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APPLICATION NUMBER:

206756Orig1s000

PHARMACOLOGY REVIEW(S)

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 ADDENDUM

Application number:	206756
Supporting document/s:	EDR SD #11
Applicant's letter date:	5/1/2015
CDER stamp date:	5/1/2015
Product:	STIOLTO RESPIMAT (tiotropium / olodaterol) (Combination of a long-acting muscarinic antagonist and long-acting β_2-adrenergic agonist)
Indication:	Long-term, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema
Applicant:	Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Drug Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Christine Ford

Template Version: September 1, 2010

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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 206756.

1 Executive Summary

1.1 Introduction

This addendum to the nonclinical primary reviews filed to NDA 206756 on January 23, 2015 and January 28, 2015 serves to provide additional labeling changes and supporting rationale for STIOLTO RESPIMAT (tiotropium-olodaterol inhalation spray).

1.3 Recommendations

1.3.3 Labeling

In addition to the labeling changes recommended in the reviews dated January 23, 2015 and January 28, 2015 the reviewer also suggests the change listed below. These most recent changes were proposed by the sponsor in their labeling submission #11 dated May 1, 2015. The proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

The text below now contains the final, complete labeling recommendations for sections 8.1, 12.1 and 13.1.

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Teratogenic Effects: Pregnancy Category C.
There are no adequate and well-controlled studies with STIOLTO RESPIMAT or its individual components, tiotropium bromide and olodaterol, in pregnant women. Animal reproduction studies were conducted with the individual components of STIOLTO RESPIMAT, tiotropium bromide and olodaterol. STIOLTO RESPIMAT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Tiotropium
No evidence of structural alterations was observed in rats and rabbits at approximately 790 (b) (4) and 8 (b) (4) times the recommended human daily inhalation dose (RHDID; on a mcg/m basis at maternal inhalation doses of 1471 and 7 mcg/kg/day in rats and rabbits, respectively). However, in rats, tiotropium caused fetal resorption, litter loss, decreases in the number of live pups at birth and the mean pup weights, and a delay in pup sexual maturation at approximately 40 (b) (4) times the RHDID (on a mcg/m^2 basis at a maternal inhalation dose of 78 mcg/kg/day). In rabbits, tiotropium caused an increase in post-implantation loss at approximately 430 (b) (4) times the RHDID (on a mcg/m^2 basis at a maternal inhalation dose of 400 mcg/kg/day). Such effects were not observed at approximately 5 (b) (4) and 95 (b) (4) times the RHDID (on a mcg/m^2 basis at maternal inhalation doses of 9 and 88 mcg/kg/day in rats and rabbits, respectively).

Olodaterol
Olodaterol was not teratogenic in rats at approximately 2731 times the RHDID (on an AUC basis at a maternal inhalation dose of 1054 mcg/kg/day). Placental transfer of olodaterol was observed in pregnant rats.

Olodaterol has been shown to be teratogenic in New Zealand rabbits at approximately 7130 times the RHDID in adults (on an AUC basis at a maternal inhalation dose of 2489 mcg/kg/day). Olodaterol exhibited the following fetal toxicities: enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum. No significant effects occurred at approximately 1353 times the RHDID in adults (on an AUC basis at a maternal inhalation dose of 974 mcg/kg/day).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

STIOLTO RESPIMAT
STIOLTO RESPIMAT contains both tiotropium and olodaterol. The properties described below for the individual components apply to STIOLTO RESPIMAT. These drugs represent 2 different classes of medication (an anticholinergic and a beta-agonist (b) (4)) that have different effects on clinical and physiological indices.

Tiotropium
Tiotropium is a long-acting, muscarinic antagonist 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-receptors 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-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The bronchodilation following inhalation of tiotropium is predominantly a site-specific effect.

Olodaterol
Olodaterol is a long-acting beta2-adrenergic agonist (LABA). The compound exerts its pharmacological effects by binding and activation of beta2-adrenoceptors after topical administration by inhalation. Activation of these receptors in the airways results in a stimulation of intracellular adenyl cyclase, an enzyme that mediates the synthesis of cyclic-3', 5' adenosine monophosphate (cAMP). Elevated levels of cAMP induce bronchodilation by relaxation of airway smooth muscle cells. *In vitro* studies have shown that olodaterol has 241-fold greater agonist activity at beta2-adrenoceptors compared to beta1-adrenoceptors and 2299-fold greater agonist activity compared to beta3-adrenoceptors. The clinical significance of these findings is unknown.

Beta-adrenoceptors are divided into three subtypes: beta1-adrenoceptors predominantly expressed on cardiac muscle, beta2-adrenoceptors predominantly expressed on airway smooth muscle, and beta3-adrenoceptors predominantly expressed on adipose tissue. Beta2-agonists cause bronchodilation. Although the

beta2-adrenoceptor is the predominant adrenergic receptor in the airway smooth muscle, it is also present on the surface of a variety of other cells, including lung epithelial and endothelial cells and in the heart. The precise function of beta2-receptors in the heart is not known, but their presence raises the possibility that even highly selective beta2-agonists may have cardiac effects.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

STIOLTO RESPIMAT

No studies of the carcinogenicity, *in vitro* mutagenicity, or impairment of fertility were conducted with STIOLTO RESPIMAT, however, studies are available for the individual components, tiotropium and olodaterol.

Tiotropium

No evidence of tumorigenicity was observed in a 104-week inhalation study in rats at tiotropium doses up to 59 mcg/kg/day, in an 83-week inhalation study in female mice at doses up to 145 mcg/kg/day, and in a 101-week inhalation study in male mice at doses up to 2 mcg/kg/day. These doses correspond to approximately 30, 40, and 0.5 times the recommended human daily inhalation dose (RHDID) on a mcg/m^2 basis, respectively.

Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assay in human lymphocytes *in vitro*, the mouse micronucleus assay *in vivo*, and the unscheduled DNA synthesis assay in primary rat hepatocytes *in vitro*.

In rats, decreases in the number of corpora lutea and the percentage of implants were noted at inhalation tiotropium doses of 78 mcg/kg/day or greater (approximately 35 times the RHDID on a mcg/m2 basis). No such effects were observed at 9 mcg/kg/day (approximately 4 times than the RHDID on a mcg/m^2 basis). The fertility index; however, was not affected at inhalation doses up to 1689 mg/kg/day (approximately 760 times the RHDID on a mcg/m^2 basis).

