Safety margins for the clinical dose of the combination drug product (500 µg GSK573719 and 25 µg GW642444) are shown in the table below. Toxicity of the combination in rats was primarily confined to the respiratory tract. The mid dose of the combination product expressed on µg/g lung weight/day basis provides adequate safety margins for clinical doses of GSK573719 and GW642444 in the combination. Toxicity in the combination in dogs was observed both locally in the respiratory tract and systemically in the heart. The low dose of the combination provides adequate safety margins for GSK573719 on a µg/kg/day or µg/g lung weight/day basis; however, safety margins are low (<6) for GW642444 on a µg/kg/day basis. Systemic exposures with the low dose of the combination product resulted in adequate safety margins for both GSK573719 and GW642444. There was no evidence of additive or synergistic toxicity with combination in either rats or dogs. Toxicology studies with GW642444 alone provide adequate safety margins for the clinical dose of GW642444 in the combination.

Exposure margins for clinical doses of 500 µg GSK573719 and 25 µg GW642444

Study	Exposure (GSK573719/GW642444)			Exposure margins for a clinical dose of 500 µg GSK573719 and 25 µg GW642444		
	Deposited µg/kg/day	Deposited µg/g LW/day	AUC ng·hr/mL	10 / 0.5 µg/kg/day	0.5 / 0.025 µg/g LW/day	4.32 / 0.16 ng·hr/mL
Rats 4-weeks	81.7 / 0.44	20.6 / 0.125	23.3 / NC	8.2 / 0.9	163 / 5	5.4 / -
	120 / 6.1	29.6 / 1.5	25.2 / 2.6	12 / 12	240 / 244	5.8 / 16.2
	106 / 10.4	27.3 / 26.8	23.9 / 53	10.6 / 21	54 / 1072	5.5 / 332
Dogs 4-weeks	249 / 1.62	26.7 / 0.174	76.7 / 9.6	24.9 / 3.2	53 / 7	17.8 / 60
	47.5 / 51.3	5.4 / 5.9	12.85 / 199	4.75 / 103	10.8 / 236	2.97 / 1244

In 4-week inhalation toxicology studies with the combination of GSK573719 and GW642444 in rats and dogs, there was no evidence of additive or synergistic toxicity. Inhalation toxicology studies with monoproducts alone in rats and dogs provide adequate safety margins for the proposed clinical doses of GSK573719 and GW642444 in the combination. Observed toxic effects with the combination product in rats or dogs were also observed with monoproducts alone. Cardiac effects observed in dogs could be attributed to the pharmacological effects of both agents. Findings of general inflammatory or irritant effects observed in various parts of the respiratory tract cannot be easily ascribed as class effects of one agent or the other.

Both GSK573719 and GSK642444 were negative in the standard battery of genotoxicity studies.

Magnesium stearate is being used as an excipient in the drug product. The maximum clinical dose of magnesium stearate in the proposed clinical trial is 200 µg/day (4 µg/kg/day for a 50 kg subject). The approved dose of magnesium stearate in the approved Foradil® Certihaler™ product is 56 µg/day. Pulmonary deposited doses of magnesium stearate in vehicle-control groups from the 4-week inhalation toxicology studies with rats and dogs were 17.7 and 8.7 µg/kg/day, respectively, which were not associated with adverse effects. Further, magnesium stearate does not appear to alter the toxicity or toxicokinetic of GSK573719 or GW642444. However, these pulmonary deposited doses do not provide adequate safety margins for the maximum clinical dose of magnesium stearate. The sponsor has rights of reference to a 6-month inhalation toxicology study with magnesium stearate in rats. The NOAEL (pulmonary deposited dose) was identified as 180 µg/kg/day, which supports a maximum clinical dose of 18 µg/kg/day.

The sponsor has proposed modifications of ongoing and planned studies with the combination drug product as described below. These studies were evaluated under IND 104,479 and more specific details can be found in reviews under that IND. Each modification is followed by an evaluation.

1. A 13 week inhaled combination study in the dog is ongoing; the design of which was submitted to the Agency as part of the initial submission for IND 104,479. Reference

was made to Division comments received on September 1 and October 9, 2009. The Division concurred with the selection of the dog as the most sensitive species and with the general design and doses of the study. The sponsor acknowledged the Division's comment [redacted] (b) (4) however, they retained this procedure for dose groups 5 and 8 in order to allow dogs to be exposed to the maximum tolerated dose of GW642444 (160 µg/kg/day). On veterinary advice, the pre-treatment adaptation was considered necessary for animal welfare reasons to avoid excessive tachycardia and physiological stress to dogs, and thereby minimize the risk of avoidable, irrelevant, pharmacological driven cardiac damage when a high (supratherapeutic) dose is administered to dogs on the first day of the study. Pre-study adaptation in dogs has been performed during the majority of toxicology studies with GW642444. This approach was previously accepted as supporting clinical trials with GW642444 (IND 74,696). It was used in the combination 13-week toxicology study with [redacted] (b) (4) in dogs, for which the Division raised no objection [redacted] (b) (4) he continued use of pre-adaptation in the proposed combination toxicity study with GSK573719 and GW642444 provides consistency with previous combination toxicity studies and to previous toxicity studies with GW642444 alone.

Evaluation: The Division maintains concerns that pre-exposure of dogs to smaller doses may provide protection against subsequent exposure to larger doses. The use of the pre-adaptation phase will be a review issue when the study is submitted.

2. A combination embryo fetal study in the rabbit by the subcutaneous route was also proposed in the IND on GSK573719. The Division concurred with the selection of the rabbit and the overall outline design for the study. Since the initial submission of IND 104,479, the M3(R2) ICH Guideline (Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and marketing Authorization for Pharmaceuticals) was finalized (reached Step 4) in June 2009. The guideline states "If nonclinical embryo-fetal studies have indicated that neither agent poses a potential human developmental risk, combination studies are not recommended unless concerns exist, based on the properties of individual components, that their combination could give rise to a hazard for humans." Based on the guideline, the sponsor has proposed that a combination rabbit embryofetal study is no longer required for the following reasons:

(a) GW642444 did not have any adverse effects on the developing fetus in rats up to inhaled doses of 33700 µg/kg/day. However, a variety of pharmacological class-related findings were seen in rabbit fetuses (e.g. open eyes, cleft palate, sternebral fusion, fissures in the frontal bone ossification, elongation of the anterior fontanelle, fissures in the frontal bones, and enlargement of the frontal/frontal bone structure). These effects are common to a number of beta-agonists, and as the changes appear to be dependant on either high exposures

or the conditions associated with snout-only exposure they are not considered to represent a potential human developmental risk.

(b) GSK573719 did not have any adverse effects on the developing fetus in either the rat or the rabbit following inhaled administration up to doses of 278 and 306 µg/kg/day, respectively.

(c) (b) (4)

(d) GSK does propose, however, to perform a rabbit embryo fetal study in the rabbit by the subcutaneous route on GSK573719 alone to improve upon the systemic exposure multiples already achieved in the inhaled rabbit embryo fetal study (3-fold AUC compared to the maximum clinical dose of 1000 µg).

Therefore, as neither GW642444 nor GSK573719 are considered to pose a potential human development risk, and in accordance with the M3(R2) Guidelines, the sponsor has proposed that a combination embryofetal study is no longer required to support clinical trials involving WOCBP and registration.

Evaluation: The Division does not concur that the rabbit findings with GW642444 are not indicative of potential human developmental risk. The ICH M3 (R2) Guidance states that "in those cases where the patient population includes WOCBP and studies with the individual agent(s) have shown findings indicative of embryo-fetal risk, combination studies are not recommended as a potential human developmental hazard has already been identified." An embryofetal development study with the combination drug product is not needed. The embryofetal development study with higher doses of GSK573719 in rabbits is acceptable although significant decrements of body weight gain (or body weight loss) and food consumption were evident with inhaled doses up to 306 µg/kg/day. If drug exposure can be increased without the significant decrements of body weight gain and food consumption, this would be beneficial to the safety assessment of GSK573719.

Internal Comments: There are potential concerns regarding the sponsor's use of a pre-adaptation phase in toxicology studies with dogs that received GW642444 alone or in combination with a long-acting muscarinic antagonist or corticosteroid. The Division "appears" to be inconsistent regarding recommendations not to use a pre-adaptation phase. However, in general, safety evaluations of these studies have essentially discarded doses where the pre-adaptation was used.

In 4-week inhalation toxicology studies with the combination of GSK573719 and GW642444 in rats and dogs, there was no evidence of additive or synergistic toxicity. Inhalation toxicology studies with monoproducts alone in rats and dogs provide adequate safety margins for the proposed clinical doses of GSK573719 and GW642444 in the combination. Toxic effects observed the combination product were consistent with monoproducts alone.

The sponsor provided full particle size distribution information of the product and PSD profile comparison to the relevant mono-component drug products. The mean drug mass on each individual NGI stage is typical for dry powder inhaler products. It is noticed that, even though it is at the same nominal dose, compared to the corresponding mono-component drug product, the combination product tends to have [redacted] (b) (4)) for both drug substances. GSK573719 FPM is [redacted] (b) (4) or [redacted] (b) (4) for the combination product, [redacted] (b) (4) for its sum on stages 3 through 5. Similarly, GW642444 FPM is [redacted] (b) (4) for the combination product, [redacted] (b) (4) for its sum on stages 3 through 5. Fine particle mass is the amount of drug substance that has the potential to deposit into the airways and lung peripheral, therefore it represents the amount responsible for therapeutic efficacy. This combination product versus mono-component product difference is also shown in the particle size distribution plots. Safety margins identified from inhalation toxicology studies with monoproducts in rats and dogs are sufficiently robust (with particular reference to µg/g lung weight/day and AUC calculations) to cover the [redacted] (b) (4) increases of fine particle mass for the individual agents in the combination product.

Recommendation: The proposed clinical trial appears reasonably safe and should be allowed to proceed.

Internal Comment: The following comments have already been conveyed to the sponsor in the 60-day letter.

1. For the ongoing 13-week inhalation toxicology study with the combination of GSK573719 and GW642444 in dogs, it is noted that the pre-adaption phase was used. The Division maintains concerns that pre-exposure of dogs to smaller doses may provide protection against subsequent exposure to larger doses. The use of the pre-adaptation phase will be a review issue when the study is submitted.

2. The Division does not concur that the rabbit findings with GW642444 are not indicative of potential human developmental risk. The ICH M3 (R2) Guidance states that "in those cases where the patient population includes WOCBP and studies with the individual agent(s) have shown findings indicative of embryo-fetal risk, combination studies are not recommended as a potential human developmental hazard has already been identified." An embryofetal development study with the combination drug product is not needed. The embryofetal development study with higher doses of GSK573719 in rabbits is acceptable although significant decrements of body weight gain (or body weight loss) and food consumption were evident with inhaled doses up to 306 µg/kg/day. If drug exposure can be increased without the significant decrements of body weight gain and food consumption, this would be beneficial to the safety assessment of GSK573719.

Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Molly E. Shea, Ph.D.

cc: list:
IND 106,616, HFD-570
ChungE, HFD-570
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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-106616	ORIG-1	GLAXO GROUP LTD DBA GLAXOSMITHKLINE	GSK573719 /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.

/s/

TIMOTHY W ROBISON
02/10/2010

MOLLY E SHEA
02/10/2010
I concur.

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:	104,479 and 106,616
Supporting document/s:	IND 104,479 #043, 044, 045, 047, 052 IND 106,616 # 070, 079, 090, and 108
Sponsor's letter date:	IND 104,479 August 27, 2010 August 30, 2010 September 21, 2010 October 6, 2010 December 14, 2010 IND 106,616 August 30, 2010 September 21, 2010 October 6, 2010 December 14, 2010
CDER stamp date:	IND 104,479 August 30, 2010 August 31, 2010 September 22, 2010 October 6, 2010 December 14, 2010 IND 106,616 August 31, 2010 September 22, 2010 October 6, 2010 December 14, 2010
Product:	Combination of GSK573719 (Muscarinic receptor antagonist) and GW642444 (LABA)
Indication:	COPD
Sponsor:	GlaxoSmithKline P.O. Box 13398 Five Moore Drive Research Triangle Park North Carolina 27709-3398
Review Division:	Division of Pulmonary, Allergy, and Rheumatology

	Products
Reviewer:	Timothy W. Robison, Ph.D., D.A.B.T.
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1 Executive Summary

1.1 Introduction

The sponsor has proposed a Phase III, 52-week clinical trial to evaluate the safety and tolerability of the long-acting muscarinic antagonist GSK573719 125mcg alone and in combination with the long-acting beta$_2$ agonist GW642444 25mcg compared with placebo in male or female subjects (≥40 years old) with COPD.

1.2 Brief Discussion of Nonclinical Findings

In support of the proposed clinical trial, the sponsor has provided nonclinical reports for the following studies: a 26-week inhalation toxicology study with rats, a 39-week inhalation toxicology study with dogs, fertility and reproductive performance studies in male and female rats, and embryofetal development studies in rats and rabbits.

In a 26-week inhalation toxicology study, rats received GSK573719 at inhaled doses of 0, 87.1, 289, or 987 µg/kg/day. Target organs of toxicity were the lung, tracheal bifurcation, nasal cavity/sinuses, nasopharynx, and larynx. The NOAEL was identified as the low dose based upon histopathological findings for the tracheal bifurcation (squamous metaplasia) in the mid and high dose groups.

In a 39-week inhalation toxicology study, dogs received GSK573719 at achieved doses of 0, 109, 421, and 1002 µg/kg/day. Potential target organs of toxicity were the heart, lungs, larynx, and nose turbinates. The NOAEL was identified as the low dose of 109 µg/kg/day based upon histopathological findings in the heart at the mid and high doses and the lung, larynx, and nose turbinates at the high dose.

Under IND 74,696, the sponsor has conducted 26-week rat and 39-week dog toxicology studies with GW642444 alone that support the clinical dose of 25 µg/day (see Review dated March 5, 2010). Target organs of toxicity identified in rats 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). Target organs of toxicity identified in the dog 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).

In a 13-week repeat dose oropharyngeal tube inhalation toxicology study, beagle dogs received GSK573719A and GW642444A alone or in combination. Achieved doses of GSK573719/GW642444 for Groups 2, 3, 4, 5, 6, and 7 were 1070/7.5, 23/29, 60/72, 177/183, 1048/0, and 0/180 µg/kg/day, respectively. Prior to giving dogs GW642444 at a target dose of 160 µg/kg/day in combination with GSK573719 or alone (i.e., Groups 5 and 7), the sponsor administered GW642444 at a target dose of 40 µg/kg/day (alone or with GSK573719) for 10 min/day for 3 consecutive days prior to escalation to the high

target dose of 160µg/kg/day. The sponsor contended that this pre-adaptation phase of dosing (Phase 1) was required to induce tachyphylaxis to the cardiovascular effects of GW642444, allowing administration of higher doses than would normally be possible in untreated dogs. The Division communicated several concerns to the sponsor that pre-exposure of dogs to smaller doses may provide protection against subsequent exposure to larger doses (see Toxicology and Applied Pharmacology 69: 199-205, 1983). It was noted that the use of the pre-adaptation phase will be a review issue when the study is submitted. Histopathological findings were observed in the lung, larynx, nose/turbinates, trachea, thyroid, testes, thymus, and heart that could potentially be attributed to treatment. In general, these findings were consistent with the individual test articles, GSK573719 and GW642444, and there was no evidence of novel toxicity with the combination.

The sponsor conducted reproductive toxicology studies with GSK573719 consisting of a fertility and reproductive performance study in male and female rats and embryofetal development studies in rats and rabbits. Mating and fertility indexes were unaffected in male rats that received subcutaneous doses up to 180 µg/kg/day. In a female fertility and early embryonic development study, female rats received estimated achieved inhalation doses of 3.37, 29.1, 100, and 294 µg/kg/day. There were no effects on fertility or reproductive performance. In an embryofetal development study, rats received estimated achieved inhalation doses of 0, 0.0317, 0.0969, and 0.278 mg/kg/day. Some potentially treatment-related minor skeletal and visceral abnormalities were observed. In an embryofetal development study, rabbits received estimated achieved inhalation doses of 0, 0.0285, 0.0889, and 0.306 mg/kg/day. Overall fetal body weights were decreased. There were some potentially treatment-related minor visceral and skeletal abnormalities. In an embryofetal development study, GSK573719 was not teratogenic in rabbits with subcutaneous doses up to 0.18 mg/kg/day although dosing may have been inadequate. GSK573719 did not appear to be teratogenic in rats and rabbits.

1.3 Recommendations

1.3.1 Clinical Study Safe to Proceed: **Yes**

The proposed clinical trial appears reasonably safe and should be allowed to proceed.

2 Drug Information

2.1 Drug

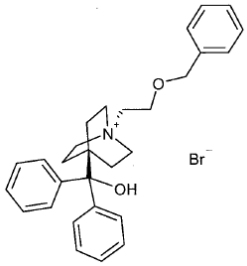
Code name: GSK573719

Chemical name: 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide

Molecular formula/molecular weight: $C_{29}H_{34}NO_2^{\cdot}Br$
508.5 (GSK573719A as quaternary ammonium bromide compound)

428.6 (as free cation, GSK573719)

Structure:

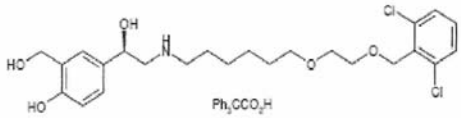


Code name: GW642444

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

Molecular formula/molecular weight: $C_{24}H_{33}Cl_2NO_5 \cdot C_{20}H_{16}O_2$ (as triphenyl acetate salt) / 774.78 g/mole (as the triphenylacetate salt), 486.43 g/mole (as the free base)

Structure:



Pharmacologic class: GSK573719 is a quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist.

GW642444 is a long-acting β_2-adrenergic agonist.

2.2 Relevant INDs, NDAs, and DMFs

IND 74,696 (GSK, GW642444 Inhalation Powder, Long-acting β_2 agonist)
IND [redacted] (b) (4)
IND 77,855 (GSK, GW642444 + Fluticasone)
IND [redacted] (b) (4)
IND 104,479 (GSK, GSK573719)

Drug Formulation

The following double-blind study medications will be used in the proposed study:

• Dry powder formulations of GSK573719/GW642444 Inhalation Powder in the nDPI administered once-daily (in the morning)
• Dry powder formulations of GSK573719 Inhalation Powder in the nDPI administered once-daily (in the morning)
• Matching placebo in the nDPI once-daily (in the morning)

The GSK573719/GW642444 Inhalation Powder in the nDPI contains two strips. One strip contains a blend of micronized GSK573719 (as the quaternary ammonium bromide salt, GSK573719A), lactose monohydrate and magnesium stearate. The second strip contains a blend of micronized GW642444X (as the triphenylacetate salt, GW642444M), lactose monohydrate, and magnesium stearate. Similarly, the placebo product consists of two strips each containing lactose monohydrate, and magnesium stearate. The nDPI will deliver, when actuated, the contents of a single blister simultaneously from each of the two blister strips. The LAMA monotherapy product consists of a single strip containing GSK573719 (as the quarternary ammonium bromide salt, GSK573719A), lactose monohydrate, and magnesium stearate.

Table 1 Formulation and Dose Information for GSK573719/GW642444, GSK573719, and matching placebo

Investigational Product	Formulation in the nDPI	
	Strip 1	Strip 2
GSK573719/GW642444 Inhalation Powder in the nDPI	125mcg per blister of GSK573719, as the bromide salt GSK573719A (micronized), blended with lactose monohydrate and magnesium stearate	25mcg per blister of GW642444X, as the triphenyl acetate salt GW642444M (micronized), blended with lactose monohydrate and magnesium stearate
GSK573719 Inhalation Powder in the nDPI	125mcg per blister of GSK573719, as the bromide salt GSK573719A (micronized), blended with lactose monohydrate and magnesium stearate	N/A[1]
Placebo DPI	Lactose monohydrate blended with magnesium stearate	Lactose monohydrate blended with magnesium stearate

1. Monotherapy nDPIs will only contain one strip

2.4 Proposed Clinical Population and Dosing Regimen

Chronic obstructive pulmonary disease (COPD)

2.6 Proposed Clinical Protocol

A 52-Week, Multicenter, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study to Evaluate the Safety and Tolerability of GSK573719 125mcg once-daily alone and in combination with GW642444 25mcg once-daily via novel Dry Powder Inhaler (nDPI) in Subjects with Chronic Obstructive Pulmonary Disease (COPD)
(Protocol DB2113359)

The sponsor has proposed a multicenter, randomized, double-blind, parallel-group, placebo-controlled study to evaluate the safety and tolerability of the long-acting muscarinic antagonist GSK573719 125mcg alone and in combination with the long-acting beta$_2$ agonist GW642444 25mcg compared with placebo over 52 weeks in male or female subjects (≥40 years old) with COPD. The target enrollment is approximately 500 randomized subjects at approximately 50 study centers globally. Eligible subjects will be randomized using a 2:2:1 ratio to GSK573719/GW642444 Inhalation Powder 125mcg/25mcg, GSK573719 Inhalation Powder 125mcg, and placebo, respectively.

A female is eligible to enter and participate in the study if she is of non-child bearing potential (i.e. physiologically incapable of becoming pregnant including any female who is post-menopausal or surgically sterile).

A female of child bearing potential is eligible to enter if she has a negative pregnancy test at screening and agrees to one of the following acceptable contraceptive methods (abstinence, oral contraceptive, either combined or progestogen alone, injectable progestogen, implants of levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, intrauterine device (IUD) or intrauterine system (IUS) that meets the SOP effectiveness criteria as stated in the product label, male partner sterilization (vasectomy with documentation of azoospermia) prior to the female subject's entry into the study, and this male is the sole partner for that subject, double barrier method: condom and an occlusive cap (diaphragm or cervical/vault caps) with a vaginal spermicidal agent (foam/gel/film/cream/suppository)).

2.7 Previous Clinical Experience

(From the sponsor's summaries)

<u>GSK573719 Dose Selection</u>

Safety and efficacy data from two Phase IIb studies conducted for GSK573719 Inhalation Powder (Studies AC4113073 and AC4113589) were considered for an overall risk to benefit analysis and selection of the lowest optimal dose of GSK573719 Inhalation Powder. Evaluation of the 12-lead ECG, 24 hour Holter monitor, and vital signs findings did not indicate that cardiovascular safety was adversely affected by any of the once daily doses evaluated (ranging from 62.5mcg to 1000mcg), nor was there any indication for an effect on clinical laboratory parameters or any indication of treatment-related effects on pulse rate. Evaluation of adverse event data indicated a dose-related increase in the incidence of adverse events, with total daily doses of 500 and 1000mcg showing the most notable increases relative to placebo. This was primarily due to a higher incidence of cough, dry mouth, and dysgeusia with 500 and

1000 mcg. The incidence of adverse events with 62.5 and 125 mcg doses was generally similar to placebo. The incidence of adverse events with the 250 mcg once-daily dose was higher compared with placebo only in Study ADC4113073. However, in both studies there was a higher incidence of cough with the 250 mcg dose favoring selection of a lower dose based on tolerability. Pharmacokinetic data showed that following once-daily treatment with GSK573719 Inhalation Powder, systemic exposure to GSK573719 was limited with rapid absorption followed by a rapid decline in plasma concentrations and achievement of steady state after 7 days of treatment. Spirometry data from the AC4113073 and AC4113589 studies showed a robust bronchodilator response for GSK573719 Inhalation Powder over 24 hours with consistent activity maintained after 28 days of treatment. Similar to that seen in clinical pharmacology studies, a relatively flat dose-response was observed across the once-daily doses. Overall, the 125 mcg and 250 mcg once-daily dose generally showed the most favorable bronchodilator efficacy over the course of 24 hours. The efficacy of the 500 mcg and 1000 mcg doses was limited by an early transient decrease from baseline in FEV1, potentially related to an increase in cough. The treatment response generally favored 125 mcg and 250 mcg once-daily over 62.5 mcg when administered either once- or twice-daily. Based on the totality of the safety and efficacy findings, the 125 mcg once-daily dose of GSK573719 Inhalation Powder was selected as the optimally effective dose for Phase III development of GSK573719 Inhalation Powder monotherapy and as a component of GSK573719/GW642444 Inhalation Powder in subjects with COPD.

GW642444 Dose Selection

Separate dose-ranging studies in asthma (B2C109575) and COPD (B2C111045) were conducted to select the optimally effective and safe dose of GW642444 Inhalation Powder to assess in Phase III, specifically with the dose-selection of GW642444 Inhalation Powder for COPD being supported from data from asthma. Additionally, a once-daily dosing interval for GW642444 Inhalation Powder was confirmed in study HZA113310 that evaluated doses of 6.25 mcg, 12.5 mcg, and 25 mcg once-daily and 6.25 mcg twice-daily for 7 days each in subjects with persistent asthma. Based upon the efficacy and safety data from these studies, 25 mcg once-daily was selected as the optimally effective and safe dose of GW642444 Inhalation Powder in COPD for both the progression of the combination of Fluticasone Furoate/GW642444 Inhalation Powder and this program, GSK573719/GW642444 Inhalation Powder.

GSK573719/GW642444

Study DB2113208 was a randomized, double-blind, placebo-controlled, four-way crossover study that evaluated the safety, tolerability, pharmacodynamics, and pharmacokinetics of a single inhaled dose of GSK573719 Inhalation Powder 500 mcg and GW642444 Inhalation Powder 50mcg delivered individually via the nDPI and concurrently from separate nDPIs in 16 healthy Japanese subjects. All the adverse events (AEs) were mild in intensity and reported as unrelated to study medication. There were no clinically significant changes in vital signs, hematology or biochemistry, which were attributed to study drug. Additionally, there were no clinically significant 12-lead ECG or 24 hour Holter ECG abnormalities observed during the study following

dosing with study medication. The safety, tolerability, pharmacokinetics and pharmacodynamics of GSK573719/GW642444 Inhalation Powder 500 mcg/25 mcg was evaluated in a multicenter, randomized, placebo-controlled, parallel-group study evaluating the combination of GSK573719 Inhalation Powder 500 mcg and GW642444 Inhalation Powder 25 mcg administered once-daily for 28 days in subjects with COPD (DB2113120). The incidence of AEs was low, and no SAEs were reported. There were no clinically significant increases in mean pulse rate compared to placebo at 28 days. In addition, there were no clinically significant changes in blood pressure (BP), ECG, or Holter findings. This study confirmed that 28 days of dosing with GSK573719/GW642444 Inhalation Powder 500 mcg/25 mcg was safe and well-tolerated in COPD subjects.

2.8 Regulatory Background

An EOP2 meeting was held with the sponsor on October 29, 2010. Responses to the sponsor's nonclinical questions and subsequent discussion are listed below.

Nonclinical

Question 9.1.1.

A list of completed, ongoing or proposed non-clinical studies for the individual components, GSK573719 and GW642444, alone and in combination is provided in attachment 2. Are the data from the non-clinical toxicology studies adequate to support initiation of the proposed Phase III clinical trials with the combination product and subsequent registration of the combination product?

FDA Response:

The data appears to be inadequate at this time with specific reference to the histopathological examinations of organs and tissues in the 13-week toxicology study with the combination of GSK573719 and GW642444 in dogs.

This study was intended to determine if the observed toxic effects observed with the combination of GSK573719 + GW642444 are consistent with toxic effects observed with monoproducts alone (i.e., there is no evidence of additive or synergistic toxic effects). Further, the study should attempt to identify a NOAEL.

Several findings were identified at increased incidences in the lungs, larynx, and trachea from dogs that received the combination of GSK573719 + GW642444 at a dose of 177/183 µg/kg/day (Group 5); however, lower dose groups were not examined in order to determine if a NOAEL could be identified. Further, it is noted that pre-adaptation or tolerance phase was used for Group 5, which might have potentially protected animals from subsequent exposure to higher doses. Groups 3 and 4 need to be examined to ensure that there were no additional findings due to the use of a tolerance phase for Group 5.

Organ/Tissue	Finding

Lung	perivascular/peribronchial/subpleural inflammatory cell infiltrate, G1
Lung	aggregates of alveolar macrophages
Larynx	mucosa: mixed inflammatory cell infiltrate, G1-G2
Trachea	mucosa: mixed inflammatory cell infiltrate, G1

Organs and tissues from dogs that received monoproducts (GSK573719 or GW642444 alone) were, in general, not submitted to the histopathological examination. This makes the direct comparison of findings with the combination product and monoproducts difficult or essentially impossible. The only potential comparisons are to rely on findings from other toxicology studies with monoproducts alone; however, these studies were conducted under differing experimental conditions and doses. This renders the comparisons inexact and inadequate.

Provide histopathological examinations of all tissues for combination groups that received 23/29 and 60/72 µg/kg/day (Groups 3 and 4). The control group (Group 1) needs to be reexamined by the pathologist along with drug treatment groups in order to have a valid comparison. Tissues, in which findings were identified for Groups 3, 4, or 5, should also be examined for monoproducts groups (Groups 6 and 7).

Question 9.1.2.

Inhaled combination toxicity studies with GSK573719 and GW642444 of up to 28 days duration in the rat and dog and up to 13 weeks duration in the dog have been completed. A further 28-day toxicity in the dog is ongoing and data will be available prior to the start of the Phase III trials. Does the Agency agree that, based on results of these combination studies, that no further nonclinical studies on the combination are required to support Phase III or registration?

FDA Response:

We cannot provide a definitive response to this question at this time. The need for any additional nonclinical studies will be assessed following review of the histopathology data for the 13-week combination toxicology study in dogs requested in Question 9.1.1 and the 28-day combination study in dogs to determine the effect of a pre-adaptation phase.

Question 9.1.3.

Interstitial, bronchoalveolar and granulomatous inflammation in the lung were observed at all doses in the dog 28-day study. Due to the histopathological appearance of the lesions, it was considered likely that this lung pathology was a result of accidental inhalation and deposition of exogenous material in the lung. This hypothesis has been further substantiated in subsequent studies in the dog with

GSK573719 alone or in combination with GW642444. Does the Division agree that the induction of granulomas in the dog has been adequately explained and resolved?

FDA Response:

We agree that the induction of granulomas in the dog has been adequately explained and resolved.

Question 9.1.4.

GSK have conducted 28-day and 13-week dog combination toxicology studies evaluating GSK573719/GW642444 in combination at various dose levels. A subsequent 28-day dog combination study is ongoing to assess the effect of a pre-adaptation phase used in the previous dog combination studies on cardiovascular function/parameters; the study report will be submitted within 30 days of initiating the long-term safety study (i.e. mid Nov 2010). Given that for the proposed Phase III clinical dose (125mcg), adequate safety margins already exists from a non-pre-adapted dose in the 13 week combination study at the 1:1 ratio, GSK believe it is safe to proceed with combination clinical studies longer than 12 weeks duration. Does the Division agree?

FDA Response:

We cannot provide a definitive response to this question at this time. The adequacy of nonclinical support for combination clinical studies longer than 12 weeks duration will be assessed following review of the histopathology data for the 13-week combination toxicology study in dogs requested in Question 9.1.1 and the 28-day combination study in dogs to determine the effect of a pre-adaptation phase.

The proposed clinical doses of GSK573719 and GW642444 in the combination product need to be adequately supported by NOAELs identified in the 6-month rat and 9-month dog toxicology studies with the individual monoproducts as well as the 13-week combination toxicology study in the dog. A NOAEL for the 13-week combination toxicity study has not yet been established.

Discussion:

The Sponsor expressed their thinking that the nonclinical information in their briefing package was comprehensive enough to start Phase 3 studies in humans. They noted that histopathological findings in the 3 month combination study were consistent with findings for each of the monoproducts alone and there was no evidence for any additive or synergistic effect with the combination. Further, the observed findings were not considered adverse. Thus, they did not believe that there was any need to examine additional groups in the combination study. FDA responded that we have expressed numerous concerns regarding the use of a pre-adaptation or tolerance phase for Group 5 as this might lead to protective effects for various tissues and mask any potential toxic effects of the combination. The primary concern was for the heart; however, protective effects for other tissues could not be excluded. FDA further stated that the sponsor should

have examined Groups 3 and 4 to ensure the pre-adaptation or tolerance phase used for Group 5 did not mask any potential toxic effects of the combination. The 28-day dog study was designed to assess the effects of the pre-adaptation phase with specific reference to the heart. It might provide supportive data for the use of Group 5 from the 3-month combination study; however, it does not alleviate the need to examine all tissues from Groups 3 and 4. The data from the 28-day study is unknown at this time.

The sponsor agreed to examine all the tissues for Groups 3 and 4 in the 13 week combination study with dogs. As stated in the response to Question 9.1.1., tissues in which findings were identified for Groups 3, 4, or 5, should also be examined for monoproducts groups (Groups 6 and 7). The FDA agreed. It was also confirmed that the 28-day dog study to assess the effects of pre-adaptation should be submitted prior to the human study protocol.

The sponsor inquired how long it would take for FDA to review the data if they submit the data by the end of November with the clinical trial. FDA discouraged the sponsor from submitting the clinical protocol without prior agreement that the nonclinical data are adequate. FDA stated that it could not pinpoint a time, however, the nonclinical reviewer will give it high priority and review the data as soon as it is submitted.

The sponsor stated that their intent is to start their study in humans as soon as possible after the animal data is complete. FDA acknowledged this.

3 Studies Submitted

3.1 Studies Reviewed

1. A 26-Week Inhalation Toxicity Study of a Powder Aerosol Formulation in the Rat followed by a 6-Week Recovery Period (Audited Draft Report)
2. Toxicity Study by Inhalation Administration to Beagle Dogs for 39 Weeks with a 6-Week Recovery Period (Audited Draft Report)
3. GSK573719A and GW642444M – A 13-Week Combination Inhalation Toxicity Study in Dogs (Audited Draft Report)

3.2 Studies Not Reviewed

1. A 4-week combination inhalation toxicity study in dogs (2010N10970_00)
2. Subcutaneous embryofetal development study in rabbits (CD2010/00253/00)*
3. Subcutaneous male fertility study in rats (CD2010/00187/00)*

[* Summary provided]

3.3 Previous Reviews Referenced

PharmTox Review of IND 104,479 dated June 3, 2009
PharmTox Review of IND 104,479 Initial Submission dated May 25, 2010
PharmTox Review of IND 106,616 Initial Submission dated February 10, 2010

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: <u>A 26-Week Inhalation Toxicity Study of a Powder Aerosol</u>

<u>Formulation in the Rat followed by a 6-Week Recovery Period</u> (Audited Draft Report)

Study no.: FD2009/00467/00
Study report location: Amendment #043 and EDR
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: August 11, 2009
GLP compliance: No signatures were included in the document (Audited Draft Report)
QA statement: No signatures were included in the document (Audited Draft Report)
Drug, lot #, and % purity: Micronized GSK573719A (purity of 1.194) was blended in vehicle at [redacted] (b) (4). GSK573719A powder was blended in Lactose monohydrate EP/USP/JP (Batch No. 626567) with a nominal 1% (w/w) magnesium stearate (batch number C811538) at concentrations of 2.5% (w/w), used to dose 87.1 and 289 µg/kg/day; or 25% (w/w) used to dose 987 µg/kg/day. The vehicle, lactose with a nominal 1% (w/w) magnesium stearate was blended at [redacted] (b) (4). GSK573719A/lactose/ magnesium stearate formulations were stable for the duration of the study.

Concentrations of test article and excipients in the formulation are expressed in terms of parent and are nominal.

Material	Input Material Batch Number	Blend Lot Number	Concentration in blend (Nominal)	Content Uniformity	Blend Expiry	Blend Storage
GSK573719A	R360975	79570-22-jul-2009-02	2.5% w/w	98.7%	22 Jul 2010	RT/dark/ desiccant
		79570-22-jul-2009-03	25% w/w	98.7%	22 Jul 2010	
		79570-10-nov-2009-05		99.5%	10 Nov 2010	
Magnesium Stearate	C811538	79570-22-jul-2009-01	1% w/w	NP	22 Jul 2010	RT/dark/ desiccant
		79570-10-nov-2009-04			10 Nov 2010	
		79570-09-dec-2009-06			09 Dec 2010	

RT Room Temperature (ie in a controlled environment set to maintain 21°C).
NP Not performed.

Key Study Findings

• In a 26-week inhalation toxicology study, rats received GSK573719 at inhaled doses of 0, 87.1, 289, or 987 µg/kg/day. Deposited doses were 0, 8.7, 28.9, or 98.7 µg/kg/day, respectively.

• Target organs of toxicity were the lung, tracheal bifurcation, nasal cavity/sinuses, nasopharynx, and larynx.

• Findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to human subjects that will receive the drug by oral inhalation. Findings in the larynx were judged to be rodent-specific (i.e., nonspecific irritant effects associated with aerosol inhalation) and irrelevant to humans.

• In the lungs, incidences of alveolar macrophage were increased for all treatment groups. These findings were reversible by the end of the 6-week recovery period. There was no evidence of inflammation in surrounding tissues.

• At the tracheal bifurcation, increased incidences of squamous metaplasia were observed for males and females in the mid and high dose groups. These findings are indicative of irritant effects induced by the test article.

• Additional findings in the kidneys, adrenal glands, uterus heart, tracheobronchial LN, salivary gland (parotid and sublingual), stomach, and subcutaneous tissue observed primarily in the high dose group were of low incidence and relationships of these findings to treatment were unclear. The incidence of tubular basophilia in the kidneys was increased for males in the 987 µg/kg/day group; however, the incidence for control females was relatively high. The incidence of angiectasis in the adrenal glands for females in the 987 µg/kg/day group was increased.

• The NOAEL was identified as the low dose based upon histopathological findings for the tracheal bifurcation (squamous metaplasia) in the mid and high dose groups. Doses for males and females in the low dose group, expressed on µg per gram lung weight basis, were 2.86 and 2.02 µg/kg/day, respectively. AUC values during week 26 for males and females in the low dose group were 47.6 and 41.1 ng·hr/mL, respectively.

Methods

Doses:	Achieved doses were 0, 87.1, 289, or 987 µg/kg/day
Frequency of dosing:	Daily (60 min/day)
Route of administration:	Nose-only inhalation
Dose volume:	NA
Formulation/Vehicle:	Control animals received lactose with a nominal 1% (w/w) magnesium stearate
Species/Strain:	Male and Female Sprague-Dawley rats were obtained from (b) (4) (b) (4)
Number/Sex/Group:	12 rats/sex/group
Age:	5 to 7 weeks old at the start of treatment
Weight:	Body weight ranges were 194 to 249 g for males and 144 to 186 g for females.
Satellite groups:	An additional 3 rats/sex/group were included in each group for toxicokinetic measurements. An additional 6 rats/sex/group were included in the control and 987 µg/kg/day groups for a 6-week recovery period.
Unique study design:	None
Deviation from study protocol:	The quantifiable levels of GSK573719 in the plasma in 13 out of a total of 126 control samples (from Weeks 4, 13 and 26) were considered likely to be a result of external contamination because of the relatively low levels of quantifiable GSK573719 in the samples (most being below 1 ng/mL) and/or lack of any corresponding toxicokinetic profiles. In addition all anti-contamination procedures were followed (with no test article detected in the control exposure chambers on these occasions). This finding is considered not to have affected the integrity of the toxicokinetic data or outcome of the study. Males given a target of 100 µg/kg/day were dosed twice on Day 61, resulting in a total estimated achieved dose of 341 µg/kg on this day is considered not to have affected the integrity of the study given the variance from target dose this represents over the entire duration of the study. Upon occasion the continuous monitoring of environmental conditions data was not available

for analysis and retention since it was not permanently captured due to a temporary system component malfunction. However, all alarm data for the duration of the malfunction was maintained in a log by the testing facilities security and is being retained in the data for any alarms documented in the rooms housing the animals for this study. The availability of the alarm data permits to establish that the environmental conditions for housing animals were in accordance with the protocol and applicable SOP; therefore, although some of the continuous environmental monitoring data was not retained this did not affect the integrity of the study.

The objectives described in the protocol were achieved, and as nothing occurred to affect adversely the quality or integrity of the study, I consider the data generated to be valid.

Study Design:
During the 26 week treatment period rats were given daily nose-only inhaled doses of GSK573719 or vehicle for 60 minutes/day. The animals were restrained in polycarbonate restraint tubes which were inserted onto the chambers. The vertical position of animals on the exposure chamber during dosing was changed weekly to minimize any bias resulting from variations in test material concentration at the 4 exposure levels of the chamber. GSK573719 (Batch number R360975) was administered as a dry powder formulation blended with a nominal 1% (w/w) magnesium stearate in lactose at nominal concentrations of 2.5% (w/w) used to dose animals given 87.1 and 289 µg/kg/day or 25% (w/w) used to dose animals given 987 µg/kg/day.

Table 1 Design of the 26-week inhalation toxicology study with rats

Study Design					
Group Number	Target Dose (µg/kg/day)[a, c]	Estimated Achieved Dose (µg/kg/day) [a, b, c]	Target Chamber Aerosol Concentration (µg/L) [a]	Achieved Chamber Aerosol Concentration (µg/L) [a, b]	Number/Sex[d, e]
1	0	0	0	0	21
2	100	87.1	2.8	2.4	15
3	300	289	8.4	7.9	15
4	1000	987	28	27	21

a. Expressed in terms of GSK573719.
b. As determined by chemical analysis
c. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
d. 3 animals/sex/group were assigned to the toxicokinetic study
e. 6 animals/sex/group (Groups 1 and 4) were retained for a 6 week treatment free recovery period

D = (RMVxTxC) / (BW)
D dose (µg/kg/day)
RMV (respiratory minute volume) $0.499 \times BW(kg)^{0.809}$ (L/min) [Bide, 2000].
T Duration of exposure/day (minutes)
C GSK573719 Aerosol concentration (µg/L)
BW Group average body weight for study (kg)

Chamber Aerosol Concentration

A measured volume of air was drawn from exposure chambers at a rate of 10 L/min through a glass fiber or Teflon filter, fitted to a sampling holder attached to the sampling port. Samples were collected at least twice during the pre-treatment period, daily during the first week of treatment, and 3 times weekly during Weeks 2 to 4. Chemical analysis was then performed 2 times/week from Week 5 onwards (including days of toxicokinetic evaluations) from each dose group. The filters were analyzed at (b) (4) by the Laboratory Sciences department for GSK573719 content using HPLC (quantification range of 0.0102 to 2.04 mg/filter) and for magnesium stearate content using atomic absorption spectroscopy (quantification range of 0.0102 to 0.0816 mg/filter).

Particle Size Distribution

A measured volume of air was drawn at a rate of 28.3 L/minute from inhalation exposure chambers containing GSK573719 or vehicle through a cascade impactor attached to a sampling port. Samples were collected at least once prior to the start of treatment and again in Week 1 and at least monthly thereafter. The glass fiber filters from stages 0 to 8 of the cascade impactor and the terminal filter were weighed. The filters were analyzed at (b) (4) by the Laboratory Sciences department for GSK573719 content using HPLC (quantification range of 0.0102 to 2.04 mg/filter) and for magnesium stearate content using AA spectroscopy (quantification range of 0.0102

to 0.0816 mg/filter). From these data the mass mean aerodynamic diameter (MMAD) and GSD was calculated using a log normal distribution of particle size.

Table 2 Estimated achieved doses of GSK573719 in the 26-week inhalation toxicology study with rats

GSK573719

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	Estimated Achieved Dose (µg/kg/day) [a]	MMAD (µm)	GSD
1	0	Males 0 Females 0 **Average** 0	(b) (4)	
2	2.4	Males 82.6 Females 91.5 **Average** 87.1		
3	7.9	Males 273 Females 304 **Average** 289		
4	27	Males 933 Females 1040 **Average** 987		

a. Expressed in terms of GSK573719 to 3 significant figures.
MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation

Table 3 Doses of GSK573719 in rats expressed on a µg/g lung weight/day basis

Deposited dose µg/kg/day	Sex	Body weight kg (Week 26)	Total deposited dose, µg/day	Lung weight G	Dose µg/g LW/day
8.7	M	0.5579	4.85	1.6966	2.86
	F	0.3105	2.70	1.3341	2.02
28.9	M	0.5396	15.59	1.7726	8.80
	F	0.2913	8.42	1.2453	6.76
98.7	M	0.5394	53.26	1.7541	30.36
	F	0.2826	27.89	1.2882	21.65

Table 4 Estimated achieved doses of magnesium stearate in the 26-week inhalation toxicology study with rats

Magnesium Stearate

Group Number	Average Achieved Chamber Aerosol Concentration (µg/L)	Estimated Achieved Dose (µg/kg/day) [a]	MMAD (µm)	GSD
1	1.8	Males 60.7 Females 68.4 **Average** 64.6	(b) (4)	
2	0.5	Males 17.2 Females 19.1 **Average** 18.2		
3	1.8	Males 62.3 Females 69.4 **Average** 65.9		
4	0.7	Males 24.2 Females 27.1 **Average** 25.7		

a. Expressed in terms of GSK573719 to 3 significant figures.
MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation

Observations and Results:

Mortality: A viability check (including a check for signs of ill health or reaction to treatment) was performed twice daily.