Olodaterol

Two-year inhalation studies were conducted in rats and mice to assess the carcinogenic potential of olodaterol. Lifetime treatment of female rats induced leiomyomas of the mesovarium at doses of 25.8 and 270 mcg/kg/day (approximately 18- and 198-fold, respectively, the RHDID on an AUC basis). No tumor findings were observed in male rats at doses up to 270 mcg/kg/day (approximately 230-fold the RHDID on an AUC basis). Lifetime treatment of female mice induced leiomyomas and leiomyosarcomas of the uterus at doses ≥76.9 mcg/kg/day (approximately 106-fold the RHDID on an AUC basis). No tumor findings were observed in male mice at doses up to 255 mcg/kg/day (approximately 455-fold the RHDID on an AUC basis). Increases in leiomyomas and leiomyosarcomas of the female rodent reproductive tract have been similarly demonstrated with other beta2-adrenergic agonist drugs. The relevance of these findings to human use is unknown.

Olodaterol was not mutagenic in the *in vitro* Ames test or in the *in vitro* mouse lymphoma assay. Olodaterol produced increased frequency of micronuclei in rats after intravenous doses. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. The mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Olodaterol did not impair male or female fertility in rats at inhalation doses up to 3068 mcg/kg/day (approximately 2322 times the RHDID on an AUC basis).

11 Integrated Summary and Safety Evaluation

The text shown in Section 1.3 above represents the final recommended nonclinical labeling for STIOLTO RESPIMAT under NDA 206756. Note that certain changes have been implemented when compared to the approved monoproduct labels, SPIRIVA RESPIMAT and STRIVERDI RESPIMAT. The goal of these edits was to conform to current division labeling practices and/or to harmonize text in the adjacent tiotropium and olodaterol sections of the STIOLTO RESPIMAT for the benefit of the prescriber.

In the labeling communication dated April 15, 2015, one of the nonclinical comments conveyed to the sponsor that the tiotropium dose ratios in Section 13.1 (e.g., "*These doses correspond to approximately 30, 40, and 0.5 times the recommended human daily inhalation dose (RHDID) on a mcg/m^2 basis, respectively*") had been edited to reflect calculations based on a 60 kg human weight. This change reflects current division labeling practice but deviates from the dose ratios present in the SPIRIVA RESPIMAT labeling.

In the submission dated May 1, 2015, the sponsor proposed to also modify the tiotropium dose ratios in Section 8.1 to reflect the 60 kg human weight value. This proposal was accepted by the review team and is reflected in the marked up text above.

One addition change was proposed by the sponsor in the May 1, 2015 submission. The proposal to replace (b) (4) with "beta-agonist" in the description of the mechanism of action in Section 12.1 was considered acceptable by the review team.

The final recommended labeling text shown above was included in the version sent to the sponsor on May 15, 2015.

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/s/

ANDREW C GOODWIN
05/18/2015

TIMOTHY W ROBISON
05/18/2015
I concur

Pharmacology and Toxicology Secondary Review for NDA 206-756

TO: NDA 206-756 (STIOLTO RESPIMAT; Combination of Tiotropium Bromide Monohydrate and Olodaterol Hydrochloride)

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

DATE: January 28, 2015

STIOLTO RESPIMAT is a combination product, containing the active pharmaceutical ingredients (APIs), tiotropium bromide monohydrate (an anticholinergic) and olodaterol hydrochloride (a long acting β_2-adrenergic agonist; LABA), with a proposed indication for the treatment of chronic obstructive pulmonary disease (COPD). One actuation delivers 2.5 µg each of tiotropium and olodaterol. The product is indicated for once daily dosing consisting of two actuations corresponding to a daily dose of 5 µg tiotropium and 5 µg olodaterol.

Dr. Goodwin's review dated January 23, 2015 focused on the nonclinical safety assessment of the combination of tiotropium and olodaterol. The Sponsor has previously received FDA approval of the two related monoproducts, SPIRIVA RESPIMAT (tiotropium inhalation spray, NDA 21-936) and STRIVERDI RESPIMAT (olodaterol inhalation spray, NDA 203-108). A tiotropium dry powder inhalation product, SPIRIVA HANDIHALER, has also received FDA approval (NDA 21-395).

I concur with the recommendations of Dr. Andrew Goodwin's review dated January 23, 2015 that the nonclinical pharmacology and toxicology of the drug product, a combination of tiotropium and olodaterol, have been adequately studied and STIOLTO RESPIMAT should be approved from the nonclinical perspective.

The toxicology of the tiotropium-olodaterol combination was evaluated in 4-week inhalation studies with rats and 4- and 13-week inhalation studies with dogs using a range of tiotropium: olodaterol dose ratios. In the pivotal 13-week inhalation toxicology studies with the combination in dogs, clinical signs consistent with the β_2-agonist and antimuscarinic activity of the APIs were observed, including tachycardia, mydriasis, and dry mouth. A synergistic increase in heart rate was observed, but this finding was considered to be monitorable in a clinical setting. Target organs of toxicity included the heart (decreased organ weights, gross discoloration, and fibrosis / necrosis / mineralization upon microscopic examination) and liver (glycogen depletion / increased storage). There were no novel histopathological findings attributed to the combination and the findings in the heart and liver were not considered to be dose-limiting. Gross and microscopic changes in the heart were attributed to the increased heart rate, and the changes in liver glycogen were considered a class effect for β_2-agonists such as olodaterol. In summary, toxicology studies conducted in rats and dogs with the

tiotropium-olodaterol combination did not reveal any novel toxicities of clinical concern. There was no evidence of any additive or synergistic toxicity between tiotropium and olodaterol beyond observed increases of heart rate. Further, there was no evidence of any interactions with respect to toxicokinetics. The completed nonclinical program adequately supports the proposed clinical use of the STIOLTO RESPIMAT product consisting of 5 µg per day tiotropium and 5 µg per day olodaterol. The No Observed Adverse Effects Level (NOAEL) in the pivotal dog study provided adequate systemic and local (pulmonary) safety margins compared to the proposed clinical dose levels.