There were four deaths during the treatment period. No deaths were attributed to treatment.

One male control (#1013), assigned to the recovery phase, was found dead on day 81 during unloading from the inhalation chamber following exposure. No adverse clinical signs were observed prior to death.

One female control (#1513), assigned to the recovery phase, was found dead on day 86 following bleeding procedures that same day. No adverse clinical signs were observed prior to death.

One toxicokinetic female (#3513) in the 289 µg/kg/day group was found dead on day 28 following blood collection that same day. No adverse clinical signs were observed prior to death. There were no further deaths in this group as treatment progressed and there were no apparent treatment-related deaths in the high dose group at 987 µg/kg/day.

Following blood collection on day 85, the condition of one toxicology male (#4015) in the 987 µg/kg/day group deteriorated (e.g., decreased activity, lying on its side). The animal was not dosed on day 86. The animal's condition did not improve and it was subsequently found dead on day. Death was attributed to the blood collection procedure. There were no further deaths in this group as treatment progressed.

Clinical Signs: During the treatment period, main and recovery animals were examined daily, pre and post dosing, for recurring clinical signs. Detailed clinical examinations were performed on animals prior to initiation of dosing, during Weeks 13 and 26, at the end of the recovery period and at necropsy, with more frequent observations being performed when deemed appropriate.

There were no treatment-related clinical signs.

Body Weights: Body weights were measured once weekly.

Absolute body weights for males in the 87.1, 289, and 987 µg/kg/day groups during week 26 were decreased to 89, 86.1, and 86.1% of the control, respectively. Absolute body weights for females in the 87.1, 289, and 987 µg/kg/day groups during week 26 were decreased to 95.6, 90, and 87% of the control, respectively. Body weight gains for male and female rats in the 987 µg/kg/day group were increased during the recovery period although absolute body weights were still decreased as compared to controls.

Table 5 Absolute body weights and body weight gains

Parameter	Males				Females			
	0	87.1	289	987	0	87.1	289	987
BW (g), Wk 1	286.6	270 8	272.9	270.9	187 9	189.5	186.9	187 0
BW (g), Wk 26	626.4	557 9	539.6	539.4	324 9	310.5	291.3	282.6
%Cont BW Wk26	100.0	89.1	86.1	86.1	100 0	95.6	89.7	87 0
Δ (g)	339.8	287.1	266.7	268.5	137 0	121.0	104.4	95.6
%Cont BW Gain	100.0	84 5	78.5	79.0	100 0	88.3	86.3	91.6

Table 6 Body weight curves for male and female rats

Figure 1 Group Mean Body Weights - Males

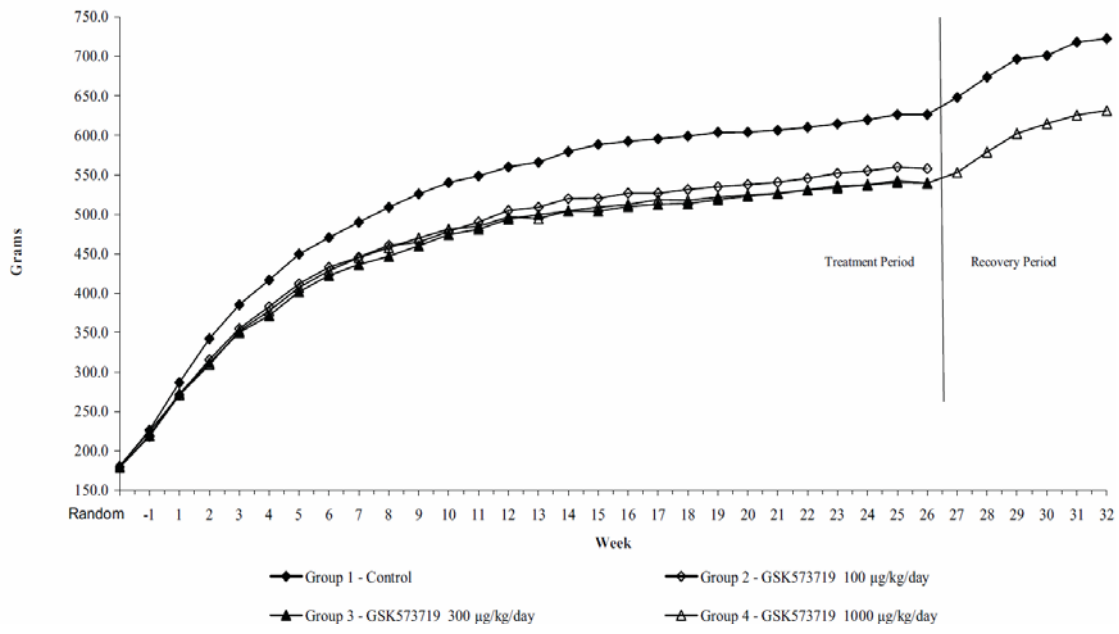
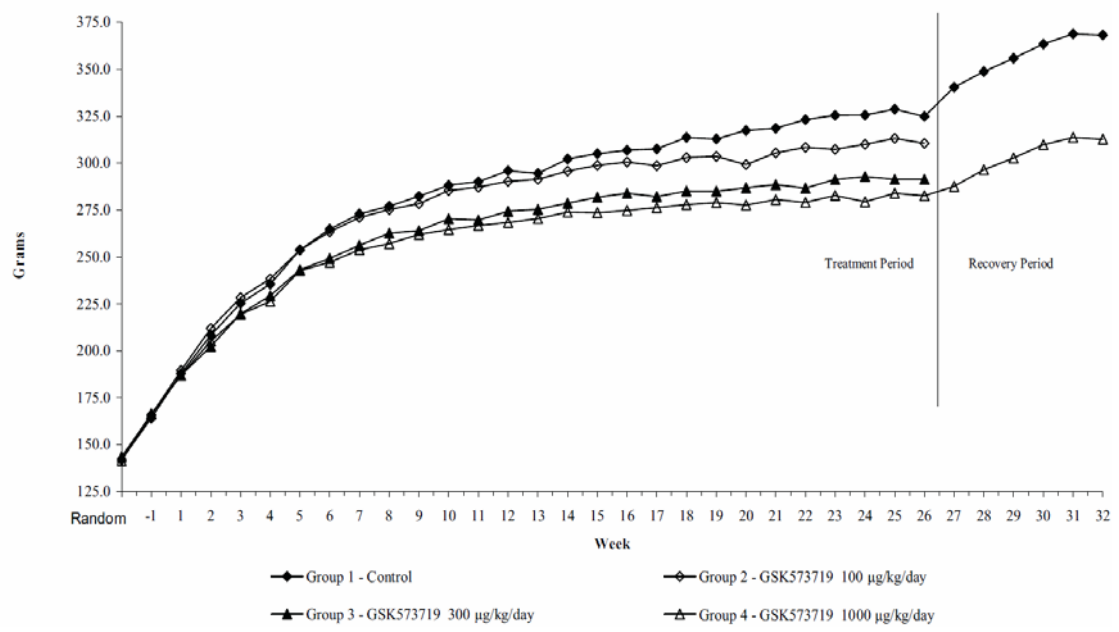


Figure 2 Group Mean Body Weights - Females



Feed Consumption: Individual food consumption was measured (cage measurements) during the week prior to treatment and weekly throughout the treatment and recovery periods.

Food consumption was decreased for male treatment groups during the dosing period.

Table 7 Food consumption during the 26-week treatment period (% of control)

Males				Females			
Control	LD	MD	HD	Control	LD	MD	HD
100	89.80	91.10	93.42	100.00	100.20	99.35	103.64

Ophthalmoscopy: Ophthalmic examinations were conducted prior to the start of dosing and during weeks 13 and 25.

There were no treatment-related ophthalmic findings at weeks 13 and 25. Observed ophthalmic findings were judged to be spontaneous in nature and unrelated to treatment.

ECG: Not conducted.

Hematology: Blood samples for measurement of a complete panel of hematology parameters were collected during weeks 4, 13, 26, and 32.

Neutrophil counts were elevated for male treatment groups during the dosing period and persisted into the recovery period. Lymphocyte, monocyte, basophil and large unstained cell (LUC) counts were decreased for female treatment groups during week 26 and persisted into the recovery period with the exception of basophil counts. The significance of these changes was unclear.

Neutrophil counts were increased up to 1.74-fold for males in all treatment groups during weeks 4 and 13 and males in the high dose group during week 26. The increase for the high dose group persisted into the recovery period. The significance of these differences was somewhat questionable given the range of neutrophil counts observed in the control group over the course of the treatment period. Neutrophil counts were increased for females in mid and high dose groups during week 13.

Lymphocyte counts were decreased for female treatment groups during week 26.

Monocyte counts were decreased for females in the high dose group during week 4, females in the mid and high dose groups during week 13, and all female treatment groups during week 26. The decrease for the high dose group persisted into the recovery group.

Basophil and LUC counts were decreased for female treatment groups during week 26. The decrease of LUC counts for the high dose group persisted into the recovery period.

Table 8 Hematology parameters at weeks 4, 13, 26, and 32

Parameter	Week	Males				Females			
		0	100	300	1000	0	100	300	1000
Neutrophils 10^9/L	4	1.057	1.448 (137%)	1.392 (132%)	1.839* (174%)	NC	NC	NC	NC
	13	1.311	1.819 (139%)	1.831 (140%)	1.983 (151%)	0.762	0.755	0.988 (130%)	0.948 (124%)
	26	1.819	1.643	1.769	2.250 (124%)	NC	NC	NC	NC
	32	1.666			2.022 (121%)	0.780			0.592 (76%)
Lymphocytes	4	NC	NC	NC	NC	6.220	7.543 (121%)	7.413 (119%)	7.557 (121%)
	13	NC	NC	NC	NC	NC	NC	NC	NC
	26	NC	NC	NC	NC	3.567	2.669* (76%)	2.979 (84%)	2.636* (97%)
	32	NC	NC	NC	NC	4.858			3.590 (74%)
Monocytes 10^9/L	4	NC	NC	NC	NC	0.221	0.193	0.201	0.143 (65%)
	13	NC	NC	NC	NC	0.175	0.170	0.156 (89%)	0.157 (90%)
	26	NC	NC	NC	NC	0.142	0.106 (75%)	0.110 (78%)	0.079* (56%)
	32	NC	NC	NC	NC	0.182			0.092* (50%)
Basophils 10^9/L	26	NC	NC	NC	NC	0.007	0.003 (43%)	0.004 (57%)	0.002* (29%)
LUC 10^9/L	26	NC	NC	NC	NC	0.030	0.017* (57%)	0.018* (60%)	0.015* (50%)
	32	NC	NC	NC	NC	0.052			0.034 (60%)

* $p \leq 0.05$
NC = No change

Clinical Chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected during weeks 4, 13, 26, and 32.

Changes of clinical chemistry parameters were generally small and their biological significance was unclear. Elevated urea and decreased potassium concentrations for male treatment groups could potentially correlate with histopathological findings of an increased incidence of renal tubular basophilia for males in the high dose group.

Urea concentrations were slightly elevated for male treatment groups during weeks 4, 13, and 26. These elevations were reversible by the end of the recovery period.

Triglyceride concentrations were decreased for male treatment groups during week 26.

Potassium concentrations were slightly decreased for males in the mid and high dose groups during week 26.

Calcium concentrations were slightly decreased for female treatment groups during week 26. Chloride concentrations were slightly increased for female treatment groups during weeks 4, 13, and 26.

Table 9 Clinical chemistry parameters at weeks 4, 13, 26, and 32

Parameter	Week	Males				Females			
		0	100	300	1000	0	100	300	1000
Urea mmol/L	4	4.66	5.18 (111%)	5.10 (109%)	5.16* (111%)	NC	NC	NC	NC
	13	4.91	5.30 (108%)	5.12 (104%)	5.44 (111%)	NC	NC	NC	NC
	26	4.84	4.95	4.99	5.47 (113%)	NC	NC	NC	NC
	32	5.46			5.26 (96%)	NC	NC	NC	NC
Triglyceride mmol/L	26	1.362	0.970 (71%)	0.807* (59%)	0.818* (60%)	NC	NC	NC	NC
Potassium mmol/L	26	5.612	5.662	5.375 (96%)	5.298* (94%)	NC	NC	NC	NC
Calcium mmol/L	26	NC	NC	NC	NC	2.728	2.674 (98%)	2.629* (96%)	2.645* (97%)
Chloride mmol/L	4	NC	NC	NC	NC	100.3	101.4	100.2	102.0 (102%)
	13	NC	NC	NC	NC	102.0	102.4	102.8	103.5* (101%)
	26	NC	NC	NC	NC	102.4	103.4	103.8	104.4* (102%)

* p≤0.05
NC = No change

Urinalysis: Urine samples for measurement of urinalysis parameters were collected overnight (approximately 16 hr) during weeks 4, 13, 26, and 32.

There were no treatment-related effects on urinalysis parameters.

Gross Pathology: After 26 weeks of treatment, surviving toxicology animals were sacrificed and submitted to detailed external and internal examinations.

Gross pathological findings were evident for the adrenal glands, lung, and thymus at the end of the treatment period.

Increased incidences of area dark in the adrenal glands were observed for females in the 987 µg/kg/day group. This finding appeared to correlate with histopathological findings of angiectasis.

Increased incidences of area pale in the lung were observed for females in the 987 µg/kg/day group. It was unclear if this finding correlated with histopathological findings of alveolar macrophage accumulation that occurred for both male and female treatment groups although with higher incidences for males.

Increased incidences of small thymus were observed for females in the 289 and 987 µg/kg/day groups. There was no apparent corresponding histopathological finding.

Table 10 Gross pathological findings at the end of the 26-week treatment and 6-week recovery periods

Organ/Tissue	Sex	End of 26-week treatment period				End of 6-week recovery period	
		0	87.1	289	987	0	987
Adrenals							
-area dark	M	0/13	0/12	0/12	0/13	0/5	0/5
	F	0/13	0/12	1/12	3/12	0/5	0/6
Lung							
-area pale	M	0/13	0/12	0/12	0/13	0/5	1/5
	F	1/13	0/12	0/12	3/12	0/5	1/6
Thymus							
-small	M	0/13	0/12	0/12	0/13	0/5	0/5
	F	0/13	0/12	2/12	2/12	0/5	0/6

Organ Weights: Absolute and relative organ weights were measured for the adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate, spleen, testes, and thymus.

There were no treatment changes of organ weights. Decreased body weights for male and female treatment groups produced a number of statistically significant increased organ weights.

Histopathology:

Adequate Battery: A complete panel of tissues was submitted to histopathological examination. Samples of the protocol-specified tissues (with the exception of animal identification, Harderian glands, preputial/clitoral glands and rectum) were processed to paraffin wax, sectioned, stained with hematoxylin and eosin, and examined microscopically for all control and high-dose animals, all unscheduled deaths (toxicology animals), and macroscopic abnormalities from all animals. Histopathological examination of the main study Groups 2 and 3 and recovery Groups 1 and 4 was limited to potential target organs (nasal cavity/sinuses, nasopharynx, larynx, tracheal bifurcation and lungs) as identified by evaluation of Groups 1 and 4.

Table 11 Tissues collected in the 26-week inhalation toxicology study with rats

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 (3 levels)	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	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

Peer Review: A peer review of selected microscopic tissue sections and pathology data interpretation was completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.

Histological Findings: Target organs of toxicity were the lung, tracheal bifurcation, nasal cavity/sinuses, nasopharynx, and larynx. Findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to human subjects that will receive the drug by oral inhalation. Findings in the larynx were judged to be rodent-specific (i.e., nonspecific irritant effects associated with aerosol inhalation) and irrelevant to humans. Additional findings in the kidneys, adrenal glands, uterus, heart, tracheobronchial LN, salivary gland (parotid and sublingual), stomach, and subcutaneous tissue observed primarily in the high dose group were of low incidence and relationships of these findings to treatment were unclear.

In the lungs, incidences of alveolar macrophage were increased for all treatment groups. These findings were reversible by the end of the 6-week recovery period. There was no evidence of inflammation in surrounding tissues.

At the tracheal bifurcation, increased incidences of squamous metaplasia were observed for males and females in the mid and high dose groups. These findings are indicative of irritant effects induced by the test article.

In the nasal cavity/sinuses, minimal to moderate squamous metaplasia of transitional epithelium and/or hyperplasia of transitional epithelium were seen in males and/or females at all doses. There was an increased incidence and severity of minimal to slight inflammation in males given ≥289 µg/kg/day and females given 987 µg/kg/day. Minimal to slight degeneration/regeneration of olfactory (predominantly in the anterior sections of the nasal cavity) and/or respiratory epithelium, squamous metaplasia of respiratory epithelium, respiratory epithelial metaplasia of olfactory epithelium, increased eosinophilic globules in olfactory epithelium and exudate were seen in males and/or females given ≥289 µg/kg/day. Minimal hyperplasia/hypertrophy of goblet cells was noted at all doses, including controls, in males and females. One control female had minimal degeneration/regeneration of the respiratory epithelium and inflammation. In general, the animals given 987 µg/kg/day had an increased severity and/or incidence of these changes. At the end of the recovery period, in the nasal cavity/sinuses, minimal squamous metaplasia and/or hyperplasia of transitional epithelium, degeneration/regeneration of olfactory epithelium were seen in one single male previously given 987 µg/kg/day and minimal exudate in a different male previously given 987 µg/kg/day. Minimal degeneration/regeneration of olfactory epithelium was also present in one control female and one female previously given 987 µg/kg/day. Minimal increased eosinophilic globules in olfactory epithelium were present in males and females previously given 987 µg/kg/day.

In the nasopharynx, increased incidence of minimal hyperplasia/hypertrophy of goblet cells was seen in males and females given 987 µg/kg/day. Minimal inflammation was seen in males and females at all doses, including controls, with an increased incidence in males given 987 µg/kg/day. Additionally, one male given 987 µg/kg/day had minimal degeneration/regeneration of respiratory epithelium. These findings were reversible at the end of the recovery period.

In the larynx, minimal to moderate squamous metaplasia/hyperplasia and minimal to severe degeneration/necrosis of the ventral cartilage was seen in males and females at all doses (≥87.1 µg/kg/day). Minimal to slight exudate and/or minimal inflammation were seen in males and females at all doses of GSK573719, as well as one control female. Due to the increased severity, the finding of ventral cartilage degeneration/necrosis was considered adverse at the doses of ≥289 µg/kg/day in males and females. At the end of the recovery period, in the larynx, minimal to slight squamous metaplasia/hyperplasia, slight to marked degeneration/necrosis of the ventral cartilage and minimal exudate were present in males and females previously given the test article. Minimal to slight

regeneration of the ventral cartilage in males and minimal inflammation in females previously given the test article were also seen. The ventral cartilage degeneration/necrosis was considered to be adverse due to the severity.

The incidence of tubular basophilia in the kidneys was increased for males in the 987 µg/kg/day group; however, the incidence for control females was relatively high.

The incidence of angiectasis in the adrenal glands for females in the 987 µg/kg/day group was increased.

The incidence of erythrocytosis/hemorrhage in the tracheobronchial LN was increased for males and females in the 987 µg/kg/day group.

Findings for single animals in the 987 µg/kg/day group were evident in uterus, heart, salivary gland (parotid and sublingual), stomach, and subcutaneous tissue.

Table 12 Histopathological findings at the end of the 26-week treatment and 6-week recovery periods

Organ/Tissue	Sex	End of 26-week treatment period				End of 6-week recovery period	
		0	87.1	289	987	0	987
Lung							
-macrophage accumulation, Grade 1-2	M	3/13	6/12	5/12	10/13	2/5	1/5
	F	2/13	5/12	4/12	4/12	1/5	2/6
Tracheal bifurcation							
-metaplasia, squamous, G1	M	0/13	0/12	3/11	6/13	0/4	0/5
	F	0/13	0/12	4/11	2/12	0/5	0/6
Nasal cavity/Sinuses							
-exudate, G1-G2	M	0/13	0/12	2/12	8/13	0/5	1/5
	F	0/13	0/12	0/12	3/12	0/5	0/5
-inflammation, G1-G2	M	0/13	0/12	3/12	11/13	0/5	0/5
	F	1/13	1/12	0/12	7/12	0/5	0/5
-metaplasia squamous: transitional epithelium, G1-G3	M	0/13	0/12	3/12	13/13	0/5	1/5
	F	0/13	1/12	2/12	9/13	0/5	0/5
-hyperplasia, transitional epithelium, G1-G2	M	0/13	5/12	4/12	11/13	0/5	1/5
	F	0/13	1/12	2/12	7/12	0/5	0/5
-degeneration/regeneration, respiratory epithelium, G1-G2	M	0/13	0/12	2/12	9/13	0/5	0/5
	F	0/13	0/12	1/12	9/13	0/5	0/5
-metaplasia squamous, respiratory epithelium, G1-G2	M	0/13	0/12	0/12	7/13	0/5	0/5
	F	0/13	0/12	1/12	9/12	0/5	0/5
-hyperplasia/hypertrophy, goblet cells, G1	M	5/13	4/12	1/12	4/13	0/5	0/5
	F	1/13	7/12	5/12	8/12	2/5	0/6
-degeneration/regeneration,	M	0/13	0/12	0/12	4/13	0/5	1/5

Organ/Tissue	Sex	End of 26-week treatment period				End of 6-week recovery period	
		0	87.1	289	987	0	987
olfactory epithelium, G1-G2	F	0/13	0/12	2/12	8/12	1/5	1/6
-metaplasia, respiratory and olfactory epithelium, G1	M	0/13	0/12	0/12	2/13	0/5	0/5
	F	0/13	0/12	0/12	0/13	0/5	0/5
-increased eosinophilic globules, olfactory epithelium, G1	M	0/13	0/12	1/12	9/13	0/5	2/5
	F	0/13	0/12	0/12	9/12	0/5	6/6
Nasopharynx							
-inflammation, G1	M	3/13	5/11	6/12	9/13	1/5	1/5
	F	2/13	1/12	2/12	1/12	0/5	0/6
-hyperplasia/hypertrophy, goblet cells, G1	M	4/13	2/11	4/12	10/13	2/5	1/5
	F	4/13	4/12	6/12	8/12	3/5	3/6
-degeneration/regeneration, respiratory epithelium, G1	M	0/13	0/11	0/12	1/13	0/5	0/5
	F	0/13	0/12	0/12	0/12	0/5	0/6
Larynx							
-metaplasia/hyperplasia, squamous, G1-G3	M	0/13	10/12	12/12	13/13	0/5	3/5
	F	0/13	7/12	11/12	12/12	0/5	4/6
-degeneration/necrosis, ventral cartilage, G1-G5	M	0/13	5/12	12/12	13/13	0/5	3/5
	F	0/13	6/12	12/12	12/12	0/5	6/6
-inflammation, G1	M	0/13	4/12	9/12	8/13	0/5	0/5
	F	0/13	2/12	0/12	12/12	0/5	2/6
-exudate, G1-G2	M	0/13	1/12	9/12	13/13	0/5	1/5
	F	1/13	1/12	2/12	5/12	0/5	1/6
-regeneration, ventral cartilage, G1-G2	M	0/13	0/12	0/12	0/13	0/5	2/5
	F	0/13	0/12	0/12	0/12	0/5	0/6
Kidney							
-basophilia, tubular, G1	M	1/13	0/3	0/2	4/13	-	-
	F	2/13	-	-	0/12	-	-
-dilatation, pelvis, G1-G2	M	1/13	1/3	2/2	2/13	-	-
	F	0/13	-	-	1/12	-	-
-pyelitis/pyelonephritis, G1	M	0/13	0/3	0/2	1/13	-	-
	F	3/13	-	-	2/12	-	-
-urolithiasis, G1	M	0/13	0/3	0/2	1/13	-	-
	F	0/13	-	-	0/12	-	-
Adrenal							
-angiectasis, G1-G2	M	1/13	-	-	0/13	-	-
	F	1/13	-	0/1	3/12	0/1	-
Tracheobronchial LN							
-erythrocytosis/hemorrhage, G1	M	0/13	-	-	2/12	-	-
	F	1/13	-	-	2/12	-	-
Uterus							
-benign granular cell tumor	F	0/13	-	-	1/12	-	-

Organ/Tissue	Sex	End of 26-week treatment period				End of 6-week recovery period	
		0	87.1	289	987	0	987
Heart -hemorrhage, G1	M F	0/13 0/13	- -	- -	1/13 0/12	- -	- -
Salivary gland Parotid -hemorrhage, G1	M F	0/13 0/13	- -	- -	0/13 1/12	- -	- -
Salivary gland, Sublingual -hemorrhage, G1	M F	0/13 0/13	- -	- -	0/13 1/12	- -	- -
Stomach -hemorrhage, G1	M F	0/13 0/13	- -	0/1 -	0/13 1/12	- -	- -
Subcutaneous tissue -hemorrhage, G2	M F	- -	- -	- -	- 1/1	- -	- -

Special Evaluation: None

Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected during weeks 4, 13, and 26. Animals were bled nominally at approximately 0, 0.5, 1, 2, 4, 8 and 23 hr after dosing. Plasma samples were analyzed for GSK573719 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) for GSK573719 was 0.1 ng/mL, using a 25 µL aliquot of rat plasma with a higher limit of quantification (HLQ) of 100 ng/mL.

Plasma C_{max} and AUC values for GSK573719 increased in an approximate dose proportional manner. C_{max} and AUC values were comparable for male and female rats. For the 89.1 and 289 µg/kg/day groups, C_{max} and AUC values were relative comparable at weeks 4, 13, and 26. However, C_{max} and AUC values for the 987 µg/kg/day group at week 26 were lower than values at weeks 4 and 13, which were relatively comparable.

Plasma samples collected from vehicle-control rats, sampled at the same time points and dates as the GSK573719A treated rats, in addition to naïve rat plasma samples, were analyzed for GSK573719. There were quantifiable concentrations of GSK573719 in 13 out of 126 control samples. The concentration in these samples ranged from 0.159 to 62.5 ng/mL although most samples were below 1 ng/mL. There were also 4 control samples with unconfirmed quantifiable concentrations of GSK573719. GSK573719 was quantifiable in 2 out of the 9 naïve plasma samples (both processed in Week 4). As levels in the control/naïve samples were generally low; close to the lower limit of quantification of the analytical method (0.1 ng/mL); distributed in no discernable pattern; as separate equipment was used for control samples; as all anti-contamination procedures were implemented; and as no contamination of the dosing apparatus samples was noted during the study, it is considered that the detection of test article in some of the control/naïve samples for toxicokinetic evaluation was most likely a result of ex-vivo contamination and had no impact upon the integrity or the validity of the study.

Table 13 Toxicokinetic parameters for GSK573719 in rats at weeks 4, 13, and 26

Parameter+	Week	Male			Female		
		Estimated Achieved Dose			Estimated Achieved Dose		
		87.1 µg/kg/day	289 µg/kg/day	987 µg/kg/day	87.1 µg/kg/day	289 µg/kg/day	987 µg/kg/day
$AUC_{(0\text{-}t)}$ (ng.h/mL)	Week 4	6.04	29.7	86.7	9.29	36.5++	83.4
	Week 13	5.64	22.9	61.4	8.50	33.4++	72.1
	Week 26	8.88	17.6	47.6	10.1	22.6++	41.1++
C_{max} (ng/mL)	Week 4	1.28	7.10	28.4	1.52	9.56	29.4
	Week 13	1.72	5.44	22.5	2.25	10.5++	27.7
	Week 26	0.885	4.21	13.9	1.66	6.33++	12.8++

+ Calculated by dose-normalising toxicokinetic data on the individual TK sampling occasions and then corrected for the overall estimated achieved dose of both sexes (refer to Appendix 4).
++ A mean of two results, due to incomplete sampling or no samples for third rat.

Dosing Formulation Analysis: The final report should be checked for details of variations of daily exposure concentrations.

Study title: **Toxicity Study by Inhalation Administration to Beagle Dogs for 39 Weeks with a 6-Week Recovery Period (Audited Draft Report)**

Study no.: FD2009/00466/00
Study report location: Amendment #043 and 045 and EDR
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: June 9, 2009
GLP compliance: No signatures were included in the document (Audited Draft Report)
QA statement: No signatures were included in the document (Audited Draft Report)
Drug, lot #, and % purity: Micronized GSK573719A, was blended with vehicle at [redacted] (b) (4). Each blend was prepared in the required proportions of GSK573719A 35% (w/w) in vehicle (Lactose [Batch No 081167512] with a nominal 1% (w/w) magnesium stearate [Batch No. 071140952]). Blends were deemed acceptable for use on the study only after blend sample analysis results were approved by the Sponsor.

Concentrations of test article and excipients in the formulation are expressed in terms of parent and are nominal.

Material	Input Material Batch number	Input Material Purity	Blend Batch Number	Blend concentration (nominal)	Content uniformity	Blend Expiry (retest)	Blend Storage
GSK573719A	081186716	TBD	BVR/P/1007/09	35% (w/w)	97.8%	2 Jun 2010	Below 30ºC, protected from light

Key Study Findings:

• In a 39-week inhalation toxicology study, dogs received GSK573719 at achieved doses of 0, 109, 421, and 1002 µg/kg/day. Deposited doses were 0, 27, 105, 250 µg/kg/day.

• Several clinical signs were evident for GSK573719-treated dogs (i.e., dry mouth, swollen neck, decreased incidences of unformed, watery, and mucoid stools, and increased incidences of vomit and vomit with food). Many of these findings are expected pharmacological effects of an M3 muscarinic antagonist.

• For ECG measurements conducted during week 39, immediately following administration of GSK573719, increased heart rates were observed at ≥109 µg/kg/day (up to 1.50- and 1.61-times predose in males and females, respectively). Respiratory sinus arrhythmia was generally absent in males given ≥421 µg/kg/day and females given ≥109 µg/kg/day.

• On day 1, elevations of serum cardiac Troponin I (cTnI) concentrations were observed for dogs that received ≥421 µg/kg/day.

• Slight increases in blood urea nitrogen (BUN) were noted during weeks 26 and 39 in both sexes given 1002 µg/kg/day (up to 1.38- and 1.27-times controls in males and females, respectively). ALP activities for females in the high dose group were increased during weeks 4, 26, and 39 (1.85-, 1.73-, and 1.24-times controls, respectively) although no change was evident at week 13.

• Potential target organs of toxicity were the heart, lungs, larynx, and nose turbinates.

• Moderate subacute inflammation of the extramural coronary arteries was seen in one female (Animal No. 4925) given 1002 µg/kg/day and in one male (Animal No. 3427) given 421 µg/kg/day.

• In one female (Animal No. 4926) given 1002 µg/kg/day, there was mild intimal thickening with minimal focal areas of mixed, predominantly mononuclear, inflammatory

cells infiltrate in an arteriole in one lobe of the lung. Pleural fibrosis was observed for one female given 1002 µg/kg/day.

• Slight epithelial erosion and/or ulceration was present in the squamous epithelium overlying the base of the vocal folds in the larynx of one male (Animal No. 4425) and one female (Animals No. 4926) given 1002 µg/kg/day. In addition, slight subacute to chronic inflammation was present in the mucosal glands adjacent to the arytenoid cartilage in the male dog (Animal #4425). Focal minimal squamous cell hyperplasia and hyperkeratosis was present in the epithelium overlying the base of the vocal folds in one male (Animal #3425) given 421 µg/kg/day. Minimal focal erosion/ulceration was also seen in one control animal at the end of the recovery period.

• Minimal squamous metaplasia of the respiratory epithelium was seen in the most cranial section of the nasal turbinates in one male (Animal No. 4427) given 1002 µg/kg/day.

• The NOAEL was identified as the low dose of 109 µg/kg/day based upon histopathological findings in the heart at the mid and high doses and the lung, larynx, and nose turbinates at the high dose. AUC values for males and females at 109 µg/kg/day during week 39 were 4.64 and 7.13 ng·hr/mL, respectively. The deposited doses, expressed on µg/g lung weight/day basis, for males and females were 3.14 and 2.86 µg/g lung weight/day, respectively.

Methods

Doses:	Achieved doses were 0, 109, 421, or 1002 µg/kg/day
Frequency of dosing:	Daily (10 min/day)
Route of administration:	Oropharyngeal tube inhalation
Dose volume:	NA
Formulation/Vehicle:	Lactose with a nominal 1% (w/w) magnesium stearate
Species/Strain:	Male and Female beagle dogs were obtained from (b) (4)
Number/Sex/Group:	4 dogs/sex/group
Age:	On day 1, dogs were 6 to 7 months old
Weight:	Body weight ranges were 7.4 to 9.9 kg for males and 5.6 to 8.1 kg for females
Satellite groups:	An additional 2 dogs/sex/group were included in the control and high dose groups for a 6-week recovery period.
Unique study design:	None
Deviation from study protocol:	Deviations were minor and did not affect the overall integrity of the study.

Study Design: During the 39 week treatment period dogs were given daily inhaled doses of GSK573719 or vehicle for 10 min/day administered using an oropharyngeal tube.

Table 14 Achieved doses of GSK573719 in the 39-week toxicology study with dogs

Study Design					
Group Number	Target Dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day) [b]	Target Tube Aerosol Concentration (µg/L)	Achieved Tube Aerosol Concentration (µg/L)	Number/Sex
1	0	0	0	0	6
2	100	109	30	34	4
3	400	421	120	130	4
4	1000	1002	300	310	6

a. Expressed in terms of GSK573719.
b. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC)/ (BW)
D Dose (µg/kg/day)
RMV $0.499 \times BW(kg)^{0.809}$ (L/min).
T Duration of exposure/day (minutes)
C Tube Aerosol concentration (µg/L)
BW Group average body weight for study (kg)

Table 15 Doses in dogs expressed on a µg/g lung weight/day basis

Deposited dose µg/kg/day	Sex	Body weight kg (Week 39)	Total deposited dose, µg/day	Lung weight g	Dose µg/g LW/day
27	M	12.2	329.4	105.011	3.14
	F	10.4	280.8	98.047	2.86
105	M	11.3	1186.5	102.322	11.6
	F	9.9	1039.5	85.314	12.18
250	M	12.1	3025.0	80.833	37.43
	F	9.8	2450	81.500	30.06

Table 16 Achieved doses of magnesium stearate in the 39-week toxicology study with dogs

Study Design			
Group Number	Estimated Achieved Dose (µg/kg/day) [a, b]	Achieved Tube Aerosol Concentration (µg/L)	Number/Sex
1	28.0	8.71	6
2	3.06	0.95	4
3	10.5	3.23	4
4	25.7	7.93	6

a. Expressed in terms of Magnesium stearate, as determined by atomic absorption spectroscopy.
b. Estimated achieved dose calculated as follows and assumes 100% deposition in the respiratory tract:

Each aerosol delivery system consisted of a dust generator (Wright Dust Feed), an aerosol conditioning chamber with a sub-chamber fitted with 4 outlets, aerosol delivery tubing, Y-piece connections with isolating ball valve, and oropharyngeal tube attached. The oropharyngeal tubes were constructed of silicone rubber and were inserted into the mouth of the dogs during dosing. A separate atmosphere generation system was employed to dose each group of animals.

The compressed air flow rate exiting the Wright Dust Feed (WDF) for all groups was set to 20 L/min and an additional dilution flow of compressed air was supplied at 10 L/min to the conditioning chamber, via an inlet that was adjacent to the aerosol entry point. The aerosol-laden air exiting the conditioning chamber (30 L/min, equal to 7.5 L/min/animal and each of 2 spare positions for sampling) was to 4 active outlets to potential animal exposure positions via clinical aerosol tubing with a Y-piece providing the attachment point for the ball valve and oropharyngeal tube. Two aerosol outlets were used to supply aerosol to the animals assigned to the study and the remaining outlets were used for the collection of aerosol samples. This arrangement was necessary due to the short exposure duration and the occasional requirement for the simultaneous collection of 2 samples (i.e., concentration and particle size distribution).

The magnesium stearate/lactose vehicle control group was exposed to a dry powder blend of magnesium stearate blended in lactose at a nominal concentration of 1% w/w. The concentration of magnesium stearate in the aerosol given to this group was targeted to be the same as the highest concentration delivered to Group 4.

Chamber Aerosol Concentration: A minimum of 2 samples per group were collected on each day of exposure. A measured volume of air was drawn from exposure chambers at a rate of approximately 2 L/min through a Pall type A/E glass fiber filter, fitted to an

open-faced filter holder attached to a spare animal breathing point. For magnesium stearate chemical analysis, a measured volume of air was drawn at a rate of 7.5 L/min from a representative animal breathing point through a Millipore Quartz fiber filter, fitted to an open-faced filter holder. Additional samples were collected from an animal breathing point of each group before daily treatment commenced and the filters weighed to demonstrate (gravimetrically) satisfactory operation of the exposure system.

On each day and from all groups, the first sample taken from the exposure system was designated for analysis of GSK573719. The second sample was then taken and designated for analysis of magnesium content. The glass fiber filters analyzed for GSK573719 content utilized a single HPLC method with Ultra Violet (UV) detection and a quantification range of 5.0 to 80 µg/mL. Magnesium stearate concentration was determined by analyzing the quartz filters for magnesium using atomic absorption spectroscopy and a magnesium lamp within a quantification range of 2.0 to 10 µg/mL. The results were expressed in terms of magnesium stearate.

Particle Size Distribution: A measured volume of air was drawn at a calibrated rate through a Marple 296 series cascade impactor. The Marple 296 impactor (flow rate 2 to 3 L/min) was employed for samples that were designated for analysis of GSK573719 and magnesium stearate, which were predicted to have a Mass Median Aerodynamic Diameter (MMAD) less than 3.5 micrometers. Samples collected for Atomic Absorption analysis of magnesium content and for gravimetric analysis of total particulate were taken using a Marple impactor employing a flow rate of 3 L/min to permit accurate assessment of MMAD values greater than 3 micrometers.

Samples were collected during Weeks 1, 4, 8 and 13 for gravimetric and chemical analyzes. Samples for chemical analysis of GSK573719 (groups given 109, 421 or 1002 µg/kg/day) and for magnesium stearate (all groups) were collected separately. The impactor stages were analyzed chemically for GSK573719 content using a single HPLC method with UV detection and quantification range(s) 5.0 to 80 µg/mL. Samples analyzed for magnesium content utilized Atomic Absorption spectroscopy and a magnesium lamp within a quantification range of 2.0 to 10 µg/mL. The results were expressed as magnesium stearate. The MMAD and its geometric standard deviation (GSD) were calculated.

Table 17 Tube Aerosol Concentration, Estimated Achieved Doses, and Particle Size Distribution Data for GSK573719

GSK573719

Group Number	Average Achieved Tube Aerosol Concentration (µg/L) [a]	Estimated achieved dose (µg/kg/day) [a]	MMAD (µm)	GSD
1	0	Males 0 Females 0 **Average 0**	(b) (4)	
2	34	Males 107 Females 111 **Average 109**		
3	130	Males 414 Females 427 **Average 421**		
4	310	Males 980 Females 1023 **Average 1002**		

a : Expressed in terms of GSK573719
MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation

Table 18 Tube Aerosol Concentration, Estimated Achieved Doses, and Particle Size Distribution Data for magnesium stearate

Magnesium stearate

Group Number	Average Achieved Tube Aerosol Concentration (µg/L) [a]	Estimated achieved dose (µg/kg/day) [a]	MMAD (µm)	GSD
1	8.71	Males 22.2 Females 28.7 **Average 28.0**	(b) (4)	
2	0.95	Males 3.00 Females 3.11 **Average 3.06**		
3	3.23	Males 10.3 Females 10.6 **Average 10.5**		
4	7.93	Males 25.1 Females 26.2 **Average 25.7**		

a : Expressed in terms of magnesium, as determined by atomic absorption spectroscopy.
MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation

Observations and Results

Mortality: Animals were checked twice daily for viability.

None

Clinical Signs: During the treatment period, all animals were examined at least twice daily. Additional detailed observations related to dosing were recorded at the following times in relation to dose administration: Week 1 to 19 (During and immediately after the dosing procedure, 0.5 to 2 hr after completion of dosing, and as late as possible in the working day) and Week 20 onward (during and immediately after the dosing, 0.5 to 2 hr after completion of dosing and twice weekly (at the middle and end of the week) and as late as possible in the working day). Animals were removed from their cages and detailed clinical examinations were performed during the pre-treatment period (Week -2) and weekly during the dosing and recovery periods until necropsy.

Incidences of swollen neck were increased for male and female treatment groups during the dosing period. Incidences of unformed, watery, and mucoid stools were decreased for male and female treatment groups (expected pharmacological effect). Incidences of vomit and vomit with food were increased for male and female treatment groups. Incidences of dry mouth were increased for male and female treatment groups (expected pharmacological effect).

Drying of mucosal secretions (dry eyes and dry mouth) is a known pharmacological response to this class of compound and has been previously reported in animals given GSK573719 (WD2006/03669/00 and WD2007/01512/00). These changes resolved during the recovery period.

Bilateral swelling of the neck seen during and after dosing generally persisted until the end of the day, but was only measurable (up to 40 mm in diameter) up to 4.5 hr after the completion of dosing. Additional investigations (see below) into this observation in selected dogs, including radiographs of the swollen areas, did not reveal additional information on the mechanism of the swellings. Cytological examination of the fluid aspirated from these swollen areas did not reveal any atypical cells. Excessive salivation, seen during and immediately after dosing was likely a response to the method of dosing and to the inhalation of a dry powder.

The frequency of abnormal stool findings (unformed, watery and/or with mucus) was decreased in animals given GSK573719 when compared to controls. This reduction in occurrence was more apparent, and also dose-related, in males. It has been documented that the M3 receptors are important in gastric emptying and colonic transit (Chiba et al, 2002). It is likely that the reduced frequency of stool findings were due to an increase in colonic transit time, resulting from exposure to the test article. These observations were no longer evident during the recovery period and considered not to be adverse.

Table 19 Clinical signs during the dosing period (total number of observations/number of animals)

Clinical signs	Males				Females			
	0	109	421	1002	0	109	421	1002
Swelling neck	3/2	26/4	154/4	204/6	6/1	17/4	41/4	208/6
Unformed stools	1245/6	131/4	93/4	86/6	387/6	43/4	175/4	105/6

Watery stool	193/6	2/2	4/2	2/2	6/4	2/2	10/2	6/6
Mucus in stool	66/6	29/4	11/4	8/6	81/6	18/4	9/4	1/1
Oral, vomit	14/4	21/3	16/4	36/6	4/4	28/4	25/4	31/6
Oral, vomit-food	32/6	71/4	51/4	200/6	52/6	63/4	46/4	64/6
Oral, Dry mouth	1/1	19/4	53/4	115/6	0/0	38/4	71/4	156/6

Body Weights: Body weights were measured during the treatment and recovery periods.

Body weight gains were unaffected.

Feed Consumption: Food consumption was measured daily, beginning 1 week prior to dosing (measured and recorded daily, 7 days/week).

There were no treatment-related effects on food consumption.

Ophthalmoscopy: Ophthalmic examinations were conducted during the pre-treatment period and at the end of the dosing period (Week 40).

There were no treatment-related ophthalmic findings.

Schirmer Tear Test: The rate of tear production was measured by placing the wick of a sterile test strip in the lower conjunctival sac for 1 min and recording the distance travelled by tear secretion in both eyes of all animals. Measurements were made pretest, on Day 1 and during Weeks 4, 13, 26 and 39 (predose and within 0.5 to 1 hr after the start of dosing) and at the end of the recovery period.

Artificial Tears were administered to the eyes of all animals immediately after dosing, or as required. On occasions when tear tests were performed (Day1 and Weeks 4, 13, 26, and 39) artificial tears were administered on completion of the test.

When compared to pretest and to controls, there was a reduction in tear production when measured prior to and after dosing during Weeks 1, 4, 13, 26, and 39 in animals given ≥109 µg/kg/day (as low as 0.11 times pretest for each sex). Measurements taken after dosing showed an even greater decrease in tear production, which was dose-related in males. At the end of the recovery period, tear production in animals previously given 1002 µg/kg/day for 39 weeks was generally comparable to both pretest and to controls.

ECG: Electrocardiograms were recorded from each animal once during the pre-treatment period, at the end of Week 39, and at the end for recovery. During the treatment period recordings were made prior to and immediately after dosing at the approximate T_{max}. Tracings were taken on unanesthetized dogs positioned in right lateral recumbency. The following leads were used at each interval: I, II, III, aVR, aVL, aVF, CV5RL (rV2), CV6LL (V2), CV6LU (V4) and V10. ECG lead cable for CV6LU (V4) was located on the left 6th intercostal space at the costochondral junction. The tracings were examined for abnormalities and heart rate.

Inhalation exposure of dogs to GSK573719 in lactose with 1% (w/w) magnesium stearate at target doses of ≥109 µg/kg/day resulted in an antimuscarinic pharmacological effect characterized by increased heart rate (HR). Higher HR with no RSA at the week 39 predose interval observed in males and females given GSK573719 at ≥400 and ≥100 µg/kg/day, respectively, indicates a persistent effect of GSK573719.

ECGs were measured during week 39. Immediately prior to dosing, heart rates were increased versus pretest values in males and females given ≥421 and ≥109 µg/kg/day, respectively (up to 1.35- and 1.42-time pretest values, respectively). There was no respiratory sinus arrhythmia (RSA) in ECGs collected from males and females given GSK573719 at 1002 and ≥421 µg/kg/day, respectively. Immediately following administration of GSK573719, increased heart rates were observed at ≥109 µg/kg/day (up to 1.50- and 1.61-timed predose in males and females, respectively). RSA was generally absent in males given ≥421 µg/kg/day and females given ≥109 µg/kg/day.

Table 20 Heart rate and respiratory sinus arrhythmia (RSA) during week 39

Group 1: Lactose/1% Magnesium Stearate Control

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 39					Pretest	Week 39	
	Pretest	Pre/Post		Predose	Postdose		Pretest	Pre/Post		Predose	Postdose
1425m	98	81/89	+	+	+	1925f	93	96/92	+	+	+
1426m	91	56/64	+	+	+	1926f	86	81/87	+	+	+
1427m	92	68/95	+	+	+	1927f	100	89/90	+	+	+
1428m	96	78/81	+	+	+	1928f	82	80/81	+	+	+
1429m	82	62/68	+	+	+	1929f	99	112/119	+	+	+
1430m	102	88/81	+	+	+	1930f	110	84/95	+	+	+
mean	**94**	**72/79**	**0/6***	**0/6***	**0/6***	**mean**	**95**	**90/94**	**0/6***	**0/6***	**0/6***

Group 2: GSK573719 100 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 39					Pretest	Week 39	
	Pretest	Pre/Post		Predose	Postdose		Pretest	Pre/Post		Predose	Postdose
2425m	90	110/149	+	+	–	2925f	100	130/163	+	+	–
2426m	105	97/99	+	+	+	2926f	102	122/171	+	+	–
2427m	111	74/70	+	+	+	2927f	103	101/115	+	+	–
2428m	99	95/100	+	+	+	2928f	115	97/96	+	+	+
mean	**101**	**94/104**	**0/4***	**0/4***	**1/4***	**mean**	**105**	**113/136**	**0/4***	**0/4***	**3/4***

Group 3: GSK573719 400 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 39					Pretest	Week 39	
	Pretest	Pre/Post		Predose	Postdose		Pretest	Pre/Post		Predose	Postdose
3425m	90	108/152	+	+	–	3925f	105	89/129	+	+	–
3426m	102	117/220	+	+	–	3926f	110	126/160	+	–	–
3427m	114	124/111	+	+	+	3927f	98	137/203	+	–	–
3428m	92	124/127	+	+	–	3928f	102	137/196	+	–	–
mean	**100**	**118/153**	**0/4***	**0/4***	**3/4***	**mean**	**104**	**122/172**	**0/4***	**3/4***	**4/4***

Group 4: GSK573719 1000 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 39					Pretest	Week 39	
	Pretest	Pre/Post		Predose	Postdose		Pretest	Pre/Post		Predose	Postdose
4425m	104	122/170	+	–	–	4925f	96	136/171	+	–	–
4426m	86	108/162	+	–	–	4926f	95	138/128	+	–	–
4427m	109	138/139	+	–	–	4927f	106	109/175	+	–	–
4428m	78	98/123	+	+	+	4928f	96	113/121	+	–	+
4429m	108	144/138	+	–	–	4929f	88	124/170	+	–	–
4430m	97	131/186	+	+	–	4930f	83	109/120	+	–	–
mean	**97**	**124/153**	**0/6[a]**	**4/6[a]**	**5/6[a]**	**mean**	**94**	**122/148**	**0/6[a]**	**6/6[a]**	**5/6[a]**

m : male
f : female
RSA : respiratory sinus arrhythmia
+ : presence of RSA
– : absence of RSA
a : number of animals with no RSA

Femoral Pulse: Femoral pulse was measured by palpation for all animals on Days 1 and 7, and during Weeks 4, 13, 26 and 39. Measurements were made predose, immediately after dose (within 10 min) and 1, 3, 7 and 24 after the start of dosing.

On all occasions up to and including week 26, pulse rates increased (up to 1.51- and 1.53-times predose in males and females) commencing immediately after dosing in males and females given ≥109 µg/kg/day and generally peaked within 1 hr after the start of dosing. A return to baseline levels was reached between 1 and 3 hours after the start of dosing. In comparison with previous occasions during the study, the increases observed in week 39 were less pronounced (up to 1.20X and 1.11X predose values in males and females, respectively).

Cardiac Troponin I: Blood samples for measurement of serum troponin I concentrations were collected from all animals on Day 1, pre-dose and 4, 8, and 24 hr after the start of dosing. Samples were shipped frozen on dry ice to the Sponsor for analysis. Concentrations of cardiac Troponin I in serum from all study animals were determined using a Bayer Centaur CP Chemiluminescence analyzer.

In females given ≥421 µg/kg/day, elevations in mean serum cardiac Troponin I (cTnI) concentrations were noted in the majority of animals at 4 and 8 hr after the start of dosing (up to 11.6 times and 7.4 times predose, respectively). A near complete return to baseline was achieved by 24 hr after the start of dosing. Male #3425 in the 421 µg/kg/day group demonstrated an increased serum cTnI concentration at 4 and 8 hr after the start of dosing (0.201 and 0.217 µg/L, respectively), with partial return to baseline levels seen at 24 hr. Slight increases appeared to evident for males in the 1002 µg/kg/day group.

Table 21 Serum troponin I concentrations on day 1 in male dogs

		[Cardiac Troponin I] µg/L			
Estimated Achieved Dose GSK573719A µg/kg/day	Animal	Predose	4h	8h	24h
0	1425	**0.006**	0.008	0.013	0.006
	1426	0.006	0.008	0.010	0.009
	1427	0.067	0.006	**0.006**	**0.006**
	1428	**0.006**	**0.006**	0.017	**0.006**
	1429	**0.006**	0.008	0.011	0.007
	1430	**0.006**	0.007	0.015	0.008
	mean	0.0162	0.0072	0.0120	0.0070
	SD	0.0249	0.0010	0.0039	0.0013
109	2425	0.006	0.008	0.008	0.019
	2426	0.009	0.011	0.009	0.008
	2427	0.016	0.013	0.040	0.009
	2428	0.006	0.032	**0.006**	0.006
	mean	0.0093	0.0160	0.0158	0.0105
	SD	0.0047	0.0109	0.0162	0.0058
421	3425	0.052	0.201	0.217	0.105
	3426	0.011	0.010	0.008	0.009
	3427	0.007	0.014	0.006	0.031
	3428	0.007	0.023	0.014	0.016
	mean	0.0193	0.0620	0.0613	0.0403
	SD	0.0219	0.0928	0.1039	0.0441
1002	4425	0.008	0.039	0.039	0.012
	4426	0.007	0.021	0.015	0.011
	4427	**0.006**	nr	0.012	**0.006**
	4428	0.028	0.046	0.049	0.010
	4429	0.006	0.027	0.022	0.011
	4430	0.008	0.016	0.016	0.019
	mean	0.0105	0.0298	0.0255	0.0115
	SD	0.0086	0.0125	0.0150	0.0042

Results in bold are below the sensitivity of the assay i.e. <0.006 µg/L
nr = no result reported due to poor duplication of results.

Table 22 Serum troponin I concentrations on day 1 in female dogs

		[Cardiac Troponin I] µg/L			
Estimated Achieved Dose GSK573719A µg/kg/day	Animal	Predose	4h	8h	24h
0	1925	0.008	0.011	0.007	0.009
	1926	**0.006**	0.006	0.007	0.010
	1927	0.016	0.009	0.029	0.009
	1928	**0.006**	**0.006**	**0.006**	0.008
	1929	0.006	0.008	0.009	0.007
	1930	**0.006**	**0.006**	**0.006**	**0.006**
	mean	0.0080	0.0077	0.0107	0.0082
	SD	0.0040	0.0021	0.0090	0.0015
109	2925	0.011	0.014	0.012	0.012
	2926	0.009	0.012	0.010	0.009
	2927	**0.006**	**0.006**	0.011	**0.006**
	2928	**0.006**	0.011	0.009	0.011
	mean	0.0080	0.0108	0.0105	0.0095
	SD	0.0024	0.0034	0.0013	0.0026
421	3925	**0.006**	0.014	0.009	0.007
	3926	0.008	0.137	0.088	0.029
	3927	**0.006**	0.061	0.035	0.007
	3928	**0.006**	0.129	0.087	0.022
	mean	0.0065	0.0853	0.0548	0.0163
	SD	0.0010	0.0585	0.0393	0.0111
1002	4925	0.006	0.233	0.259	0.085
	4926	0.007	0.037	0.021	0.022
	4927	0.010	0.087	0.046	0.017
	4928	0.007	0.089	0.062	0.027
	4929	0.009	0.018	0.021	0.017
	4930	0.008	0.072	0.066	0.029
	mean	0.0078	0.0893	0.0792	0.0328
	SD	0.0015	0.0759	0.0902	0.0260

Results in bold are below the sensitivity of the assay i.e. <0.006 µg/L

Hematology: Blood samples for measurement of clinical chemistry were obtained from all dogs once pre-treatment (Week -2), during Weeks 4, 13, 26, and 39, and at the end of the recovery period.

There were slight decreases of red blood cell mass (hemoglobin, hematocrit, and red blood cell count; decreased up to 9% as compared to control values) in males given ≥109 µg/kg/day at week 39. No changes of hematology parameters were observed for female treatment groups. APTT for males in the 1002 µg/kg/day group during weeks 26 and 39 were decreased to 85.3 and 84.3% of control values (16.6-18.8 sec), respectively.

Clinical Chemistry: Blood samples for measurement of clinical chemistry were obtained from all dogs once pre-treatment (Week -2), during Weeks 4, 13, 26, and 39, and at the end of the recovery period.

Slight increases in blood urea nitrogen (BUN) were noted during weeks 4, 13, 26, and 39 in males and females that received 1002 µg/kg/day (up to 1.43 times and 1.50 times control values in males and females, respectively). ALP activities for females in the high dose group were increased during weeks 4, 26, and 39 (1.87, 1.73, and 1.24 times control values, respectively) although no change was evident at week 13. Glyceraldehyde dehydrogenase activity was slightly increased for females in the 421 and 1002 µg/kg/day group during week 13 (up to 1.2 times the control). Phosphorus levels were slightly increased for female treatment groups during weeks 4, 26, and 39 although there was no change during week 13. Cholesterol levels were slightly increased for females in the 1002 µg/kg/day group during weeks 4, 13, 26, and 39. The significance of clinical chemistry changes was unclear as there were no corresponding histopathological findings in the kidneys or liver.

Table 23 Changes of clinical chemistry parameters

Parameter	Week	Males				Females			
		0	109	421	1002	0	109	421	1002
BUN mg/dL	4	15	17	16	18 (120%)	14	16 (114%)	18 (128%)	21 (150%)
	13	17	18	18	20 (122%)	NC	NC	NC	NC
	26	20	20	22	25 (125%)	19	18	20	23 (121%)
	39	16	17	18	22 (143%)	15	16	15	19 (127%)
ALP U/L	4	NC	NC	NC	NC	86	97	89	161 (187%)
	13	NC	NC	NC	NC	NC	NC	NC	NC
	26	NC	NC	NC	NC	66	71	78 (118%)	114 (173%)
	39	NC	NC	NC	NC	85	59	68	105 (124%)
GLDH U/L	13	NC	NC	NC	NC	7.6	7.4	9.3 (122%)	9.0 (118%)
Phosphorus mg/dL	4	NC	NC	NC	NC	5.8	6.8 (117%)	6.5 (112%)	7.0 (121%)
	13	NC	NC	NC	NC	NC	NC	NC	NC
	26	NC	NC	NC	NC	4.6	4.9 (107%)	5.2 (113%)	5.4 (117%)
	39	NC	NC	NC	NC	4.2	5.1 (121%)	4.5 (107%)	5.0 (119%)
Cholesterol mg/dL	4	NC	NC	NC	NC	132	146	165 (125%)	160 (121%)
	13	NC	NC	NC	NC	185	169	169	212 (115%)
	26	NC	NC	NC	NC	199	166	251 (126%)	229 (115%)
	39	NC	NC	NC	NC	196	182	204	217 (111%)

Urinalysis: Urine samples for measurement of urinalysis parameters were collected overnight (approximately 16 hr) from pans placed under the animal cages once pre-treatment (Week -2) and during Weeks 4, 13, 26 and 39. Food and water were removed during urine collection.

During week 26, creatinine and urinary glucose for female treatment groups were transiently increased up to 1.8- and 1.75-fold, respectively. No changes of these parameters were evident at week 39. The significance of these changes was unclear as there were no treatment-related histopathological findings in the kidneys.

Gross Pathology: After 39 weeks of treatment, 4 animals/sex/group from Groups 1 – 4 or following a 6-week recovery period, 2 animals/sex/group from Groups 1 and 4 were sacrificed and submitted to gross necropsy examination.

There were no treatment-related gross pathological findings.

Organ Weights: Absolute and relative organ weights were measured for the adrenals, (paired), brain, heart, kidneys, liver, lung, ovaries (paired), prostate, testes (paired), and thymus.

Dose-related decreases of thymus weights were observed for male treatment groups (decreased to 0.54 times control). Thymus weights were increased for female treatment groups (up to 1.29 times control). Liver weights were decreased for male treatment groups (decreased to 0.87 times controls). Liver weights were increased for females in the high dose group (up to 1.17 times controls). Ovary weights were decreased in female treatment groups although a dose-response relationship was not present.

Table 24 Organ weights at the end of the treatment and recovery periods

Parameter	Week	Males				Females			
		0	109	421	1002	0	109	421	1002
Thymus g	26	9.633	8.205 (85%)	7.07 (73%)	5.239 (54%)	5.544	6.830 (123%)	6.332 (114%)	7.143 (129%)
	R	15.033			10.521 (70%)	9.822			6.394 (65%)
Thymus %BW	26	0.081	0.068 (84%)	0.062 (77%)	0.047 (58%)	0.054	0.069 (128%)	0.065 (120%)	0.071 (131%)
	R	0.100			0.075 (75%)	0.102			0.073 (72%)
Thymus %BrW	26	12.010	9.589 (80%)	8.707 (73%)	6.581 (55%)	7.334	9.517 (130%)	8.611 (117%)	9.675 (132%)
	R	18.819			12.569 (67%)	14.237			8.296 (58%)
Liver g	26	363.136	337.721 (93%)	325.318 (90%)	316.951 (87%)	312.459	277.149	274.940	358.425 (115%)
	R	411.042			360.72 (88%)				
Liver %BW	26	3.034	2.839 (94%)	2.907 (96%)	2.905 (96%)	3.032	2.7221	2.863	3.589 (118%)

	R	2.769			2.517 (91%)				
Liver %BrW	26 R	455.564 513.936	404.228	393.889	400.738 424.500 (83%)	412.456	384.090	374.781	485.514 (118%)
Ovaries g	26 R					0.976 0.950	1.858 (190%)	1.186 (122%)	1.568 (161%) 1.686 (177%)
Ovaries %BW	26 R					0.009 0.010	0.018 (200%)	0.012 (133%)	0.016 178%) 0.019 (190%)
Ovaries %BrW	26 R					1.289 1.356	2.637 (205%)	1.616 (125%)	2.113 (164%) 2.194 (162%)

Histopathology:

Adequate Battery: A complete panel of tissues was submitted to histopathological examination. Samples of the tissues indicated were processed to paraffin wax, sectioned, stained with hematoxylin and eosin, and examined microscopically for all animals.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Ovaries	X
Adrenals	X	Pancreas	X
Aorta (thoracic)	X	Pituitary	X
Brain (cerebellum, cerebrum, midbrain, medulla)	X	Prostate	X
Cecum	X	Rectum	
Colon	X	Salivary gland[b]	
Duodenum	X	mandibular	X
Epididymides	X	parotid	X
Esopagus (distal portion)	X	Sciatic nerve[b]	X
Eyes	X	Skeletal muscle[b] (thigh)	X
		Skin (dorsal)	X
Femur (Femoral head)	X	Spinal cord	
Gallbladder	X	Lumber level (transverse and longitudinal section)	X
Heart (left and right Auricle, left and right Ventricle and Interventricular septum)	X	cervical	
Ileum	X	Spleen	X
Jejunum	X	Sternum with bone marrow	X
Kidneys	X	Stomach (cardiac, fundic and pyloric)	X
Larynx (2 levels)	X	Testes	X
Liver (section from left lateral and right medial lobes)	X	Thymus	X
Lungs including bronchi (left and right cardiac, left and right apical, left and right diaphragmatic)[a]	X	Thyroids with Parathyroids	X
Lymph node -		Tongue	X
mandibular	X	Tonsils	X
mesenteric	X	Trachea (top, middle, bottom)	X
popliteal		Tracheal Bifurcation (with main stem bronchi)	X
tracheo-bronchial	X	Urinary bladder	X
Mammary gland (inguinal)	X	Uterus with cervix	X
Nasal cavities and nasopharynx with skull (4 levels)	X	Vagina	X
Optic nerves	X		

[a]At least 4 lobes were examined, left and right, to include proximal and distal areas.
[b]Only 1 examined.

Peer Review: A peer review of selected microscopic tissue sections, and pathology data interpretation was completed. An additional peer review was performed by the Sponsor. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.

Histological Findings: Potential target organs of toxicity were the heart, lungs, larynx, and nose turbinates. There were additional findings in testes, tonsils, and adrenals of uncertain relationship to treatment.

Heart: Moderate subacute inflammation of the extramural coronary arteries was seen in one female (#4925) given 1002 µg/kg/day and in one male (#3427) given 421 µg/kg/day. The change was characterized by intimal proliferation, medial necrosis and mononuclear inflammatory cell infiltrate predominantly in the adventitia. No similar changes were present in the concurrent controls or have been reported in control dogs from studies at this laboratory.

The coronary arterial change was similar in morphology and distribution to spontaneous idiopathic extramural coronary arteritis, a well-recognized arterial lesion described in beagle dogs which generally affects small to large muscular arteries. The pulmonary arterial change could be part of the spectrum of this change.

Drug-induced vascular injury has been reported in laboratory animals with various vasoactive drugs of different pharmacological classes, and generally considered to be a consequence of hemodynamic changes. Drug-induced vascular changes of this nature are generally acute in onset and show morphological changes such as hemorrhage and necrosis in the media and/or adventitia, perivascular edema and mixed inflammatory cell infiltrate with multifocal distribution affecting blood vessels in various organ; they may or may not display a dose-response relationship.

While there are no consistent pathognomonic histologic features that differentiate the spontaneous from the drug-induced vascular changes of this nature, features such as intimal proliferation and marked mononuclear inflammatory cell infiltrate and absence of hemorrhage are more consistent with spontaneous change than with drug-induced vascular injury.

There are a number of reasons to consider the arterial changes in this study to be incidental, or an exacerbation of an underlying idiopathic disease. These include the morphologic similarity of the lesion to spontaneous idiopathic coronary arteritis in the dog, the absence of exposure-relationship in the current study, and the absence of vascular changes in dogs in shorter term studies with GSK573719 at significantly higher systemic exposures (WD2005/01423/00). However, based on the occurrence of the vascular lesions in dogs given 421 or 1002 µg/kg/day and the absence of similar findings in concurrent controls or in the historical data base at this laboratory, a conservative approach has been taken to set the NOAEL for this finding at 109 µg/kg/day.

Lung: In one female (#4926) given 1002 µg/kg/day, there was mild intimal thickening with minimal focal areas of mixed, predominantly mononuclear, inflammatory cells infiltrate in an arteriole in one lobe of the lung.

Larynx: GSK573719 related changes were present in the mucosa of the vocal folds and glands adjacent to the arytenoid cartilage in dogs given 1002 µg/kg/day.

Slight epithelial erosion and/or ulceration was present in the squamous epithelium overlying the base of the vocal folds in the larynx of one male (#4425) and one female (#4926) given 1002 µg/kg/day. This finding was characterized by focal necrosis of the epithelium with mild acute to subacute inflammation in the underlying mucosa. In addition, slight subacute to chronic inflammation was present in the mucosal glands adjacent to the arytenoid cartilage in the male dog (#4425). Focal minimal squamous cell hyperplasia and hyperkeratosis was present in the epithelium overlying the base of the vocal folds in one male (Animal #3425) given 421 µg/kg/day. Minimal focal erosion/ulceration was also seen in one control animal at the end of the recovery period.

The most rostral areas of the larynx receive greater exposure to inhaled substances and are more likely to show inflammation and erosion in response to direct contact with irritant material. Prolonged irritation may also induce an adaptive change in the stratified squamous epithelium lining this region of the larynx, which is characterized by hyperplasia and increased thickness of the overlying keratinized layer (hyperkeratosis).

Nasal turbinates: Minimal squamous metaplasia of the respiratory epithelium was seen in the most cranial section of the nasal turbinates in one male (#4427) given 1002 µg/kg/day. It was not completely clear how the nasal turbinates were exposed to GSK573719 from oropharyngeal tube other than through indirect leakage.

There were additional findings in testes (seminiferous tubules, hypospermatogenesis), tonsils, and adrenals of uncertain relationship to treatment. Findings in the testes were not dose responsive. The finding in the tonsils (abscess) was confined to single animals. The findings in the adrenal glands (nodules) were observed in two high dose dogs at the end of the treatment period and one control dog at the end of the recovery period.

Table 25 Histopathological findings at the end of the treatment and recovery periods

Organ/Tissue	Sex	End of the 39-week treatment period				End of the 6-week recovery period	
		0	109	421	1002	0	1002
Heart							
-extramural coronary arteries: arteritis necrotizing, G3	M	0/4	0/4	1/4	0/4	0/2	0/2
	F	0/4	0/4	0/4	1/4	0/2	0/2
-atrioventricular valve: hemorrhage, G1	M	0/4	0/4	1/4	0/4	0/2	0/2
	F	0/4	0/4	0/4	0/4	0/2	0/2
Lungs							
-perivascular/peribronchial/subpleural mixed inflammatory cell infiltrate, G1-2	M	3/4	2/4	1/4	2/4	0/2	0/2
	F	0/4	2/4	3/4	4/4	0/2	1/2
-subacute/chronic inflammation, G1-2	M	1/4	2/4	3/4	0/4	1/2	0/2
	F	1/4	2/4	1/4	2/4	1/2	0/2
-increased alveolar macrophages, G1-2	M	0/4	0/4	1/4	1/4	0/2	0/2
	F	0/4	2/4	2/4	1/4	0/2	0/2
-pleural fibrosis, G1	M	0/4	0/4	0/4	0/4	0/2	0/2
	F	0/4	0/4	0/4	1/4	0/2	0/2
-arterial wall: focal mononuclear inflammatory cell infiltrate, G1	M	0/4	0/4	0/4	0/4	0/2	0/2
	F	0/4	0/4	0/4	1/4	0/2	0/2
Larynx							
-mucosal epithelium: erosion/ulceration, G2	M	0/4	0/4	0/4	1/4	1/2	0/2
	F	0/4	0/4	0/4	1/4	0/2	0/2

-mucosal glands: subacute/chronic inflammation, G2	M F	0/4 0/4	0/4 0/4	0/4 0/4	1/4 0/4	0/2 0/2	0/2 0/2
-squamous epithelium: hyperplasia, G1	M F	0/4 0/4	0/4 0/4	1/4 0/4	0/4 0/4	0/2 0/2	0/2 0/2
-submucosa: increased inflammatory cell infiltrate	M F	0/4 0/4	0/4 0/4	0/4 0/4	1/4 0/4	0/2 0/2	0/2 0/2
Nose Turbinates -respiratory epithelium: squamous metaplasia, G1	M F	0/4 0/4	0/4 0/4	0/4 0/4	1/4 0/4	0/2 0/2	0/2 0/2
Testes -seminiferous tubules: hypospermatogenesis, G1-2	M	1/4	2/4	0/4	3/4	0/2	1/2
Tonsil -abscess	M F	0/4 0/4	0/4 0/4	0/4 0/4	0/4 1/4	0/2 0/2	0/1 0/2
Adrenals -cortex: capsular/extra-capsular nodules, G1-2	M F	0/4 0/4	0/4 0/4	0/4 0/4	1/4 1/4	1/2 0/2	0/2 0/2

Special Evaluation: During Week 37 of the treatment period, 1 animal each, showing the greatest apparent degree of neck swelling, from Groups 1, 3 and 4 were selected for further evaluations of the neck swellings. After completion of dosing, the necks of the fasted animals were shaved and the animals anesthetized with propofol (7 mg/kg), which was administered via the cephalic or saphenous vein. Additional propofol was administered as needed to maintain anesthesia throughout the procedure. The shaven area was cleansed with povidone iodine, followed by saline and alcohol. Using a needle of an appropriate gauge, the swollen area was punctured and any fluid that was present within the affected tissue(s) beneath the skin was withdrawn. With the needle still in place, an X-ray of the sampled area was taken and evaluated by the Testing Facility Veterinarian. A Note to the Study File detailing the results of the evaluations was placed in the study raw data file. Fluid collected from the dogs was submitted to the Testing Facility's Clinical Pathology department for slide preparation and staining with modified Wright's stain.

Radiographs of swellings in the neck of a male given 421 µg/kg/day (animal #3425) and a male given 1002 µg/kg/day (animal #4427) showed nothing remarkable.

One slide each from Animal #3425 and from Animal #4427 were examined.

Animal #3425 (421 µg/kg/day): Slide from Animal #3425 had very low cellularity and a pale, smooth eosinophilic background. There were few scattered erythrocytes that were interpreted to be minimal blood contamination secondary to the fine needle aspiration procedure. No nucleated cells were observed. No atypical cells were present. The examination was judged to be inconclusive due to absence of nucleated cells.

Animal No. 4427 (1002 µg/kg/day): Slide from Animal #4427 had low cellularity and a pale granular basophilic background, with few scattered erythrocytes. Few intact

nucleated cells were present in small loose clusters or individually but associated with clusters. These were consistent with epithelial cells from the salivary gland. The cells had round nuclei and variable quantities of pale basophilic, granular to vacuolated cytoplasm. Nuclei were small to medium and uniform, with smooth chromatin. Nucleoli were not detectable. Cells that tended to be present in clusters had large quantities of cytoplasm and were interpreted to be secretory cells. Cells that tended to be present individually had small amounts of cytoplasm. There were numerous broken nuclear remnants representing lysed cells. Basophilic granular background material was consistent with cytoplasm from these lysed cells. Rare neutrophils were seen. No other evidence of inflammation was present. No atypical cells were present.

Toxicokinetics: Blood samples were for measurement of plasma drug concentrations were collected on Day 1, immediately after dosing (within 10 min) and during Weeks 4, 13, 26, and 39, immediately after dosing (within 10 min), and at 20, 30 and 45 minutes and 1, 3, 7 and 24 hours after the start of dosing. All sample tubes were stored frozen (approximately -80°C or lower) and shipped (frozen, on dry ice) to the Sponsor for analysis.

Naïve blood samples (approximately 2.0 or 3.0 mL) were collected from 16 animals. On the day that toxicokinetic blood sampling occurred, the naïve control samples were removed from the freezer and placed in a dedicated labeled container. One naïve plasma sample was then placed in a rack alongside the concurrent control blood samples collected at the first and last sampling points on each day of sampling [i.e., immediately after completion of dosing (all occasions) and 7 hours after the start of dosing (during Weeks 4, 13, 26 and 39)] and at 24 hours after the start of dosing (during Weeks 4, 13, 26 and 39). Naïve samples were analyzed at the same time as the study samples.

Concentrations of GSK573719 in plasma from all animals (including controls) were determined by HPLC-MS/MS by GSK PCD DMPK (Ware). The lower limit of quantification (LLQ) for GSK573719 was 0.1 ng/mL, using a 25 or 50 µL aliquot of dog plasma with a higher limit of quantification (HLQ) of 100 ng/mL.

C_{max} and AUC values for GSK573719 increased in an approximate dose proportional manner. There were significant decreases in drug exposure for all male treatment groups during week 39 as compared to earlier time points. There were decreases in drug exposure for females in the high dose group during weeks 26 and 39 as compared to earlier time points. There were no differences in drug exposure between males and females.

Toxicokinetic Parameter+	Week	Males				Females			
		Overall Estimated Achieved Dose GSK573719				Overall Estimated Achieved Dose GSK573719			
		0 µg/kg/day	109 µg/kg/day	421 µg/kg/day	1002 µg/kg/day	0 µg/kg/day	109 µg/kg/day	421 µg/kg/day	1002 µg/kg/day
$AUC_{(0-t)}$ (ng.h/mL)	4	NQ	11.6	39.5	85.6	NQ	13.3	44.6	114
	13	NQ	9.89	32.2	98.5	NQ	11.2	46.9	86.2
	26	NQ	16.4	39.5	94.4	NQ	15.2	34.8	43.0
	39	NQ	4.64	23.0	46.9	NQ	7.13	29.5	41.2
C_{max} (ng/mL)	4	NQ	11.1	45.0	86.0	NQ	12.2	44.2	128
	13	NQ	5.78	29.9	94.4	NQ	5.73	46.2	96.8
	26	NQ	10.2	39.6	83.4	NQ	9.12	30.2	21.4
	39	NQ	3.13	14.3	17.9	NQ	3.92	16.5	35.2

+ These values have been derived by dose normalising the AUC and C_{max} using the estimated achieved dose on the day of sampling for each sex and then re-normalising the AUC and C_{max} using the overall combined dose.
NQ Not quantifiable due to plasma results being below the LOQ.

Dosing Formulation Analysis: The final report should be checked for details of variations of daily exposure concentrations.

Study title: <u>GSK573719A and GW642444M – A 13-Week Combination Inhalation Toxicity Study in Dogs</u> (Audited Draft Report)

Study no.:	WD2010/00677/01
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	September 22, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See table below. Micronized GSK573719A and GW642444M were blended at [redacted] (b) (4). Test article blends were prepared at [redacted] (b) (4). Each blend was prepared in the required proportions of test articles in vehicle (Lactose [Batch/Lot No 626567/081167512] with a nominal 1% (w/w) magnesium stearate [Batch No. 081175758]). Blends were deemed acceptable for use on the study only after blend sample analysis results were approved by the Sponsor.

Concentrations of test article and excipients in the formulation are expressed in terms of parent and are nominal.

Material	Input Material Batch number	Input Material Purity	Blend Batch Number	Blend test article concentration (nominal)	Content uniformity	Blend Expiry/ Retest	Blend Storage
GSK573719A GW642444M (Group 2)	081168732 071137021	99.8% 64.2%	BVR/P/1012/09	40% w/w 0.2% w/w	96.9% 97.0%	21 Sep 2010	2 to 8°C, protect from light
GSK573719A GW642444M (Groups 3 & 4)	081168732 071137021	99.8% 64.2%	BVR/P/1015/09	3% w/w 3% w/w	95.5% 99.3%	23 Sep 2010	2 to 8°C, protect from light
GSK573719A GW642444M (Group 5)	081168732 071137021	99.8% 64.2%	BVR/P/1014/09	15% w/w 15% w/w	98.2% 101.8%	22 Sep 2010	2 to 8 °C, protect from light
GSK573719A (Group 6)	081168732	99.8%	BVR/P/1011/09	40% w/w	96.9%	21 Sep 2010	Up to 30 °C, protect from light
GW642444M (Group 7)	071137021	64.2%	BVR/P/1013/09	15% w/w	100.8%	22 Sep 2010	2 to 8°C, protect from light

Key Study Findings

• In a 13 week repeat dose oropharyngeal tube inhalation toxicology study, beagle dogs received GSK573719A and GW642444A alone or in combination for 10 min/day. Achieved doses of GSK573719/GW642444 for Groups 2, 3, 4, 5, 6, and 7 were 1070/7.5, 23/29, 60/72, 177/183, 1048/0, and 0/180 µg/kg/day, respectively.

• Prior to giving dogs GW642444 at a target dose of 160 µg/kg/day in combination with GSK573719 or alone (i.e., Groups 5 and 7), the sponsor administered GW642444 at a target dose of 40 µg/kg/day (alone or with GSK573719) for 10 min/day for 3 consecutive days prior to escalation to the high target dose of 160µg/kg/day. The sponsor contended that this pre-adaptation phase of dosing (Phase 1) was required to induce tachyphylaxis to the cardiovascular effects of GW642444, allowing administration of higher doses than would normally be possible in untreated dogs. The Division communicated several concerns to the sponsor that pre-exposure of dogs to smaller doses may provide protection against subsequent exposure to larger doses (see Toxicology and Applied Pharmacology 69: 199-205, 1983). It was noted that the use of the pre-adaptation phase will be a review issue when the study is submitted. The sponsor has submitted a 4-week dog study to directly assess the effects of pre-adaptation that will evaluated in a future review.

• Increased incidences of excessive salivation were observed in all male and female treatment groups during dosing and were still evident in a few treatment groups immediately postdose. Increased incidences of bilateral neck swelling were observed in all male and female treatment groups during dosing and /or immediately postdose.

• Immediately after dosing (IAD) on day 91/92, heart rates increases (up to 1.86- and 1.76-fold times higher than mean pretest values in males and females, respectively) were observed in dogs that received either GSK573719 or GW642444 alone or in combination.

• Both prior to and immediately after dosing on days 91/92, respiratory sinus arrhythmia (RSA) was absent in 15/16 animals from Groups 2 and 6 that received GSK573719 at a target dose of 1000 µg/kg/day either in combination with GW642444 or alone, respectively, suggesting that the effect was persistent at this target dose of GSK573719. RSA was absent immediately after dosing on days 91/92 for several male and female dogs in Groups 3, 4, and 5 that received the combination of GSK573719 and GW642444 (up to target doses of 160 µg/kg/day for each component).

• During Phase1 (Pre-adaptation), elevations of serum cTnI concentrations were observed for females in Group 5 (44/47 µg/kg/day) and males and females in Group 7 (49 µg/kg/day GW642444). During Phase 2 (treatment period), fewer females in Group 5 (177/183 µg/kg/day) were observed with elevated serum cTnI concentrations despite the 4-fold increase of dose. Only 1 male in Group 5 was observed with elevated serum cTnI concentrations. For dogs in Group 7 (180 µg/kg/day GW642444), 1 male and 3 females were observed with elevated serum cTnI concentrations. Based upon the response of females in Group 5, the pre-adaptation phase induced tachyphylaxis or tolerance to potential cardiac injury (as measured by serum cTnI concentrations) induced by the combination of GSK573719 and GW642444.

• Histopathological findings were observed in the lung, larynx, nose/turbinates, trachea, thyroid, testes, thymus, and heart that could potentially be attributed to treatment. In general, these findings were consistent the individual test articles, GSK573719 and GW642444, and there was no evidence of novel toxicity with the combination.

• In the lungs, marked, localized, subacute/chronic inflammation was seen in 1 male dog (#2452) in Group 2 (1000/5 µg/kg/day). This change was associated with bronchial/bronchiolar ectasia with luminal cellular debris, hyperplasia of the bronchial goblet cells, and pleural adhesions. In addition, plant/food material and bacterial colonies were present.

• In the larynx, slight increase in incidence and/or severity of mixed inflammatory cell infiltrates was observed for male dogs in Groups 2 (1000/5 µg/kg/day) and 5 (160/160 µg/kg/day) and females in Group 3 (23/29 µg/kg/day).

• Increased inflammatory cells in the nasal mucosa were observed for single dogs in Groups 3 (23/29 µg/kg/day), 5 (177/183 µg/kg/day), 6 (1048 µg/kg/day), and 7 (180 µg/kg/day). Inflammatory cells and cell debris were observed for single dogs in Groups 6 (1048 µg/kg/day) and 7 (180 µg/kg/day).

• Mixed inflammatory cell infiltrate in the tracheal mucosa was observed for 1 male dog in Group 2 (1000/5 µg/kg/day).

• There was no evidence of additive or synergistic toxicity with the combination of GSK573719 and GW642444. Observed toxic effects were consistent with the individual agents.

Methods

Doses:	Prior to giving dogs GW642444 at a target dose of 160 µg/kg/day in combination with GSK573719 or alone (i.e., Groups 5 and 7), the sponsor administered GW642444 at a target dose of 40 µg/kg/day (alone or with GSK573719) for 10 min/day for 3 consecutive days prior to escalation to the high target dose of 160µg/kg/day. The sponsor contended that this pre-adaptation phase of dosing (Phase 1) was required to induce tachyphylaxis to the cardiovascular effects of GW642444, allowing administration of higher doses than would normally be possible in untreated dogs. Target doses of GSK573719/GW642444 for Phase 2 were 0/0, 1000/5, 20/20, 60/60, 160/160, 1000/0, and 0/160 µg/kg/day.
Frequency of dosing:	Daily (10 min/day)
Route of administration:	Oropharyngeal tube inhalation
Dose volume:	NA
Formulation/Vehicle:	Control received Lactose/1% magnesium stearate
Species/Strain:	28 male and 28 female beagle dogs obtained from (b) (4)
Number/Sex/Group:	4 dogs/sex/group
Age:	Dogs were 11-12 months old at the start of treatment
Weight:	Body weight ranges for males were 6.9 to 10.1 kg (Phase 1) and 7.1 to 10.8 kg (Phase 2). Body weight ranges for females were 6.6 to 9.5 kg (Phase 1) and 6.7 to 9.7 kg (Phase 2).
Satellite groups:	None
Unique study design:	The study included a pre-adaptation phase.
Deviation from study protocol:	Deviations were minor and did not influence the integrity of the study.

Study Design:

Phase 1 (Tolerance Phase, Days -3 to -1; Groups 5 and 7 only)

Tachycardia is a known and expected pharmacological effect following administration of β_2-agonists, the magnitude and duration of which diminishes upon repeat dosing. In order to induce tachyphylaxis to the cardiovascular effects of GW642444 (a β_2-agonist) expected at a target dose of 160 µg/kg/day (maximum tolerated dose for GW642444) prior to the 4-week dosing period, animals (4/sex/group) in Groups 5 and 7 were given a lower dose for 3 consecutive days (Phase 1) immediately prior to Phase 2 as follows:

Group	Blend	Target aerosol concentration	Average achieved aerosol concentration	Target dose	Estimated achieved dose
		(µg/L)[1]	(µg/L)[1,2]	(µg/kg/day)[1,3]	(µg/kg/day)[1,4]
5	GSK573719/ GW642444	12/12	13.2/14.1	40/40	44/47
7	GW642444	12	14.6	40	49

1. Expressed in terms of GSK573719 or GW642444 (the parent compound), as appropriate.
2. Based on chemical analysis.
3. The dosage is calculated as the total inhaled body dose assuming an exposure duration of 10 minutes and a body weight of 8.5 kg (inhalable fraction F not included in calculation).

$$\text{Dose (µg/kg/day)} = \frac{\text{C (µg/L)} \times \text{RMV (L/min)} \times \text{D (min)}}{\text{BW (kg)}}$$

Where C = Concentration (µg/L) in air inhaled of GSK573719 or GW642444
RMV = Respiratory minute volume (L/minute).
The RMV is calculated from the formula
$\text{RMV (L/minute)} = 0.499 \times \text{BW (kg)}^{0.809}$ (Bide, 2000)
D = Duration of exposure in minutes
BW = Bodyweight (kg)

4. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation above; the body weights used were group averages for Phase 1.

Phase 2 (13-Week Treatment Period, Days 1 to 92; all groups)

Beginning on Day 1 and throughout the 13-week dosing period, animals in Groups 1 to 7 (4/sex/group) were administered doses as detailed in the following table:

Group	Blend	Target aerosol concentration	Average achieved aerosol concentration	Target dose	Estimated achieved dose	MMAD	GSD
		(µg/L)[1]	(µg/L)[1,2]	(µg/kg/day)[1,3]	(µg/kg/day)[1,4]	(µm)	
1	Lactose/1% Magnesium Stearate Control	0	0	0	0	(b) (4)	
2	GSK573719/ GW642444	300/1.5	328/2.30	1000/5	1070/7.5		
3	GSK573719/ GW642444	6/6	7.19/8.85	20/20	23/29		
4	GSK573719/ GW642444	18/18	18.6/22.1	60/60	60/72		
5	GSK573719/ GW642444	48/48	54.6/56.2	160/160	177/183		
6	GSK573719	300	319	1000	1048		
7	GW642444	48	55.4	160	180		

1. Expressed in terms of GSK573719 or GW642444 (the parent compound), as appropriate.
2. Based on chemical analysis.
3. The dosage is calculated as the total inhaled body dose assuming an exposure duration of 10 minutes and a body weight of 8.5 kg (inhalable fraction F not included in calculation).

$$\text{Dose (µg/kg/day)} = \frac{C\ (\mu g/L) \times RMV\ (L/min) \times D\ (min)}{BW\ (kg)}$$

Where C = Concentration (µg/L) in air inhaled of GSK573719 or GW642444
RMV = Respiratory minute volume (L/minute).
The RMV is calculated from the formula
RMV (L/minute) = 0.499 x BW (kg) $^{0.809}$ (Bide, 2000)
D = Duration of exposure in minutes
BW = Bodyweight (kg)

4. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation above; however, the body weights used were group averages for the study.

NA = Not applicable.

Aerosol delivery system: Each aerosol delivery system consisted of a dust generator (Wright Dust Feed), an aerosol conditioning chamber with a sub-chamber fitted with 4 outlets, aerosol delivery tubing, Y-piece connections with isolating ball valve and oropharyngeal tube attached. The aerosol exhaust system included a scavenger exhaust system consisting of a chamber of identical dimensions to the sub-chamber used in the delivery system, further aerosol tubing and a series of particulate filters, a flow meter with integral needle valve and an extract pump. The oropharyngeal tubes were constructed of silicone rubber and were inserted into the mouth of the dogs during dosing.

A separate atmosphere generation system was employed to dose each group of animals.

The compressed air flow rate exiting the Wright Dust Feed (WDF) for all groups was set to 20 liters/minute (L/min) and an additional dilution flow of compressed air was supplied at 10 L/min to the conditioning chamber, via an inlet that was adjacent to the aerosol entry point. The aerosol-laden air exiting the conditioning chamber (30 L/min, equal to 7.5 L/min/animal and each of 2 spare positions for sampling) was to 4 active outlets to potential animal exposure positions via clinical aerosol tubing with a Y-piece providing the attachment point for the ball valve and oropharyngeal tube. Two aerosol outlets were used to supply aerosol to the animals assigned to the study and the remaining outlets were used for the collection of aerosol samples. This arrangement was necessary due to the short exposure duration and the occasional requirement for the simultaneous collection of 2 samples (i.e., concentration and particle size distribution).

Chamber Aerosol Concentration: A minimum of 2 samples per group were collected on each day of exposure. A measured volume of air was drawn from exposure chambers at a rate of approximately 2 Liters/minute through a Pall type A/E glass fiber filter, fitted to an open-faced filter holder attached to a spare animal breathing point. For magnesium stearate chemical analysis, a measured volume of air was drawn at a rate of 7.5 L/min from a representative animal breathing point through a Millipore Quartz fiber filter, fitted to an open-faced filter holder. Additional samples were collected from an animal breathing point of each group before daily treatment commenced to demonstrate (gravimetrically) satisfactory operation of the exposure system. Data generated from these samples were not reported, but were retained in the study file.

On each day and from all groups, the first sample taken from the exposure system was designated for analysis of GSK573719 and/or GW642444, as appropriate. The second sample was then taken and designated for analysis of magnesium content.

The glass fiber filters analyzed for GSK573719 and for GW642444 content utilized a single High Performance Liquid Chromatography (HPLC) method with Ultra Violet (UV) detection and a quantification range of 1.0 to 16 µg/mL.

Magnesium stearate concentration was determined by analyzing the quartz filters for magnesium using atomic absorption spectroscopy and a magnesium lamp within a

quantification range of 2.0 to 10 µg/mL. The results were expressed in terms of magnesium stearate.