The Established Pharmacological Class (EPC) for STIOLTO RESPIMAT is a combination of tiotropium, an anticholinergic, and olodaterol, a long-acting beta$_2$-adrenergic agonist (LABA).

Dr. Goodwin's reviews dated January 23 and 28, 2015 recommend changes to product labeling in Indications and Usage under Highlights of Prescribing Information, Section 1.1 (Indications and Usage), Section 8.1 (Pregnancy), Section 8.3 (Nursing Mothers), Section 10 (Overdosage), Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility). I concur with Dr. Goodwin's recommendations for changes to the product label. See Dr. Goodwin's reviews for additional details of changes to the product labeling.

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
01/29/2015

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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION ADDENDUM

Application number:	206756
Supporting document/s:	EDR SD #1 (New NDA)
Applicant's letter date:	5/22/2014
CDER stamp date:	5/22/2014
Product:	STIOLTO RESPIMAT (tiotropium / olodaterol) (Combination of a long-acting muscarinic antagonist and long-acting β_2-adrenergic agonist)
Indication:	Long-term, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema
Applicant:	Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Drug Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Christine Ford

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

This addendum to the nonclinical primary review filed to NDA 206756 on January 23, 2015 serves to provide additional recommended labeling changes for the sponsor's STIOLTO RESPIMAT (tiotropium-olodaterol inhalation spray) product.

1.3 Recommendations

1.3.3 Labeling

In addition to the labeling changes recommended in the review dated January 23, 2015, the reviewer also suggests the change listed below. The sponsor's proposed text is taken from NDA 206756 Electronic Data Room (EDR) Supporting Document (SD) #1 dated May 22, 2014. The reviewer's proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

INDICATIONS AND USAGE (under Highlights of Prescribing Information)

STIOLTO RESPIMAT is a combination of tiotropium, an anticholinergic [(b) (4)] and olodaterol, a long-acting beta$_2$-adrenergic agonist (LABA) indicated for:

The long-term, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), [(b) (4)]

1 INDICATIONS AND USAGE

1.1 Maintenance Treatment of COPD

STIOLTO RESPIMAT is a combination of tiotropium [(b) (4)] and olodaterol [(b) (4)] indicated for long-term, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.

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/s/

ANDREW C GOODWIN
01/28/2015

TIMOTHY W ROBISON
01/28/2015
I concur

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PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206756
Supporting document/s:	EDR SD #1 (New NDA)
Applicant's letter date:	5/22/2014
CDER stamp date:	5/22/2014
Product:	STIOLTO RESPIMAT (tiotropium / olodaterol) (Combination of a long-acting muscarinic antagonist and long-acting β_2-adrenergic agonist)
Indication:	Long-term, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema
Applicant:	Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Drug Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
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List of Abbreviations

ACh	Acetylcholine
ADME	Absorption, Distribution, Metabolism, Excretion
ALD	Approximate Lethal Dose
API	Active Pharmaceutical Ingredient
AUC	Area Under the Curve
BAC	Benzalkonium Chloride
BM	bone marrow
cAMP	cyclic-3', 5' Adenosine Monophosphate
CHO	Chinese Hamster Ovary
COPD	Chronic Obstructive Pulmonary Disease
DNA	Deoxyribonucleic Acid
ECAC	Executive Carcinogenicity Assessment Committee
ECG	Electrocardiography
EDR	Electronic Document Room
EDTA	Edetate Disodium
EXC	Excipients
FDA	Food and Drug Administration
FDA	Female
g	gram
GD	Gestation Day
GLP	Good Laboratory Practices
h	hour(s)
HD	High-dose
HEK	Human Embryonic Kidney
hERG	human Ether-à-go-go-Related Gene
HPLC	High-Performance Liquid Chromatography
HR	Heart Rate
ICH	International Conference on Harmonization
ICS	Inhaled Corticosteroids
IH	Inhalation
IMP	Impurities
IND	Investigational New Drug application
IV	intravenous
Kd	Dissociation constant
kg	kilogram
Ki	Inhibitor constant
LABA	Long-acting Beta-2 Adrenergic Receptor Agonist
LAMA	Long-acting Muscarinic Antagonist
LD	Low-dose
LW	Lung weight
M	Molar

M	Male
m2	square meter
mcg	microgram
MD	Mid-dose
mg	milligram
mL	milliliter
MMAD	Mass Median Aerodynamic Diameter
mol	mole
MS	Mass Spectrometry
NDA	New Drug Application
NF	National Formulary
NOAEL	No Observed Adverse Effect Level
O	Olodaterol
PDD	Pulmonary Deposited Dose
PK	Pharmacokinetics
PP	Post Partum
RHDID	Recommended Human Daily Inhalation Dose
SD	Supporting Document
T	Tiotropium
uL	microliter
USP	United States Pharmacopeia
UV	Ultraviolet

1 Executive Summary

1.1 Introduction

Boehringer Ingelheim submitted New Drug Application (NDA) 206756 on May 22, 2014 seeking approval for STIOLTO RESPIMAT. STIOLTO RESPIMAT is a combination product containing the active pharmaceutical ingredients (APIs) tiotropium bromide monohydrate and olodaterol hydrochloride with a proposed indication for the treatment of chronic obstructive pulmonary disease (COPD). The product consists on an aqueous solution delivered via inhalation using the Respimat (b) (4) device, with a proposed daily dose of 5 micrograms (mcg) tiotropium and 5 mcg olodaterol.

Throughout the review, the APIs are referred to as tiotropium and olodaterol, and dose levels are expressed in terms of the free bases. For consistency, all references to the combination dose levels and dose ratios will be expressed as tiotropium followed by olodaterol. This practice differs from certain sponsor study reports and prior reviews.

Tiotropium, code name BA 679 BR, is an anticholinergic (more specifically referred to as a long-acting muscarinic antagonist [LAMA]) that was developed by the sponsor under Investigational New Drug application (IND) (b) (4) and IND 65127 (spray). Olodaterol, code name BI 1744 CL, is a long-acting β_2-adrenergic receptor agonist (LABA) that was developed by the sponsor under IND 76362. The tiotropium-olodaterol combination product was developed under IND 76397.