Summary of Average Achieved Tube Aerosol Concentration and Estimated Achieved Doses of GSK573719

Group Number	Target tube aerosol concentration (µg/L) [1]	Average achieved tube aerosol concentration (µg/L) [1, 2]		% change from target tube aerosol concentration		Estimated achieved dose (µg/kg/day) [1, 3]	
		Males	Females	Males	Females	Males	Females
1[4]	0	0	0	0	0	0.00	0.00
						Average = 0.00	
2[5]	300	326	329	+8.67	+9.67	1056	1084
						Average = 1070	
3[6]	6	7.20	7.17	+20.0	+19.5	22.9	23.7
						Average = 23.3	
4[6]	18	18.6	18.5	+3.33	+2.78	58.9	61.2
						Average = 60.2	
5[7]	Phase 1 [10]						
	12	13.0	13.3	+8.33	+10.8	42.8	44.8
						Average = 43.8	
	Phase 2 [11]						
	48	54.7	54.4	+14.0	+13.3	176	178
						Average = 177	
6[8]	300	317	321	+5.67	+7.00	1021	1074
						Average = 1048	
7[9]	Phase 1 [10]						
	0	0	0	0	0	0.00	0.00
						Average = 0.00	
	Phase 2 [11]						
	0	0	0	0	0	0.00	0.00
						Average = 0.00	

1. Expressed in terms of GSK573719 (the parent compound).
2. As determined by chemical analysis.
3. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation below.
4. Exposed to vehicle (1% [w/w] magnesium stearate in lactose) only.
5. Exposed to 40% (w/w) GSK573719 and 0.20% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.

6. Exposed to 3% (w/w) GSK573719 and 3% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
7. Exposed to 15% (w/w) GSK573719 and 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.

8. Exposed to 40% (w/w) GSK573719 in 1% (w/w) magnesium stearate in lactose.
9. Exposed to 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
10. In order to induce tachyphylaxis to the cardiovascular effects of GW642444 prior to the 13-week dosing period, either a dose of 40 µg/kg/day GW642444 combined with 40 µg/kg/day GSK573719 (Group 5), or a dose of 40 µg/kg/day GW642444 alone (Group 7) was administered for 3 consecutive days (Days –3 to –1) prior to Day 1.
11. Beginning on Day 1 and throughout the 13-week dosing period, a dose of 160 µg/kg/day GW642444 combined with 160 µg/kg/day GSK573719 (Group 5), or a dose of 160 µg/kg/day GW642444 alone (Group 7) was administered.

$D = (RMV \times T \times C)/BW$

D Dose (µg/kg)
RMV Respired minute volume (L/min) $0.499 \times BW(kg)^{0.809}$ [Bide et al, 2000]
T Duration of exposure/day (minutes)
C Aerosol concentration of GSK573719 (µg/L)
BW Group average body weight for the treatment period (kg)

Calculation of Estimated Achieved GSK573719 Inhaled Doses

$$\text{Estimated dose to animals (µg/kg/day)} = \frac{RMV \times T \times C}{BW}$$

Where:
T - Time of exposure (minutes/day)
C - Aerosol concentration (µg GSK573719/L)
BW - The average of the group mean body weight for the treatment period (kg)
RMV - Respired minute volume (L/min) ($0.499 \times BW^{0.809}$) [Bide et al, 2000]

Group	Average achieved tube aerosol concentration (µg/L) [1, 2]		Time of exposure (min)	Body weight (kg) [3]		Estimated achieved dose (µg/kg/day) [1, 2]	
	Male	Female		Male	Female	Males	Females
1[4]	0.00	0.00	10	9.2	7.7	0.00	0.00
						Average = 0.00	
2[5]	326	329	10	9.6	8.8	1056	1084
						Average = 1070	
3[6]	7.20	7.17	10	10.5	8.7	22.9	23.7
						Average = 23.3	
4[6]	18.6	18.5	10	10.8	8.6	58.9	61.5
						Average = 60.2	
5[7]	54.7	54.4	10	9.8	9.1	176	178
						Average = 177	
6[8]	317	321	10	9.9	8.1	1021	1074
						Average = 1048	
7[9]	0.00	0.00	10	10.0	8.9	0.00	0.00
						Average = 0.00	

1. Expressed in terms of GSK573719 (the parent compound).
2. As determine by chemical analysis.
3. Average of the group mean weekly body weight values taken during the treatment period.
4. Exposed to vehicle (1% [w/w] magnesium stearate in lactose) only.
5. Exposed to 40% (w/w) GSK573719 and 0.20% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
6. Exposed to 3% (w/w) GSK573719 and 3% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
7. Exposed to 15% (w/w) GSK573719 and 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
8. Exposed to 40% (w/w) GSK573719 in 1% (w/w) magnesium stearate in lactose.
9. Exposed to 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.

Summary of Average Achieved Tube Aerosol Concentration and Estimated Achieved Doses of GW642444

Group Number	Target tube aerosol concentration (µg/L) [1]	Average achieved tube aerosol concentration (µg/L) [1, 2]		% change from target tube aerosol concentration		Estimated achieved dose (µg/kg/day) [1, 3]	
		Males	Females	Males	Females	Males	Females
1[4]	0	0	0	0	0	0.00	0.00
						Average = 0.00	
2[5]	1.5	2.29	2.30	+52.7	+53.3	7.42	7.58
						Average = 7.50	
3[6]	6	8.86	8.83	+47.7	+47.2	28.2	29.1
						Average = 28.7	
4[6]	18	22.1	22.1	+22.8	+22.8	70.0	73.1
						Average = 71.6	
5[7]	Phase 1 [10]						
	12	13.9	14.3	+15.8	+19.2	45.8	48.2
						Average = 47.0	
	Phase 2 [11]						
	48	56.3	56.0	+17.3	+16.7	182	183
						Average = 183	
6[8]	0	0	0	0	0	0.00	0.00
						Average = 0.00	
7[9]	Phase 1 [10]						
	12	15.1	14.0	+25.8	+16.7	49.7	47.2
						Average = 48.5	
	Phase 2 [11]						
	48	55.4	55.4	+15.4	+15.4	178	182
						Average = 180	

1. Expressed in terms of GW642444 (the parent compound).
2. As determined by chemical analysis.
3. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation below.
4. Exposed to vehicle (1% [w/w] magnesium stearate in lactose) only.
5. Exposed to 40% (w/w) GSK573719 and 0.20% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.

6. Exposed to 3% (w/w) GSK573719 and 3% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
7. Exposed to 15% (w/w) GSK573719 and 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
8. Exposed to 40% (w/w) of GSK573719 in 1% (w/w) magnesium stearate in lactose.
9. Exposed to 15% (w/w) of GW642444 in 1% (w/w) magnesium stearate in lactose.
10. In order to induce tachyphylaxis to the cardiovascular effects of GW642444 prior to the 13-week dosing period, either a dose of 40 µg/kg/day GW642444 combined with 40 µg/kg/day GSK573719 (Group 5), or a dose of 40 µg/kg/day GW642444 alone (Group 7) was administered for 3 consecutive days (Days –3 to –1) prior to Day 1.
11. Beginning on Day 1 and throughout the 13-week dosing period, a dose of 160 µg/kg/day GW642444 combined with 160 µg/kg/day GSK573719 (Group 5), or a dose of 160 µg/kg/day GW642444 alone (Group 7) was administered.

D = (RMVxTxC)/BW

D Dose (µg/kg)
RMV Respired minute volume (L/min) $0.499 \times BW(kg)^{0.809}$ [Bide et al, 2000]
T Duration of exposure/day (minutes)
C Aerosol concentration of GW642444 (µg/L)
BW Group average body weight for the treatment period (kg)

Calculation of Estimated Achieved GW642444 Inhaled Doses

$$\text{Estimated dose to animals } (\mu g/kg/day) = \frac{RMV \times T \times C}{BW}$$

Where:

T - Time of exposure (minutes/day)
C - Aerosol concentration (µg GW642444/L)
BW - The average of the group mean body weight for the treatment period (kg)
RMV - Respired minute volume (L/min) ($0.499 \times BW^{0.809}$) [Bide et al, 2000]

Group	Average achieved tube aerosol concentration (µg/L) [1, 2]		Time of exposure (min)	Body weight (kg) [3]		Estimated achieved dose (µg/kg/day) [1, 2]	
	Male	Female		Male	Female	Males	Females
1[4]	0.00	0.00	10	9.2	7.7	0.00	0.00
						Average = 0.00	
2[5]	2.29	2.30	10	9.6	8.8	7.42	7.58
						Average = 7.50	
3[6]	8.86	8.83	10	10.5	8.7	28.2	29.1
						Average = 28.7	
4[6]	22.1	22.1	10	10.8	8.6	70.0	73.1
						Average = 71.6	
5[6]	56.3	56.0	10	9.8	9.1	182	183
						Average = 183	
6[7]	0.00	0.00	10	9.9	8.1	0.00	0.00
						Average = 0.00	
7[8]	55.4	55.4	10	10.0	8.9	178	182
						Average = 180	

1. Expressed in terms of GW642444 (the parent compound).
2. As determined by chemical analysis.
3. Average of the group mean weekly body weight values taken during the treatment period.
4. Exposed to vehicle (1% [w/w] magnesium stearate in lactose) only.
5. Exposed to 40% (w/w) GSK573719 and 0.20% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
6. Exposed to 3% (w/w) GSK573719 and 3% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
7. Exposed to 15% (w/w) GSK573719 and 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
8. Exposed to 40% (w/w) GSK573719 in 1% (w/w) magnesium stearate in lactose.
9. Exposed to 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.

Summary of Average Achieved Tube Aerosol Concentration and Estimated Achieved Doses of Magnesium Stearate

Group Number	Average achieved tube aerosol concentration (µg/L) [1, 2]		Estimated achieved dose (µg/kg/day) [1, 3]	
	Males	Females	Males	Females
1[4]	7.89	8.01	25.8	27.1
			Average = 26.5	
2[5]	6.64	6.68	21.5	22.0
			Average = 21.8	
3[6]	2.18	2.20	6.94	7.26
			Average = 7.10	
4[6]	5.72	5.72	18.1	18.9
			Average = 18.5	
5[7]	Phase 1[10]			
	0.76	0.72	2.50	2.43
			Average = 2.47	
	Phase 2[11]			
	2.46	2.46	7.94	8.05
			Average = 8.00	
6[8]	6.24	6.29	20.1	21.0
			Average = 20.6	
7[9]	Phase 1[10]			
	0.78	0.68	2.56	2.30
			Average = 2.43	
	Phase 2[11]			
	2.25	2.26	7.23	7.43
			Average = 7.33	

1. Dose levels and tube aerosol concentrations are expressed in terms of Magnesium Stearate.

2. Magnesium was quantified using an atomic absorption spectroscopy method.
3. Estimated inhaled dose calculated and assumes 100% deposition in the respiratory tract per equation below.
4. Exposed to vehicle (1% [w/w] magnesium stearate in lactose) only.
5. Exposed to 40% (w/w) GSK573719 and 0.20% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
6. Exposed to 3% (w/w) GSK573719 and 3% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
7. Exposed to 15% (w/w) GSK573719 and 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
8. Exposed to 40% (w/w) GSK573719 in 1% (w/w) magnesium stearate in lactose.
9. Exposed to 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
10. In order to induce tachyphylaxis to the cardiovascular effects of GW642444 prior to the 13-week dosing period, either a dose of 40 µg/kg/day GW642444 combined with 40 µg/kg/day GSK573719 (Group 5), or a dose of 40 µg/kg/day GW642444 alone (Group 7) was administered for 3 consecutive days (Days –3 to –1) prior to Day 1.
11. Beginning on Day 1 and throughout the 13-week dosing period, a dose of 160 µg/kg/day GW642444 combined with 160 µg/kg/day GSK573719 (Group 5), or a dose of 160 µg/kg/day GW642444 alone (Group 7) was administered.

D = (RMVxTxC)/BW

D Dose (µg/kg)
RMV Respired minute volume (L/min) $0.499 \times BW(kg)^{0.809}$ [Bide et al, 2000]
T Duration of exposure/day (minutes)
C Aerosol concentration of magnesium stearate (µg/L)
BW Group average body weight for the treatment period (kg)

Calculation of Estimated Achieved Magnesium Stearate Inhaled Doses

$$\text{Estimated dose to animals } (\mu g/kg/day) = \frac{RMV \times T \times C}{BW}$$

Where:
T - Time of exposure (minutes/day)
C - Aerosol concentration (µg magnesium stearate/L)
BW - The average of the group mean body weight for the treatment period (kg)
RMV - Respired minute volume (L/min) ($0.499 \times BW^{0.809}$) [Bide et al. 2000]

Group	Average achieved tube aerosol concentration (µg/L) [1,2]		Time of exposure (min)	Body weight (kg) [3]		Estimated achieved dose (µg/kg/day) [1,2]	
	Male	Female		Male	Female	Males	Females
1[4]	7.89	8.01	10	9.2	7.7	25.8	27.1
						Average = 26.5	
2[5]	6.64	6.68	10	9.6	8.8	21.5	22.0
						Average = 21.8	
3[6]	2.18	2.20	10	10.5	8.7	6.94	7.26
						Average = 7.10	
4[6]	5.72	5.72	10	10.8	8.6	18.1	18.9
						Average = 18.5	
5[6]	2.46	2.46	10	9.8	9.1	7.94	8.05
						Average = 8.00	
6[7]	6.24	6.29	10	9.9	8.1	20.1	21.0
						Average = 20.6	
7[8]	2.25	2.26	10	10.0	8.9	7.23	7.43
						Average = 7.33	

1. Dose levels and tube aerosol concentrations are expressed in terms of magnesium stearate.
2. Magnesium was quantified using an atomic absorption spectroscopy method.
3. Average of the group mean weekly body weight values taken during the treatment period.
4. Exposed to vehicle (1% [w/w] magnesium stearate in lactose) only.
5. Exposed to 40% (w/w) GSK573719 and 0.20% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
6. Exposed to 3% (w/w) GSK573719 and 3% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
7. Exposed to 15% (w/w) GSK573719 and 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.
8. Exposed to 40% (w/w) GSK573719 in 1% (w/w) magnesium stearate in lactose.
9. Exposed to 15% (w/w) GW642444 in 1% (w/w) magnesium stearate in lactose.

Particle Size Distribution

A measured volume of air was drawn at a calibrated rate through a Marple 296 series cascade impactor. The Marple 296 impactor (flow rate 2 to 3 Liters/minute) was used for samples that were designated for analysis of GSK573719, GW642444 and magnesium stearate, which were predicted to have a mass median aerodynamic diameter (MMAD) less than 3.5 micrometers. Samples collected for Atomic Absorption analysis of magnesium content and for gravimetric analysis of total particulate were taken using a

Marple impactor employing a flow rate of 3 Liters/minute to permit accurate assessment of MMAD values greater than 3 micrometers.

Samples were collected during Weeks 1, 4, 8 and 13 for gravimetric and chemical analyses. Samples for chemical analysis of GSK573719 (Groups 2, 3, 4, 5 and 6) and GW642444 (Groups 2, 3, 4, 5 and 7), and for magnesium stearate (all groups) were collected separately.

The impactor stages were analyzed chemically for GSK573719 and GW642444 content using a single HPLC method with UV detection and quantification range(s) 1.0 to 16 µg/mL. Samples analyzed for magnesium content utilized Atomic Absorption spectroscopy and a magnesium lamp within a quantification range of 2.0 to 10 µg/mL and the results were expressed as magnesium stearate.

The MMAD and its geometric standard deviation (GSD) were calculated.

Summary of Group Particle Size Distribution Results for GSK573719 Aerosols

Group number	Average achieved tube aerosol concentration (µg/L) [1]		MMAD (µm) [2]	GSD [3]
	Males	Females		
2	326	329	(b) (4)	
3	7.20	7.17		
4	18.6	18.5		
5	54.7	54.4		
6	317	321		

1. Tube aerosol concentrations are expressed in terms of GSK573719 (the parent compound)
2. Mass Median Aerodynamic Diameter
3. Geometric Standard Deviation

Summary of Group Particle Size Distribution Results for GW642444 Aerosols

Group number	Average achieved tube aerosol concentration (µg/L) [1]		MMAD (µm) [2]	GSD [3]
	Males	Females		
2	2.29	2.30	(b) (4)	
3	8.86	8.83		
4	22.1	22.1		
5	56.3	56.0		
7	55.4	55.4		

1. Tube aerosol concentrations are expressed in terms of GW642444 (the parent compound)
2. Mass Median Aerodynamic Diameter
3. Geometric Standard Deviation

Summary of Group Particle Size Distribution Results for Magnesium Stearate

Group number	Average achieved tube aerosol concentration (µg/L) [1, 2]		MMAD (µm) [3]	GSD [4]
	Males	Females		
1	7.89	8.01	(b) (4)	
2	6.64	6.68		
3	2.18	2.20		
4	5.72	5.72		
5	2.46	2.46		
6	6.24	6.29		
7	2.25	2.26		

1. Average achieved aerosol concentration throughout the treatment period
2. Tube aerosol concentrations are expressed in terms of Magnesium (as determine by atomic absorption spectroscopy)
3. Mass Median Aerodynamic Diameter
4. Geometric Standard Deviation

Observations and Results

Mortality: A viability check was performed twice daily.

None

Clinical Signs: Clinical observations were performed at least twice daily during the treatment period. Detailed examinations (during and immediately after dosing, 0.5 to 2 hr postdose, and as late as possible during the day) were conducted daily during daily during Phase 1, daily during the first week of Phase 2, twice weekly during weeks 2 to 4 of Phase 2, and once weekly during weeks 5 to 13 of Phase 2.

Increased incidences of excessive salivation were observed in all male and female treatment groups during dosing and were still evident in a few treatment groups immediately postdose. Increased incidences of bilateral neck swelling were observed in all male and female treatment groups during dosing and /or immediately postdose.

Incidences of oral vomit of food were increased for males and females in Groups 2 and 6. Incidences of unformed stool were decreased for males in Group 2 and females in Groups 2, 3, 4, 5, and 6.

Table 26 Summary of Daily Observations – Exposure Observations during Dosing (Data expressed as number of observations/number of animals)

Clinical observations	Males							Females						
	G1	G2	G3	G4	G5	G6	G7	G1	G2	G3	G4	G5	G6	G7
Excessive Salivation	16/4	164/4	57/4	104/4	95/4	183/4	102/4	7/2	232/4	11/2	13/3	225/4	243/4	103/4
Neck Swelling-	15/4	149/4	33/3	73/4	23/3	200/4	22/4	21/4	182/4	21/4	13/4	114/4	251/4	74/4

Bilateral														
Neck Swelling-Unilateral	1/1	2/2	1/1	1/1	0/0	5/4	1/1	0/0	3/2	0/0	0/0	2/2	2/1	1/1
Neck Swelling	0/0	1/1	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	3/1	0/0	1/1

Table 27 Summary of Daily Observations – Exposure Observations Immediately Postdose (Data expressed as number of observations/number of animals)

Clinical observations	Males							Females						
	G1	G2	G3	G4	G5	G6	G7	G1	G2	G3	G4	G5	G6	G7
Excessive Salivation	5/3	32/3	9/2	41/3	19/4	33/4	19/2	3/3	94/3	0/0	1/1	83/4	101/4	27/3
Neck Swelling-Bilateral	29/4	174/4	55/4	97/4	33/4	244/4	39/4	32/4	212/4	31/4	29/4	165/4	283/4	115/4
Neck Swelling-Unilateral	3/2	4/3	2/2	2/2	0/0	6/3	0/0	1/1	3/2	0/0	2/2	5/3	1/1	4/1
Neck Swelling	0/0	0/0	0/0	0/0	0/0	2/2	1/1	0/0	0/0	0/0	0/0	2/2	0/0	2/2

Table 28 Summary of Clinical Observations – Daily Viability Checks and Post-Dose Observations (Cage-Side) (Data expressed as number of observations/number of animals)

Clinical observations	Males							Females						
	G1	G2	G3	G4	G5	G6	G7	G1	G2	G3	G4	G5	G6	G7
Swelling, neck	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Activity, convulsions	0/0	0/0	0/0	0/0	0/0	2/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Oral Vomit	2/2	14/4	6/4	10/4	0/0	2/2	2/2	2/2	6/4	2/2	7/4	6/4	6/4	3/3
Oral Vomit, Food	11/4	54/4	10/4	10/3	6/4	54/4	14/4	10/4	40/4	28/4	2/2	6/4	50/4	8/4
Unformed stool	67/4	38/2	69/4	72/4	83/4	92/4	24/4	153/4	28/4	35/4	22/4	75/4	16/4	166/4
Watery stools	0/0	0/0	2/2	2/2	0/0	14/2	0/0	4/2	0/0	2/2	0/0	2/2	2/2	14/2
Mucus in stools	20/4	0/0	18/4	2/2	4/2	2/2	6/2	19/4	11/2	4/4	0/0	11/2	0/0	37/4

Body Weights: Dogs were weighed twice during the pre-treatment phase, the day that dosing commenced, and weekly during the treatment period.

Body weight gains were generally increased for dogs that received GW642444 alone or in combination with GSK573719. The increased body weight gains were attributed to the pharmacological action of GW642444, a long-acting β_2-adrenergic agonist.

Table 29 Body weight gains (kg) from weeks 1 to 13

BW Gain kg	Control	GSK573719+GW642444				GSK573719	GW642444
		1070/7.5	23/29	60/72	177/183	1048	180

Male	0.7	1.5	2.6	2.4	1.6	0.9	1.6
Females	0.8	1.2	1.7	1.6	1.8	1.1	1.6

Feed Consumption: Each dog was offered approximately 400 g of Certified Canine Diet, No. 2025C mixed with warm water (at least 800 mL) once daily approximately 3 to 4 hr after completion of dosing during the dose phase. There were issues of aspiration of the diet into the lung in the 4-week inhalation toxicology study with dogs. Food consumption was measured daily, beginning 1 week prior to dosing (measured and recorded daily, 7 days/week). To obtain the average food consumed per week (g/dog/week) the amount of total food consumed for the week was divided by the number of days in the week/interval.

Food consumption was slightly increased by 3.2 to 5.9% for male dogs in Groups 5, 6, and 7. Food consumption was increased by 11.1 to 12.7% for female dogs in Groups 2, 3, 4, and 5 and by 25.6% for female dogs in Group 7. Increased food consumption for female dogs could be attributed to the pharmacological action of GW642444.

Ophthalmoscopy: The eyes of all animals were examined during the pre-treatment period and at the end of the dosing period (Week 13). Artificial Tears were administered to the eyes of all animals immediately after dosing, (after the tear test being performed on Days 5, 23 and 86) during the Phase 2 dosing period or as needed.

Conjunctivitis (possibly secondary to dry eye) was present on day 88/89 in three dogs from Group 6 (GSK573719 only), #6453 (Right eye), #6950F (Bilateral), and #6953F (Left eye). This was more than likely associated with administration of GSK573719.

Anterior uveitis was present in the left eye of one dog (#7950F) on day 88 from Group 7 (GW642444 only). This may have been associated with administration of GW642444.

Schirmer Tear Test (Weeks 1, 4, and 13): The rate of tear production was measured by placing the wick of a sterile test strip in the lower conjunctival sac for 1 minute and recording the distance travelled by tear secretion in both eyes of all animals. Measurements were made pretest and during Phase 2 (Days 5, 23, and 86) predose and within 5 minutes after completion of dosing.

Decreased tear production was observed for male and female dogs in Groups 2, 5, and 6 during weeks 1, 4, and 13. These effects were persistent throughout the treatment period and frequently evident at the predose time point. This effect was attributed to the pharmacological action of GSK573719.

Table 30 Schirmer Test (mm/min) - Group Means for Right and Left Eyes Combined

Group	Males							Females						
	Wk -1	Wk1		Wk 4		Wk 13		Wk -1	Wk1		Wk 4		Wk 13	
	PT	PreD	0.5-1hr PD	PreD	0.5-1hr PD	PreD	0.5-1hr PD	PT	PreD	0.5-1hr PD	PreD	0 5-1hr 16.5PD	PreD	0.5-1hr PD
1	17.5	20.5	23	19	17.5	17.5	18.5	16	19.5	18	20	16.5	20.5	18.5
2	17.5	7.5	2.5	8	4	10	2.5	20.5	13.5	4	8.5	7.5	11	4
3	15.5	16	16.5	14	14.5	13	16.5	16.5	17.5	16.5	14.5	14.5	17	10.5
4	18	14.5	17	13	11	10	12.5	17	16.5	14.5	12	12	14	12
5	17.5	13.5	4	7	5.5	6.5	6.5	18	14	7.5	12	3.5	15	10.5
6	20.5	11	4.5	6.5	6.5	14	5.5	15	6.5	1.5	1	1	0	2.5
7	15.5	16.5	12	12	12	18	12	15	20	15.5	15.5	12	16	17

PT = Pretest; PreD = Predose; and PD = Postdose

ECG: Electrocardiograms were recorded from each animal once during the pre-treatment period and at the end of Week 13. During the treatment period recordings were made prior to and immediately after dosing at the approximate T_{max}. Tracings were taken on unanesthetized dogs positioned in right lateral recumbancy. The following leads were used at each interval: I, II, III, aVR, aVL, aVF, CV5RL (rV2), CV6LL (V2), CV6LU (V4) and V10. ECG lead cable for CV6LU (V4) was located on the left 6th intercostal space at the costochondral junction. The tracings were examined for abnormalities, and heart rate.

Prior to dosing on Day 92 (males) and 91 (females), increased heart rates were observed in dogs from Groups 2 and 6 that received GSK573719 at a target dose of 1000 µg/kg/day either in combination with GW642444 or alone, respectively. For these dogs, increased heart rate was approximately 1.3 times the pretest values for both males and females.

Immediately after dosing (IAD) on day 91/92, heart rate increases (up to 1.86- and 1.76-fold times higher than mean pretest values in males and females, respectively) were observed in dogs that received either GSK573719 or GW642444 alone or in combination. Peak increases of heart rate were observed in males and females that received GSK573719/GW642444 at 1070/7.5 µg/kg/day (158 and 165 bpm, respectively) and in females that received GSK573719 alone at 1048 µg/kg/day (170 bpm).

Both prior to and immediately after dosing on days 91/92, respiratory sinus arrhythmia (RSA) was absent in 15/16 animals from Groups 2 and 6 that received GSK573719 at a target dose of 1000 µg/kg/day either in combination with GW642444 or alone, respectively, suggesting that the effect was persistent at this target dose of GSK573719. RSA was absent immediately after dosing on days 91/92 for several male and female dogs in Groups 3, 4, and 5 that received the combination of GSK573719 and GW642444 (up to target doses of 160 µg/kg/day for each component).

Table 31 Semiquantitative heart rate (pretest/week 13 pre/post) and presence of respiratory sinus arrhythmia (RSA)

Group 1: Lactose/1% Magnesium Stearate Control

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
1450m	97	95 / 120	+	+	+	1950f	103	146 / 135	+	+	+
1451m	76	92 / 80	+	+	+	1951f	87	111 / 136	+	+	+
1452m	76	66 / 83	+	+	+	1952f	98	80 / 82	+	+	+
1453m	83	70 / 86	+	+	+	1953f	89	64 / 66	+	+	+
mean	**83**	**81 / 92**	**0/4 [a]**	**0/4 [a]**	**0/4 [a]**	**mean**	**94**	**100 / 105**	**0/4 [a]**	**0/4 [a]**	**0/4 [a]**

Group 2: GSK573719/GW642444 1000/5 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
2450m	83	106 / **160**	+	–	–	2950f	100	132 / **177**	+	–	–
2451m	73	115 / 107	+	–	–	2951f	76	106 / **166**	+	–	–
2452m	93	118 / **176**	+	–	–	2952f	108	139 / **202**	+	–	–
2453m	92	115 / **187**	+	–	–	2953f	92	113 / 115	+	–	–
mean	**85**	**114 / 158**	**0/4 [a]**	**4/4 [a]**	**4/4 [a]**	**mean**	**94**	**122 / 165**	**0/4 [a]**	**4/4 [a]**	**4/4 [a]**

Group 3: GSK573719/GW642444 20/20 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
3450m	91	88 / 98	+	+	+	3950f	101	101/ **139**	+	+	+
3451m	113	113 / **130**	+	+	–	3951f	112	86 / **121**	+	+	+
3452m	85	94 / **161**	+	+	–	3952f	81	74 / 78	+	+	+
3453m	99	98 / **130**	+	+	+	3953f	109	82 / **137**	+	+	+
mean	**97**	**98 / 130**	**0/4 [a]**	**0/4 [a]**	**2/4 [a]**	**mean**	**101**	**85 / 119**	**0/4 [a]**	**0/4 [a]**	**0/4 [a]**

Group 4: GSK573719/GW642444 60/60 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
4450m	88	95 / 115	+	+	+	4950f	89	83 / **182**	+	+	–
4451m	104	127 / **151**	+	+	–	4951f	105	71 / 115	+	+	+
4452m	83	99 / **140**	+	+	–	4952f	85	92 / **147**	+	+	+
4453m	81	80 / **146**	+	+	–	4953f	94	74 / **165**	+	+	–
mean	**89**	**100 / 138**	**0/4 [a]**	**0/4 [a]**	**3/4 [a]**	**mean**	**93**	**80 / 152**	**0/4 [a]**	**0/4 [a]**	**2/4 [a]**

Group 5: GSK573719/GW642444 160/160 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
5450m	72	87 / **159**	+	+	–	5950f	92	88 / **123**	+	+	+
5451m	128	116 / **151**	+	+	–	5951f	96	120 / **165**	+	–	–
5452m	86	75 / 115	+	+	+	5952f	99	71 / 72	+	+	+
5453m	71	76 / **158**	+	+	–	5953f	112	81 / **208**	+	+	–
mean	**89**	**89 / 146**	**0/4 [a]**	**0/4 [a]**	**3/4 [a]**	**mean**	**100**	**90 / 142**	**0/4 [a]**	**1/4 [a]**	**2/4 [a]**

Group 6: GSK573719 1000 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
6450m	94	109 / 93	+	–	+	6950f	97	124 / **151**	+	–	–
6451m	96	110 / 93	+	–	–	6951f	95	130 / **177**	+	–	–
6452m	93	123 / **189**	+	–	–	6952f	88	110 / **176**	+	–	–
6453m	96	150 / **170**	+	–	–	6953f	102	102 / **177**	+	–	–
mean	**95**	**123 / 136**	**0/4 [a]**	**4/4 [a]**	**3/4 [a]**	**mean**	**96**	**116/ 170**	**0/4 [a]**	**4/4 [a]**	**4/4 [a]**

Group 7: GW642444 160 µg/kg/day

Animal No.	HR		RSA			Animal No.	HR		RSA		
			Pretest	Week 13					Pretest	Week 13	
	Pretest	Pre / Post		Predose	Postdose		Pretest	Pre / Post		Predose	Postdose
7450m	75	77 / **139**	+	+	+	7950f	95	77 / **136**	+	+	+
7451m	74	57 / **109**	+	+	+	7951f	107	89 / **134**	+	+	+
7452m	77	50 / 79	+	+	+	7952f	100	78 / **151**	+	+	+
7453m	96	59 / **155**	+	+	–	7953f	102	79 / **126**	+	+	+
mean	**80**	**61 / 121**	**0/4 [a]**	**0/4 [a]**	**1/4 [a]**	**mean**	**101**	**81 / 137**	**0/4 [a]**	**0/4 [a]**	**0/4 [a]**

m : male
f : female
RSA : respiratory sinus arrhythmia
+ : presence of RSA
– :absence of RSA
a :number of animals with no RSA

Femoral Pulse

During Phase 1 (pre-adaptation), femoral pulse was measured by palpation for all dog in Groups 5 and 7 on days 1, 2, and 3. Measurements were made predose, within 5 min after completion of dosing and 0.5, 1, 2, 4, 8 and 23 hr 50 min after the completion of dosing. During Phase 2, femoral pulse was measured by palpation for all animals on Day 1 and during Weeks 4 and 13. Measurements were made predose, within 5 min after completion of dosing, and at 0.5, 1, 2, 4, 8 and 23 hr 50 min after the completion of dosing (after each toxicokinetic bleed at these time points).

Phase 1 (Pre-adaptation period)
During Phase 1, animals given GSK573719/GW642444 at 44/47 µg/kg/day and 0/49 µg/kg/day showed increased pulse rates starting immediately after dosing (IAD) and peak increases were generally reached within 1 hr following completion of dosing.

From IAD to 1 hr after completion of dosing on days 1 to 3 of the pre-adaptation period, males and females that received GSK573719/GW642444 at 44/47 µg/kg/day showed maximal increases of pulse rates that ranged from 1.82X to 2.14X and 1.53X to 2.18X mean predose values, respectively. Pulse rates in both sexes returned to predose values between 8 hr and 23 hr and 50 min after the completion of dosing. From IAD to 1 hr after completion of dosing on days 1 to 3 of the pre-adaptation period, males and females given GSK573719/GW642444 at 0/49 µg/kg/day showed increased pulse rates ranging from 1.41X to 1.92X and 1.34X to 1.67X mean predose values, respectively. Pulse rates generally returned to predose values between 4 and 23 hr and 50 min after the completion of dosing.

Phase 2 (Treatment period)
On Day 1, animals given GSK573719 or GW642444 alone or in combination showed increased pulse rates starting immediately after dosing (IAD) and peak increases were generally reached within 1 hr following completion of dosing. The largest increases of pulse rates were observed for males and females that received GSK573719/GW642444 at 177/183 µg/kg/day (2.23X and 2.17X predose, respectively). Pulse rates in both sexes returned to predose values between 8 hours and 23 hours and 50 minutes after the completion of dosing.

During Week 4, increases of pulse rates were not as marked when compared to day 1 and values returned to predose levels more quickly suggesting induction of tachyphylaxis. The largest increases of pulse rates during week 4 were observed for males and females that received GSK573719/GW642444 at 177/183 µg/kg/day (1.54X and 1.30X predose, respectively).

During Week 13, increases of pulse rates were minimal and not as marked when compared to day 1 or Week 4. For groups where slight increases of pulse rates were evident, values returned to predose levels more quickly. It was clear that tachyphylaxis had occurred.

Cardiac Troponin I
Blood was collected from all animals in Groups 5 and 7 on Day -3 of Phase 1 and from all animals on Day 1 of Phase 2 pre-dose and 4, 8 and 23 hr 50 min after the completion of dosing. Concentrations of cardiac Troponin I in serum from all study animals were determined using a Bayer Centaur CP Chemiluminescence analyzer and currently approved methods by GSK Clinical Pathology, Safety Assessment, Ware, Hertfordshire.

During Phase1 (Pre-adaptation), elevations of serum cTnI concentrations were observed for females in Group 5 (44/47 µg/kg/day) and males and females in Group 7

(49 µg/kg/day GW642444). During Phase 2 (treatment period), fewer females in Group 5 (177/183 µg/kg/day) were observed with elevated serum cTnI concentrations despite the 4-fold increase of dose. Only 1 male in Group 5 was observed with elevated serum cTnI concentrations. For dogs in Group 7 (180 µg/kg/day GW642444), 1 male and 3 females were observed with elevated serum cTnI concentrations. Based upon the response of females in Group 5, the pre-adaptation phase induced tachyphylaxis or tolerance to potential cardiac injury (as measured by serum cTnI concentrations) induced by the combination of GSK573719 and GW642444. The Division communicated several concerns to the sponsor that pre-exposure of dogs to smaller doses may provide protection against subsequent exposure to larger doses (see Toxicology and Applied Pharmacology 69: 199-205, 1983). It was noted that the use of the pre-adaptation phase will be a review issue when the study is submitted.

Elevations of serum cTnI concentrations were also observed for dogs in Groups 3 and 4. Minimal increases of serum cTnI concentrations with questionable biological significance were observed for dogs in Groups 2 and 6.

Phase 1 (Pre-adaptation period)
All female dogs in the 44/47 µg/kg/day group (Group 5) were observed with increased serum cTnI concentrations (up to 23 times the predose baseline of 0.186 µg/L) at 4 and 8 hr after completion of dosing with return to baseline by 23 hr 50 min after the completion of dosing. Male dogs in the 44/47 µg/kg/day group (Group 5) had slight elevations of serum cTnI at 4 and 8 hr after completion of dosing that were of questionable toxicological significance.

For dogs in the 49 µg/kg/day group (Group 7), 2 of 4 males and 2 of 4 females were observed with increased cTnI concentrations at 4 and 8 hr after the completion of dosing (up to 40 times the predose baseline of 0.241 µg/L). Concentrations for all dogs except female #7952 (cTnI concentration 0.436 µg/L) were returning to baseline by 23 hr 50 min after the completion of dosing.

Phase 2 (Treatment Period)
For female dogs in the 177/183 µg/kg/day group (Group 5) that received pre-adaptation prior to start of the treatment period, only 1 female (#5953) demonstrated marked increases of serum cTnI concentrations (up to 198 times the predose baseline of 0.010 µg/L) at 4 and 8 hr after the completion of dosing and remained elevated at 23 hr 50 min (1.093 µg/L). Fewer females responded to the treatment during Phase 2 than during Phase 1, despite the 4-fold increase in administered dose, which demonstrated induction of tachyphylaxis by exposure to lower doses during the pre-adaptation period. One male (#5451) in the 177/183 µg/kg/day group (Group 5) was observed with increases of serum cTnI concentrations (11 times the predose baseline of 0.006 µg/L) at 4 and 8 hr after completion of dosing. No elevations were evident for this dog during Phase 1.

For dogs in the 180 µg/kg/day (GW642444 alone) group (Group 7) that received pre-adaptation prior to start of the treatment period, 1 of 4 males (male #7451 that did not

show any changes of cTnI concentrations during Phase 1) and 3 of 4 females were observed with increases of serum cTnI concentrations (up to 43 time the pre-study baseline) at 4 and 8 hr. A return toward baseline was observed for all animals by 23 hr 50 min except for female #7952 (2.1 times its predose baseline).

For dogs in the 60/72 µg/kg/day group (Group 4), 3 of 4 males and 3 of 4 females were observed with increased serum cTnI concentrations (22 and 35 times predose baselines for males and females, respectively) at 4 and 8 hr after the completion of dosing. It should be noted that one male animal (#4452) displayed an increased serum cTnI concentration at predose (0.164 µg/L). Females were observed with a return toward baseline by 23 hr 50 min; however, only partial recovery was evident for males (9 times the predose baseline) at 23 hr 50 min.

For dogs in the 23/29 µg/kg/day group (Group 3), 3 of 4 females were observed with increased serum cTnI concentrations (up to 35 times the pre-dose baseline) at 4 and 8 hr post dosing. Two females still displayed elevated concentrations (0.072 and 0.060 µg/mL) at 23 hr 50 min postdose. Only one male (#3451) showed increased cTnI concentrations (up to 13 times its own predose baseline at 8 hr postdose).

For dogs in the 1070/7.5 µg/kg/day group (Group 2), 2 of 4 males showed minimal increases of serum cTnI concentrations. No changes were evident for females in Group 2.

For dogs in the 1048 µg/kg/day (GSK573719 only) group (Group 6), one female (#6952) demonstrated increased serum cTnI concentration (up to 27 times its own predose baseline) at 4 and 8 hr with recovery seen by 23 hr 50 min.

Table 32 Cardiac troponin concentrations for male dogs

Table 1 Phase 1 and 2 Cardiac Troponin I results for all timepoints in Males

		concentration Cardiac Troponin I µg/L							
Group and Estimated Achieved Dose	Animal No	Phase 1				Phase 2			
		Predose	4 h	8 h	23h 50m	Predose	4 h	8 h	23h 50m
1 Control	1450					**0.006**	**0.006**	**0.006**	**0.006**
	1451					**0.006**	0.006	**0.006**	**0.006**
	1452					**0.006**	**0.006**	**0.006**	**0.006**
	1453					**0.006**	**0.006**	0.006	0.006
	mean					0.006	0.006	0.006	0.006
	SD					0.000	0.000	0.000	0.000
2 1070µg/kg/day GSK573719 plus 7.5µg/kg/day GW642444	2450					0.006	0.008	0.007	0.008
	2451					**0.006**	**0.006**	0.043	**0.006**
	2452					**0.006**	0.048	0.050	0.035
	2453					**0.006**	**0.006**	**0.006**	**0.006**
	mean					0.006	0.017	0.027	0.014
	SD					0.000	0.021	0.023	0.014
3 23µg/kg/day GSK573719 plus 29µg/kg/day GW642444	3450					**0.006**	**0.006**	**0.006**	**0.006**
	3451					**0.006**	0.063	0.079	0.023
	3452					**0.006**	0.009	0.009	**0.006**
	3453					**0.006**	0.006	**0.006**	**0.006**
	mean					0.006	0.021	0.025	0.010
	SD					0.000	0.028	0.036	0.009
4 60µg/kg/day GSK573719 plus 72µg/kg/day GW642444	4450					**0.006**	0.268	0.546	0.274
	4451					**0.006**	0.012	0.013	0.008
	4452					0.164	0.979	0.823	0.382
	4453					**0.006**	2.791	2.522	1.002
	mean					0.046	1.013	0.976	0.417
	SD					0.079	1.254	1.084	0.421
5 44/177µg/kg/day GSK573719 plus 47/183µg/kg/day GW642444 (phase 1/phase 2)	5450	**0.006**	0.029	0.026	**0.006**	**0.006**	0.033	0.035	0.011
	5451	**0.006**	**0.006**	**0.006**	**0.006**	**0.006**	0.068	0.053	0.008
	5452	0.014	0.018	0.022	0.016	0.008	0.016	0.015	0.011
	5453	**0.006**	0.016	0.011	**0.006**	**0.006**	0.014	0.020	0.007
	mean	0.008	0.017	0.016	0.009	0.007	0.033	0.031	0.009
	SD	0.004	0.009	0.009	0.005	0.001	0.025	0.017	0.002
6 1048µg/kg/day GSK573719	6450					0.045	0.008	0.010	0.007
	6451					0.009	0.022	0.013	0.008
	6452					**0.006**	0.018	0.028	0.019
	6453					0.008	0.012	0.011	0.015
	mean					0.017	0.015	0.016	0.012
	SD					0.019	0.006	0.008	0.006
7 49/180µg/kg/day GW642444 (phase 1/phase2)	7450	0.009	0.393	0.431	0.056	0.026	0.021	0.020	0.017
	7451	0.007	0.010	0.007	**0.006**	0.008	0.193	0.129	0.019
	7452	**0.006**	0.213	0.179	0.025	0.007	0.017	0.015	0.007
	7453	**0.006**	0.013	0.015	**0.006**	**0.006**	0.006	0.037	0.010
	mean	0.007	0.157	0.158	0.023	0.012	0.059	0.050	0.013
	SD	0.001	0.184	0.199	0.024	0.010	0.089	0.053	0.006

Bolded results indicate the result is below the sensitivity of the assay and should be reported as <0.006 µg/L.

Table 33 Cardiac troponin concentrations for female dogs

Table 2 Phase 1 and 2 Cardiac Troponin I results for all timepoints in Females

Group and Estimated Achieved Dose	Animal No	concentration Cardiac Troponin I µg/L							
		Phase 1				Phase 2			
		Predose	4 h	8 h	23h 50m	Predose	4 h	8 h	23h 50m
1 Control	1950					**0.006**	**0.006**	**0.006**	**0.006**
	1951					**0.006**	**0.006**	**0.006**	**0.006**
	1952					**0.006**	0.010	**0.006**	0.006
	1953					0.020	0.023	0.008	**0.006**
	Mean					0.010	0.011	0.007	0.006
	SD					0.007	0.008	0.001	0.000
2 1070µg/kg/day GSK573719 plus 7.5µg/kg/day GW642444	2950					**0.006**	**0.006**	**0.006**	**0.006**
	2951					0.006	**0.006**	0.010	0.012
	2952					**0.006**	0.016	0.006	**0.006**
	2953					**0.006**	0.024	0.024	0.012
	Mean					0.006	0.013	0.012	0.009
	SD					0.000	0.009	0.009	0.003
3 23µg/kg/day GSK573719 plus 29µg/kg/day GW642444	3950					**0.006**	0.427	0.458	0.072
	3951					**0.006**	0.614	0.484	0.060
	3952					0.012	0.073	0.075	0.013
	3953					**0.006**	0.006	0.009	**0.006**
	Mean					0.008	0.280	0.257	0.038
	SD					0.003	0.289	0.249	0.033
4 60µg/kg/day GSK573719 plus 72µg/kg/day GW642444	4950					**0.006**	0.282	0.301	0.040
	4951					**0.006**	**0.006**	**0.006**	**0.006**
	4952					**0.006**	0.214	0.270	0.055
	4953					**0.006**	0.357	0.376	0.061
	Mean					0.006	0.215	0.238	0.041
	SD					0.000	0.151	0.161	0.025
5 44/177µg/kg/day GSK573719 plus 47/183µg/kg/day GW642444 (phase 1/phase 2)	5950	0.008	0.095	0.086	0.016	**0.006**	0.037	0.047	0.011
	5951	0.012	0.161	0.181	0.051	0.016	0.016	0.026	0.018
	5952	**0.006**	0.277	0.231	0.038	0.006	0.014	**0.006**	0.008
	5953	**0.006**	0.212	0.089	0.013	0.013	1.984	2.580	1.093
	Mean	0.008	0.186	0.147	0.030	0.010	0.513	0.665	0.283
	SD	0.003	0.077	0.071	0.018	0.005	0.981	1.277	0.540
6 1048µg/kg/day GSK573719	6950					**0.006**	**0.006**	**0.006**	**0.006**
	6951					**0.006**	0.038	0.043	0.035
	6952					**0.006**	0.163	0.118	0.037
	6953					**0.006**	**0.006**	**0.006**	**0.006**
	Mean					0.006	0.053	0.043	0.021
	SD					0.000	0.075	0.053	0.017
7 49/180µg/kg/day GW642444 (phase 1/phase2)	7950	0.007	0.065	0.073	0.029	**0.006**	0.156	0.105	0.026
	7951	**0.006**	0.009	0.016	**0.006**	**0.006**	0.102	0.071	0.056
	7952	**0.006**	0.879	0.842	0.436	0.074	0.734	0.476	0.156
	7953	**0.006**	0.010	0.006	**0.006**	0.007	0.028	0.039	0.018
	Mean	0.006	0.241	0.234	0.119	0.023	0.255	0.173	0.064
	SD	0.001	0.426	0.406	0.211	0.034	0.324	0.204	0.063

Bolded results indicate the result is below the sensitivity of the assay and should be reported as <0.006 µg/L.

Hematology: Blood samples for measurement of hematology parameters were collected from all dogs once pre-treatment (Week -2) and during Weeks 4 and 13.

Changes of hematology parameters were generally small (<15% change from control) and appeared to lack toxicological significance.

Clinical Chemistry: Blood samples for measurement of clinical chemistry parameters were collected from all dogs once pre-treatment (Week -2) and during Weeks 4 and 13.

Increases of glutamate dehydrogenase (GLDH) were observed for females in Group 2 (1070/7.5 µg/kg/day) and males and females in Group 6 (1048 µg/kg/day) during weeks 4 and 13.

Slight increases of phosphorus were observed for males and females that received GSK573719 alone, GW642444 alone, and in combination during week 4. These increases were still evident for males during week 13, but not for female treatment groups.

Slight increases of potassium levels were observed for males and females that received GW642444 alone (180 µg/kg/day) or in combination with GSK573719 (Groups 3, 4, and/or 5).

Alkaline phosphatase (ALKP) activity was increased approximately 1.9-fold for females in Group 3 (23/29 µg/kg/day) during week 4 although it did not appear to be a dose-responsive change. Slight increases of ALP were observed for males in Groups 3 (23/29 µg/kg/day) and 4 (60/72 µg/kg/day) during week 13 although these changes did not appear to be dose-responsive.

Slight increases of blood urea nitrogen (BUN) were observed for males and females that received GSK573719 alone, GW642444 alone, or in combination (up to 1.28X and 1.71X controls in males and females, respectively).

Creatinine concentrations were increased for males in Group 4 (60/72 µg/kg/day) and females in Groups 3 (23/29 µg/kg/day), 4 (60/72 µg/kg/day), and 5 (177/183 µg/kg/day) during week 13.

There were no histopathological changes in the liver or kidneys that correlated with observed changes of clinical chemistry parameters.

Table 34 Clinical chemistry parameters at weeks 4 and 13

Parameter	Time	Males							Females						
		G1	G2	G3	G4	G5	G6	G7	G1	G2	G3	G4	G5	G6	G7
GLDH U/L	Wk4	3 8	4.3	3.8	3.3	3.5	6 0 (158%)	4 2	5 0	6.9 (138%)	4.2	4.7	3.9	7.8 (156%)	5 3 (106%)
	Wk13	6 0	6.8	5.7	4.6	5.5	7 0 (117%)	5.4	6 5	7.8 (120%)	4.9	5.1	7.4 (114%)	9.8 (151%)	6.4
Phosphorus mg/dL	Wk4	4.7	5.0	5.3 (113%)	5.6 (119%)	5.4 (115%)	5.4 (115%)	5 0	4.4	5.9 (134%)	5.3 (120%)	5.4 (123%)	5.3 (120%)	5.1 (116%)	5 6 (127%)
	Wk13	4.1	5.0 (112%)	4.9 (120%)	4.9 (120%)	5.2 (127%)	4 8 (117%)	4 6 (112%)	5 2	5.4	4.7	5.4	5.4	4.9	4.7
K mEq/L	Wk4	4 6	4.8	5.0 (109%)	5.2 (113%)	5.0 (109%)	4 8	5.1 (111%)	4.7	4.9	5.1 (109%)	5.2 (111%)	5.1 (109%)	4.9	5 0
	Wk13	4.4	4.7	4.8 (109%)	5.0 (114%)	4.9 (111%)	4.7	5 0 (114%)	4 6	4.8	4.9	5.1 (111%)	5.0 (109%)	4.7	5.1 (111%)
ALKP U/L	Wk4	NC	NC	NC	NC	NC	NC	NC	67	64	130 (194%)	87	53	51	70
	Wk13	88	60	134 (152%)	113 (128%)	89	70	104 (118%)	NC	NC	NC	NC	NC	NC	NC
BUN mg/dL	Wk4	18	21 (117%)	24 (133%)	22 (122%)	24 (133%)	20	19	14	22 (157%)	20 (143%)	20 (143%)	21 (150%)	17 (121%)	23 (164%)
	Wk13	18	22 (122%)	21 (117%)	23 (128%)	23 (128%)	22 (122%)	20	17	24 (141%)	21	20	29 (171%)	20	19
Creatinine mg/dL	Wk13	0 5	0.6	0.6	0.9 (140%)	0.5	0 6	0.7	0.4	0.5	0.6 (150%)	0.6 (150%)	0.7 (175%)	0.4	0 6 (150%)

NC = no change

Urinalysis: Urine samples were also collected overnight (over approximately 16 hr) from pans placed under the animal cages once pre-treatment (Week -2) and during Weeks 4 and 13. Parameters measured/calculated included volume, pH, specific gravity, appearance, glucose, protein, creatinine, glucose total output, creatinine total output, and protein total output. There were no measurements for blood or microscopic examination of the urinary sediment.

Creatinine was increased for females in Groups 3, 4, 5, and 7 during week 4 and females in Groups 3 and 7 during week 13. Protein out was increased for females in Groups 2, 3, and 7 during week 4. Creatinine out and protein out were increased for males in Groups 2, 3, 4, and 5 during week 13. The significance of these changes was unclear. There were no corresponding histopathological findings in the kidneys.

Table 35 Urinalysis parameters at weeks 4 and 13

Parameter	Time	Males							Females						
		G1	G2	G3	G4	G5	G6	G7	G1	G2	G3	G4	G5	G6	G7
Creatinine Out, mg	Wk4	NC	NC	NC	NC	NC	NC	NC	82.6	87.8	172.2 (209%)	138 0 (167%)	133 6 (162%)	105.5	162.6
	Wk13	121.5	194.2 (160%)	188.7 (155%)	274.7 (226%)	192 2 (158%)	52.4 (43%)	125.8	NC	NC	NC	NC	NC	NC	NC
Protein Out, mg	Wk4	NC	NC	NC	NC	NC	NC	NC	11.7	16.5 (141%)	24.7 (211%)	13.2	13.8	15 0	32.7 (280%)
	Wk13	15.5	25.4 (164%)	24.4 (157%)	34.8 (225%)	23.3 (150%)	11.4	16.7	NC	NC	NC	NC	NC	NC	NC

NC = No change

Gross Pathology: After 13 weeks of treatment, dogs were sacrificed and submitted to a detailed external and internal examination.

At necropsy, several dogs throughout treated groups presented with discoloration of some lung lobes. Tan or red discolored areas, affecting mainly the most cranial lobes, were observed in four treated dogs (Male #2452 in Group 2, Female #2950 in Group 2, Female #3952 in Group 3, and Male #6450 in Group 6). In addition, white foci affecting all lobes were seen in three treated animals (Female #3950 in Group 3, Male #7451 in Group 7, and Female #7951 in Group 7). These changes, which often correlated with areas of minimal subacute/chronic inflammation or aggregates of foamy macrophages seen microscopically, were mostly considered incidental and not related to test article.

Organ Weights: Organ weights were measured for the adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, testes, and thymus.

Absolute and relative prostate weights of males in Group 5 (177/183 µg/kg/day) and Group 7 (180 µg/kg/day) were reduced compared with controls (as low as 0.60 times and 0.70 times controls, respectively).

Females given GSK573719, alone or in combination, had reduced absolute and relative heart weights when compared to controls. The change was most notable in animals given a target dose of 1000 µg/kg/day, alone and in combination.

Absolute and relative testes of males in Group 5 (177/183 µg/kg/day) and Group 7 (180 µg/kg/day) were increased as compared to controls although changes expressed as percent of body weight were minimal.

Table 36 Organ weights

Organ	Males							Females						
	G1	G2	G3	G4	G5	G6	G7	G1	G2	G3	G4	G5	G6	G7
Prostate g	9 246	9.390	8.219	9.982	6.527 (71%)	9.083	7.382 (79 8%)							
Prostate %BW	0.106	0.094	0.073	0.088	0.064 (60%)	0.092	0.072 (68%)							
Prostate %BrW	13.278	12.697	10 954	12.955	8.700 (66%)	11.983	10 071 (76%)							
Heart g	78.746	68.977 (88%)	78 254	79.527	81.147	78.976	85.764	73.487	62 522 (85%)	67.701	68.855	64.453 (88%)	61.145 (83%)	75.464
Heart %BW	0 841	0.703 (84%)	0.703 (84%)	0.714 (85%)	0.791	0.787	0.828	0 887	0.684 (77%)	0.753	0.751	0.684 (77%)	0.724 (82%)	0.802
Heart %BrW	112.539	93.333 (83%)	104.942	104 047	108.033	104 905	116.454	107 688	88 222 (82%)	92.317	97.231	92.243 (86%)	91.567 (85%)	104.825
Testes g	10.722	12.243 (114%)	11.154	12.547 (117%)	13 554 (126%)	12.829 (120%)	14 898 (139%)							
Testes %BW	0.128	0.123	0.101	0.112	0.133	0.130	0.150 (117%)							
Testes %BrW	15.388	16.493	15.170	16.356	18 063 (117%)	16.805	20 667 (134%)							

Histopathology:

Adequate Battery: In the initial histopathological examination, all protocol specified tissues were examined microscopically from terminal kill animals in Group 1 (vehicle control), Group 2 (1000/5 µg/kg/day), and Group 5 (160/160 µg/kg/day). At the EOP2 meeting with the sponsor on October 29, 2010, the sponsor was asked to conduct histopathological examinations of protocol specified tissues from all groups (i.e., Groups 3, 4, 6, and 7 should also be examined, see final meeting minutes dated November 17, 2010).

An amended report was provided in the sponsor's submission dated December 14, 2010 that included results of histopathological examination of protocol specified tissues from all dogs (Groups 1 through 7). An adequate panel of organs and tissues was submitted to histopathological examinations. Samples of the tissues indicated were processed to paraffin wax, sectioned (4-5 microns), stained with hematoxylin and eosin, and examined microscopically for all groups. Due to the original study pathologist (Dr. (b) (4)) having left the employment of the contracting laboratory prior to the request for additional microscopic evaluation of tissues, a new study pathologist (Dr. (b) (4) was assigned to the study to conduct the additional examinations. To ensure consistency of recording thresholds and diagnostic criteria for the target tissues in this study, the new study pathologist reread all the respiratory tissues (nasal turbinates, larynx, trachea, tracheal bifurcation and lungs) that had already been examined (Groups 1, 2, and 5) as well as all tissues from the groups that had not previously been examined (Groups 3, 4, 6, and 7). The original diagnostic terminology was retained and, where necessary, new findings were added to allow for changes in the previously unexamined slides. Following re-evaluation of the respiratory tissues by

the new study pathologist, there were a few minor differences in recording of findings by the current pathologist in tissues previously read by the original pathologist. However, these had no impact on the overall conclusions of the study or identification of target tissues. The accompanying text in the amended report reflected the evaluation and interpretation of the study based on the amended data.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Ovaries	X
Adrenals	X	Pancreas	X
Aorta (thoracic)	X	Pituitary	X
Brain (cerebellum, cerebrum, midbrain, medulla)	X	Prostate	X
Caecum	X	Rectum	
Colon	X	Salivary gland[c]-	
Duodenum	X	mandibular	X
Epididymides	X	parotid	X
Esophagus (distal portion)	X	Sciatic nerve[b]	X
Eyes	X	Skeletal muscle[b]	X
Femoral bone marrow	X	Skin	X
Femur (Femoral head)	X	Spinal cord (transverse and longitudinal section at the lumbar level) -	
Gallbladder	X	cervical	
Heart (left and right Auricle, left and right Ventricle 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 (2 levels)	X	Testes	X
Liver (section from left lateral and right medial lobes)	X	Thymus	X
Lungs including bronchi (left and right cardiac, left and right apical, left and right diaphragmatic)[a]	X	Thyroids with Parathyroids	X
Lymph node -		Tongue	X
mandibular	X	Tonsils	X
mesenteric	X	Trachea (top, middle, bottom)	X
popliteal		Tracheal Bifurcation (with main stem bronchi)	X
tracheo-bronchial	X	Urinary bladder	X
Mammary gland (inguinal)	X	Uterus with cervix	X
Nasal cavities and nasopharynx with skull (4 levels)	X	Vagina	X
Optic nerves	X		

[a]At least 6 lobes were examined, left and right, to include proximal and distal areas.
[b]Only 1 taken per animal.
[c]Only 1 examined.

Peer Review: A peer review of selected microscopic tissue sections, and pathology data interpretation was performed by the Sponsor. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data. The Peer Review Statement is not presented in the report but is maintained in the raw data.

Histological Findings: Histopathological findings were observed in the lung, larynx, nose/turbinates, trachea, thyroid, testes, thymus, and heart that could potentially be attributed to treatment. In general, these findings were consistent the individual test articles, GSK573719 and GW642444, and there was no evidence of novel toxicity to the combination. There were additional findings in the liver, mandibular salivary gland, lymph nodes, and uterus of unclear relation to treatment.

Lungs: In the lungs, marked, localized, subacute/chronic inflammation was seen in 1 male dog (#2452) in Group 2 (1000/5 µg/kg/day). This change was associated with bronchial/bronchiolar ectasia with luminal cellular debris, hyperplasia of the bronchial goblet cells, and pleural adhesions. In addition, plant/food material and bacterial colonies were present. The microscopic changes correlated to the slight to moderate discoloration of the apical and cardiac lobes and severe adhesion of the pleura seen at necropsy.

Larynx: In the larynx, slight increase in incidence and/or severity of mixed inflammatory cell infiltrates was observed for male dogs in Groups 2 (1000/5 µg/kg/day) and 5 (160/160 µg/kg/day) and females in Group 3 (23/29 µg/kg/day). The finding for females in Group 3 was not dose responsive.

Nose/Turbinates: Increased inflammatory cells in the nasal mucosa were observed for single dogs in Groups 3 (23/29 µg/kg/day), 5 (177/183 µg/kg/day), 6 (1048 µg/kg/day), and 7 (180 µg/kg/day). Inflammatory cells and cell debris were observed for single dogs in Groups 6 (1048 µg/kg/day) and 7 (180 µg/kg/day).

Trachea: Mixed inflammatory cell infiltrate in the tracheal mucosa was observed for 1 male dog in Group 2 (1000/5 µg/kg/day).

Thyroid: C cell hyperplasia was observed for 1 male and 1 female in Group 5 (177/183 µg/kg/day).

Testes: Dilatation of the seminiferous tubules was observed for 1 male in Group 5 (177/183 µg/kg/day).

Thymus: Incidences and severity of thymic involution/atrophy were increased for Groups 2, 3, 4, 5, and 7

Heart: Inflammation of the epicardium was observed for 1 male in Group 5 (177/183 µg/kg/day) and epicardial proliferation was observed for 1 female in Group 7 (180 µg/kg/day).

Liver: Herniation of the liver was observed for 1 male in Group 2 (1000/5 µg/kg/day).

Mandibular, Mesenteric, and Tracheobronchial LN: Free erythrocytes and/or erythrocytosis were observed for single dogs in Groups 2, 4, 5, and 6.

Mandibular salivary gland: Mononuclear cell infiltrates were observed for single dogs in Groups 3, 4, and 5.

Uterus w/Cervix: Pseudopregnancy was observed for 1 female in Group 2 (1000/5 µg/kg/day).

Histopathological findings at the end of the 13-week dosing period

Organ/Tissue	Sex	Control	GSK573719 + GW642444				GSK573719	GW642444
			1070/7.5	23/29	60/72	177/183	1048	180
Lungs								
perivascular/peribronchial/subpleural inflammatory cell infiltrate, G1	M	1/4	0/4	2/4	2/4	2/4	1/4	2/4
	F	2/4	2/4	2/4	2/4	3/4	2/4	2/4
-aggregates of alveolar macrophages	M	0/4	0/4	0/4	1/4	1/4	1/4	0/4
	F	0/4	0/4	0/4	1/4	1/4	1/4	1/4
-bronchial/bronchiolar lumen: ectasia, G3	M	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
-bronchial epithelium: goblet cell hyperplasia, G2	M	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
-bronchial/bronchiolar lumen: inflammatory cells/cellular debris, G1-G3	M	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	F	2/4	1(G3)/4	0/4	1/4	1/4	1/4	2/4
-pleural adhesion, G3	M	0/4	0/4	0/4	0/4	0/4	0/4	0/4
	F	0/4	1/4	0/4	0/4	0/4	0/4	0/4
-subacute/chronic inflammation, G1-G4	M	1/4	1(G4)/4	0/4	1/4	0/4	1/4	1/4
	F	2/4	2(G1,3)/4	2(G1,3)/4	1/4	2/4	2/4	1/4
-bronchial/bronchiolar lumen: inflammatory cells/cellular debris G1-G3	M	0/4	1(G3)/4	1/4	0/4	0/4	0/4	0/4
	F	2/4	0/4	0/4	1/4	1/4	1/4	2/4
Larynx								
-mucosa: mixed inflammatory cell infiltrate, G1-G2	M	1/4	4/4	0/4	0/4	2/4	1/4	1/4
	F	1/4	0/4	2/4	0/4	1/4	2/4	1/4
-lumen: inflammatory cells/cell debris, G1-G2	M	1/4	1/4	0/4	0/4	0/4	0/4	0/4
	F	1/4	0/4	1/4	0/4	0/4	0/4	0/4
Nose/Turbinates								
-nasal mucosa (respiratory) increased inflammatory cells, G1	M	0/4	0/4	0/4	0/4	0/4	1/4	1/4
	F	0/4	0/4	1/4	0/4	1/4	0/4	0/4
-nasal lumen: inflammatory cells/cell debris, G1	M	0/4	0/4	0/4	0/4	1/4	1/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
-nasal mucosa (respiratory) epithelium erosions, G1	M	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Trachea								
-mucosa: mixed inflammatory cell infiltrate, G1	M	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Thyroid								
-C cell hyperplasia, G2	M	0/4	0/4	0/4	0/4	1/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	1/4	0/4	0/4
Testes								

-seminiferous tubules: tubular dilatation, G2	M	0/4	0/4	0/4	0/4	1/4	0/4	0/4
Thymus								
-involution/atrophy, G2-G4	M	0/3	1/4	2/4	3(G2,3,4)/4	2/4	1/4	2/4
	F	1/4	2/4	1 (G4)/4	1/4	2/4	0/4	1/4
Heart								
-epicardium: chronic inflammation, G2	M	0/4	0/4	0/4	0/4	1/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
-epicardial proliferation, G2	M	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Liver								
-mixed mononuclear cell infiltrate, G1	M	0/4	1/4	0/4	0/4	0/4	1/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
-herniation	M	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Mandibular Salivary Gland								
-mononuclear cell infiltrate, G1	M	0/4	0/4	1/4	1/4	1/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Mandibular LN								
-sinuses: free erythrocytes/erythrocytosis, G2	M	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Mesenteric LN								
-sinuses: erythrophagocytosis, G1-G3	M	0/4	0/4	0/4	0/4	1(G3)/4	0/4	1/4
	F	0/4	0/4	0/4	0/4	1/4	0/4	0/4
Tracheobronchial LN								
-sinuses: free erythrocytes/erythrocytosis, G2	M	0/4	0/4	0/4	1/4	0/4	1/4	0/4
	F	0/4	0/4	0/4	0/4	1/4	0/4	0/4
Uterus w/Cervix								
-pseudopregnancy	F	0/4	1/4	0/4	0/4	0/4	0/4	0/4

Toxicokinetics: In Phase 2 during Weeks 4, 8 and 13, blood samples for measurement of plasma concentrations of GSK573719 and GW642444 were obtained from all animals in each group. Samples were obtained immediately after completion of dosing (within 5 min), and 0.5, 1, 2, 4, 8 and 23 hr 50 min after the completion of dosing. Concentrations of GSK573719 and GW642444 in plasma from all animals (including controls) were determined by HPLC-MS/MS by GSK PCD DMPK (Ware). The lower limit of quantification (LLQ) for GSK573719 and GW642444 was 0.1 ng/mL, using a 50 µL aliquot of dog plasma with a higher limit of quantification (HLQ) of 100 ng/mL.

C_{max} and AUC values for GSK573719 in Groups 2 (1070/7.5 µg/kg/day) and 6 (1048 µg/kg/day) during weeks 4, 8, and 13 were relatively comparable. C_{max} and AUC values for GSK573719 in Groups 3, 4, and 5 increased in an approximate dose proportional manner. Exposures to GSK573719 were relatively comparable between males and females with the exception of Group 2 where C_{max} and AUC values were higher in females as compared to males.

C_{max} and AUC values for GW642444 in Group 7 (180 µg/kg/day) were slighter than values in Group 5 (183/180 µg/kg/day) during weeks 4, 8, and 13. C_{max} and AUC values for GW642444 in females from Groups 2, 3, 4, and 5 increased with elevating doses; however, increases were less than dose proportional. C_{max} and AUC values in males increased from 7.5 µg/kg/day (Group 2) to 72 µg/kg/day (Group 4); however, values at 183 µg/kg/day (Group 5) were less than or equal to values at 72 µg/kg/day.

Table 37 AUC and C_{max} values for GSK573719 and GW642444 at weeks 4, 8, and 13

Toxicokinetic Parameter+	Phase 2	Male							Female						
		Overall Estimated Achieved Dose GSK573719 (µg/kg/day)							Overall Estimated Achieved Dose GSK573719 (µg/kg/day)						
		0	1070	23	60	177	1048	0	0	1070	23	60	177	1048	0
$AUC_{(0-t)}$ (ng.h/mL)	Week 4	NC	55.6	0.445	4.32	11.2	59.7	NC	NC	52.4	2.36	4.92	8.32	81.7	NC
	Week 8	NC	50.3	0.445	8.76	9.03	57.6	NC	NC	65.3	3.00	5.22	9.74	110	NC
	Week 13	NC	83.5	0.468	7.86	10.6	53.4	NC	NC	61.0	2.01	4.44	9.38	115	NC
C_{max} (ng/mL)	Week 4	NC	55.6	0.679	2.76	8.50	55.5	NC	NC	34.2	1.99	3.48	8.14	87.0	NC
	Week 8	NC	33.2	0.819	5.52	5.49	44.0	NC	NC	59.9	2.81	3.84	8.14	102	NC
	Week 13	NC	59.9	0.913	3.60	4.43	28.3	NC	NC	26.8	1.38	2.40	2.48	104	NC

+Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.
NC Not calculated due to plasma results being below the limit of quantification (LOQ).

Toxicokinetic Parameter+	Phase 2	Male							Female						
		Overall Estimated Achieved Dose GW642444 (µg/kg/day)							Overall Estimated Achieved Dose GW642444 (µg/kg/day)						
		0	7.5	29	72	183	0	180	0	7.5	29	72	183	0	180
$AUC_{(0-t)}$ (ng.h/mL)	Week 4	NC	8.10	33.3	97.5	141	NC	207	NC	14.5	96.0	129	198	NC	156
	Week 8	NC	8.03	37.6	204	134	NC	232	NC	11.5	147	169	313	NC	365
	Week 13	NC	8.78	41.9	174	168	NC	223	NC	13.3	93.8	163	199	NC	205
C_{max} (ng/mL)	Week 4	NC	3.70	15.7	46.8	90.6	NC	119	NC	5.96	43.0	75.3	115	NC	79.9
	Week 8	NC	2.72	18.7	87.5	65.3	NC	101	NC	6.26	56.4	96.8	126	NC	157
	Week 13	NC	4.13	17.9	72.4	74.1	NC	67.1	NC	4.04	30.5	59.8	50.0	NC	75.4

+Calculated values derived by multiplying the dose-normalized data by the overall estimated achieved dose.
NC Not calculated due to plasma results being below the limit of quantification (LOQ).

Dosing Formulation Analysis: Variations of daily exposure concentrations were not clearly described in this draft report. The final report should be checked for details of variations of daily exposure concentrations.

11 Integrated Summary and Safety Evaluation

The sponsor has proposed a Phase III, 52-week clinical trial to evaluate the safety and tolerability of the long-acting muscarinic antagonist GSK573719 125mcg alone and in combination with the long-acting $beta_2$ agonist GW642444 25mcg compared with placebo in male or female subjects (≥40 years old) with COPD. In support of the proposed clinical trial, the sponsor has provided nonclinical reports for the following studies: a 26-week inhalation toxicology study with rats, a 39-week inhalation toxicology study with dogs, fertility and reproductive performance studies in male and female rats, and embryofetal development studies in rats and rabbits.

In a 26-week inhalation toxicology study, rats received GSK573719 at inhaled doses of 0, 87.1, 289, or 987 µg/kg/day. Deposited doses were 0, 8.7, 28.9, or 98.7 µg/kg/day, respectively. Target organs of toxicity were the lung, tracheal bifurcation, nasal cavity/sinuses, nasopharynx, and larynx. Findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to human subjects that will receive the drug by oral inhalation. Findings in the larynx were judged to be rodent-specific (i.e., nonspecific irritant effects associated with aerosol inhalation) and irrelevant to humans. In the lungs, incidences of alveolar macrophage were increased for all treatment groups. These findings were reversible by the end of the 6-week recovery period. There was no evidence of inflammation in surrounding tissues. At the tracheal bifurcation, increased incidences of squamous metaplasia were observed for males and females in

the mid and high dose groups. These findings are indicative of irritant effects induced by the test article. The NOAEL was identified as the low dose based upon histopathological findings for the tracheal bifurcation (squamous metaplasia) in the mid and high dose groups. Doses for males and females in the low dose group, expressed on µg per gram lung weight basis, were 2.86 and 2.02 µg/kg/day, respectively. AUC values during week 26 for males and females in the low dose group were 47.6 and 41.1 ng·hr/mL, respectively.

In a 39-week inhalation toxicology study, dogs received GSK573719 at achieved doses of 0, 109, 421, and 1002 µg/kg/day. Deposited doses were 0, 27, 105, 250 µg/kg/day. Several clinical signs were evident for GSK573719-treated dogs (i.e., dry mouth, swollen neck, decreased incidences of unformed, watery, and mucoid stools, and increased incidences of vomit and vomit with food. Many of these findings were expected pharmacological effects of an M3 muscarinic antagonist. For ECG measurements conducted during week 39, immediately following administration of GSK573719, increased heart rates were observed at ≥109 µg/kg/day (up to 1.50- and 1.61-times predose in males and females, respectively). Respiratory sinus arrhythmia was generally absent in males given ≥421 µg/kg/day and females given ≥109 µg/kg/day. On day 1, elevations of serum cardiac Troponin I (cTnI) concentrations were observed for dogs that received ≥421 µg/kg/day.

Potential target organs of toxicity were the heart, lungs, larynx, and nose turbinates. Moderate subacute inflammation of the extramural coronary arteries was seen in one female given 1002 µg/kg/day and in one male (Animal No. 3427) given 421 µg/kg/day. In one female given 1002 µg/kg/day, there was mild intimal thickening with minimal focal areas of mixed, predominantly mononuclear, inflammatory cells infiltrate in an arteriole in one lobe of the lung. Pleural fibrosis was observed for one female given 1002 µg/kg/day. For the larynx, slight epithelial erosion and/or ulceration was present in the squamous epithelium overlying the base of the vocal folds in the larynx of one male and one female given 1002 µg/kg/day. In addition, slight subacute to chronic inflammation was present in the mucosal glands adjacent to the arytenoid cartilage in the male dog. Focal minimal squamous cell hyperplasia and hyperkeratosis was present in the epithelium overlying the base of the vocal folds in one male given 421 µg/kg/day. Minimal focal erosion/ulceration was also seen in one control animal at the end of the recovery period. Minimal squamous metaplasia of the respiratory epithelium was seen in the most cranial section of the nasal turbinates in one male (Animal No. 4427) given 1002 µg/kg/day. The NOAEL was identified as the low dose of 109 µg/kg/day based upon histopathological findings in the heart at the mid and high doses and the lung, larynx, and nose turbinates at the high dose. AUC values for males and females at 109 µg/kg/day during week 39 were 4.64 and 7.13 ng·hr/mL, respectively. The deposited doses, expressed on µg/g lung weight/day basis, for males and females were 3.14 and 2.86 µg/g lung weight/day, respectively.

In a 13 week repeat dose oropharyngeal tube inhalation toxicology study, beagle dogs received GSK573719A and GW642444A alone or in combination for 10 min/day. Achieved doses of GSK573719/GW642444 for Groups 2, 3, 4, 5, 6, and 7 were

1070/7.5, 23/29, 60/72, 177/183, 1048/0, and 0/180 µg/kg/day, respectively. Prior to giving dogs GW642444 at a target dose of 160 µg/kg/day in combination with GSK573719 or alone (i.e., Groups 5 and 7), the sponsor administered GW642444 at a target dose of 40 µg/kg/day (alone or with GSK573719) for 10 min/day for 3 consecutive days prior to escalation to the high target dose of 160µg/kg/day. The sponsor contended that this pre-adaptation phase of dosing (Phase 1) was required to induce tachyphylaxis to the cardiovascular effects of GW642444, allowing administration of higher doses than would normally be possible in untreated dogs. The Division communicated several concerns to the sponsor that pre-exposure of dogs to smaller doses may provide protection against subsequent exposure to larger doses (see Toxicology and Applied Pharmacology 69: 199-205, 1983). It was noted that the use of the pre-adaptation phase will be a review issue when the study is submitted.

Increased incidences of excessive salivation were observed in all male and female treatment groups during dosing and were still evident in a few treatment groups immediately postdose. Increased incidences of bilateral neck swelling were observed in all male and female treatment groups during dosing and /or immediately postdose. Immediately after dosing (IAD) on day 91/92, heart rates increases (up to 1.86- and 1.76-fold times higher than mean pretest values in males and females, respectively) were observed in dogs that received either GSK573719 or GW642444 alone or in combination. Both prior to and immediately after dosing on days 91/92, respiratory sinus arrhythmia (RSA) was absent in 15/16 animals from Groups 2 and 6 that received GSK573719 at a target dose of 1000 µg/kg/day either in combination with GW642444 or alone, respectively, suggesting that the effect was persistent at this target dose of GSK573719. RSA was absent immediately after dosing on days 91/92 for several male and female dogs in Groups 3, 4, and 5 that received the combination of GSK573719 and GW642444 (up to target doses of 160 µg/kg/day for each component).

During Phase1 (Pre-adaptation), elevations of serum cTnI concentrations were observed for females in Group 5 (44/47 µg/kg/day) and males and females in Group 7 (49 µg/kg/day GW642444). During Phase 2 (treatment period), fewer females in Group 5 (177/183 µg/kg/day) were observed with elevated serum cTnI concentrations despite the 4-fold increase of dose. Only 1 male in Group 5 was observed with elevated serum cTnI concentrations. For dogs in Group 7 (180 µg/kg/day GW642444), 1 male and 3 females were observed with elevated serum cTnI concentrations. Based upon the response of females in Group 5, the pre-adaptation phase induced tachyphylaxis or tolerance to potential cardiac injury (as measured by serum cTnI concentrations) induced by the combination of GSK573719 and GW642444. The sponsor conducted a 4-week inhalation toxicology study with dogs to assess the effects of the pre-adaptation phase (Study number 2010N109790_00); this study will be reviewed at a later date.

Histopathological findings were observed in the lung, larynx, nose/turbinates, trachea, thyroid, testes, thymus, and heart that could potentially be attributed to treatment. In general, these findings were consistent the individual test articles, GSK573719 and GW642444, and there was no evidence of novel toxicity with the combination. In the lungs, marked, localized, subacute/chronic inflammation was seen in 1 male dog

(#2452) in Group 2 (1000/5 µg/kg/day). This change was associated with bronchial/bronchiolar ectasia with luminal cellular debris, hyperplasia of the bronchial goblet cells, and pleural adhesions. In addition, plant/food material and bacterial colonies were present. In the larynx, slight increase in incidence and/or severity of mixed inflammatory cell infiltrates was observed for male dogs in Groups 2 (1000/5 µg/kg/day) and 5 (160/160 µg/kg/day) and females in Group 3 (23/29 µg/kg/day). Increased inflammatory cells in the nasal mucosa were observed for single dogs in Groups 3 (23/29 µg/kg/day), 5 (177/183 µg/kg/day), 6 (1048 µg/kg/day), and 7 (180 µg/kg/day). Inflammatory cells and cell debris were observed for single dogs in Groups 6 (1048 µg/kg/day) and 7 (180 µg/kg/day). Mixed inflammatory cell infiltrate in the tracheal mucosa was observed for 1 male dog in Group 2 (1000/5 µg/kg/day). There was no evidence of additive or synergistic toxicity with the combination of GSK573719 and GW642444. Observed toxic effects were consistent with the individual agents.

In a female fertility and early embryonic development study, rats received estimated achieved inhalation doses of 3.37, 29.1, 100, and 294 µg/kg/day (See review dated May 25, 2010). Body weight gains were unaffected. Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day (Reviewed in Review #01 dated June 3, 2009). Body weight gains for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group (-2.9%). Dose selection in the present study appeared to be adequate. There were no effects on fertility or reproductive performance for female rats treated with inhalation doses up to 294 µg/kg/day (equivalent to a pulmonary deposited dose of 29.4 µg/kg/day). A detailed review of this study will be conducted at a later time.

In a male fertility and reproductive performance study (Study number CD2010/00187/00), male rats received GSK573719 at subcutaneous doses of 0, 30, 60, or 180 µg/kg/day for a total of 49 to 53 daily doses. Body weight gains for males in the 30, 60, and 180 µg/kg/day groups were decreased to 84.6, 86.8, and 81.6% of the control, respectively. After 14 days of treatment, male rats were mated with untreated female rats. Mating and fertility indexes were unaffected with subcutaneous doses up to 180 µg/kg/day. A detailed review of this study will be done later.

GSK573719A was administered by nose-only inhalation for 1 hr each day from gestation days 6 to 17 to pregnant female Sprague-Dawley rats at estimated achieved doses of 0, 0.0317, 0.0969, and 0.278 mg/kg/day (See review dated May 25, 2010). Body weight gains from gestation days 6 to 18 were unaffected. Dose selection was based upon the 4-week inhalation toxicology study with rats (WD2005/01422/00) that received doses of 0, 26.1, 243, and 1829 µg/kg/day (Reviewed in Review #01 dated June 3, 2009). Body weight gains for females in the low and mid dose groups were reduced to 69.8 and 47.3% of the control, respectively. Body weight loss occurred for the high dose group. Dose selection in the present study appeared to be adequate. There were no external, skeletal, or visceral malformations. Increased incidences of minor skeletal and visceral abnormalities/variants were observed for treatment groups. The incidence of ventral arch of the 1st cervical vertebra was increased for all treatment

groups in a dose-related manner. Incidences of metatarsals (incomplete ossification/unossified) and phalanges (none ossified) bilateral hindlimb were increased for all treatment groups although dose-response relationships were not present. Subdural hemorrhages were evident in the mid and high dose groups. Hemorrhage in the cerebellum was observed in the mid and high dose groups although a dose-response relationship was not present. Hemorrhage within cerebral hemispheres was observed in the high dose group. GSK573719 was not teratogenic in rats at inhaled doses up to 0.278 mg/kg/day (equivalent to a pulmonary deposited dose of 0.028 mg/kg/day or 27.8 µg/kg/day).

In an embryofetal development study, time-mated female New Zealand white rabbits received GSK573719 at estimated inhaled doses of 0, 0.0285, 0.0889 or 0.306 mg/kg/day from gestation days 7 to 19 (See review dated May 25, 2010). Body weight gains from gestation days 7 to 20 for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups were reduced as compared to the control although a dose-response relationship was not evident. Food consumption for females in the 0.0285, 0.0899, and 0.306 mg/kg/day groups from gestation days 7 to 19 was reduced to 77.3, 78.15, and 73.1% of the control, respectively. Gravid uterine weights for the 0.0899 and 0.305 mg/kg/day groups were reduced to 94.2 and 92.3% of the control (0.52 kg) although the toxicological significance of these differences was not clear. Fetal body weights (male, female, and overall) were slightly reduced for all treatment groups although the significance of these differences was not clear. There were no treatment-related external, visceral, or skeletal malformations. Two fetuses in the high dose group were observed with thymus-undescended horn. The incidence of caudal vertebrae-incomplete ossification/unossified was increased for the high dose group. The incidence of sternebrae-bipartite ossified/offset alignment was increased for the high dose group and slightly exceeded the historical control incidence. GSK573719 was not teratogenic in rabbits with inhaled doses up to 0.306 mg/kg/day.

In an embryofetal development study, mated female rabbits received GSK573719 at subcutaneous doses of doses of 0, 0.04, 0.1 and 0.18 mg/kg/day once daily from gestation days 7 to 19 (Study number CD2010/00253/00). Mated females and their litters were euthanized on gestation day 29. There were signs of elongated feces, soft feces and decreased fecal output at 0.18 mg/kg/day. Elongated feces were also observed at doses of 0.1 and 0.04 mg/kg/day. Body weight gains from gestation days 7 to 20 were unaffected. Food consumption for low, mid, and high dose groups from gestation days 7 to 20 were decreased to 96, 88, and 87% of the control, respectively. There were no treatment-related external, visceral, or skeletal malformations. The incidence of centrum incompletely ossified, T13 supernumerary, and hyoid incompletely ossified were increased for all treatment groups. The incidence talus not ossified was increased for the mid and high dose groups. The incidence of sternebrae variation in shape was increased for the high dose group. GSK573719 was not teratogenic in rabbits with subcutaneous doses up to 0.18 mg/kg/day although dosing may have been inadequate. A detailed review of this study will be done later.

Safety margins for proposed clinical doses of GSK573719 and GW642444 on AUC and µg/gram lung weight/day bases are shown in the tables below. NOAELs in the 26-week rat and 39-week dog toxicology studies provide adequate safety margins for the proposed clinical dose of GSK573719 on AUC and µg/gram lung weight/day bases. In the 13-week inhalation toxicology study with the combination of GSK573719 and GW642444 in dogs, there was no evidence of additive or synergistic toxicity. Observed toxic effects were consistent with the individual agents. The proposed Phase III, 52-week clinical trial to evaluate the safety and tolerability of the long-acting muscarinic antagonist GSK573719 125mcg alone and in combination with the long-acting $beta_2$ agonist GW642444 25mcg appears reasonably safe and should be allowed to proceed.

Under IND 74,696, the sponsor has conducted 26-week rat and 39-week dog toxicology studies with GW642444 alone that support that support the clinical dose of 25 µg/day (see Review dated March 5, 2010). Target organs of toxicity identified in rats 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).Target organs of toxicity identified in the dog 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).

Table 38 Safety margins for the proposed clinical dose of GSK573719 at 125 µg/day on an AUC basis

Safety margins for GSK573719			
Study	NOAEL		Safety margins for clinical dose of 125 µg/day
	Achieved dose	AUC	AUC = 0.07 ng*hr/mL
	µg/kg/day	ng*hr/mL	
26-week rat	987	44.35	633.57
39-week dog	109	5.88	84.00
13-week dog	G2/1070	72.25	1032.14
	G3/23	1.239	17.70
	G4/60	6.15	87.86
	G5/177	9.99	142.71
	G6/1048	84.2	1202.86

Table 39 Safety margins for the proposed clinical dose of GSK573719 at 125 µg/day on µg/g lung weight/day basis

Safety margins for GSK573719			
Study	NOAEL		Safety margins for clinical dose of 125 µg/day
	Achieved dose	Deposited dose	0.125 µg/g LW/day
	µg/kg/day	µg/g LW/day	
26-week rat	987	2.44	19.52
39-week dog	109	3	24.00
13-week dog	1070	28.11	224.88
	23	0.61	4.88
	60	1.71	13.68
	177	5.04	40.32
	1048	27.42	219.36

Table 40 Safety margins for the proposed clinical dose of GW642444 at 25 µg/day on an AUC basis from the 13-week combination toxicology study with dogs

Safety margins for GW642444			
Study	Achieved dose	AUC	Safety margins for clinical dose of 25 µg/day
			AUC = 0.163 ng*hr/mL
13-week dog	µg/kg/day	ng*hr/mL	
	G2/7.5	11.04	67.73
	G3/29	67.85	416.26
	G4/72	168.5	1033.74
	G5/183	183.5	1125.77
	G7/180	214	1312.88

Table 41 Safety margins for the proposed clinical dose of GW642444 at 25 µg/day on a µg/g lung weight/day basis from the 13-week combination toxicology study with dogs

Safety margins for GW642444			
Study	NOAEL		Safety margins for clinical dose of 25 µg/day
	Achieved dose	Deposited dose	0.025 µg/g LW/day
	µg/kg/day	µg/g LW/day	
13-week dog	7.5	0.2	8.00
	29	0.77	30.80
	72	2.06	82.40
	183	5.22	208.80
	180	5.17	206.80

Table 42 Safety margins for the proposed clinical dose of GW642444 at 25 µg/day on a µg/g lung weight/day basis from the 26-week rat and 39-week dog toxicology studies (from IND 74,696)

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 0.025	B 0.025		

[a] 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).

Table 43 Safety margins for the proposed clinical dose of GW642444 at 25 µg/day on an AUC basis from the 26-week rat and 39-week dog toxicology studies (from IND 74,696)

Species	NOAEL, mcg/kg				Safety Margin			
	mcg/kg A		AUC $_{0-t}$ ng.h/ml C		mcg/kg A/B		AUC $_{0-t}$ 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 AUC: 0.165[a] ng.h/ml								

[a] Obtained from the 6/26/08 review of IND 77,855 by Huiqing Hao, Ph.D.

<u>Use of the Excipient, Magnesium Stearate</u>: Magnesium stearate is being used as an excipient in the drug product. The approved dose of magnesium stearate in the

approved Foradil® Certihaler™ product is 56 µg/day. Pulmonary deposited doses of magnesium stearate in vehicle-control groups from the 26-week rat and 39-week inhalation toxicology studies were 6.5 and 7.0 µg/kg/day, respectively. From other studies, magnesium stearate does not appear to alter the toxicity or toxicokinetics of GSK573719. The sponsor has rights of reference to a 6-month inhalation toxicology study with magnesium stearate in rats. The NOAEL (pulmonary deposited dose) was identified as 180 µg/kg/day, which supports a maximum clinical dose of 18 µg/kg/day. The clinical dose of magnesium stearate is 200 µg/day or 3.3 µg/kg/day, which is covered by the 6-month study with rats.

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/s/

TIMOTHY W ROBISON
02/14/2011

MOLLY E TOPPER
02/14/2011
I concur.

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:	104,479 and 106,616
Supporting document/s:	IND 104,479 #043, 044, 045, 047, 052 IND 106,616 # 070, 079, 090, and 108
Sponsor's letter date:	IND 104,479 August 27, 2010 August 30, 2010 September 21, 2010 October 6, 2010 December 14, 2010 IND 106,616 August 30, 2010 September 21, 2010 October 6, 2010 December 14, 2010
CDER stamp date:	IND 104,479 August 30, 2010 August 31, 2010 September 22, 2010 October 6, 2010 December 14, 2010 IND 106,616 August 31, 2010 September 22, 2010 October 6, 2010 December 14, 2010
Product:	Combination of GSK573719 (Muscarinic receptor antagonist) and GW642444 (LABA)
Indication:	COPD
Sponsor:	GlaxoSmithKline P.O. Box 13398 Five Moore Drive Research Triangle Park North Carolina 27709-3398
Review Division:	Division of Pulmonary, Allergy, and Rheumatology

	Products
Reviewer:	Timothy W. Robison, Ph.D., D.A.B.T.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Angela Ramsey

Template Version: September 1, 2010

This is an addendum to the review dated February 14, 2011.