The sponsor has previously received FDA approval of the two related monoproducts, SPIRIVA RESPIMAT (tiotropium inhalation spray, NDA 21936) and STRIVERDI RESPIMAT (olodaterol inhalation spray, NDA 203108). A tiotropium dry powder inhalation product, SPIRIVA HANDIHALER, has also received FDA approval (NDA 21395).

1.2 Brief Discussion of Nonclinical Findings

The nonclinical evaluation of NDA 206756 relied on data from individual studies of tiotropium and olodaterol that were previously reviewed under the INDs and NDAs referenced above. In addition, pharmacology and toxicology studies evaluating the combination of tiotropium and olodaterol were submitted to the NDA for review.

Pharmacology studies were conducted in guinea pig and dog models of acetylcholine-induced bronchospasm. Results indicated that the combination of a LAMA (tiotropium) plus a LABA (olodaterol) results in a synergistic bronchoprotection.

Tiotropium was evaluated in 52-week general toxicology studies in rats and dogs via the inhalation route of administration. Drug-related effects included inhibition of salivation / lacrimation, tachycardia and decreased gastrointestinal motility. These findings were largely attributable to exaggerated pharmacology that was consistent with other muscarinic antagonists.

Likewise, olodaterol was evaluated in 52-week inhalation toxicology studies in rats and dogs. Test article-related findings consistent with LABA class effects included anabolic (body weight gains and skeletal muscle hypertrophy / necrosis) and cardiac (tachycardia, increased organ weights, congestion / scarring) effects as well as histopathological findings in the liver (dogs) and ovaries (rats). In addition, the pancreas (rats) and trachea (rats and dogs) were identified as target organs of toxicity.

The toxicology of the tiotropium-olodaterol combination was evaluated in 4-week rat studies and 4- and 13-week dog inhalation studies at a range of tiotropium: olodaterol dose ratios. Drug-related findings were typical of the pharmacological effects of the monoproduct constituents and no novel safety concerns were identified. A synergistic increase in heart rate was observed but this finding is considered to be clinically monitorable. The No Observed Adverse Effects Level (NOAEL) in the pivotal dog study provided adequate systemic and local (pulmonary) safety margins compared to the proposed clinical dose levels.

Combination studies were not performed with respect to genotoxicity, carcinogenicity, or reproductive and developmental toxicity. Results of these studies conducted with the monoproducts are briefly highlighted below.

Tiotropium was negative in a standard battery of genotoxicity assays and no evidence of tumorigenicity was noted in two-year carcinogenicity studies in rats and mice. Tiotropium was not teratogenic but did induce embryo-fetal toxicity in both rats and rabbits.

Olodaterol was negative in two in vitro genotoxicity assays, and interpretation of the positive result in an in vivo micronucleus test was complicated by the apparent stimulation of erythropoiesis by the test article. However, the finding was considered unlikely to be relevant at clinical exposure levels. In two-year carcinogenicity studies, olodaterol induced leiomyomas and/or leiomyosarcomas in the female reproductive tract of both mice and rats. Olodaterol had no effect on fertility, embryo-fetal, or postnatal development in rats but was teratogenic in rabbits.

In summary, no new clinically relevant safety concerns were identified with regard to the combination of tiotropium and olodaterol. The sponsor has adequately characterized the pharmacology and toxicology of the STIOLTO RESPIMAT product in support of the proposed dose level of 5 mcg tiotropium and 5 mcg olodaterol per day.

1.3 Recommendations

1.3.1 Approvability

STIOLTO RESPIMAT (tiotropium-olodaterol inhalation spray) is recommended for approval from the nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

Labeling recommendations are provided in Section **1.3.3**. There are no other nonclinical recommendations or outstanding issues at this time.

1.3.3 Labeling

The reviewer's recommended labeling is provided below. The sponsor's proposed text is taken from NDA 206756 Electronic Data Room (EDR) Supporting Document (SD) #1 dated May 22, 2014. The reviewer's proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

INDICATION AND USAGE

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C.
There are no adequate and well-controlled studies with STIOLTO RESPIMAT or its individual components, tiotropium bromide and olodaterol, in pregnant women. STIOLTO RESPIMAT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Tiotropium
No evidence of structural alterations was observed in rats and rabbits at (b) (4) approximately (b) (4) times the recommended human daily inhalation dose (RHDID (b) (4) on a mcg/m^2 basis at maternal inhalation doses of 1471 and 7 mcg/kg/day in rats and rabbits, respectively). However, in rats, tiotropium caused fetal resorption, litter loss, decreases in the number of live pups at birth and the mean pup weights, and a delay in pup sexual maturation at (b) (4) approximately (b) (4) times the RHDID (on a mcg/m^2 basis at a maternal inhalation dose of 78 mcg/kg/day). In rabbits, tiotropium caused an increase in post-implantation loss at (b) (4) ~~of~~ approximately (b) (4) times the RHDID (on a mcg/m^2 basis at a maternal inhalation dose of 400 mcg/kg/day). Such effects were not observed at (b) (4) approximately (b) (4) times the RHDID (on a mcg/m^2 basis at maternal inhalation doses of 9 and 88 mcg/kg/day in rats and rabbits, respectively). (b) (4)

Olodaterol
Olodaterol was not teratogenic in rats at (b) (4) approximately 2731 times the (b) (4) RHDID (b) (4) (on an AUC basis (b) (4) t a (b) (4) maternal inhalation dose of 1054 mcg/kg/day). Placental transfer of olodaterol was observed in pregnant rats.

Olodaterol has been shown to be teratogenic in New Zealand rabbits at [redacted] approximately 7130 times the [redacted]RHDID in adults (on an AUC basis [redacted] at a [redacted] maternal inhalation dose of 2489 mcg/kg/day). Olodaterol exhibited the following fetal toxicities: enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum. No significant effects occurred at [redacted] approximately 1353 times the [redacted]RHDID in adults (on an AUC basis [redacted] at a [redacted] maternal inhalation dose of 974 mcg/kg/day).

8.3 Nursing Mothers

Clinical data from nursing women or infants exposed to STIOLTO RESPIMAT or its individual active components are not available. Tiotropium, olodaterol, and metabolites of olodaterol are excreted into the milk of lactating rats.