With reference to the 26-week inhalation toxicology study in rats (Study number FD2009/00467/00), rat to human exposure (AUC) ratios are corrected in the table below. The values reported in Table 38 in the review dated February 14, 2011 were in error as AUC values from the high dose group rather than the low dose group were used in the calculation. It is noted that there was no evidence of systemic toxicity in rats at all inhaled doses in this study as all drug-related toxicity was localized to the respiratory tract. AUC values from the low dose group still provide large safety margins for the maximum proposed clinical dose of 125 µg/day. The calculation error had no impact on the conclusions of the review.

The calculations in Table 39 for safety margins on a µg/g lung weight/day basis were correct. The high dose on a µg/kg/day basis was listed in the table; however, the µg/g lung weight/day value listed in the table was from the low dose group.

In a 26-week inhalation toxicology study (study number FD2009/00467/00), rats received GSK573719 at inhaled doses of 0, 87.1, 289, or 987 µg/kg/day. Deposited doses were 0, 8.7, 28.9, or 98.7 µg/kg/day, respectively. Target organs of toxicity were the lung, tracheal bifurcation, nasal cavity/sinuses, nasopharynx, and larynx. Findings in the nasal cavity/sinuses and nasopharynx were judged to be irrelevant to human subjects that will receive the drug by oral inhalation. Findings in the larynx were judged to be rodent-specific (i.e., nonspecific irritant effects associated with aerosol inhalation) and irrelevant to humans. In the lungs, incidences of alveolar macrophage were increased for all treatment groups. These findings were reversible by the end of the 6-week recovery period. There was no evidence of inflammation in surrounding tissues. At the tracheal bifurcation, increased incidences of squamous metaplasia were observed for males and females in the mid and high dose groups. These findings are indicative of irritant effects induced by the test article. The NOAEL was identified as the low dose based upon histopathological findings for the tracheal bifurcation (squamous metaplasia) in the mid and high dose groups. Doses for males and females in the low dose group, expressed on µg per gram lung weight basis, were 2.86 and 2.02 µg/kg/day, respectively. AUC values during week 26 for males and females in the low dose group were 8.88 and 10.1 ng·hr/mL, respectively.

Table 38 Safety margins for the proposed clinical dose of GSK573719 at 125 µg/day on an AUC basis

Safety margins for GSK573719			
Study	NOAEL		Safety margins for clinical dose of 125 µg/day
	Achieved dose	AUC	AUC = 0.07 ng*hr/mL
	µg/kg/day	ng*hr/mL	
26-week rat	87.1	9.49	135.6

Table 39 Safety margins for the proposed clinical dose of GSK573719 at 125 µg/day on µg/g lung weight/day basis

Safety margins for GSK573719			
Study	NOAEL		Safety margins for clinical dose of 125 µg/day
	Achieved dose	Deposited dose	0.125 µg/g LW/day
	µg/kg/day	µg/g LW/day	
26-week rat	87.1	2.44	19.52

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/s/

TIMOTHY W ROBISON
09/06/2012

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REVIEW

General Toxicology:

1. **One-month Inhalation Toxicity Study with Magnesium Stearate in Rats.** (b) (4) (b) (4) **Vol. 3, page 8-39.**

Testing Laboratory:	(b) (4)
Study Number:	
GLP Statement	Yes
Study Date:	4/21/99 –6/29/99; Final Report date: 9/15/1999
Lot Number:	96114/2 (expiration date 10/16/99)
End Point:	Toxicity of inhaled Mg stearate on respiratory system

Reference ID: 3330354

Method

Wistar rats (5 rats/sex/dose, 7 –11 weeks of age) were exposed by nose-only inhalation to 0 or 26 µg/kg/day (pulmonary deposition[1]) magnesium stearate for 30 days. The control group received room air only. The duration of exposure was 1 hour/day for both groups. The mean aerosol concentration for magnesium stearate was 8.1±2 µg/l air. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for the treatment group were 0.92 µm ± 3.15.

The following parameters were monitored during the study:

Clinical Signs:	Weekly
Body Weight:	Weekly
Food Consumption:	Weekly
Ophthalmology:	Pre-exposure and Week 4
Clinical Pathology:	Hematology, blood chemistry, and urinalysis examinations on day 30 of the exposure
Pathology:	Time of sacrifice
Organ Weights:	Adrenals, brain, heart, kidneys, liver, lungs, spleen, testes/ovaries, thymus, thyroids
Necropsy:	All animals
Histopathology:	Respiratory tract only: nasal cavity, nasopharynx, larynx, lungs, lymph-nodes, trachea and bronchi.

Results

Clinical signs: No deaths or treatment-related clinical signs were observed.
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 26 µg/kg/day did not result in any treatment-related findings. The no-observed-adverse-effect-level (NOAEL) for magnesium stearate can be tentatively identified as 26 µg/kg/day in rats, as the study had a relatively small sample size.

1. The study report cites a magnesium stearate dose of 258 µg/kg/day. This is an expected amount in the inhaled air (8.1 µg/l x 0.134 l/min x 60 min ÷ 0.25 kg = 258 µg/kg/day). The Division uses the expected pulmonary dose for the safety evaluation of inhalation drugs indicated for asthma. The pulmonary dose of magnesium stearate in this study would be 26 µg/kg/day, using a pulmonary deposition factor of 0.1 in rats.

Reference ID: 3330354

1. **Six-month Inhalation Toxicity Study with Magnesium Stearate in Rats.** [redacted] (b) (4) **Vol. 4, page 8-260.**

Testing Laboratory: [redacted] (b) (4)

Study Number:
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	Estimated exposure (µg/kg/day)	
	MMAD	GSD	(µg/L air)	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[2] 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: 56 mg (per day of lactose) x 1000 µg/mg x 1% magnesium stearate ÷ 60 kg body weight = 9.3 µg/kg/day. Using a body weight of 50 kg, the expected human exposure of magnesium stearate would be 11.2 µg/kg/day. This was based on the proposed device delivering 7 mg of lactose per actuation and the expected daily clinical dose being 8 actuations.

Reference ID: 3330354

The following parameters were monitored during the study:

Clinical Signs:	Daily
Clinical exams:	Weekly
Body Weight:	Weekly
Food Consumption:	Weekly
Ophthalmology:	Pre-exposure and Week 26
Clinical Pathology:	Hematology, blood chemistry, and urinalysis examinations in weeks 6 and 26 of the exposure
Pathology:	Time of sacrifice
Organ Weights:	Adrenals, brain, heart, kidneys, liver, lungs, spleen, testes/ovaries, thymus, thyroids
Necropsy:	All animals
Histopathology:	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 µg/kg/day did not result in any treatment related findings. The no-observed-adverse-effect-level (NOAEL) for magnesium stearate can be identified as 180 µg/kg/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 1- and 6-month, respectively. The doses (pulmonary deposition) were 26 µg/kg/day for the 1-month study and 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 µg/kg/day with the inhalation route of exposure.

Reference ID: 3330354

3. Use of Magnesium Stearate Excipient in Lactose Formulations, (b) (4) (b) (4), 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^3 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

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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 a (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

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has conducted and submitted two inhalation toxicity studies with exposure durations of one- and 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 (11.2 µg/kg/day). It is concluded that there is sufficient margin of safety to support the safety of magnesium stearate in the proposed protocol.

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Luqi Pei, D.V.M., Ph.D. Pharmacologist/Toxicologist	Robin Huff, Ph.D. Pharm/Tox Team Leader

Reference ID: 3270883

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/s/

JANE J SOHN
06/24/2013

TIMOTHY W ROBISON
06/25/2013
I concur.

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 203975
Supporting document/s:	SD-1, SD-12 (Response to information request)
Applicant's letter date:	12/18/12, 3/25/13
CDER stamp date:	12/18/12, 3/25/13
Product:	Umeclidinium-Vilanterol
Indication:	Treatment of airflow obstruction in COPD
Applicant:	GlaxoSmithKline
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Jane J. Sohn, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., DABT
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.
Project Manager:	Leila P. Hann

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203975 are owned by GlaxoSmithKline or are data for which GlaxoSmithKline has obtained a written right of reference.
Any information or data necessary for approval of NDA 203975 that GlaxoSmithKline does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203975.

Reference ID: 3313292

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Reference ID: 3313292

1 Executive Summary

1.1 Introduction

GlaxoSmithKline (GSK) has submitted this NDA for registration of Anoro-Ellipta for treatment of airflow obstruction in chronic obstructive pulmonary diseases (COPD). Anoro-Ellipta is a dry powder inhaler that delivers a dry powder mixture of umeclidinium bromide salt (umeclidinium) and vilanterol triphenylacetate salt (vilanterol) in magnesium stearate and lactose monohydrate. The sponsor proposes that each actuation deliver 62.5/25 mcg or 125/25 mcg of umeclidinium/vilanterol, respectively, with one actuation proposed per day.

Two-year carcinogenicity studies with umeclidinium in mouse and rat are reviewed here. Special protocol assessments for both carcinogenicity protocols were conducted under IND 104479, for umeclidinium. All studies supporting vilanterol are reviewed under NDA 204275. Umeclidinium is known by the code name GSK573719A, and vilanterol is known by the code name GW642444M.

1.2 Brief Discussion of Nonclinical Findings

Two-year carcinogenicity studies with umeclidinium in mice and rats were analyzed. Both the mouse and rat protocols were reviewed through special protocol assessments conducted under IND 104479.

Umeclidinium was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation assay, *in vitro* mouse lymphoma assay, and *in vivo* rat micronucleus test.

The sponsor conducted a 2-year bioassay in CD-1 mice with GSK573719A by inhalation. Initial doses were based on MTD determined in a 13 week toxicology study (ECAC minutes 6/3/09). Female mice received nominal doses of 0 (vehicle), 20, 60, and 200 mcg/kg/day throughout the study. Males initially received nominal doses of 0, 60, 200, and 600 mcg/kg/day. Doses were decreased at Week 67 for males only to 0, 30, 100, and 300 mcg/kg/day, based on loss of body weight in dosed males, with concurrence from the ECAC (minutes 2/18/11). There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis. Histiocytic sarcomas (whole body) were noted in females dosed with the test article, but the findings were not statistically significant by trend analysis and pair-wise analysis, and were within historical control data for females, based on data submitted by the sponsor from 13 studies conducted at (b) (4) (b) (4) Additional historical control data from Crl: CD1 mice from (b) (4) (b) (4) (updated March 2010) showed that 1.67 to 11.67% of animals were diagnosed with histiocytic sarcomas in 9 studies conducted in the US or Europe.

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The sponsor conducted a 2-year bioassay in Crl: CD (Sprague Dawley) rats with GSK573719A by inhalation. Initial doses were based on the MTD determined from a 13-week inhalation toxicology study (ECAC minutes 6/3/09). The recommended doses were 0, 30, 100, and 300 mcg/kg/day (60 minute exposures). Effective from Week 73, doses were reduced to target doses of 15, 50, and 150 mcg/kg/day with ECAC concurrence (minutes 2/18/11). Based on data up to Week 70, the sponsor noted decreases in body weight gains and proposed lower doses. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD. There were no treatment-related neoplastic findings based on the lack of statistical significance. Neoplastic findings observed with increased incidences in test article-treated groups were basal cell tumors (skin) in females and lymphomas in males, but these did not achieve statistical significance for trend and pair-wise comparison, and were within historical control values.

Bilateral opacity on the posterior position of the lens was increased in both the mouse and rat bioassays. In male rats, this finding was increased in a dose-dependent manner (control 2/39, LD 5/46, MD 5/43, HD 8/48). The sponsor provided historical control data showing that the incidence of was within the historical control range. Bilateral opacity of the posterior lens was also increased in a male mice in a dose-dependent manner (control 9/41, LD 12/33, MD 15/33, HD 27/41), and was increased in LD and HD females (control 16/31, LD 35/45, MD 13/29, HD 27/35), but was not dose-dependent. The incidence for male mice was within historical controls, but that for females was not. In female mice, the lack of dose-response makes it difficult to determine if this finding is test-article related. These findings might be attributed to direct exposure of the lens to the test article rather than through systemic exposure

In conclusion, umeclidinium was determined to not be tumorigenic based on evaluation in mice and rats. Exposures achieved, based on the top dose in carcinogenicity studies for umeclidinium, provide safety margins greater than 20-fold over clinical exposure in COPD patients for the lower proposed dose of 62.5 mcg/day of umeclidinium.

1.3 Recommendations

Conclusions from Executive CAC meeting conducted on April 9, 2013:

Rat:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.

Mouse:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

Reference ID: 3313292

- The Committee concurred that there were no drug-related neoplasms in either male or female mice.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 869113-09-7 (Umeclidinium bromide salt), 503068-34-6 (vilanterol)

Generic Name:
1) Umeclidinium bromide salt
2) Vilanterol triphenylacetate salt

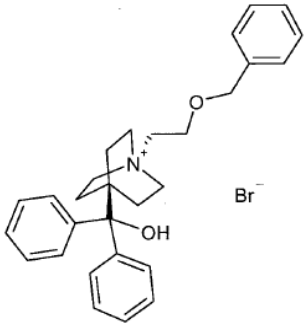
Code Name:
1) GSK573719A (umeclidinium bromide salt)
2) GW642444M (vilanterol triphenylacetate salt)

Chemical Name:
1) 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide
2) Triphenylacetic acid-4-{(1R)-2-[(6-{2-[2,6-dicholorobenzyl)oxy]ethoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol (1:1)

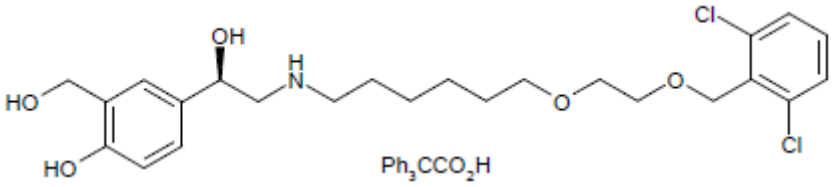
Molecular Formula/Molecular Weight:
1) $C_{29}H_{34}NO_2 \cdot Br$
508.5 (GSK573719A as quaternary ammonium bromide compound)
428.6 (as free cation, GSK573719);
2) $C_{24}H_{33}Cl_2NO_5.C_{20}H_{16}O_2$
774.78 (as the triphenylacetate salt)
486.43 (as the free base)

Reference ID: 3313292

Structure:



Umeclidinium bromide salt



Vilanterol triphenylacetate salt

Pharmacologic Class:
1) muscarinic acetylcholine receptor (mAChR) antagonist
2) long-acting β_2-adrenergic agonist.

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 104,479 (GSK, GSK573719 (umeclidinium))
IND 106,616 (GSK, GSK573719/GW642444 (vilanterol))
IND 74,696 (GSK, GW642444)
IND [redacted] (b) (4)
IND 77,855 (GSK, GW642444 + Fluticasone)
IND [redacted] (b) (4)
DMF 025,906 (GSK, GW642444)

3 Studies Submitted

3.1 Studies Reviewed

1. GSK573719A: Carcinogenicity Study by Inhalation Administration to CD-1 Mice for 104 weeks (study BVR1374/M28976)
2. GSK573719A: Carcinogenicity Study by Inhalation Administration to Crl:CD(SD) Rats for 104 weeks (study BVR1357/R28862)

Reference ID: 3313292

4 Carcinogenicity

2-year Mouse Study

Study title: GSK573719A: CARCINOGENICITY STUDY BY INHALATION ADMINISTRATION TO CD-1 MICE FOR 104 WEEKS

Study no.:	BVR1374/M28976
Study report location:	SD-1 (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12 November 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK573719, R360974/081168623, 99.4%
CAC concurrence:	Yes

Key Study Findings

- In a 2-year carcinogenicity study, mice received GSK573719A by inhalation (nose-only). Female mice received nominal doses of 0 (vehicle), 20, 60, and 200 mcg/kg/day throughout the study. Male mice initially received nominal doses of 0, 60, 200, and 600 mcg/kg/day (ECAC minutes 6/3/09). Doses were decreased at Week 67 for males to 0, 30, 100, and 300 mcg/kg/day based on non-dose related increases of mortality and reduced body weight in dosed males, with concurrence from the ECAC (minutes 2/18/11).
- Treatment with the test article was associated with increased mortality in males by week 66; however, following the dose reduction, there were no dose-dependent effects on mortality at Week 104.
- Midway through the study, at Week 52, males dosed with test article showed an overall decrease in absolute body weight, which was not dose-dependent. A decrease of doses in males resulted in a reversal of the decrease in absolute body weight. By Week 104, there were no notable differences in absolute body weight in dosed animals compared to control in either sex.
- Incidences of bilateral opacity of the posterior lens were increased in a male mice in a dose-dependent manner (control 9/41, LD 12/33, MD 15/33, HD 27/41), and were increased in LD and HD females (control 16/31, LD 35/45, MD 13/29, HD 27/35), but was not dose-dependent. The incidence for male mice was within historical controls, but that for females was not. In female mice, the lack of dose-response makes it difficult to determine if this finding is test-article related. These findings might be attributed to direct exposure of the lens to the test article rather than through systemic exposure.

Reference ID: 3313292

- There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis. Increased incidences of histiocytic sarcomas were noted in females dosed with the test article, but the findings were within historical background incidences, and were not statistically significant by trend and pair-wise analysis.
- Non-neoplastic findings were identified in the lungs and bronchi, kidneys, thymus, larynx, tracheal bifurcates, and nasal turbinates. Findings in the nasal turbinates are not clinically relevant. The majority of findings were related to local toxicity from the inhalation route of administration. The larynx showed increased incidence and severity in squamous hyperplasia/metaplasia in the ventral epithelium. The tracheal bifurcates showed a loss of cilia at the point of bifurcation. In the lungs and bronchi, males showed an increase in bronchioloalveolar hyperplasia, with associated peribronchioloar lymphoid aggregates.

  With respect to systemic toxicity, female kidneys showed a test article related increased incidence and severity of cortical tubules with hyaline droplets. In the thymus, females showed an increase in lymphoid hyperplasia.
- Systemic exposure based on $AUC_{(0\text{-}t)}$ generally increased with dose. C_{max} generally increased with dose. T_{max} was reported for TK samples collected at Weeks 4 and 26, and occurred between 1 and 3 hours after dosing.
- GSK573719A was not tumorigenic in mice.

Adequacy of Carcinogenicity Study

- The duration of treatment at 104 weeks was adequate.
- The maximum tolerated dose appears to have been achieved in males based on the need to decrease doses due to increased mortality and body weight loss prior to Week 67. There appears to have been no affect on females, based on mortality, body weight, or feed consumption.

Appropriateness of Test Models

- The ECAC concurred with the protocol for the presented 2-year carcinogenicity study in CD-1 mice with drug administered by inhalation. The CD-1 mouse is a standard model for assessment of carcinogenic potential.

Evaluation of Tumor Findings

- There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis. Increased incidences of histiocytic sarcomas were noted in females dosed with the test article, but the findings were within historical background incidence, and were not statistically significant by trend and pair-wise analysis.

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Methods

Doses:	Target doses: Males: 0 (1% magnesium stearate in lactose), 60, 200, and 600 mcg/kg/day, from week 67 doses of 0, 30, 100, and 300 mcg/kg/day; females: 0, 20, 60, and 200 mcg/kg day
Frequency of dosing:	Daily
Dose volume:	Males: 60 min, from week 67 exposure time of 30 min; females: 60 min
Route of administration:	Inhalation (nose-only)
Formulation/Vehicle:	1% magnesium stearate in lactose
Basis of dose selection:	13-week inhalation toxicology study in mice. MTD was identified as 600 mcg/kg/day for males, and 200 mcg/kg/day for females. (ECAC minutes 6/3/09). Pulmonary deposited doses are 60 mcg/kg/day and 20 mcg/kg/day, respectively, assuming pulmonary deposition of 10%. Effective from Week 67, doses were reduced for males to target doses of 30, 100, and 300 mcg/kg/day, based on a non-dose-related reduction in percentage body weight gain (compared to control), accompanied by a slight reduction in food consumption. (ECAC minutes 2/18/11).
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	75/sex/group main study, 45/sex/group test article group (satellite; TK), 15/sex/group vehicle (satellite; TK)
Age:	22-28 days
Animal housing:	Mice were group housed in polypropylene cages with wood flakes as bedding.
Paradigm for dietary restriction:	None.
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	45/sex/group test article group (satellite), and 15/sex/group vehicle (satellite) for TK.
Deviation from study protocol:	In Week 3 there was a 6 day period of overdosing (4-fold) of the low dose females (animals were given approximately 80 mcg/kg/day, instead of 20 mcg/kg/day). No obvious effects were noted in the animals associated with this overdosing. There was no evidence of tumorigenic response at the higher doses and toxicokinetic evaluation 2 weeks

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after this incident showed no unexpected changes. Therefore this is considered not to have affected the integrity or outcome of the study.

Note: The estimated achieved doses, particle size distribution, and animal numbers are shown in the table below. The initial (weeks 1-66) and reduced doses (weeks 67-104) are shown for males. The target dose was constant for females throughout the study.

The pulmonary deposited dose was calculated as 10% of the estimated achieved dose, and is listed in the tables below as "pulmonary deposited dose."

The particle size distribution ranged from [redacted] (b) (4) (MMAD ± GSD) based on chamber measurements shown below. This is within respirable range.

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Group Number	Week	Duration of Exposure (minutes)	Achieved Chamber Aerosol Concentration (µg/L) [a]	Estimated achieved dose (µg/kg/day)[a]	MMAD (µm)	GSD
Males						
1	1-66	60	0	0	(b) (4)	
	67-104	30	0	0		
2	1-66	60	1.05	58.6		
	67-104	30	1.17	32.2		
3	1-66	60	3.38	188		
	67-104	30	3.68	102		
4	1-66	60	9.50	533		
	67-104	30	10.6	295		
Females						
5	1-104	60	0	0	(b) (4)	
6	1-104	60	0.361	20.8		
7	1-104	60	1.10	63.7		
8	1-104	60	3.45	200		

a : Expressed in terms of GSK573719.
MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation
NA = Not Applicable

Observations and Results

Mortality

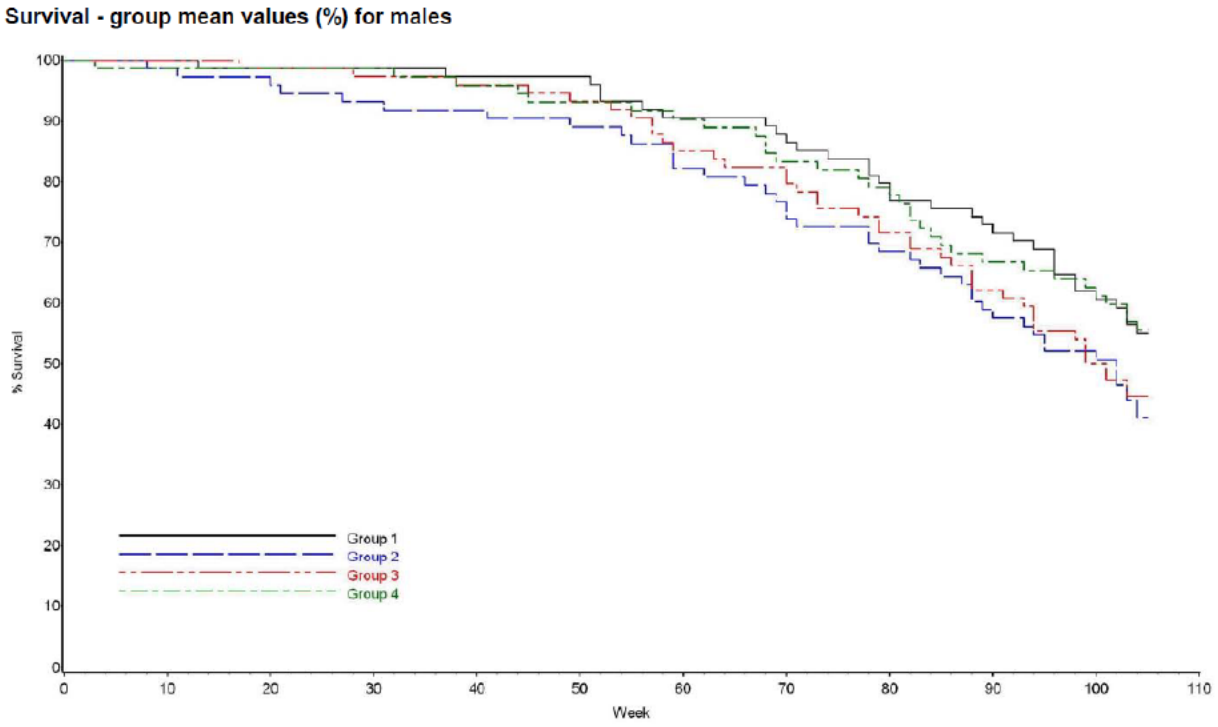
Animals were checked for morbidity/mortality 2 times per day.

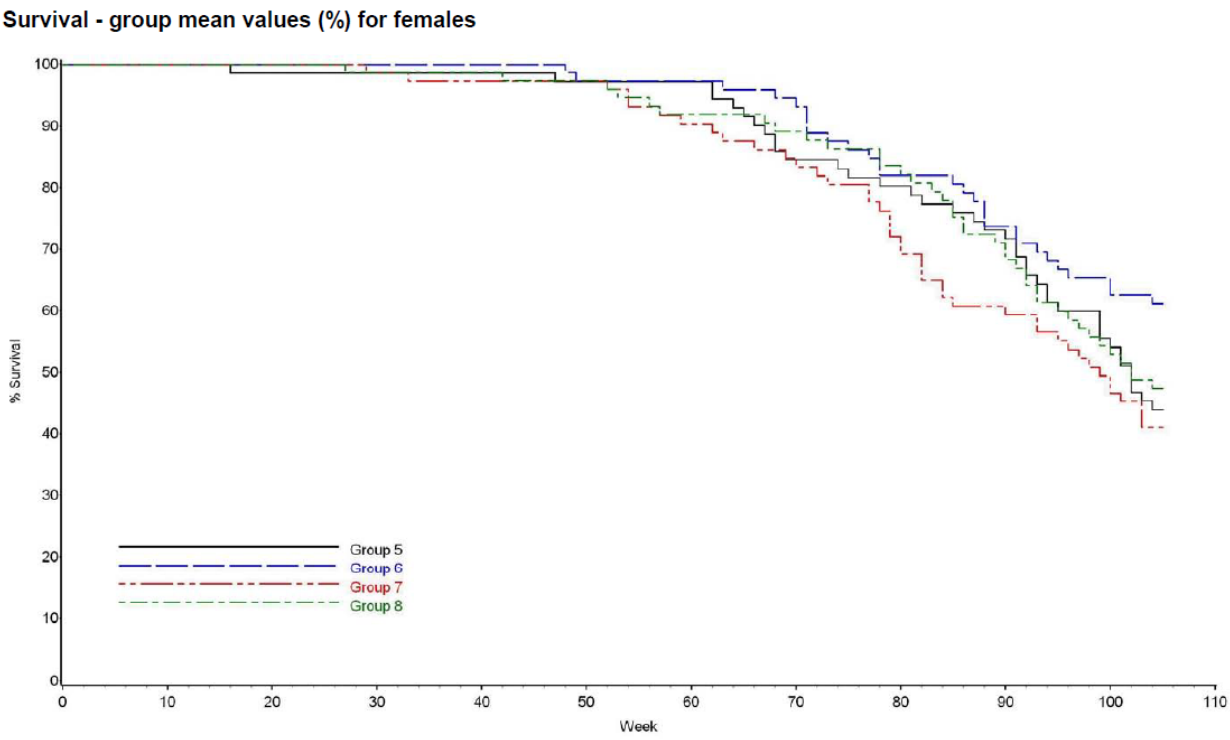
There were no dose-dependent effects on mortality at Week 104; however, treatment with the test article was associated with increased mortality in males by week 66, and the ECAC agreed to the sponsor's dose reduction for males.

Table 1: Mouse carcinogenicity study: Mortality and survival

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	5.86/3.22	18.8/10.2	53.3/29.5	0	2.08	6.37	20
Died/killed moribund								
Week 66 (dose level change for males)	8	17	14	11	11	6	14	8
Week 104	35	45	42	35	45	31	46	41
Survival %								
Week 66 (dose level change for males)	89	77	81	85	85	92	81	89
Week 104	53	40	44	53	40	59	39	45

Figure 1: Mouse carcinogenicity study: Survival of mice during 104-week treatment period





Clinical Signs

Animals were observed for morbidity two times per day. Instances of wet fur as a result of dosing were recorded daily. Detailed observations were recorded daily during Week 1, twice weekly during Weeks 2 to 4, weekly during Weeks 5 to 13, once every two weeks during Weeks 14-52, and once every four weeks thereafter. Palpations were performed weekly, with new masses recorded with respect to mass number, location, date, size and description.

There were no test article related clinical signs. Incidental observations were observed in 1 or 2 out of 75 animals/sex.

Body Weights

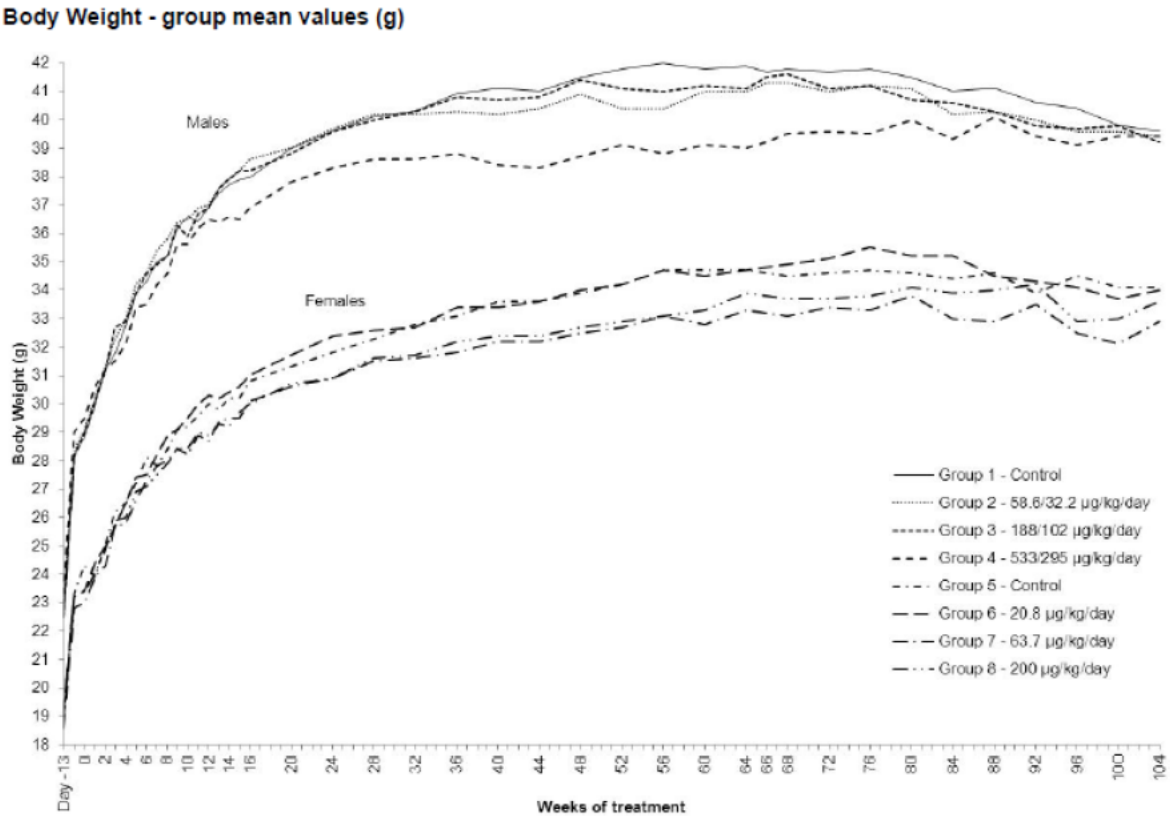
Body weights were measured twice weekly for Weeks -2 and -1, on Day 1 of dosing, weekly for the first 16 weeks, once every 4 weeks from Week 17 to the end of treatment, and on the day of necropsy.

Midway through the study, at Week 52, males dosed with test article showed an overall decrease in absolute body weight, which was not dose-dependent. At Week 52, there was also an overall decrease in body weight gain compared to control, which was not dose-dependent. A reduction of doses in males at week 67 resulted in a reversal of the decrease in absolute body weight; at Week 104, there were no notable differences in absolute body weight in dosed animals compared to control in either sex. There was partial reversal of the decrease in body weight gain, with males showing a slight dose-dependent decrease compared to control (LD 95%, MD 94%, HD 92%).

Table 2: Mouse carcinogenicity study: Body weight analyses

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	5.86/ 3.22	18.8/ 10.2	53.3/ 29.5	0	2.08	6.37	20
Week 0	28.8	29.1	29	29.5	24.3	23.4	23	23.4
Week 24	39.6	39.7	39.6	38.3	31.8	32.4	30.9	30.9
Absolute BW, % Control	100%	100%	100%	97%	100%	102%	97%	97%
Δ (g)	10.8	10.6	10.6	8.8	7.5	9	7.9	7.5
BW gain, % initial	38%	36%	37%	30%	31%	38%	34%	32%
BW gain, % control	100%	98%	98%	81%	100%	120%	105%	100%
Week 52	42	40.4	41.1	38.8	34.2	34.2	32.7	32.9
Absolute BW, % Control	100%	96%	98%	92%	100%	100%	96%	96%
Δ (g)	13.2	11.3	12.1	9.3	9.9	10.8	9.7	9.5
BW gain, % initial	46%	39%	42%	32%	41%	46%	42%	41%
BW gain, % control	100%	86%	92%	70%	100%	109%	98%	96%
Week 76	41.8	41.2	41.2	39.5	34.7	35.5	33.3	33.8
Absolute BW, % Control	100%	99%	99%	94%	100%	102%	96%	97%
Δ (g)	13	12.1	12.2	10	10.4	12.1	10.3	10.4
BW gain, % initial	45%	42%	42%	34%	43%	52%	45%	44%
BW gain, % control	100%	93%	94%	77%	100%	116%	99%	100%
Week 104	39.6	39.4	39.2	39.4	34.1	34	32.9	33.6
Absolute BW, % Control	100%	99%	99%	99%	100%	100%	96%	99%
Δ (g)	10.8	10.3	10.2	9.9	9.8	10.6	9.9	10.2
BW gain, % initial	38%	35%	35%	34%	40%	45%	43%	44%
BW gain, % control	100%	95%	94%	92%	100%	108%	101%	104%

Figure 2: Mouse carcinogenicity study: Body weight



Feed Consumption

Mouse No. 1 Maintenance Diet (supplied by Special Diets Services Ltd., Witham, Essex, England) and water were available ad libitum, except during noted procedures. Feed consumption was measured weekly during the week before treatment, for the first 16 weeks, and every 4 weeks thereafter until the end of the study.

There was a decrease in absolute amount of feed consumed from the beginning to the end of the study (Week 1-104) in males at the HD. The percent change in feed consumption in HD males was -7%, compared to controls. This decrease in feed consumption correlated with decreased body weight gain in HD males.

Table 3: Mouse carcinogenicity study: Percent change in feed consumption

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	5.86/3.22	18.8/10.2	53.3/29.5	0	2.08	6.37	20
Absolute	4067	4030	4083	3793	3679	3721	3635	3608
Percent change		-1%	0%	-7%		1%	-1%	-2%

Ophthalmology

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Main study mice were examined by indirect ophthalmoscopy and a slit lamp during Weeks 13, 52, 78, and 103. Mydriatic solution was administered, and structures were examined including the adnexa, conjunctiva, cornea and sclera, anterior chamber and iris (pupil dilated) lens and vitreous and the ocular fundus.

Incidences of bilateral opacity of the posterior lens were increased in a dose-dependent manner in males (control 9/41, LD 12/33, MD 15/33, HD 27/41), and were associated with dosing in females, although not dose-dependent (control 16/31, LD 35/45, MD 13/29, HD 27/35). The sponsor provided historical control data from two studies initiated in September 2007 and August 2009, utilizing the same strain of mouse and the same recording equipment. HD males that showed an incidence of 27/41 (66%) were within the historical control range of 26% and 70%. Females, however, at the LD (35/45; 78%) and HD (27/35; 77%) were not within the historical control range of 37% and 70%. In female mice, the lack of dose-response makes it difficult to determine if this finding is test-article related. These findings might be attributed to direct exposure of the lens to the test article rather than through systemic exposure.

Table 4: Mouse carcinogenicity study: Ophthalmic lesions

				mcg/kg/d (pulmonary deposited dose)							
				Male				Female			
Time point	Structure	Finding	Involvement	0	5.86/ 3.22	18.8/ 10.2	53.3/ 29.5	0	2.08	6.37	20
Predose	N examined			75	75	75	75	75	75	75	75
	Lens	Opacity	posterior bilateral	1	0	0	0	0	0	2	2
Week 103	N examined			41	33	33	41	31	45	29	35
	Lens	Opacity	posterior bilateral	9	12	15	27	16	35	13	27
			-Faint	1	0	4	1	4	1	3	0
			-Slight	6	12	11	26	11	31	10	25
			-Moderate	2	0	0	0	1	3	0	2

Table 5: Historical control data for ophthalmic lesion

	Study Identity	
	A-control	B-control
Route of administration	Inhalation	Oral gavage
Start date	Sep-07	Aug-09
Finding (Week 100-103)		
Males:		
Lens, Bilateral Posterior Subcapsular opacity (all severities)		
Number with finding	14	9
Number examined	20	34
% with finding	70	26
Females		
Lens, Bilateral Posterior Subcapsular opacity (all severities)		
Number with finding	14	11
Number examined	20	30
% with finding	70	37

From studies comparable with strain of mouse and recording equipment

(Sponsor's table)

Hematology

Blood was collected from animals under light general anesthesia induced with isoflurane. Blood was obtained from the retro-orbital sinus at scheduled termination. A blood film was collected for possible analysis, but analysis was not performed and is not required for a carcinogenesis study.

Clinical Chemistry

Blood was collected from animals under light general anesthesia induced with isoflurane. Blood was obtained from the retro-orbital sinus at scheduled termination. Blood was not analyzed for clinical chemistry markers; this is not required for a carcinogenesis study.

Gross Pathology

Main study animals were sacrificed after 104 weeks of treatment by intraperitoneal injection of pentobarbitone and exsanguinated.

There was a dose-dependent distension of the preputial glands. There were no microscopic findings that correlated with this gross finding.

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Table 6: Mouse carcinogenicity study: Gross finding

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	5.86/3.22	18.8/10.2	53.3/29.5	0	2.08	6.37	20
Organ/N examined	75	75	75	75	75	75	75	75
Preputial glands Distended	3	5	4	11	-	-	-	-

Histopathology

Tissues were examined in main study animals. A full battery of tissues was examined, and tissues were preserved in 10% neutral buffered formalin except as noted. The testes and epididymides were fixed in Bouin's solution, and transferred to 70% industrial methylated spirit, trimmed and processed. Eyes were fixed in Davidson's solution before trimming and processing.

Peer Review: Yes

Neoplastic:

Neoplasias were analyzed by organ, and tumors that shared common cell origins in the same organ were combined for analysis. Specific tumors that arise from systemically distributed cells were identified by incidence per animal. Specifically, hemangioma and hemangiosarcoma, lymphomas, leukemias, mesotheliomas, and histiocytic sarcomas were analyzed by incidence per animal. Brix *et al.* state, "Such systemic neoplasms that might be considered in this fashion would include all blood cell neoplasms, including histiocytic sarcoma, as well as neoplasms in which the cell type is present in many different organs, such as hemangiosarcomas and malignant mesotheliomas[1].

Histiocytic sarcomas, which are of hematopoietic origin, showed a roughly dose-dependent increase in females, with an incidence of 5/75 (6.7%) animals at both the MD and HD. This finding was not significant by either trend or pair-wise statistical analysis. The sponsor submitted historical data in control CD-1 mice from thirteen 104 week studies conducted by [redacted] (b) (4) initiated from April 2007 to June 2008, with different routes of administration (inhalation, oral gavage, subcutaneous injection, dermal and an additional route abbreviated as "dt"). For females, studies showed 1.7 to 11.7% of females were diagnosed with histiocytic sarcomas in each study, for a total of 48/767 females (6.26%) diagnosed overall. For males, studies showed 0.0 to 5.0% of males were diagnosed with histiocytic sarcomas in each study, for a total of 9/767 males (1.18%) diagnosed overall. Therefore, the incidence of histiocytic sarcomas in this study is consistent with the reported background incidence of histiocytic sarcomas

[1] Brix, A.E. J.F. Hardisty, and E.E. McConnell. Combining neoplasms for evaluation of rodent carcinogenesis studies. In: Hsu and Stedeford (eds.) Cancer Risk Assessment, John Wiley and Sons, Inc., 2010: 699-715.

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In addition, data from Crl:CD1 mice from [redacted] (b) (4) (March 2010) was examined. Data was complied from 9 studies of 78-104 weeks duration in 5 different facilities (in the US and Europe) using CD1 mice sourced from [redacted] (b) (4) The studies showed that 1.67 to 11.67% of animals were diagnosed with histiocytic sarcomas. Histiocytic sarcomas were categorized as a "whole body" tumor. Upon close inspection of these percentages in the relevant 9 studies, 8 out of 9 studies had an incidence of 1-3 out of 50-70 animals that developed histiocytic sarcomas. Only 1 out of the 9 studies showed a notably higher incidence of 7 out of 60 animals that developed histiocytic sarcomas[2].

Table 7: Mouse carcinogenicity study: Neoplastic finding

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	5.86/3.22	18.8/10.2	53.3/29.5	0	2.08	6.37	20
Whole body/N examined	75	75	75	75	75	75	75	75
Histiocytic sarcoma	1	0	1	0	1	1	5	5

Figure 3: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons in Mice (Statistician's Review)

Sex	Organ Name	Tumor Name	Cont N=75	Low N=75	Med N=75	High N=75	P-Value Dose Resp	C vs. L	C vs. M	C vs. H
Female	H-POIETIC TUMOU	HISTIOCYTIC SARCOMA	1	1	5	5	0.0478	0.2708	0.0896	0.1206
	LUNGS + BRONCHI	BRONCHIOLOALVEOLAR AD	0	2	5	3	0.1729	0.2708	0.0269*	0.1356
	WHOLE_BODY	HISTIOCYTIC_SARCOMA	1	1	5	5	0.0478	0.2708	0.0896	0.1206

Non Neoplastic:

Test article related non-neoplastic findings were identified in the lungs and bronchi, kidneys, thymus, larynx, tracheal bifurcates, and nasal turbinates. Findings in the nasal turbinates are shown in the table below, but are not clinically relevant as mice were dosed by nose-only inhalation, and this is not the route of administration in humans.

The majority of findings were related to local toxicity from the inhalation route of administration. The larynx of both sexes showed a test article related increased incidence and severity in squamous hyperplasia/metaplasia in the ventral epithelium.

2 [redacted] (b) (4)

The tracheal bifurcates of both sexes showed a dose dependent loss of cilia at the point of bifurcation. In the lungs and bronchi, males showed an increase in bronchioloalveolar hyperplasia, with associated peribronchioloar lymphoid aggregates.

With respect to systemic toxicity, female kidneys showed a test article related increased incidence and severity of cortical tubules with hyaline droplets. In the thymus, females showed an increase in lymphoid hyperplasia.