It is not known whether these compounds are excreted in human milk, but because many drugs are excreted in human milk and given these findings in rats, caution should be exercised if STILTO RESPIMAT is administered to a nursing woman.

10 OVERDOSAGE

[redacted]

STIOLTO RESPIMAT contains both tiotropium bromide and olodaterol; therefore, the risks associated with overdosage for the individual components described below apply to STIOLTO RESPIMAT.

Tiotropium
High doses of tiotropium may lead to anticholinergic signs and symptoms. However, there were no systemic anticholinergic adverse effects following a single inhaled dose of up to 282 mcg tiotropium in 6 healthy volunteers. In a study of 12 healthy volunteers, bilateral conjunctivitis and dry mouth were seen following repeated once-daily inhalation of 141 mcg of tiotropium.

No relevant adverse reactions, beyond dry mouth/throat and dry nasal mucosa in a dose-dependent [10-40 mcg daily] manner, were observed following 14-day dosing of up to 40 mcg tiotropium bromide inhalation solution in healthy subjects [redacted]

[redacted]

(b) (4)

Olodaterol
The expected signs and symptoms with overdosage of olodaterol are those of excessive beta-adrenergic stimulation and occurrence or exaggeration of any of the signs and symptoms, e.g., myocardial ischemia, angina pectoris, hypertension or hypotension, tachycardia, arrhythmias, palpitations, dizziness, nervousness, insomnia, anxiety, headache, tremor, dry mouth, muscle spasms, nausea, fatigue, malaise, hypokalemia, hyperglycemia, and metabolic acidosis. As with all inhaled sympathomimetic medications, cardiac arrest and even death may be associated with an overdose of olodaterol.

Treatment of overdosage consists of discontinuation of STIOLTO RESPIMAT together with institution of appropriate symptomatic and supportive therapy. The judicious use of a cardioselective beta-receptor blocker may be considered, bearing in mind that such medication can produce bronchospasm. There is insufficient evidence to determine if dialysis is beneficial for overdosage of STIOLTO RESPIMAT. Cardiac monitoring is recommended in cases of overdosage.

12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action

STIOLTO RESPIMAT
STIOLTO RESPIMAT contains both tiotropium and olodaterol. The mechanisms of action (b) (4) described below for the individual components apply to STIOLTO RESPIMAT. These drugs represent 2 different classes of medication (an anticholinergic (b) (4) and a (b) (4)) that have different effects on clinical and physiological indices. (b) (4)

Tiotropium
Tiotropium is a long-acting, muscarinic antagonist which is often referred to as an (b) (4) anticholinergic (b) (4). It has similar affinity to the subtypes of muscarinic receptors, M_1 to M_5. In the airways, it exhibits pharmacological effects through inhibition of M_3-receptors 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-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The bronchodilation following inhalation of tiotropium is predominantly a site-specific effect.

Olodaterol
Olodaterol is a long-acting $beta_2$-adrenergic agonist (LABA). The compound exerts its pharmacological effects by binding and activation of beta2-adrenoceptors after topical administration by inhalation. Activation of these receptors in the airways results in a stimulation of intracellular adenyl cyclase, an enzyme that mediates the synthesis of cyclic-3', 5' adenosine monophosphate (cAMP). Elevated levels of cAMP induce bronchodilation by relaxation of airway smooth muscle cells. *In vitro* studies have shown that olodaterol has 241-fold greater agonist activity at $beta_2$-adrenoceptors compared to $beta_1$-adrenoceptors and 2299-fold greater agonist activity compared to $beta_3$-adrenoceptors. The clinical significance of these findings is unknown.

Beta-adrenoceptors are divided into three subtypes: $beta_1$-adrenoceptors predominantly expressed on cardiac muscle, $beta_2$-adrenoceptors predominantly expressed on airway smooth muscle, and $beta_3$-adrenoceptors predominantly expressed on adipose tissue. $Beta_2$-agonists cause bronchodilation. Although the $beta_2$-adrenoceptor is the predominant adrenergic receptor in the airway smooth muscle, it is also present on the surface of a variety of other cells, including lung epithelial and endothelial cells and in the heart. The precise function of $beta_2$-receptors in the heart is not known, but their presence raises the possibility that even highly selective $beta_2$-agonists may have cardiac effects.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

STIOLTO RESPIMAT
No studies of the carcinogenicity, *in vitro* mutagenicity, or impairment of fertility were conducted with STIOLTO RESPIMAT, however, studies are available for the individual components, tiotropium and olodaterol.

(b) (4)

Tiotropium

No evidence of tumorigenicity was observed in a 104-week inhalation study in rats at tiotropium doses up to (b) (4) 59 mcg/kg/day, in an 83-week inhalation study in female mice at doses up to (b) (4) 145 mcg/kg/day, and in a 101-week inhalation study in male mice at doses up to (b) (4) 2 mcg/kg/day. These doses correspond to approximately 30 (b) (4), 40 (b) (4), and 0.5 (b) (4) times the recommended human daily inhalation dose (RHDID) on a mcg/m^2 basis, respectively. (b) (4)

Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assay (b) (4) in human lymphocytes *in vitro*, (b) (4) the mouse micronucleus assay (b) (4) *in vivo*, and the unscheduled DNA synthesis assay in primary rat hepatocytes *in vitro* (b) (4).

In rats, decreases in the number of corpora lutea and the percentage of implants were noted at inhalation tiotropium doses of (b) (4) 78 mcg/kg/day or greater (approximately 35 (b) (4) times the RHDID on a mcg/m^2 basis). No such effects were observed at (b) (4) 9 mcg/kg/day (approximately 4 (b) (4) times than the RHDID on a mcg/m^2 basis). The fertility index; however, was not affected at inhalation doses up to 1 (b) (4) 689 mcg/kg/day (approximately 760 (b) (4) times the RHDID on a mcg/m^2 basis). (b) (4).

Olodaterol

Two-year inhalation studies were conducted in rats and mice to assess the carcinogenic potential of olodaterol. Lifetime treatment of female rats induced leiomyomas of the mesovarium at doses of 25.8 and 270 mcg/kg/day (approximately 18- and 198-fold, respectively, the (b) (4) RHDID on an AUC basis). No tumor findings were observed in male rats at doses up to 270 mcg/kg/day (approximately 230-fold the (b) (4) RHDID on an AUC basis). Lifetime treatment of female mice induced leiomyomas and leiomyosarcomas of the uterus at doses ≥76.9 mcg/kg/day (approximately 106-fold the (b) (4) RHDID on an AUC basis). No tumor findings were observed in male mice at doses up to 255 mcg/kg/day (approximately 455-fold the (b) (4) RHDID on an AUC basis). Increases in leiomyomas and leiomyosarcomas of the female rodent reproductive tract have been similarly demonstrated with other beta$_2$-adrenergic agonist drugs. The relevance of these findings to human use is unknown.

Olodaterol was not mutagenic in the *in vitro* Ames test or in the *in vitro* mouse lymphoma assay. Olodaterol produced increased frequency of micronuclei in rats after intravenous doses. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. The mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Olodaterol did not impair male or female fertility in rats at inhalation doses up to 3068 mcg/kg/day (approximately 2322 times the (b) (4) RHDID on an AUC basis).

2 Drug Information

2.1 Drug

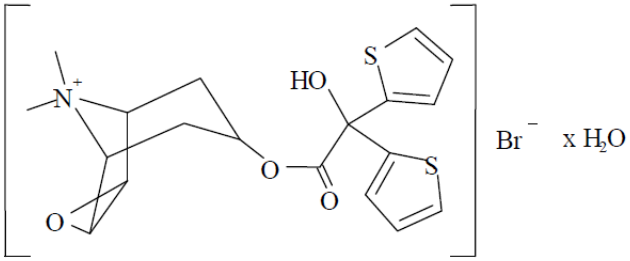
Generic Name: **Tiotropium bromide monohydrate**

Code Name: BA 679 BR

Chemical Name: (1α, 2β, 4β, 5α, 7β)-[(hydroxydi-2-thienylacetyl)-9,9-dimethyl-3-oxa-9-azoniatrcyclo[3.3.1.0]nonane
bromide monohydrate

Molecular Formula/Molecular Weight: $C_{19}H_{22}NO_4S_2Br.H_2O$ / 490.(b)(4) g/mol [salt]

Structure or Biochemical Description



Pharmacologic Class: Anticholinergic

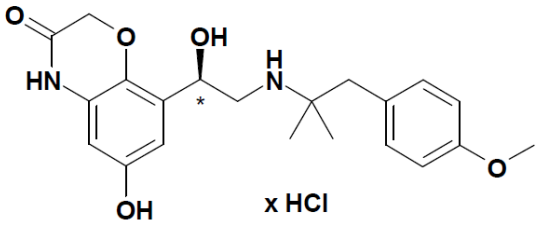
Generic Name: **Olodaterol hydrochloride**

Code Name: BI 1744 CL

Chemical Name: 2H-1,4-Benzoxazin-3H(4H)-one,6-hydroxy-8-[(1R)-1-hydroxy-2-[[2-(4-methoxyphenyl)-1,1-dimethylethyl]amino]ethyl]-, monohydrochloride

Molecular Formula/Molecular Weight: $C_{21}H_{26}N_2O_5$ x HCl / or 422.9 (b)(4) g/mol

Structure or Biochemical Description



Pharmacologic Class: β_2-adrenergic agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND (b)(4)
- IND 65127 (tiotropium spray)
- IND 76362 (olodaterol spray)
- IND 76397 (tiotropium / olodaterol combination development program)
- NDA 21395 (SPIRIVA HANDIHALER, tiotropium dry powder formulation)

- NDA 21936 (SPIRIVA RESPIMAT, tiotropium spray formulation)
- NDA 203108 (STRIVERDI RESPIMAT, olodaterol spray formulation)

2.3 Drug Formulation

STIOLTO RESPIMAT consists of a sterile aqueous solution containing two APIs, tiotropium and olodaterol. The solution is packaged in 4.5 mL cartridges (60 actuations plus technically required overfill) for delivery via inhalation using the Respimat (b) (4) inhaler device. Each actuation delivers 2.5 mcg each tiotropium and olodaterol. The product is indicated for once daily dosing consisting of two actuations, corresponding to a daily dose of 5 mcg tiotropium and 5 mcg olodaterol. Each dose represents a (b) (4) volume of (b) (4) uL and a delivered volume ex mouthpiece of 22.1 uL.

Table 1. Quantitative formulation of STIOLTO RESPIMAT Inhalation Spray

Ingredient	mg per actuation	% of formula (w/v)[4]	mg per 4.5 mL cartridge	Function	Reference standard
Tiotropium bromide monohydrate	0.003124	(b) (4)	(b) (4)	Drug substance	In-house
Tiotropium equivalent[1]	*0.0025*				
Olodaterol hydrochloride	0.002736			Drug substance	In-house
Olodaterol equivalent[2]	*0.0025*				
Benzalkonium chloride[3]	(b) (4)			(b) (4)	NF
Edetate disodium					USP
Hydrochloric acid (b) (4)					In-house[5]
Water for injection					USP
Total mass		*100.0*			(b) (4)

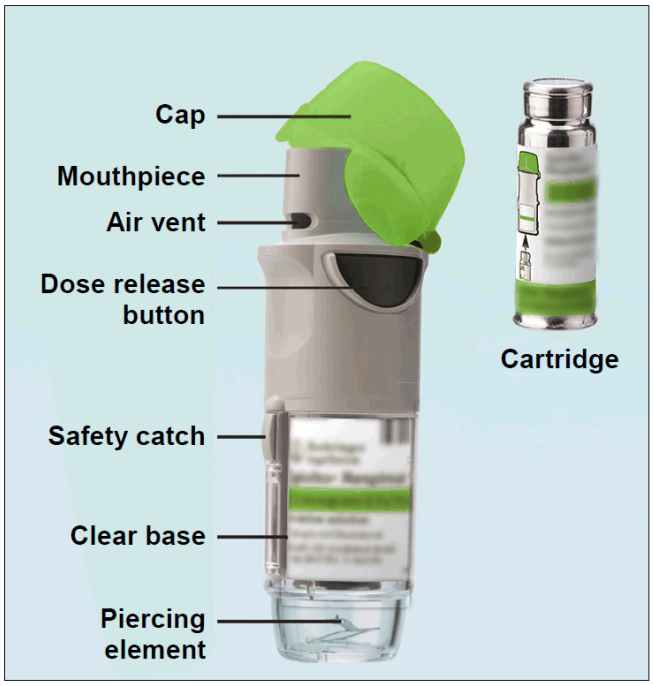
Table adapted from applicant material (Quality Overall Summary, Table 2, page 9, q00204190-04)
[1]Salt conversion factor 1.2494
[2]Salt conversion factor 1.0945
(b) (4)
Equivalent to grams per 100 mL
(b) (4)