Table 8: Mouse carcinogenicity study: Non-neoplastic findings

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
Organ/N examined	0	5.86/3.22	18.8/10.2	53.3/29.5	0	2.08	6.37	20
Lungs + Bronchi	75	75	75	75	75	75	75	75
Bronchioloalveolar Hyperplasia								
Minimal:	5	5	1	9	4	4	3	2
Slight:	1	1	1	2	1	0	0	2
Moderate:	0	1	0	0	0	0	1	0
Total:	6	7	2	11	5	4	4	4
Peribronchiolar Lymphoid Aggregates								
Minimal:	0	4	2	2	3	2	4	2
Slight:	1	1	1	6	4	1	0	1
Total:	1	5	3	8	7	3	4	3
Kidneys	75	75	75	75	75	75	75	75
Cortical Tubules with Hyaline Droplets								
Minimal:	0	0	0	0	0	0	4	0
Slight:	0	0	0	0	0	0	1	0
Moderate:	0	0	0	0	1	4	3	5
Marked:	0	0	0	0	0	0	2	2
Total:	0	0	0	0	1	4	10	7
Thymus	71	63	64	65	70	67	69	71
Lymphoid Hyperplasia								
Minimal:	3	1	0	1	2	2	4	5
Slight:	1	1	1	0	6	2	5	7
Moderate:	0	0	0	0	1	6	3	3
Marked:	0	0	0	0	0	2	0	1
Total:	4	2	1	1	9	12	12	16
Larynx	73	75	75	75	75	74	75	75
Ventral Epithelium - Squamous Hyperplasia/Metaplasia								
Minimal:	0	0	4	15	0	2	15	25
Slight:	0	0	0	4	0	0	1	2
Total:	0	0	4	19	0	2	16	27
Tracheal Bifurc.	73	73	72	72	73	75	75	75
Point of Bifurcation-Loss of Cilia								
Minimal:	5	12	13	24	6	13	10	13

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Slight:	0	0	0	0	0	1	0	0
Total:	5	12	13	24	6	14	10	13
Nasal turbinates	75	75	75	75	75	75	75	75
Olfactory Epithelium - Atrophy/Disorganisation								
Minimal:	2	7	7	22	8	13	6	11
Slight:	0	1	0	1	1	0	1	0
Moderate:	0	0	0	0	0	0	0	1
Total:	2	8	7	23	9	13	7	12
Olfactory Epithelium - Eosinophilic Inclusions								
Minimal:	23	16	13	24	20	24	27	22
Slight:	0	1	2	9	3	7	5	15
Moderate:	0	0	0	1	1	0	3	7
Total:	23	17	15	34	24	31	35	44
Respiratory Epithelium - Eosinophilic Inclusions								
Minimal:	10	7	9	18	15	27	22	22
Slight:	2	1	2	3	3	3	6	0
Moderate:	0	0	0	0	0	1	0	1
Total:	12	8	11	21	18	31	28	23
Subepithelial Glands - Dilation								
Minimal:	21	23	19	32	20	25	16	20
Slight:	5	5	5	4	3	10	3	10
Moderate:	1	0	0	1	0	0	0	0
Total:	27	28	24	37	23	35	19	30
Subepithelial Glands - Eosinophilic Inclusions								
Minimal:	12	9	13	19	13	13	12	14
Slight:	1	3	1	4	4	10	2	14
Moderate:	1	0	0	2	0	1	1	1
Marked:	0	1	0	1	0	0	0	0
Total:	14	13	14	26	17	24	15	29
Subepithelial Glands - Inflammation								
Minimal:	15	18	17	22	14	16	13	16
Slight:	0	0	0	1	0	0	0	1
Total:	15	18	17	23	14	16	13	17
Subepithelial Glands - Inflammation								
Minimal:	15	18	17	22	14	16	13	16
Slight:	0	0	0	1	0	0	0	1
Total:	15	18	17	23	14	16	13	17

Toxicokinetics

Blood samples were collected at Week 4 and Week 26 from 3 animals/sex at each time point (0.5, 1, 2, 4, 8, 23.5 hrs after dosing) for test article treated groups, and from 3 animals/sex at a select time point (4 hrs after dosing) for animals receiving vehicle. At

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Week 76 (Week 4 after the commencement of reduced doses in males only) blood was collected from 3 males/group at each time point (0.5, 1, 2, 4, 8, 23.5 hrs after dosing) for animals receiving the test article, and from 3 vehicle treated males at a select time point (4 hrs after dosing). Blood samples (~ 0.3 mL) were collected by cardiac puncture, collected into tubes containing EDTA, and plasma was isolated after centrifugation. Samples were analyzed for GSK573719 using a method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) was 0.1 ng/mL, with a higher limit of quantification (HLQ) of 100 ng/mL. After sampling, TK animals were sacrificed by intraperitoneal injection of pentobarbitone sodium.

Test article was detected in one control animal at Week 26 (1 hr time point) at a level of 0.132 ng/mL. Based on the low level of this positive result, and the lack of any test article in other control animals during the study, this does not affect the validity of the study.

In dosed groups, systemic exposure based on $AUC_{(0-t)}$ generally increased with dose. Exceptions to increasing exposure with increasing dose were noted in samples collected during Week 4, in which LD and MD males had fluctuating levels of exposure. C_{max} generally increased with dose, with an exception noted again at Week 4, which correlated with fluctuating $AUC_{(0-t)}$in LD and MD males. T_{max} was reported for TK samples collected at Weeks 4 and 26, but not for samples collected at Week 76 (4 weeks after reduced dosing in males.) T_{max} occurred between 1 and 3 hours post dose.

Table 9: Mouse carcinogenicity study: TK parameters

Dosing Period Weeks 4, 26:

		Male			Female		
		Estimated Achieved Dose					
Parameter	Week	58.6 μg/kg/day	188 μg/kg/day	533 μg/kg/day	20.8 μg/kg/day	63.7 μg/kg/day	200 μg/kg/day
$AUC_{(0-t)}$ (ng.h/mL)	4	4.15	1.50	16.0	1.01	7.10	9.65
	26	NC	1.78	8.47	NC	1.51	6.87
C_{max} (ng/mL)	4	2.49	1.27	4.14	0.429	1.59	3.16
	26	0.430	0.387	2.75	0.244	0.935	3.64
T_{max} (h)	4	1.5	1.0	1.0	1.0	3.0	1.5
	26	1.5	2.0	1.5	1.0	1.0	1.0

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Dosing Period Week 76:

		Male		
		Estimated Achieved Dose		
Parameter	Week	32.2 µg/kg/day	102 µg/kg/day	295 µg/kg/day
$AUC_{(0-t)}$ (ng.h/mL)	76 (4 weeks after reduced dose)	1.53	3.28	8.21
C_{max} (ng/mL)	76 (4 weeks after reduced dose)	0.611	0.781	4.03

Dosing Solution Analysis

Gravimetric analysis was conducted daily in samples collected from each group throughout the study.

Chemical analysis was conducted daily during Week 1 to 12, three times weekly for Weeks 13-14, and twice weekly for the remainder of the study. Quartz filters were analyzed for GSK573719 using ultra performance liquid chromatography (UPLC) within a quantification range of 0.1 to 2 mcg/mL.

Particle size distribution was conducted on a measured volume of air that was drawn at a rate of 3 liters/minute from inhalation exposure chambers through a cascade impactor (Marple Model 296 Personal Cascade Impactor, Andersen Instruments, Smyrna, Georgia, USA) attached to an exposure chamber. Samples were collected in Week 1, then every 3 months. Samples were analyzed for GSK573719

The estimated achieved doses are within 15% of the target doses. The target doses for males were 0, 60, 200, and 600 mcg/kg/day initially, and 0, 30, 100, and 300 mcg/kg/day from Week 67. For females, the target doses were 0, 20, 60, and 200 mcg/kg day. The estimated achieved doses in males were 58.6/32.2, 188/102, and 533/295 mcg/kg/day for males in the LD, MD, and HD groups, respectively, with initial and Week 67-104 doses shown for each group. The estimated achieved doses in females were 20.8, 63.7, and 200 mcg/kg/day for females in the LD, MD, and HD groups, respectively.

Table 10: Mouse carcinogenicity study: Estimated and Target Inhaled Doses

Group	Week	Duration of exposure (minutes)	Estimated achieved dose (mcg/kg/d)	Target dose (mcg/kg/d)	Percent of target dose
Males					
Control	1-66	60	0	0	-
	67-104	30	0	0	-

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LD	1-66	60	58.6	60	98%
	67-104	30	32.2	30	107%
MD	1-66	60	188	200	94%
	67-104	30	102	100	102%
HD	1-66	60	533	600	89%
	67-104	30	295	300	98%
Females					
Control	1-104	60	0	0	-
LD	1-104	60	20.8	20	104%
MD	1-104	60	63.7	60	106%
HD	1-104	60	200	200	100%

2- year Rat Study

Study title: GSK573719A: Carcinogenicity Study by Inhalation Administration to Crl:CD(SD) Rats for 104 weeks

Study no.: BVR1357/R28862

Study report location: SD-1 (EDR)

Conducting laboratory and location: (b) (4)

Date of study initiation: 16 September 2009

GLP compliance: Yes.

QA statement: Yes

Drug, lot #, and % purity: GSK573719A (bromide salt of GSK573719), batch/lot R360972/ 081168132, 99.8%

CAC concurrence: Yes.

Key Study Findings

- In the 2-year carcinogenicity study in rats, animals received umeclidinium by inhalation. Rats initially received doses of 0 (vehicle), 30, 100, and 300 mcg/kg/day, based on MTD identified in 13-week rat studies. The sponsor noted decreases in body weight gains and proposed to reduce doses. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD. Thus, effective from Week 73,

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doses were reduced to target doses of 15, 50, and 150 mcg/kg/day. The ECAC agreed with the sponsor's proposed dose reduction.

- There were no negative dose-dependent effects on mortality by Week 104
- There were no treatment-related neoplastic findings based on the lack of statistical significance. Neoplastic findings observed with increased incidences in test article-treated groups were lymphomas (whole body), and basal cell tumors (skin), but these did not achieve statistical significance for trend and pair-wise comparison.
- Non-neoplastic findings were identified in the nasal turbinates, larynx, tracheal bifurcates, lungs and bronchi, Harderian glands, liver, spleen, stomach, adrenals, lachrymal glands, and uterine cervix. Findings in the nasal turbinates are not clinically relevant as rats are obligate nose breathers, and humans will be dosed via oral inhalation.
- Bilateral opacity on the posterior position of the lens was increased in a dose-dependent manner in males (control 2/39, LD 5/46, MD 5/43, HD 8/48), with most lesions slight in severity. The sponsor provided historical control data showing that the incidence of 8/48 is within the historical control range, and therefore it is not considered to be test article related.
- At the higher doses administered with 60 minutes of exposure, systemic exposure based on $AUC_{(0-t)}$ was generally dose proportional. There were no notable differences in exposure between the sexes. The maximum plasma exposure was observed between 1-2 hrs post dose. At the lower doses starting at Week 76, with 30 minutes of exposure, systemic exposure based on AUC was approximately dose proportional. There were no notable differences in exposure between the sexes.
- GSK573719A was not tumorigenic in SD rats.

Adequacy of Carcinogenicity Study

- The duration of treatment at 104 week was adequate.
- The initial top dose was based on MTD identified in 13-week rat studies. The sponsor requested a reduction in dose based on decreases in body weight gain. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD.

Appropriateness of Test Models

- The ECAC concurred with the protocol for the presented 2-year carcinogenicity study in SD rats with drug administered by inhalation.
- The SD rat is a standard model for assessment of carcinogenic potential.

Evaluation of Tumor Findings

- There were no treatment-related neoplastic findings based on the lack of statistical significance. Neoplastic findings that were observed with increased incidences in test article-treated groups were lymphomas (whole body), and basal cell tumors (skin), but these did not achieve statistical significance for trend

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or pair-wise comparison. Basal cell tumors were also within historical control data.

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Methods

Doses:	0 (vehicle), 30.1, 101 or 276 mcg/kg/day once daily for Weeks 1 to 72, and then 0 (vehicle), 14.7, 45.0 or 137 mcg/kg/day once daily from Week 73 onwards.
Frequency of dosing:	Daily
Dose volume:	60 minutes/day for Weeks 1-72, 30 minutes/day for Weeks 73-104
Route of administration:	Inhalation (nose-only)
Formulation/Vehicle:	1% (w/w) magnesium stearate in lactose
Basis of dose selection:	13-week inhalation toxicology study in rats. MTD was identified as 300 mcg/kg/day (ECAC minutes 6/3/09). The recommended doses were 30, 100, and 300 mcg/kg/day. The top dose equals a pulmonary deposited dose of 30 mcg/kg/day. Effective from Week 73, doses were reduced to target doses of 15, 50, and 150 mcg/kg/day with ECAC concurrence (minutes 2/18/11). Based on data up to Week 70, the sponsor noted decreases in body weight gains and proposed lower doses. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD.
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	65/sex/group. 9/sex/group (satellite;TK)
Age:	6 to 7 weeks
Animal housing:	Polypropylene cages with Lignocel 3/4 wood flakes as bedding. For environmental enrichment the rats were given Aspen chew blocks (Datasand, Manchester, England) and a polycarbonate shelter.
Paradigm for dietary restriction:	*Ad libitum* except during designated procedures (i.e. dosing)
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	9/sex/group (TK)
Deviation from study protocol:	There were no deviations that affected the validity of the study. The following deviations are noted in this review:

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1) Additional animals for TK sampling were added during Week 73 to allow for sampling following 4 weeks of dosing at the reduced study doses.
2) In Week 34, the dosing of animals was prematurely terminated because of air compression issues in the HD group resulting in a shorter duration of exposure (and hence under dosing) of 43 minutes versus 60 minutes.
3) In Week 41, equipment failure for exposure generation occurred. On Day 2 of Week 41, LD/MD/HD groups had exposures of 46, 28 and 8 minutes respectively and on Day 3 of Week 41 LD/MD/HD groups had exposures of 43, 31 and 20 minutes respectively. No dosing was undertaken for any group on Days 4 and 5 of Week 41.
4) In Week 63, problems with the compressor supplying air for exposure generation resulted in some of the animals not receiving the correct duration of exposure. All groups had exposures in Week 63 on Day 3 of 60, 38, 17 and 2 minutes, respectively. On Day 4 of Week 63, the control group had an exposure of 30 minutes.
5) The dose levels were changed effective from Week 73 for males. Due to the staggered start, the change was effective for females from the last day of Week 72 (Week 72). For reporting purposes, males and females are identified as having the higher dose for Weeks 1 to 72 and the lower dose from Week 73.

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Weeks 1 to 72

Study Design						
Group Number	Target Dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day)	Target Chamber Aerosol Concentration (µg/L)	Achieved Chamber Aerosol Concentration (µg/L)[b]	Duration of Exposure (mins)	Number/Sex[c]
1	0	0	0	0	60	Main: 65M & 65F Satellites: 9M & 9F
2	30	30.1	0.77	0.846	60	Main: 65M & 65F Satellites: 9M & 9F
3	100	101	2.6	2.83	60	Main: 65M & 65F Satellites: 9M & 9F
4	300	276	7.7	7.70	60	Main: 65M & 65F Satellites: 9M & 9F

a. Expressed in terms of GSK573719
b. As determined by chemical analysis
c. Number of main study animals at start of study. Number of satellites for toxicokinetic assessment in Weeks 4 and 26

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC) / BW

D Dose (µg/kg)
RMV(L) Respiratory Minute Volume $0.499 \times BW(kg)^{0.809}$ [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol GSK573719 concentration (µg/L)
BW Group average body weight for study (kg)

Weeks 73 to 104

Study Design						
Group Number	Target Dose (µg/kg/day)[a]	Estimated Achieved Dose (µg/kg/day)	Target Chamber Aerosol Concentration (µg/L)	Achieved Chamber Aerosol Concentration (µg/L)[b]	Duration of Exposure (mins)	Number/Sex[c]
1	0	0	0	0	30	Main: 65M & 65F Satellites: 9M & 9F
2	30	14.7	0.77	0.857	30	Main: 65M & 65F Satellites: 9M & 9F
3	100	45.0	2.6	2.62	30	Main: 65M & 65F Satellites: 9M & 9F
4	300	137	7.7	7.90	30	Main: 65M & 65F Satellites: 9M & 9F

a. Expressed in terms of GSK573719
b. As determined by chemical analysis
c. Number of main study animals at start of study. Number of satellites for toxicokinetic assessment following 4 weeks exposure at lower doses

Estimated inhaled dose calculated as follows and assumes 100% deposition in the respiratory tract:
D = (RMVxTxC) / BW

D Dose (µg/kg)
RMV(L) Respiratory Minute Volume $0.499 \times BW(kg)^{0.809}$ [Bide, 2000]
T Duration of exposure/day (minutes)
C Aerosol GSK573719 concentration (µg/L)
BW Group average body weight for study (kg)

Note: The estimated achieved doses, particle size distribution, and animal numbers are shown in the table below. The initial (weeks 1-72) and reduced doses (weeks 73-104) are both shown.

The pulmonary deposited dose was calculated as 10% of the estimated achieved dose, and is listed in the tables below as "pulmonary deposited dose."

The particle size distribution ranged from [redacted] (b) (4) (MMAD ± GSD) based on chamber measurements shown below. This is within respirable range.

Group	Achieved aerosol concentration (µg/L)	Estimated achieved dose (µg//kg/day)		Duration of exposure (Minutes)	MMAD (µg/m)	GSD
Weeks 1-72						
2	0.846	Males	28.8	60	(b) (4)	
		Females	32.0			
		Average	30.1			
3	2.83	Males	96.6	60		
		Females	106			
		Average	101			
4	7.69	Males	265	60		
		Females	291			
		Average	276			
Weeks 73-104						
2	0.857	Males	14.1	30	(b) (4)	
		Females	15.5			
		Average	14.7			
3	2.62	Males	43.4	30		
		Females	47.1			
		Average	45.0			
4	7.90	Males	132	30		
		Females	144			
		Average	137			

MMAD Mass Median Aerodynamic Diameter.
GSD Geometric Standard Deviation

Observations and Results

Mortality

A viability check was performed near the start and end of each working day. A necropsy was performed on those animals that died or were killed during the treatment period (including toxicokinetic animals). Any animal that died or was sacrificed up to the end of study Week 2, or was rejected during predose ophthalmic examination, was replaced with a spare animal of the same sex.

There were no test-article related effects on mortality.

Table 11: Rat carcinogenicity study: Mortality and survival

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	2.01/1.47	10.1/4.5	27.6/13.7	0	2.01/1.47	10.1/4.5	27.6/13.7
Died/killed moribund								
Week 105	38	42	41	47	40	34	30	25
Survival %								
Week 105	58	65	63	72	38	48	54	62

Clinical Signs

Detailed observations in relation to dose administration were recorded daily during the first week of treatment, a minimum of twice weekly during Weeks 2 to 4, weekly during Weeks 5 to 13, once every two weeks during Weeks 14 to 52 and once every four weeks thereafter during the treatment period at predosing, after dosing upon return to the cage, and as late as possible during the work day. Weekly palpations were performed with physical examination. New masses were recorded at detection in respect to mass number and location, date, size and description.

There were no consistent test article related clinical signs. There were dose-dependent increase in males for hair loss and encrustation on the head, but these were not observed in females. The sponsor stated that these were related to restraint during dosing, which is reasonable.

Table 12: Rat carcinogenicity study: Clinical signs

		mcg/kg/d (pulmonary deposited dose)							
		Male				Female			
	Observation	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Category	N	65	65	65	65	65	65	65	65
Coat	Hair loss, Head	20	21	25	31	40	38	36	30
Skin	Encrustation, Head	2	6	8	8	14	8	3	5

Body Weights

The animals were weighed twice during Week -1, on the day that treatment commenced, weekly for the first 16 weeks and thereafter once every 4 weeks throughout the treatment period and on the day of necropsy. Toxicokinetic animals were also weighed on the day of toxicokinetic sampling.

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There was a dose-dependent trend for decreased body weight throughout the study, which was most pronounced at Week 104 in females versus males (males LD 97%, MD 91%, HD 88%; females LD 95%, MD 96%, HD 87%).

Table 13: Rat carcinogenicity study: Body weight analyses

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	2.01/1.47	10.1/4.5	27.6/13.7	0	2.01/1.47	10.1/4.5	27.6/13.7
Week 0	236	230	233	225	177	178	179	176
Week 24	521	510	506	487	303	289	298	288
Absolute BW, % Control	100%	98%	97%	93%	100%	95%	98%	95%
Δ (g)	285	280	273	262	126	111	119	112
BW gain, % initial	121%	122%	117%	116%	71%	62%	66%	64%
BW gain, % control	100%	98%	96%	92%	100%	88%	94%	89%
Week 52	602	580	565	546	359	323	336	321
Absolute BW, % Control	100%	96%	94%	91%	100%	90%	94%	89%
Δ (g)	366	350	332	321	182	145	157	145
BW gain, % initial	155%	152%	142%	143%	103%	81%	88%	82%
BW gain, % control	100%	96%	91%	88%	100%	80%	86%	80%
Week 76	640	612	591	570	406	362	372	357
Absolute BW, % Control	100%	96%	92%	89%	100%	89%	92%	88%
Δ (g)	404	382	358	345	229	184	193	181
BW gain, % initial	171%	166%	154%	153%	129%	103%	108%	103%
BW gain, % control	100%	95%	89%	85%	100%	80%	84%	79%
Week 104	635	614	580	559	412	391	394	359
Absolute BW, % Control	100%	97%	91%	88%	100%	95%	96%	87%
Δ (g)	399	384	347	334	235	213	215	183
BW gain, % initial	169%	167%	149%	148%	133%	120%	120%	104%
BW gain, % control	100%	96%	87%	84%	100%	91%	91%	78%

Feed Consumption

Feed consumption was recorded weekly during the week before treatment commenced, weekly for the first 16 weeks and thereafter once every 4 weeks throughout the treatment period.

There was a slight dose-dependent decrease in feed consumption in females that correlated with a decrease in body weight.

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Table 14: Rat carcinogenicity study: Percent change in feed consumption

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Absolute	6400	6415	6329	6199	4846	4622	4659	4530
Percent change		0%	-1%	-3%		-5%	-4%	-7%

Ophthalmology

The eyes of all main study rats were examined by indirect ophthalmoscopy and a slit lamp prior to initiation of dosing (including spares) and during Weeks 14, 51 (Week 50 for Group 1F only), 78 (Week 77 for Group 1F only), Week 101 (females only) and Week 103 (males only). Prior to examination, a mydriatic (0.5% tropicamide ophthalmic solution (Mydriacyl, Alcon Laboratories Ltd.)) was instilled into each eye. The structures examined included the adnexa, conjunctiva, cornea and sclera, anterior chamber and iris (pupil dilated) lens and vitreous and the ocular fundus.

Incidences of bilateral opacity on the posterior position of the lens were increased in a dose-dependent manner in males (control 2/39, LD 5/46, MD 5/43, HD 8/48). In response to an Information Request sent on March 15, 2013, the sponsor provided a summary table of severities for the bilateral opacity (see below), showing that most lesions were slight in severity. The sponsor also provided historical control data from at Week 98 or Week 103 from studies in the same laboratory using the same strain and supplier [redacted] (b) (4) showing that the incidence of 8/48 is within the historical control range. Based on the incidence being within historical control range, this was determined to not be test article related. There was no corresponding finding for female test article-treated groups.

Table 15: Rat carcinogenicity study: Ophthalmic finding

				mcg/kg/d (pulmonary deposited dose)							
				Male				Female			
Time point	Structure	Finding	Involvement	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Predose	N examined			65	65	65	65	65	65	65	65
	Lens	Opacity posterior	bilateral	0	0	0	0	0	0	1	0
Week 103	N examined			39	46	43	48#	26#	34	41	43
	Lens	Opacity posterior	bilateral	2	5	5	8	1	0	1	1

Table 16: Severities of bilateral opacity and historical control data, provided by sponsor in response to IR (SD-12):

Group/Sex Dose (mcg/kg/day)	1M 0	2M 30.1/ 14.7	3M 101/ 45	4M 276/ 137	1F 0	2F 30.1/ 14.7	3F 101/ 45	4F 276/ 137
No. Animals Examined	39	46	43	48	26	34	41	43
No. Animals with Bilateral Posterior Lens Opacity								
Faint		1		1	1			
Slight	2	3	5	7			1	1
Moderate		1						
Total No. Animals (Percentage Animals Affected)	2 (5.1)	5 (10.9)	5 (11.6)	8 (16.7)	1 (3.8)	0 (0)	1 (2.4)	1 (2.3)

Historical control data from Sponsor:

Study ID	Start	Ophthalmoscopy Method	Number Examined	Number (Percent) Affected in Category			
				Faint	Slight	Moderate	Total
1	2005	I/S	40		2 (5)		2 (5)
2	2008	I	20	4 (20)	3 (15)		7 (35)
3	2008	I	20		1 (5)		1 (5)
4	2009	I	20	1 (5)	4 (20)		5 (25)
5	2010	I/S	20	1 (5)	2 (10)	1 (5)	4 (20)
6	2010	I	20		2 (10)		2 (10)
Total			140	6 (4)	14 (10)	1 (1)	21 (15)

Key:
I = Binocular indirect ophthalmoscope.
S = Slitlamp biomicroscope.

Sponsor's table 2

Clinical Pathology

Blood samples were obtained from a tail vein before dosing during Week 101 for female animals or during Week 104 for male animals. These samples were not assayed.

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Hematology

Blood samples were obtained from a tail vein before dosing during Week 101 for female animals or during Week 104 for male animals. A blood film for possible evaluation was prepared and processed for storage but was not examined. These samples were not assayed.

Gross Pathology

After 104 weeks of treatment, surviving toxicology animals were sacrificed by intraperitoneal injection of pentobarbitone sodium and exsanguinated; this method of euthanasia was also used for animals killed at unscheduled intervals. On completion of sampling and toxicokinetic analysis, surviving toxicokinetic animals were sacrificed by intraperitoneal injection of pentobarbitone sodium without further examination.

Macroscopic lesions in the adrenals, lungs/bronchi, uterine cervix and lachrymal glands were noted in animals dosed with test article. Adrenal glands in males showed a dose-dependent increase in dark areas (control 7, LD 12, MD 13, HD 16), with an overall increase in dosed females (control 25, LD 38, MD 31, HD 36). The lungs and bronchi showed pale areas in both males (control 24, LD 36, MD 34, HD 40) and females (control 23, LD 35, MD 37, HD 38). The uterine cervix was swollen in females dosed with test article (control 3, LD 5, MD 10, HD 9). The lachrymal glands were pale in males only, in a dose-dependent manner (control 0, LD 6, MD 9, HD 10). Analysis of microscopic lesions shows corresponding findings in the adrenals (cortical hypertrophy, focal), lungs/bronchi (foamy alveolar macrophages), uterine cervix (mural hypertrophy, epithelial mucification), and lachrymal glands (cytomegaly, harderianisation, lymphoid aggregates; males only).

Table 17: Rat carcinogenicity study: Gross findings

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Number examined	65	65	65	65	65	65	65	65
Adrenals Dark area(s)	7	12	13	16	25	38	31	36
Lungs + bronchi Pale area(s)	24	36	34	40	23	35	37	38
Uterine cervix Swollen	-	-	-	-	3	5	10	9
Lachrymal glands Pale	0	6	9	10	0	0	0	0

Histopathology

Adequate Battery: Yes

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Pancreas	X
Adrenals	X	Pituitary	X
Animal identification		Preputial/clitoral gland	X
Aorta (thoracic)	X	Prostate	X
Brain	X	Rectum	X
Caecum	X	Salivary gland+	
Cervix	X	mandibular	X
Colon	X	sublingual	X
Duodenum	X	parotid	X
Epididymides	X	Sciatic nerve+	X
Eyes#	X	Seminal vesicles	X
Femur (Femoro-tibial joint)	X	Skeletal muscle+	X
Harderian glands	X	Skin with mammary glands	X
Heart	X	Spinal cord	
Ileum	X	whole	
Jejunum	X	lumbar	X
Kidneys	X	Spleen	X
Larynx	X	Sternum with bone marrow	X
Liver (all major lobes)	X	Stomach	X
Lung including bronchi[a]	X	Testes	X
Lymph node -		Thymus	X
cervical	X	Thyroid with parathyroids	X
mesenteric	X	Tongue	X
tracheo-bronchial	X	Trachea	X
Nasal cavities and nasopharynx with skull	X	Tracheal Bifurcation (with main stem bronchi)	X
Oesophagus	X	Urinary bladder	X
Optic nerves	X	Uterus	X
Ovaries	X	Vagina	X

a All major lobes examined, left and right, including proximal and distal area
\+ Only one examined
\# Eyes retained separately with left and right identified and the north pole of each eye marked for orientation at histopathology.

Peer Review Yes.

Neoplastic

Neoplasias were analyzed by organ, and tumors that shared common cell origins in the same organ were combined for analysis. Specific tumors that arise from systemically distributed cells were identified by incidence per animal. Specifically, hemangioma and hemangiosarcoma, lymphomas, leukemias, mesotheliomas, and histiocytic sarcomas were analyzed by incidence per animal. Brix *et al.* state, "Such systemic neoplasms that might be considered in this fashion would include all blood cell neoplasms, including histiocytic sarcoma, as well as neoplasms in which the cell type is present in many different organs, such as hemangiosarcomas and malignant mesotheliomas[3].

Statistical analysis revealed neoplasms that were significant for trend analysis, or pair-wise analysis at the LD or MD only. Basal cell tumors (skin) in females, and lymphomas (whole body) in males had increased dose-dependent incidence, but did not achieve statistical significance.

For basal cell tumors of the skin, the sponsor provided historical control data from 12 studies initiated between April 2004 and September 2009, conducted with different routes of administration (inhalation, oral gavage, subcutaneous injection, and an additional route abbreviated as "dt"). In females, basal cell tumors ranged from 0.0 to 4.6%, with an overall incidence of 12/779 (1.5%). The incidence of 3/65 (4.6%) HD females, in the rat study reviewed here, is within historical control data, although the pathology report does not state if the data is from the same laboratory.

For lymphomas (whole body), historical control data from [redacted] (b) (4) (updated March 2004)[4] showed that 0.91 to 6.00% Crl: CD rats were diagnosed with lymphocytic lymphomas (whole body) in their facility. The incidence of 3/65 (4.6%) HD males, in the rat study reviewed here, is within historical control data.

Figure 4: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons in Rats (Statician's review)

Sex	Organ Name	Tumor Name	Cont N=65	Low N=65	Med N=65	High N=65	P-Value Dose Resp	C vs. L	C vs. M	C vs. H
Male	BRAIN	GRANULAR CELL TUMOUR	0	5	1	0	0.9287	0.0312*	0.5047	.
	H-POIETIC TUMOU	MALIGNANT LYMPHOMA	0	1	0	3	0.0395	0.5138	.	0.1391
	PITUITARY	ADENOMA, PARS DISTA	16	16	27	15	0.6510	0.5824	0.0315	0.5227
		ADN[PRS_DST+PRS_INT]	16	17	28	15	0.6766	0.5237	0.0205	0.5227
	WHOLE_BODY	LYMPHOMA	0	1	0	3	0.0395	0.5138	.	0.1391
Female	SKIN	BASAL CELL TUMOUR	0	0	0	3	0.0181*	.	.	0.1403

[3] Brix, A.E. J.F. Hardisty, and E.E. McConnell. Combining neoplasms for evaluation of rodent carcinogenesis studies. In: Hsu and Stedeford (eds.) Cancer Risk Assessment, John Wiley and Sons, Inc., 2010: 699-715.

[4] [redacted] (b) (4)

Table 18: Rat carcinogenicity study: Neoplastic findings

	mcg/kg/d (pulmonary deposited dose)							
	Male				Female			
	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Organ/N examined	65	65	65	65	65	65	65	65
Whole body Lymphoma	0	1	0	3	1	0	0	0
Skin B-Basal Cell Tumor	0	1	0	0	0	0	0	3

Non Neoplastic

Test article related non-neoplastic findings were identified in the nasal turbinates, larynx, tracheal bifurcates, lungs and bronchi, Harderian glands, liver, spleen, stomach, adrenals, lachrymal glands, and uterine cervix. Findings in the nasal turbinates are shown in the table below, but are not clinically relevant as rats are obligate nose breathers, and humans will be dosed via oral inhalation.

The majority of findings were related to local toxicity from the inhalation route of administration. The larynx of both sexes showed a test article related increased incidence and severity in epithelial hyperplasia (arytenoid), keratinisation (arytenoid, ventral), necrosis of the ventral cartilage, and squamous metaplasia/hyperplasia (lateral/ventral/ventrolateral). The tracheal bifurcates of only males showed an increased incidence in loss of cilia at the point of bifurcation in dosed animals, but this was not dose-dependent (as seen in mice dosed for 104 weeks). In the lungs and bronchi, dosed animals showed an increase in foamy alveolar macrophages that was not clearly dose-dependent, but was associated with pigmentation of the alveolar macrophages in males.

With respect to systemic toxicity, findings were observed in organs for only one of two sexes, and often lacked dose-dependence. In the Harderian glands, females at the MD and HD showed increased porphyrin concretions compared to controls, but this was not dose-dependent. Males developed findings in the liver (focal hepatocyte necrosis), spleen (increased pigmented macrophages), stomach (dilated glands), adrenals (focal cortical hypertrophy), lachrymal glands (cytomegaly, Harderianisation, lymphoid aggregates), but these lesions were not strongly dose-dependent, and were not reproduced in females. In the uterine cervix, test-article dosed females had an increased severity in mural hypertrophy and epithelial mucification.

Table 19: Rat carcinogenicity study: Non-neoplastic findings

mcg/kg/d (pulmonary deposited dose)	
Male	Female

Reference ID: 3313292

		0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Organ/Number examined	Severity	65	65	65	65	65	65	65	65
Nasal turbinates		65	65	65	65	65	65	65	64
Olfactory Epithelium - Eosinophilic Inclusions, Increased	Minimal:	9	11	13	7	21	19	11	10
	Slight:	6	9	5	18	5	4	7	17
	Moderate:	0	1	5	9	2	0	9	14
	Marked:	0	0	0	0	0	0	0	1
Larynx		65	65	65	65	65	64	65	64
Epithelial Hyperplasia - Arytenoid	Minimal:	2	10	13	27	5	12	13	24
	Slight:	0	2	2	3	0	0	6	6
Keratinisation, Arytenoid	Minimal:	0	3	4	18	0	1	6	12
	Slight:	0	0	0	0	0	0	1	0
Keratinisation, Ventral	Minimal:	0	0	4	11	0	0	10	7
	Severe:	0	0	1	0	0	0	0	0
Necrosis of Ventral Cartilage	Minimal:	0	3	3	2	0	4	7	2
	Slight:	0	3	10	1	0	2	13	6
	Moderate:	0	0	22	13	0	2	23	35
	Marked:	0	0	9	21	0	0	6	10
	Severe:	0	0	2	21	0	0	2	6
Squamous Metaplasia/Hyperplasia - Lateral	Minimal:	0	0	7	11	0	1	2	5
	Slight:	0	1	6	11	0	0	5	9
	Moderate:	0	0	0	1	0	0	0	0
Squamous Metaplasia/Hyperplasia - Ventral	Minimal:	0	22	24	16	2	21	36	32
	Slight:	0	2	18	33	0	3	20	21
	Moderate:	0	0	1	0	0	0	0	1
	Marked:	0	0	0	0	0	0	1	0
Squamous Metaplasia/Hyperplasia - Ventrolateral	Minimal:	0	1	6	15	0	1	6	6
	Slight:	0	0	3	9	0	0	1	4
Tracheal Bifurc.		64	65	62	62	65	65	64	62
Loss of Cilia at Bifurcation	Minimal:	10	21	22	21	23	21	23	24
	Slight:	1	0	0	0	0	0	0	1
Lungs + Bronchi		65	65	65	65	65	65	65	65
Foamy Alveolar Macrophages	Minimal:	27	32	35	37	26	34	41	41
	Slight:	3	7	5	10	5	3	2	5
	Moderate:	0	0	0	1	1	1	0	0

		mcg/kg/d (pulmonary deposited dose)							
		Male				Female			
		0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Organ/Number examined	Severity	65	65	65	65	65	65	65	65
Pigmented Alveolar Macrophages	Minimal:	0	1	2	5	3	1	0	2
	Slight:	0	0	0	1	0	0	0	0
Harderian Glands		65	65	65	65	65	65	65	64
Porphyrin Concretions, Increased	Minimal:	22	27	35	28	13	13	34	34
	Slight:	9	9	9	23	3	4	8	6
	Moderate:	0	3	0	1	0	0	0	0
Liver		65	65	65	65	65	65	65	65
Hepatocyte Necrosis, Focal	Minimal:	1	2	8	8	5	1	4	3
	Slight:	1	1	0	2	1	0	1	2
	Moderate:	0	1	1	0	0	0	0	0
	Marked:	0	0	1	0	0	0	0	0
Spleen		65	65	65	65	65	65	65	64
Increased Pigmented Macrophages	Minimal:	9	16	8	15	8	11	7	4
	Slight:	15	19	25	21	27	27	27	25
	Moderate:	0	3	3	3	11	5	8	15
	Marked:	0	0	0	0	0	0	1	0
Stomach		65	65	65	65	65	65	65	65
Dilated Glands	Minimal:	2	0	2	8	1	3	1	2
	Slight:	0	0	0	3	0	0	0	0
Adrenals		3	10	21	13	0	0	0	0
Cortical Hypertrophy, Focal	Minimal:	11	13	13	15	7	6	13	14
	Slight:	2	7	3	9	10	0	3	5
	Moderate:	0	0	1	1	0	0	0	0
Lachrymal Glands		3	10	21	13	0	0	0	0
Cytomegaly	Minimal:	0	1	2	3	0	0	0	0
	Slight:	2	8	12	6	0	0	0	0
	Moderate:	0	0	1	0	0	0	0	0
Harderianisation	Minimal:	0	0	0	2	0	0	0	0
	Slight:	1	2	3	0	0	0	0	0
	Moderate:	1	3	10	6	0	0	0	0
	Marked:	1	4	4	4	0	0	0	0
	Severe:	0	0	3	0	0	0	0	0
Lymphoid Aggregates	Minimal:	0	2	5	3	0	0	0	0
	Slight:	0	3	2	2	0	0	0	0
	Moderate:	0	0	1	0	0	0	0	0
Uterine cervix		-	-	-	-	65	65	65	65
Mural Hypertrophy	Minimal:	-	-	-	-	1	0	2	0
	Slight:	-	-	-	-	2	1	5	11
Epithelial Mucification	Minimal:	-	-	-	-	3	0	0	0

		mcg/kg/d (pulmonary deposited dose)							
		Male				Female			
		0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7	0	2.01/ 1.47	10.1/ 4.5	27.6/ 13.7
Organ/Number examined	Severity	65	65	65	65	65	65	65	65
	Slight:	-	-	-	-	5	11	7	8
	Moderate:	-	-	-	-	5	5	11	14
	Marked:	-	-	-	-	0	2	0	0

Toxicokinetics

Blood samples were obtained from toxicokinetic study animals (60 minute exposure) in Weeks 4 and 26 of dosing and from additional toxicokinetic study animals (30 minute exposure) in Week 4 of dosing at the lower doses. Blood samples were obtained from Control and GSK573719 treated animals immediately after dosing (IAD, in practice approximately 10 minutes after dosing), 30 minutes, and 1, 2, 4, 8 and 23 hours after completion of exposure. Following confirmation of toxicokinetic results, the animals were sacrificed with no necropsy. Approximately 0.4 ml of whole blood was collected from the tail vein at each time point.

A single sample at week 76, 4 weeks after the dose reduction, in the control group had a quantifiable concentration of test article (0.0257 ng/mL). All other control or naïve rat plasma samples were below the lower limit of quantification (0.1 ng/mL or 0.02 ng/mL). Due the low concentration at only one time point, and the lack of test article in other control animals at the same time point, it is possible that ex vivo contamination occurred in the one positive control sample. For the sake of this review, this animal is not considered to be dosed with test article.

At the higher doses administered with 60 minutes of exposure, systemic exposure based on AUC was generally dose proportional. At the HD, drug exposure appeared comparable between Weeks 4 and 26, while at the MD, there was a slight decrease in exposure at Week 26. There were no notable differences in exposure between the sexes. The maximum plasma exposure was observed between 1-2 hrs post dose.

At the lower doses starting at Week 76, with 30 minutes of exposure, systemic exposure based on AUC was approximately dose proportional. There were no notable differences in exposure between the sexes.

Table 20: Rat carcinogenicity study: TK parameters

Dosing Period Weeks 4, 26 (60 minutes exposure):

Parameter+	Week	Male			Female		
		Estimated Achieved Dose (µg/kg/day)					
		30.1	**101**	**276**	**30.1**	**101**	**276**
AUC (0-t) (ng.h/mL)	Week 4 Week 26	NC NC	4.42 2.09	15.5 11.5	NC 0.451	6.15 3.53	13.6 13.3

C_{max} (ng/mL)	Week 4 Week 26	0.161 0.156	0.603 0.438	2.22 1.71	0.172 0.316	0.750 0.969	1.54 2.32
Tmax (h)	Week 4 Week 26	1.5 1.5	1.0 2.0	1.0 1.5	1.0 1.0	1.0 1.0	1.0 1.0

Dosing Period Week 76 (4 weeks after reduced dose of 30 minutes exposure):

Parameter+	Week	Male			Female		
		Estimated Achieved Dose (µg/kg/day)					
		14.7	**45.0**	**137**	**14.7**	**45.0**	**137**
AUC (0-t) (ng.h/mL)	Week 76 (Week 4 reduced dose)	0.921	2.10	6.26	0.353	2.09	7.23
C_{max} (ng/mL)	Week 76 (Week 4 reduced dose)	0.102	0.319	1.22	0.104	0.317	1.02

Dosing Solution Analysis

Gravimetric analysis was conducted daily in samples collected from each group throughout the study.

Chemical analysis was conducted daily during Week 1 to 5, three times for Week 6, and twice weekly for the remainder of the study. Quartz filters were analyzed for GSK573719 using ultra performance liquid chromatography (UPLC) within a quantification range of 0.1 to 2 mcg/mL.

Particle size distribution was conducted on a measured volume of air was drawn at a rate of 3 liters/minute from inhalation exposure chambers through a cascade impactor (Marple Model 296 Personal Cascade Impactor, Andersen Instruments, Smyrna, Georgia, USA) attached to an exposure chamber. Samples were collected in Week 1, then every 3 months. Samples were analyzed for GSK573719.

The estimated achieved doses are within 15% of the target doses. The target doses were initially 0, 30, 100, and 300 between Weeks 1-72, and 0, 15, 50, and 150 mcg/kg/day from Week 73.

Table 21: Rat carcinogenicity study: Estimated and Target Inhaled Doses

Group	Week	Duration of exposure (minutes)	Estimated achieved dose (mcg/kg/d)	Target dose (mcg/kg/d)	Percent of target dose
Males					
Control	1-72	60	0	0	-
	73-104	30	0	0	-
LD	1-72	60	29	30	96%

	73-104	30	14	15	94%
MD	1-72	60	97	100	97%
	73-104	30	43	50	87%
HD	1-72	60	265	300	88%
	73-104	30	132	150	88%
Females					
Control	1-72	60	0	0	-
	73-104	30	0	0	-
LD	1-72	60	32	30	107%
	73-104	30	16	15	103%
MD	1-72	60	106	100	106%
	73-104	30	47	50	94%
HD	1-72	60	291	300	97%
	73-104	30	144	150	96%

5 Integrated Summary and Safety Evaluation

GlaxoSmithKline (GSK) has submitted this NDA for registration of Anoro-Ellipta for chronic obstructive pulmonary diseases (COPD) in adult patients. Anoro-Ellipta is a dry powder inhaler that delivers a dry powder mixture of umeclidinium bromide salt (umeclidinium) and vilanterol triphenylacetate salt (vilanterol) in magnesium stearate and lactose monohydrate. The sponsor proposes that each actuation deliver 62.5/25 mcg or 125/25 mcg of umeclidinium/vilanterol, respectively, with one actuation per day.

Two-year carcinogenicity studies with umeclidinium in mouse and rat are reviewed here. Special protocol assessments for both carcinogenicity protocols were conducted under IND 104479, for umeclidinium. All studies supporting vilanterol are reviewed under NDA 204275. Umeclidinium is known by the code name GSK573719A, and vilanterol is known by the code name GW642444M.

Umeclidinium was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation assay, *in vitro* mouse lymphoma assay, and *in vivo* rat micronucleus test.

The sponsor conducted a 2-year bioassay in CD-1 mice (75/sex/group) with GSK573719A by inhalation (nose only). Initial doses were based on MTD determined in a 13 week toxicology study (ECAC minutes 6/3/09). Female mice received nominal doses of 0 (1% magnesium stearate in lactose), 20, 60, and 200 mcg/kg/day throughout the study (60 minute exposures). Males initially received nominal doses of 0 (1% magnesium stearate in lactose), 60, 200, and 600 mcg/kg/day (60 minute exposures). Doses were decreased at Week 67 for males only to 0, 30, 100, and 300 mcg/kg/day (30 minute exposures), based on loss of body weight in dosed males, with concurrence from the ECAC (minutes 2/18/11). There were no treatment-related neoplastic findings

based on the lack of statistical significance for both trend and pair-wise statistical analysis. Histiocytic sarcomas (whole body) were noted in females dosed with the test article (control 1, LD 1, MD 5, HD 5), but the findings were not statistically significant by trend analysis and pair-wise analysis, and were within historical control data (1.7 to 11.7%) for females, based on data submitted by the sponsor from 13 studies conducted at [redacted] (b) (4) Additional historical control data from Crl: CD1 mice from [redacted] (b) (4) (updated March 2010) showed that 1.67 to 11.67% of animals were diagnosed with histiocytic sarcomas in 9 studies conducted in the US or Europe. Thus, umeclidinium was determined to not be tumorigenic in mice.