(b) (4)

The Respimat (b) (4) device produces an (b) (4) The inhaler is used for several FDA-approved products

including the STIOLTO RESPIMAT monoproduct constituents SPIRIVA RESPIMAT (tiotropium, NDA 21936) and STRIVERDI RESPIMAT (olodaterol, NDA 203108), as well as COMBIVENT RESPIMAT (albuterol / ipratropium, NDA 21747).

Figure 1. Illustration of the Respimat Cartridge and Inhaler



Note the light green color specifically denotes the tiotropium/olodaterol combination
Sponsor's Figure 1, page 7, Quality Overall Summary (2.3).

2.4 Comments on Novel Excipients

STIOLTO RESPIMAT contains the excipients benzalkonium chloride and edetate disodium (EDTA) at concentrations of (b) (4)% each. These levels are equal to the concentrations in the approved monoproducts and are less than or equal to the amount present in other approved products. There are no nonclinical concerns with regards to excipients in the proposed product.

2.5 Comments on Impurities/Degradants of Concern

The toxicological qualification of tiotropium and olodaterol impurities and degradants has been addressed previously under NDA 21395 (see reviews dated August 28, 2002, December 8, 2003, May 13, 2004 and January 23, 2006), NDA 21936 (see review dated May 6, 2008) and NDA 203108 (see review dated June 27, 2012).

There are no tiotropium or olodaterol drug substance impurities present at levels that exceed the ICH Q3A qualification threshold of 0.15%. No new tiotropium or olodaterol active ingredient degradation products were observed in STIOLTO RESPIMAT

compared to the individual monoproducts. The specifications for the degradants listed in the table below are consistent with the approved monoproducts and the corresponding daily exposure levels to these compounds have been considered qualified (see reviews listed above).

Table 2. Summary of STIOLTO RESPIMAT Active Ingredient Degradants

Parent	Degradant	Specification	Daily Limit	Method
Tiotropium	(b) (4)	≤ (b) (4) %	(b) (4) mcg	HPLC-UV
	(b) (4)	≤ (b) (4) %	(b) (4) mcg	HPLC-UV
	(b) (4)	≤ (b) (4) %	(b) (4) mcg	HPLC-MS
	(b) (4)	≤ (b) (4) %	(b) (4) mcg	HPLC-MS
	Sum of All	≤ (b) (4) %	(b) (4) mcg	
Olodaterol	(b) (4)	≤ (b) (4) %	(b) (4) mcg	HPLC-UV
	(b) (4)	≤ (b) (4) %	(b) (4) mcg	HPLC-UV
	Unspecified	≤ (b) (4) %	(b) (4) mcg	HPLC-UV
	Sum of all	≤ (b) (4) %	(b) (4) mcg	

(b) (4)

Table generated by reviewer from sponsor's data.

In summary, there are no nonclinical concerns with regard to impurities or degradants in STIOLTO RESPIMAT.

2.6 Proposed Clinical Population and Dosing Regimen

The sponsor proposes that STIOLTO RESPIMAT be indicated for "the longterm, once-daily maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema." The proposed dosing regimen is a single daily dose, consisting of two actuations, corresponding to 5 mcg tiotropium and 5 mcg olodaterol. The product is delivered via the inhalation route of administration using the Respimat (b) (4) inhaler device.

2.7 Regulatory Background

Tiotropium (BA 679 BR) powder for inhalation was developed under (b) (4), opened November 30, 1994. The corresponding NDA 21395 was originally filed on December 12, 2001 and the product was ultimately approved on January 30, 2004 with the tradename SPIRIVA HANDIHALER.

Tiotropium inhalation spray was developed under IND 65127, opened June 27, 2002. The corresponding NDA 21936 was originally filed on November 16, 2007 and the product was ultimately approved on September 24, 2014 with the tradename SPIRIVA RESPIMAT.

Olodaterol (BI 1744 CL) inhalation spray was developed under IND 76362, opened January 26, 2007. The corresponding NDA 203108 was originally filed May 14, 2012

and the product was ultimately approved on July 31, 2014 with the tradename STRIVERDI RESPIMAT.

The tiotropium-olodaterol combination product inhalation spray has been developed under IND 76397. A pre-IND meeting was held November 27, 2007 (meeting minutes dated December 31, 2007) and the IND was opened March 27, 2008. An End of Phase 2 meeting was held July 20, 2011 (meeting minutes dated August 4, 2011). NDA 206756 was submitted on May 22, 2014 and the sponsor's proposed tradename of STIOLTO RESPIMAT was granted on September 2, 2014.

3 Studies Submitted

3.1 Studies Reviewed

The studies listed in the following table have been reviewed under NDA 206756. Each of these studies evaluated the combination of tiotropium and olodaterol.