Non-neoplastic findings in mice were identified in the lungs and bronchi, kidneys, thymus, larynx, tracheal bifurcates, and nasal turbinates. Findings in the nasal turbinates are not clinically relevant. The majority of findings were related to local toxicity from the inhalation route of administration. The larynx showed increased incidence and severity in squamous hyperplasia/metaplasia in the ventral epithelium. The tracheal bifurcates showed a loss of cilia at the point of bifurcation. In the lungs and bronchi, males showed an increase in bronchioloalveolar hyperplasia, with associated peribronchioloar lymphoid aggregates. With respect to systemic toxicity, female kidneys showed a test article related increased incidence and severity of cortical tubules with hyaline droplets. In the thymus, females showed an increase in lymphoid hyperplasia. Systemic exposure based on $AUC_{(0-t)}$ generally increased with dose. C_{max} generally increased with dose. T_{max} was reported for TK samples collected at Weeks 4 and 26, and occurred between 1 and 3 hours after dosing.

The sponsor conducted a 2-year bioassay in Crl: CD (Sprague Dawley) rats (65/sex/group) with GSK573719A by inhalation (nose only). Initial doses were based on the MTD determined from a 13-week inhalation toxicology study (ECAC minutes 6/3/09). The recommended doses were 0 (1% magnesium stearate in lactose), 30, 100, and 300 mcg/kg/day (60 minute exposures). Effective from Week 73, doses were reduced to target doses of 15, 50, and 150 mcg/kg/day (30 minute exposures) with ECAC concurrence (minutes 2/18/11). Based on data up to Week 70, the sponsor noted decreases in body weight gains and proposed lower doses. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD. There were no treatment-related neoplastic findings based on the lack of statistical significance. Neoplastic findings observed with increased incidences in test article-treated groups were basal cell tumors in females (skin; control/LD/MD 0, HD 3) and lymphomas in males (whole body; control 0, LD 1, MD 0, HD 3), but these did not achieve statistical significance for trend and pair-wise comparison, and were within historical control values. For basal cell tumors of the skin, the sponsor provided historical control data (0.0 to 4.6%) that covered the incidence of 3/65 (4.6%) in the reviewed study. For lymphomas, historical control data (0.91 to 6.00%) for Crl: CD rats diagnosed with lymphocytic lymphomas (whole body) from [redacted] (b) (4) covered the incidence of 3/65 (4.6%) in the reviewed study.

Non-neoplastic findings in rats were identified in the nasal turbinates, larynx, tracheal bifurcates, lungs and bronchi, Harderian glands, liver, spleen, stomach, adrenals, lachrymal glands, and uterine cervix. Findings in the nasal turbinates are not clinically relevant as rats are obligate nose breathers, and humans will be dosed via oral inhalation. At the higher doses administered with 60 minutes of exposure, systemic exposure based on $AUC_{(0-t)}$ was generally dose proportional. There were no notable differences in exposure between the sexes. The maximum plasma exposure was observed between 1-2 hrs post dose. At the lower doses starting at Week 76, with 30 minutes of exposure, systemic exposure based on AUC was approximately dose proportional. There were no notable differences in exposure between the sexes.

Bilateral opacity on the posterior position of the lens was increased in both the mouse and rat bioassays. In male rats, this finding was increased in a dose-dependent manner (control 2/39, LD 5/46, MD 5/43, HD 8/48). The sponsor provided historical control data showing that the incidence of was within the historical control range. Bilateral opacity of the posterior lens was also increased in a male mice in a dose-dependent manner (control 9/41, LD 12/33, MD 15/33, HD 27/41), and was increased in LD and HD females (control 16/31, LD 35/45, MD 13/29, HD 27/35), but was not dose-dependent. The incidence for male mice was within historical controls, but that for females was not. In female mice, the lack of dose-response makes it difficult to determine if this finding is test-article related. These findings might be attributed to direct exposure of the lens to the test article rather than through systemic exposure.

In conclusion, umeclidinium was determined to not be tumorigenic based on evaluation in mice and rats. Conclusions from Executive CAC meeting conducted on April 9, 2013 are listed below.

Exposure ratios for clinical exposures for 62.5 mcg/day of umeclidinium are shown in the table below. The sponsor is proposing clinical doses of 62.5 mcg/day and 125 mcg/day of umeclidinium for the combination product. Exposures achieved, based on the top dose in carcinogenicity studies for umeclidinium, provide safety margins greater than 20-fold over clinical exposure in COPD patients (email from Clinical Pharmacology reviewer Dr. Jianmeng Chen, 1/24/13).

Table 22: Exposure ratios for clinical exposure to umeclidinium

				Clinical dose of umeclidinium (62.5 mcg)
Study	Sex	Pulmonary deposited dose* (mcg/kg/day)	AUC (ng.h/mL)	AUC = 0.3124 (ng.h/mL)
2-year mouse	Males	3.22	1.53	4.9
		10.2	3.28	10.5
		29.5	8.21	26.3

	Females	2.08	NC	NC
		6.37	1.51	4.8
		20	6.87	22.0
2-year rat	Both	1.47	0.637	2.0
		4.5	2.095	6.7
		13.7	6.745	21.6

*lower dose, after dose change
**NC = not calculated

Conclusions from Executive CAC meeting conducted on April 9, 2013:

Rat:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.

Mouse:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female mice.

6 Appendix/Attachments

1) ECAC concurrence: Fax of June 2, 2009 minutes (see below).
2) IND 104479, Nonclinical review (February 18, 2011), supporting ECAC concurrence to decrease doses in mouse and rat 2-year carcinogenicity studies.
3) Executive CAC meeting minutes (April 9, 2013).

Reference ID: 3313292

Executive CAC
Date of Meeting: June 2, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Chuck Resnick, Ph.D., DCRP, Alternate Member
Molly Shea, Ph.D., DPAP, Acting Team Leader
Tim Robison, Ph.D., DPAP, Presenting Reviewer

Author of Draft: Tim Robison, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND# 104,479
Drug Name: GSK573719
Sponsor: GlaxSmithKline

Background:
GSK573719 is a quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist.

In vitro plasma protein binding of GSK573719 in mice, rats, and humans was comparable at 88, 86, and 88%, respectively. In vitro metabolism was examined with hepatocytes from mice, rats, and humans. Studies with human hepatocytes and microsomes found that the main routes of metabolism of GSK573719 were O-dealkylation and hydroxylation. Other routes were conjugation with glutathione and methylation and/or glucuronidation of the hydroxylated metabolite. The major routes of metabolism in mouse hepatocytes were O-dealkylation, hydroxylation followed by glucuronide conjugation, and formation of two hydrated glutathione conjugates. These represented approximately 18%, 10%, 47%, and 3% of total metabolism, respectively. All other metabolites were glucuronide conjugates of hydroxylated metabolites. The main route of metabolism in human hepatocytes (O-dealkylation) was also a major route of metabolism in rat (38% of total metabolism). For hydroxy metabolites in humans (M33/M34), M33 in rats was only a very minor route and M34 was absent in rats. The major metabolic routes in the rat were O-dealkylation, hydrated glutathione and cysteine conjugation, and hydroxy, O-glucuronide and cysteine conjugation.

Reference ID: 3313292

GSK573719A was negative in the in vitro bacterial reverse mutation assay, in vitro mouse lymphoma assay, and in vivo rat micronucleus assay.

Mouse Carcinogenicity Study Protocol and Dose Selection:
In a 13-week inhalation toxicology study, mice received GSK573719 at estimated achieved doses of 0, 0.0923, 0.287, 1.06, and 2.85 mg/kg/day. Pulmonary deposited doses were calculated to be 0, 0.009, 0.03, 0.1, and 0.3 mg/kg/day, respectively. Examination of body weight curves over the 13-week dosing period for male and female control and treatment groups do not appear to suggest any significant decrements of body weight gain. Incidences of slight or moderate retinal folds were increased for male and female treatment groups. Treatment-related histopathological findings were observed in the tracheal bifurcation, larynx, nasal turbinates, and nasopharynx. Potential treatment-related findings were observed in the liver and uterus. Histopathological findings were evident in the larynx at all doses and appeared to be nonspecific irritant effects associated with inhalation exposure. Histopathological findings were observed in the nasal turbinates at doses ≥0.287 mg/kg/day. Histopathological findings were observed in the nasopharynx at doses of 1.06 and 2.85 mg/kg/day. Histopathological findings in the tracheal bifurcation, liver, and uterus were observed at 2.85 mg/kg/day. A potential dose-limiting finding in the larynx was ventral cartilage necrosis observed for males at 1.06 and 2.85 mg/kg/day and females at all doses. This finding for females at 0.092 and 0.287 mg/kg/day was of relatively low incidence (i.e., 1 of 10 and 2 of 12, respectively). Further, the finding for 1 female at 0.092 mg/kg/day was graded as minimal. A potential dose-limiting finding in the nasal turbinates was slight ulceration of the olfactory epithelium for 1 male at 2.85 mg/kg/day. Based upon findings of ventral cartilage necrosis in larynx for males at 1.06 and 2.85 mg/kg/day and females at 0.092, 0.287, 1.06, and 2.85 mg/kg/day, maximum tolerated doses were identified as 0.6 mg/kg/day for males and 0.2 mg/kg/day for females. Doses of 0.6 and 0.2 mg/kg/day for males and females, respectively, provide extrapolated 2.0 and 0.6 exposure margins on an AUC basis for the highest proposed clinical dose.

Rat Carcinogenicity Study Protocol and Dose Selection:
In a 13-week inhalation toxicology study, rats received GSK573719 at estimated achieved doses of 0, 38, 102, 288, and 924 µg/kg/day. Pulmonary deposited doses were 0, 0.4, 10.2, 28.8, and 92.4 µg/kg/day, respectively. Body weight gains for males in the 102, 288, and 924 µg/kg/day groups were decreased to 84.8, 82.9, and 78.7% of the control, respectively. It is noted that decreased body weight gains for male treatment groups were relatively flat across the range of doses although the decrease for males at 924 µg/kg/day appeared to be excessive for a 2-year study. Body weight gains for females in the 288 and 924 µg/kg/day groups were decreased to 89.3 and 81.3% of the control, respectively. The decrease for females in the 288 µg/kg/day group did not appear to be toxicologically significant. Treatment-related histopathological findings were observed in the larynx, nasal cavity/sinuses, and nasopharynx. For the larynx, minimal to slight squamous metaplasia was seen in the ventrolateral aspect of the larynx and was often seen in combination with minimal to slight necrosis of the ventral pouch cartilage in both sexes at doses ≥38 µg/kg/day. These changes were not reversible. Minimal focal necrosis observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study. However, minimal to slight necrosis observed at 924 µg/kg/day was considered dose-limiting. Given that minimal focal necrosis in the larynx observed at doses ≤288 µg/kg/day was not considered dose-limiting for a 2-year carcinogenicity study, identification of maximum tolerated doses for males and females were based primarily on decreases of body weight gain. Based upon

decreased body weight gains for males and females at 924 µg/kg/day, the MTD was identified as 288 µg/kg/day. The dose of 288 µg/kg/day provides an approximate 1.7 exposure margin on an AUC basis for the highest proposed clinical dose.

Executive CAC Recommendations and Conclusions:

Mouse:

- The Committee recommended doses of 0.06, 0.2, and 0.6 mg/kg/day in males and 0.02, 0.06, and 0.2 mg/kg/day in females, by inhalation, based on MTD (effects in the larynx).

Rat:

- The Committee concurred with the sponsor's proposed doses of 30, 100, and 300 µg/kg/day, by inhalation, based on MTD (body weight effects).

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
(b) for statistically significant or otherwise remarkable findings in the high dose group, the sponsor will need to look at the affected tissues in all of the dose groups.
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

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

cc:\
/Division File, DPAP
/MShea, DPAP
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/MRaggio, DPAP
/ASeifried, OND IO

Reference ID: 3313292

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:	104,479
Supporting document/s:	#058
Sponsor's letter date:	February 14, 2011
CDER stamp date:	February 14, 2011
Product:	GSK573719 (Muscarinic receptor antagonist)
Indication:	COPD
Sponsor:	GlaxoSmithKline P.O. Box 13398 Five Moore Drive Research Triangle Park North Carolina 27709-3398
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Timothy W. Robison, Ph.D., D.A.B.T.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Miranda Raggio

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

GSK573719, a M3 muscarinic antagonist, is under development alone (IND 104,479) and in combination (IND 106,616) with GW642444, a β_2 adrenergic agonist, for the treatment of COPD. Two-year rat and mouse carcinogenicity studies with GSK573719 are currently ongoing. In the present submission, the sponsor has requested to lower doses used in these ongoing carcinogenicity studies based upon observed decreases of body weight gains.

1.2 Brief Discussion of Nonclinical Findings

The sponsor has conducted chronic toxicology studies with rats up to 6 months and dogs up to 9 months.

Two year carcinogenicity studies with GSK573719 in mice and rats are in progress.

1.3 Recommendations

Internal comments: In the present submission, the sponsor has requested to lower doses used in these ongoing carcinogenicity studies based upon observed decreases of body weight gains.

<u>2-year carcinogenicity study with rats</u>: The sponsor has proposed dose reduction for male and female treatment groups from target doses of 30, 100 and 300µg/kg/day to target doses of 15, 50 and 150 µg/kg/day, respectively.

Treatment of rats with GSK573719 at doses up to 300 µg/kg/day for 70 weeks had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains. The reduced doses would still provide large multiples over systemic exposure achieved with the MRHD. The ECAC, in emails dated February 18, 2010, agreed with the sponsor's proposed dose reduction for the 2-year carcinogenicity study with rats.

<u>2-year carcinogenicity study with mice</u>: The sponsor has proposed dose reduction for male treatment groups from target doses of 60, 200, and 600 µg/kg/day to target doses of 30, 100, and 300 µg/kg/day, respectively.

Treatment of mice with GSK573719 at doses up to 600 µg/kg/day in males and 200 µg/kg/day in females up to week 70 had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains for male treatment groups. The reduced doses would still provide large multiples over systemic exposure achieved with the MRHD. The ECAC, in emails dated February 18, 2010, agreed with the sponsor's proposed dose reduction for males in the 2-year carcinogenicity study with mice.

External comments: The sponsor's proposed dose reductions for male and female rats and male mice in ongoing 2-year carcinogenicity studies are acceptable based upon ECAC concurrence. Miranda Raggio communicated this information to the sponsor by telephone.

2 Drug Information

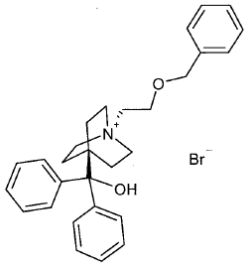
2.1 Drug

Code name: GSK573719

Chemical name: 4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl} -1-azoniabicyclo[2.2.2]octane bromide

Molecular formula/molecular weight: $C_{29}H_{34}NO_2 \cdot Br$
508.5 (GSK573719A as quaternary ammonium bromide compound)
428.6 (as free cation, GSK573719)

Structure:



Pharmacologic class: GSK573719 is a quinuclidine derivative that is a pan active muscarinic acetylcholine receptor (mAChR) antagonist.

2.2 Relevant INDs, NDAs, and DMFs

IND 106,616 (GSK, GSK573719 + GW642444)

2.8 Regulatory Background

Dose selection for the 2-year carcinogenicity studies with GSK573719 in mice and rats were reviewed by the ECAC on June 3, 2009.

3 Studies Submitted

3.1 Studies Reviewed

The sponsor submitted a proposal to reduce doses for male and female rats and male mice in the 2-year carcinogenicity studies based upon observe decreased of body weight gain.

3.3 Previous Reviews Referenced

Pharmacology and Toxicology Review of IND 104,479 dated June 3, 2009
ECAC recommendations and conclusions for 2-year carcinogenicity studies with mice and rats dated June 3, 2011

11 Integrated Summary and Safety Evaluation

GSK573719, a M3 muscarinic antagonist, is under development alone (IND 104,479) and in combination (IND 106,616) with GW642444, a β_2 adrenergic agonist, for the treatment of COPD. Two-year rat and mouse carcinogenicity studies with GSK573719 are currently ongoing. In the present submission, the sponsor has requested to lower doses used in these ongoing carcinogenicity studies based upon observed decreases of body weight gains.

2-Year inhalation carcinogenicity study with rats

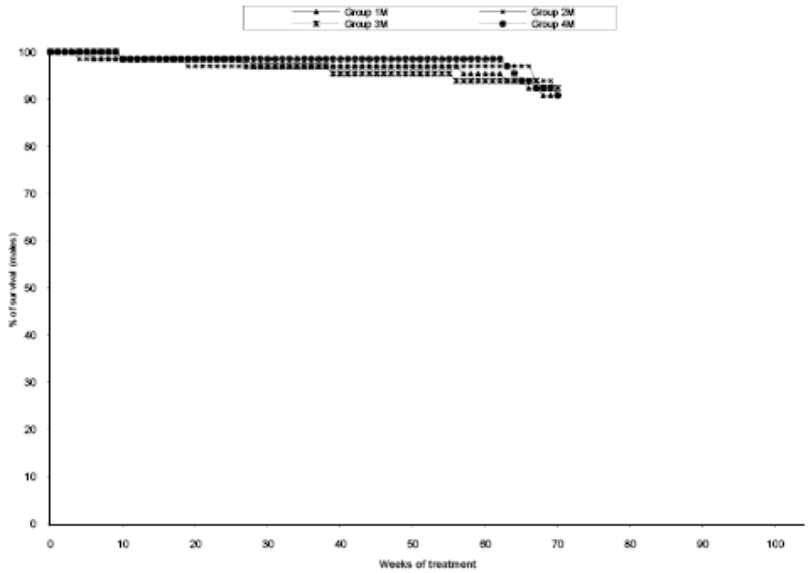
The target doses of GSK573719 for the carcinogenicity study with rats, as agreed with the ECAC in June 2009, are 30, 100 and 300 µg/kg/day. Dose selection was based on MTD (body weight effects). Dosing in this study has completed Week 70. Survival was unaffected for both male and female treatment groups.

For female treatment groups, there were non dose-related reductions of percent body weight gain compared to controls (21, 16 and 24% reduction in body weight gain for the low, mid, and high doses, respectively, compared to the concurrent control). This was accompanied by a minimal, dose related reduction of percent food consumption (range 7 10%). Absolute body weights for females in the low, mid, and high dose groups were decreased to approximately 88.5, 91, and 87.2% of the control, respectively.

In male treatment groups, there were small dose related reductions of percent body weight gain (5, 12 and 14% reduction in body weight gain for the low, mid, and high doses, respectively, compared to the concurrent control). This was accompanied by minimal, dose related reductions of food consumption (range 1 8%). Absolute body weights for males in the low, mid, and high dose groups were decreased to approximately 97, 92, and 89% of the control, respectively.

Figure 1 Survival in male and female rats through week 70

Graph 1: Current survival curve for male rats (main study rats, weeks 1-70)



Graph 2: Current survival curve for female rats (main study rats, weeks 1-70)

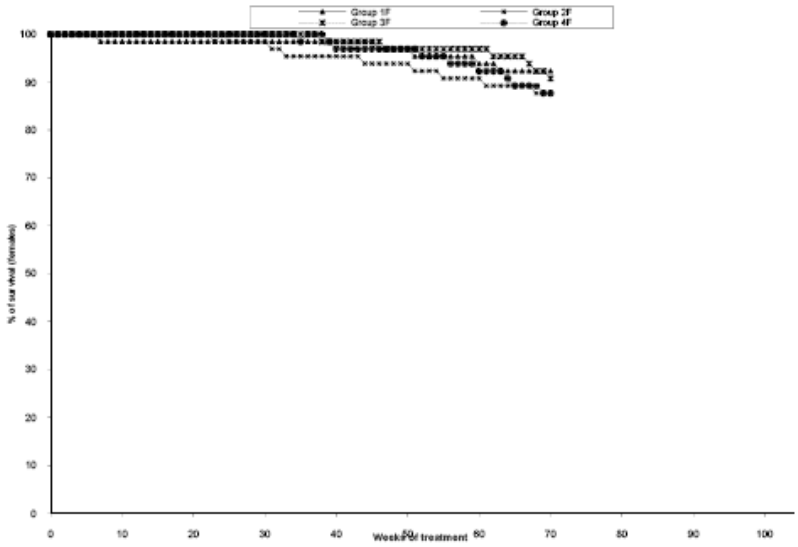
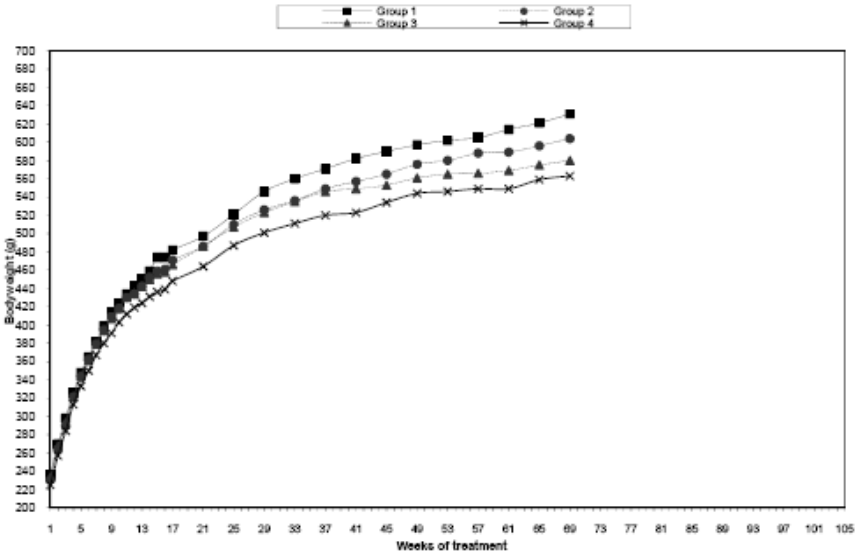


Figure 2 Body weights for male and female rats through week 68

Graph 3: Current bodyweight curve for male rats (main study rats. Weeks 1-68)



Graph 4: Current bodyweight curve for female rats (main study rats, weeks 1-68)

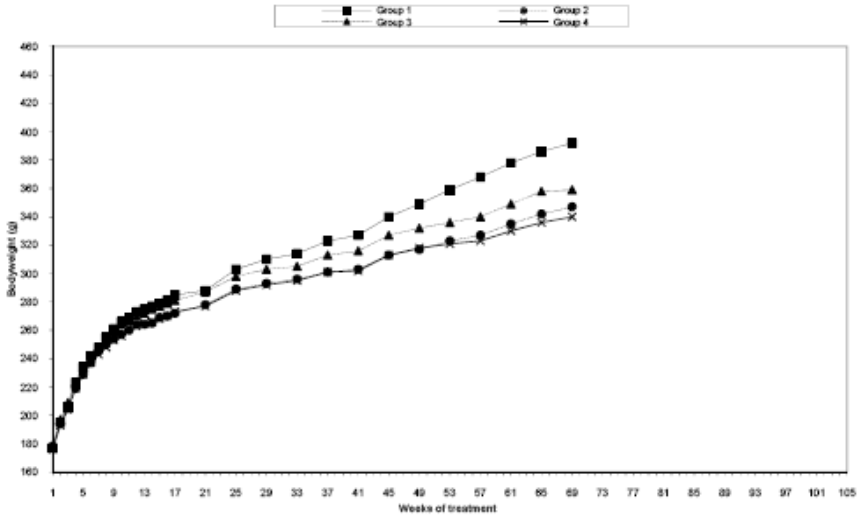


Table 1 Body weight gain, food consumption, and survival for male and female rats through week 70

Current Data on Body weight gain, food consumption and survival:

Group Number	MALE				FEMALE			
	1	2	3	4	1	2	3	4
Target dose (µg/kg/day) GSK573719	0	30	100	300	0	30	100	300
Mean body weight gain (g) Day 1 to Week 68	395	374	347	338	215	169	180	164
% of control	-	95	88	86	-	79	84	76
Mean food consumption at Week 68(g/animal/wk)	177	176	167	163	137	127	124	123
% of control	-	99	94	92	-	93	91	90
% survival at Week Number 70	91	91	92	91	92	88	91	88

Plasma C_{max} and AUC values for GSK573719 increased in an approximate dose proportional manner. There were no notable differences in systemic exposure to GSK573719 between the sexes within any of the dose groups and the data is consistent with previous data in the rat. Estimated systemic overage compared to the maximum recommended human dose (MRHD) of 125mcg is given below.

Table 2 Exposure multiples in male and female rats as compared to the MRHD

Estimated systemic overage compared to the MRHD of 125mcg.

Target Dose (µg/kg/day)	Estimated AUC exposure overage compared to the maximum clinical dose (125mcg)		Estimated Cmax exposure overage compared to the maximum clinical dose (125mcg)	
	Males	Females	Males	Females
30	NC	6	1.7	3.1
100	32	50	4.7	9.5
300	174	182	18.1	22.3
Exposure overages calculated using 1) human exposure data for 125µg/day based on repeat dose data (14 days) in COPD patients in study AC4113073 (Day 14 AUC_{0-t}- 0.07ng.h/mL and Cmax = 0.1ng/ml (geometric mean) and 2) Week 26 toxicokinetic exposure data from the ongoing carcinogenicity study (Table 2, Appendix 1)				

Sponsor's Proposal: The sponsor proposes to implement an immediate dose reduction in both male and female rats by reducing the exposure time on the inhalation chambers from 60 to 30 min. The sponsor considers this change the most efficient way to implement a dose reduction at this stage given the current stage of the study (week 71) and will result in a dose reduction from target doses of 30, 100 and 300µg/kg/day to target doses of 15, 50 and 150 µg/kg/day. This will allow immediate exploration of the sensitive part of the dose response curve based on multiple of the MHRD for the remainder of the study period. GSK573719 is expected to achieve approximately 3, 20 and 90 fold margins over human AUC at the MRHD for the proposed new target doses of 15, 50 and 150µg/kg/day, respectively. To implement any lower doses would require preparation and calibration of new blend strengths, which would take approximately 8 weeks to implement (i.e. to prepare, analyze the new blends and calibrate new aerosol concentrations on the inhalation chambers). Additional satellite animals will be introduced to the study to monitor the systemic exposure at the new target doses. Work is ongoing to validate a lower level of quantification at 20 pg/mL for GSK573719 in rat plasma to assist in better defining the plasma concentration profile of GSK573719 at lower inhaled doses (e.g. 15 µg/kg/day).

Evaluation: Treatment of rats with GSK573719 at doses up to 300 µg/kg/day up to week 70 had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains for male and female treatment groups. The reduced doses would still provide large multiples over systemic exposure

achieved with the MRHD. It is noted that the LOQ for the analytical method to measure GSK573719 might be inadequate to detect lower concentrations of GSK573719, possibly at ≤50 µg/kg/day. Exposure margins would decrease 2-fold with respect to local toxicity although this is not a typical parameter for dose selection in a carcinogenicity study and there is still a relatively large exposure margin at the high dose. The ECAC, in emails dated February 18, 2011, agreed with the sponsor's proposed dose reduction for male and female rats in the 2-year carcinogenicity study.

Table 3 Exposure margins for pulmonary deposited dose (Rat/Human)

Proposed doses µg/kg/day	PDD µg/kg/day	Exposure margins for clinical dose of 125 µg (2.1 µg/kg/day)
15	1.5	0.7
50	5	2.4
150	15	7.1

2-Year inhalation carcinogenicity study with GSK573719 in mice

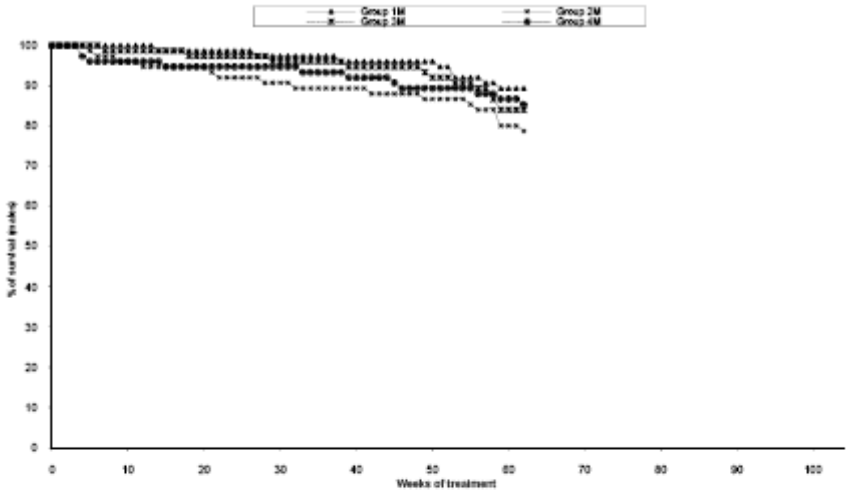
The target doses of GSK573719 for the carcinogenicity study with mice, as agreed with the ECAC in June 2009, are 60, 200 and 600µg/kg/day for males and 20, 60 and 200 µg/kg/day for females. Dose selection was based on MTD (effects in the larynx). Dosing in this study has completed Week 63. Survival appears to be >80% in all male and female treatment groups.

In males, there was a non dose related reduction in bodyweight gain compared to controls (8, 6, and 26% in the low, mid, and high doses, respectively, compared to the concurrent control). This was accompanied by a minimal reduction in food consumption (range 7 10%). Absolute body weight for males in the high dose was reduced to 90.5% of the control.

There was no effect on body weight gain in females at the low dose and only minor reductions at the mid and high doses (6 and 5%, respectively), accompanied by only minor reductions of food consumption at the mid and high doses (3%) compared to the concurrent control.

Figure 3 Survival for male and female mice through week 62

Graph 7: Current survival curve for male mice (main study mice, weeks 1-62)



Graph 8: Current survival curve for female mice (main study mice, weeks 1-62)

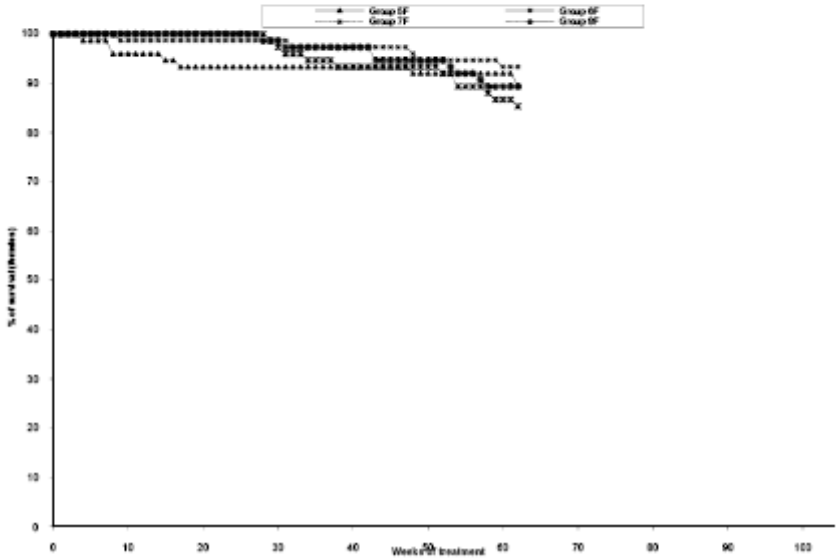
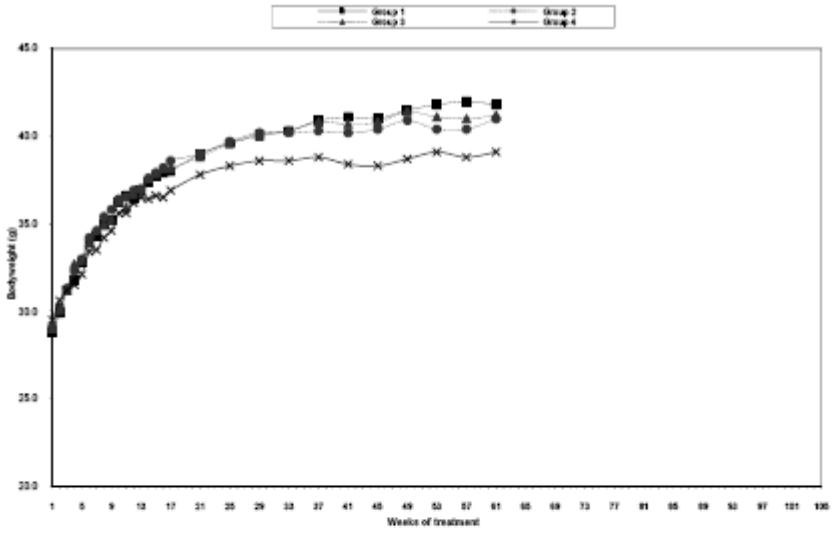


Figure 4 Body weights for male and female mice through week 60

Graph 9: Current bodyweight curve for male mice (main study mice, weeks 1-60)



Graph 10: Current bodyweight curve for female mice (main study mice, weeks 1-56)

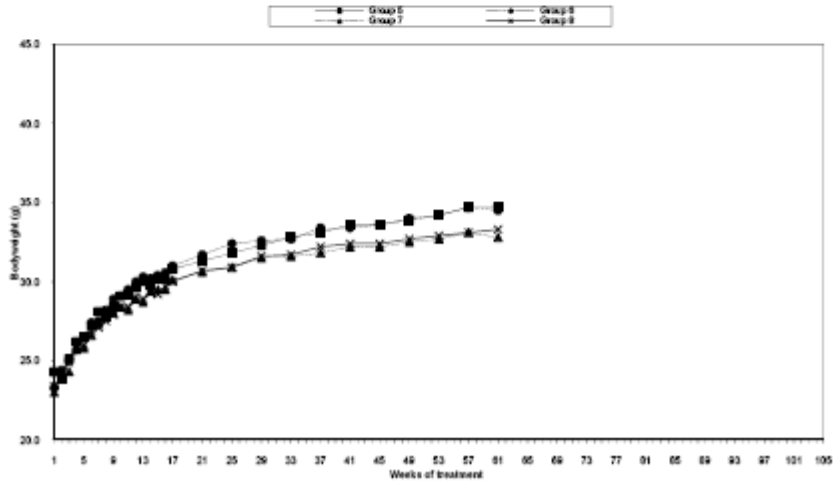


Table 4 Body weight gains, food consumption, and survival for male and female mice

Current Data on Body weight gain, food consumption and survival:

Group Number	MALE				FEMALE			
	1	2	3	4	5	6	7	8
Target dose (µg/kg/day) GSK573719	-	60	200	600	-	20	60	200
Mean body weight gain (g) Day 1 to Week 60	13.0	11.9	12.2	9.6	10.4	11.2	9.8	9.9
% of control	-	92	94	74	-	108	94	95
Mean food consumption at Week 60 (g/animal/wk)	41	38	40	37	35	37	34	34
% of control	-	93	98	90	-	106	97	97
% survival at start of Week 63	89	79	84	85	89	95	85	89

AUC and C_{max} values for GSK573719 generally increased in an approximate dose proportional manner. Exposures appeared to be relatively comparable between males and females. Estimated systemic overage compared to the MRHD of 125mcg is given below.

Table 5 Exposure multiples in male and female mice as compared to the MRHD

Estimated systemic overage compared to the MRHD of 125mcg.

Males:		
Target Dose (µg/kg/day)	Estimated AUC exposure overage compared to the maximum clinical dose (125mcg)	Estimated Cmax exposure overage compared to the maximum clinical dose (125mcg)
60	NC	4.4
200	26	11.5
600	121	27.5
Females:		
Target Dose (µg/kg/day)	Estimated AUC exposure overage compared to the maximum clinical dose (125mcg)	Estimated Cmax exposure overage compared to the maximum clinical dose (125mcg)
20	NC	2.5
60	21	9.2
200	96	36
Exposure overages calculated using 1) human exposure data for 125µg/day based on repeat dose data (14 days) in COPD patients in study AC4113073 (Day 14 AUC_{0-t}- 0.07ng.h/mL and Cmax = 0.1ng/ml (geometric mean) and 2) Week 26 toxicokinetic exposure data from the ongoing carcinogenicity study (Table 4, Appendix 2)		

Proposal: The sponsor proposes to implement an immediate dose reduction in male mice. The percent reduction in body weight gain only exceeded 10% for high dose males; however, the reduction in body weight gain for low dose males is currently 8%. The sponsor considers it prudent to implement a dose reduction across all male groups. The sponsor proposes to implement an immediate dose reduction for male treatment groups by reducing the exposure time on the inhalation chambers from 60 to 30 min. This is considered the most efficient way to implement the dose reduction at this stage given the current stage of the study (week 63) and will result in a dose reduction for male treatment groups from target doses of 60, 200, and 600 µg/kg/day to target doses of 30, 100, and 300 µg/kg/day, respectively. This will allow immediate exploration of the sensitive part of the dose response curve in males based on multiples of the MHRD for the remainder of the study period. GSK573719 is expected to achieve approximately 3.5, 3, and 60 fold exposure margins over the human AUC at the MRHD for the proposed new target doses of 30, 100 and 300 µg/kg/day, respectively. To implement any lower doses would require preparation and calibration of new blend strengths, which would take approximately 8 weeks to implement (i.e. to prepare, analyze the new blends and calibrate new aerosol concentrations on the inhalation chambers). Additional satellite animals will be introduced to the study to monitor the systemic exposure at the

new target doses. As for the rat study, work is ongoing to validate a lower level of quantification at 20 pg/mL for GSK573719 in mouse plasma to assist in better defining the plasma concentration profile of GSK573719 at lower inhaled doses.

Evaluation: Treatment of mice with GSK573719 at doses up to 600 µg/kg/day in males and 200 µg/kg/day in females up to week 70 had minimal effects on survival and absolute body weights. The sponsor has focused on trends of decreased body weight gains for male treatment groups. The reduced doses would still provide large multiples over systemic exposure achieved with the MRHD. It is noted that the LOQ for the analytical method to measure GSK573719 might be inadequate to detect lower concentrations of GSK573719 (≤60 µg/kg/day). Exposure margins would decrease 2-fold with respect to local toxicity although this is not a typical parameter for dose selection in a carcinogenicity study and there is still a relatively high exposure margin at the high dose. The ECAC, in emails dated February 18, 2011, agreed with the sponsor's proposed dose reduction for male mice in the 2-year carcinogenicity study.

Table 6 Exposure margins for pulmonary deposited dose (Male Mouse/Human)

Proposed doses µg/kg/day	PDD µg/kg/day	Exposure margins for clinical dose of 125 µg (2.1 µg/kg/day)
30	3	1.4
100	10	4.8
300	30	14.3

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/s/

TIMOTHY W ROBISON
02/18/2011

Executive CAC
Date of Meeting: April 9, 2013

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Karen Davis Bruno, Ph.D., DMEP, Alternate Member
Tim Robison, Ph.D., DPARP, Team Leader
Jane J. Sohn, Ph.D., DPARP, Presenting Reviewer

Author of Draft: Jane J. Sohn, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 203975
Drug Name: Umeclidinium-Vilanterol
Sponsor: GlaxoSmithKline

Background:
The sponsor is developing umeclidinium (GSK573719A), a muscarinic acetylcholine receptor (mAChR) antagonist, as part of a combination product with vilanterol, a long acting beta-2 agonist (LABA) for the treatment of airflow obstruction in chronic obstructive pulmonary disease (COPD). Results from 2-year carcinogenicity studies in mice and rats administered inhaled GSK573719A were submitted by the sponsor. Carcinogenicity studies with vilanterol alone were previously reviewed under NDA 204275, which is for vilanterol in combination with fluticasone furoate, a corticosteroid.

Mouse Carcinogenicity Study
The sponsor conducted a 2-year bioassay in CD-1 mice (75/sex/group) with GSK573719A by inhalation (nose only). Initial doses were based on MTD determined in a 13 week toxicology study (ECAC minutes 6/3/09). Female mice received nominal doses of 0 (1% magnesium stearate in lactose), 20, 60, and 200 mcg/kg/day throughout the study (60 minute exposures). Males initially received nominal doses of 0 (1% magnesium stearate in lactose), 60, 200, and 600 mcg/kg/day (60 minute exposures). Doses were decreased at Week 67 for males only to 0, 30, 100, and 300 mcg/kg/day (30 minute exposures), based on loss of body weight in dosed males, with concurrence from the ECAC (minutes 2/18/11).

There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis. Histiocytic sarcomas (whole body) were noted in females dosed with the test article (control 1, LD 1, MD 5, HD 5), but the findings were not statistically significant by trend analysis and pair-wise analysis, and were within historical control data (1.7 to 11.7%) for females, based on data

Reference ID: 3313292

submitted by the sponsor from 13 studies conducted at [redacted] (b) (4)
Additional historical control data from Crl: CD1 mice from [redacted] (b) (4)
(updated March 2010) showed that 1.67 to 11.67% of animals were diagnosed with histiocytic sarcomas in 9 studies conducted in the US or Europe.

Rat Carcinogenicity Study

The sponsor conducted a 2-year bioassay in Crl: CD (Sprague Dawley) rats (65/sex/group) with GSK573719A by inhalation (nose only). Initial doses were based on the MTD determined from a 13-week inhalation toxicology study (ECAC minutes 6/3/09). The recommended doses were 0 (1% magnesium stearate in lactose), 30, 100, and 300 mcg/kg/day (60 minute exposures). Effective from Week 73, doses were reduced to target doses of 15, 50, and 150 mcg/kg/day (30 minute exposures) with ECAC concurrence (minutes 2/18/11). Based on data up to Week 70, the sponsor noted decreases in body weight gains and proposed lower doses. The nonclinical reviewer determined that data up to 70 weeks showed minimal effects on survival and absolute body weights, but that reduced doses would provide large multiples over systemic exposure achieved with the MRHD.

There were no treatment-related neoplastic findings based on the lack of statistical significance. Neoplastic findings observed with increased incidences in test article-treated groups were basal cell tumors in females (skin; control/LD/MD 0, HD 3) and lymphomas in males (whole body; control 0, LD 1, MD 0, HD 3), but these did not achieve statistical significance for trend and pair-wise comparison, and were within historical control values. For basal cell tumors of the skin, the sponsor provided historical control data (0.0 to 4.6%) that covered the incidence of 3/65 (4.6%) in the reviewed study. For lymphomas, historical control data (0.91 to 6.00%) for Crl: CD rats diagnosed with lymphocytic lymphomas (whole body) from [redacted] (b) (4)
covered the incidence of 3/65 (4.6%) in the reviewed study.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.

Mouse:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

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- The Committee concurred that there were no drug-related neoplasms in either male or female mice.

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

cc:\
/Division File, DPARP
/Timothy Robison, DPARP
/Jane J. Sohn, DPARP
/Leila P. Hann, DPARP
/ASeifried, OND IO

Reference ID: 3313292

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/s/

JANE J SOHN
05/23/2013

TIMOTHY W ROBISON
05/23/2013
This review replaces the version entered into DARRTS on April 25, 2013.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 203975 **Applicant:** GSK **Stamp Date:** Dec 18, 2012

Drug Name: Umeclidinium bromide/vilanterol trifenatate **NDA/BLA Type:** Original NDA

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		Yes. Studies are submitted in eCTD format.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		Yes.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		Yes.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		Yes.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		Chronic studies with umeclidinium in rats (26 weeks) and dogs (39 weeks) were conducted with magnesium stearate and lactose. For the combination of umeclidinium and vilanterol, 4-week inhalation studies in rats and dogs and a 13-week inhalation study in dogs were conducted with magnesium stearate and lactose. Chronic studies with vilanterol in magnesium stearate and lactose are being reviewed under another NDA.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		Yes. Studies are via inhalation unless otherwise justified.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		Yes. The sponsor stated in their written summary that all pivotal toxicology studies were carried out in compliance with GLP regulations.

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		No additional studies were recommended based on minutes from the pre-NDA meeting (1/18/12).
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		The proposal labeling is in the PLR format.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		To be determined in consultation with the reviewing chemist.
11	Has the applicant addressed any abuse potential issues in the submission?	NA		There appear to be no issues regarding abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	NA		Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.
None.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
None.

Dr. Jane J. Sohn 1/23/13
Reviewing Pharmacologist Date

Dr. Timothy W. Robison 1/23/13
Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

JANE J SOHN
01/23/2013

TIMOTHY W ROBISON
01/23/2013
I concur