Table 3. Nonclinical Studies Reviewed Under NDA 206756

Document Number	Study Number	Review Section	Study Title
U05-2588	2005/LUI/Lab 3/ Report 1	4.1	Bronchoprotective effects of BI 1744 CL in combination with tiotropium bromide in a model of acetylcholine-induced bronchoconstriction in anaesthetized Beagle dogs over 24 hours
U06-2153	2006/LUI/Lab 5/ Report 5	4.1	Investigation of combination between BI 1744 CL and tiotropium bromide, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm for 24 hours in guinea pigs
N00173793-03	2005/LUIII/Lab 5/ Report 8	4.1	Investigation of BI 1744 CL in combination with tiotropium bromide, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm in anaesthetized dogs for 3 h
666455 AM1	666455 AM1	4.3	BI 1744 CL and Ba 679 BR (1:1, 1:2 and 6:1 Combinations): Cardiovascular Safety Pharmacology in Telemetered Dogs Report Amendment 1
U06-1184	05B099	6.1	BI 1744 CL and tiotropium bromide (Ba 679 BR): Acute toxicity - Acute Toxic Class Method - in mice by inhalation
U06-1201	05B194	6.1	BI 1744 CL and tiotropium bromide (Ba 679 BR) (1:2): Acute toxicity - Acute Toxic Class Method - in mice by inhalation
U06-1197	05B192	6.1	BI 1744 CL and tiotropium bromide (Ba 679 BR) (6:1): Acute toxicity - Acute Toxic Class Method - in mice by inhalation
U06-1182	05B098	6.1	BI 1744 CL and tiotropium bromide (Ba 679 BR): Acute toxicity - Acute Toxic Class Method - in rats by inhalation
U06-1199	05B193	6.1	BI 1744 CL and tiotropium bromide (Ba 679 BR) (1:2): Acute toxicity - Acute Toxic Class Method - in rats by inhalation
U06-1185	05B191	6.1	BI 1744 CL and tiotropium bromide (Ba 679 BR) (6:1): Acute toxicity - Acute Toxic Class Method - in rats by

			inhalation

3.2 Studies Not Reviewed

The studies listed in the following table were submitted to or cross-referenced in NDA 206756 but were not reviewed, as they were not pivotal to the nonclinical assessment of the tiotropium-olodaterol inhalation spray product.

Document Number	Study Number	Test Article	Description	Report Location
4.2.1.4 Pharmacodynamic Drug Interactions				
n00145337	2010-rdr1-lab4-report2	T+O+ICS	Triple combo IH study for antagonism of acetylcholine-induced bronchospasm in anesthetized dogs	NDA 206756
U11-1148-02	2011-rdr1-lab4-report1	T+O+ICS	Triple combo study for antagonism of ovalbumin-induced bronchospasm in guinea pigs	NDA 206756
4.2.2.1 Analytical Methods and Validation Reports				
U04-1855-03	V221/04HJ	O	HPLC-MS/MS for quantification in dog plasma	NDA 203108
U04-1874-03	V222/04BD	O	HPLC-MS/MS for quantification in rat plasma	NDA 203108
U05-2164-02	V270/05HJ	T	HPLC-MS/MS for quantification in dog plasma	NDA 21936
U05-2212-02	V282/05HR	T	HPLC-MS/MS for quantification in rat plasma	NDA 206756
4.2.2.6 Pharmacokinetic Drug Interactions				
U05-2446	O5B135, B2630, B2662	T+O	PK interaction after single IH dose in dog	NDA 203108
U05-2470	05B136, B2629, B2663	T+O	PK interaction after single IH dose in rat	NDA 203108
4.2.3.2 Repeat-dose Toxicity				
n00177290	05B064	T+O	4-week escalating dose Dog IH (non-GLP, 1:1, 2:1, 1:6 ratios)	NDA 206756
4.2.3.7.7 Toxicology - Other				
U09-2354	08B070	T+O+ICS	4-week Dog IH (ICS = BI 54903)	NDA 206756
U10-1530	08B071	T+O+ICS	4-week Rat IH (ICS = BI 54903)	NDA 206756
U07-1397	422786	T+O	Analytical method for measuring T+O in IH studies	NDA 206756

T = tiotropium bromide = BA 679 BR
O = olodaterol hydrochloride = BI 1744 CL
ICS = inhaled corticosteroid
PK = pharmacokinetics
IH = inhalation

3.3 Previous Reviews Referenced

The following table summarizes studies that were submitted to or cross-referenced in NDA 206756 and have been previously reviewed by the Division under the INDs and NDAs for tiotropium, olodaterol or the combination program.

Document Number	Study Number	Test Article	Description	Review Reference
4.2.1.1 Primary Pharmacodynamics				
U04-1553	2004/LUIII/Lab 5/ Report 1	O	IH: Antagonistic effects against acetylcholine-induced bronchospasms for 5h in guinea pigs	NDA 203188 1/17/2013
4.2.1.3 Safety Pharmacology				
U01-1720	GP2001/254/275/PH2	T	HEK293 hERG assay and effect on action potential configuration in isolated guinea pig papillary muscle	NDA 21396 7/29/2008
U04-1115	GP2003-0175/0181/PH2	O	HEK293 hERG assay and effect on action potential configuration in isolated guinea pig papillary muscle	NDA 203188 1/17/2013
4.2.3.2 Repeat-dose Toxicity				
U06-1893	665912	T+O	4-week Rat IH (1:1 ratio)	IND 76397 4/25/2008
U06-1892	665928	T+O	4-week Rat IH (2:1 and 1:6 ratios)	IND 76397 4/25/2008
U06-1894	665933	T+O	4-week Dog IH (1:1 ratio)	IND 76397 4/25/2008
U06-1895	665949	T+O	4-week Dog IH (2:1 and 1:6 ratios)	IND 76397 4/25/2008
U09-2249	07B077	T+O	13-week Dog IH (1:1 ratio)	IND 76397 6/24/2010
U10-1388	07B183	T+O	13-week Dog IH (2.5:1 and 1:2 ratio)	IND 76397 6/24/2010
4.2.3.7.6 Other Toxicity Studies - Impurities/ Excipients				
[redacted] (b) (4)				
U98-2224	i96003	EXC	2-week Rat IH (BAC, EDTA)	NDA 21747 4/14/2009
U97-2076	i52	EXC	2-week Dog IH (BAC, EDTA)	NDA 21747 4/14/2009

U06-1186	664458-23459	EXC	13-week Rat IH (BAC, EDTA)	NDA 21747 4/14/2009
U98-2727	653315	EXC	24-month Rat Carcinogenicity IH (BAC)	NDA 21395 9/20/2002
U97-2570	i96028	EXC	13-week Rat IH (BAC, EDTA)	NDA 21747 4/14/2009

T = tiotropium bromide = BA 679 BR
O = olodaterol hydrochloride = BI 1744 CL
IMP = Impurities
EXC = excipients
BAC = benzalkonium chloride
EDTA = edetate disodium
BM = bone marrow

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology of the constituents of STIOLTO RESPIMAT has been characterized and reviewed previously under NDAs 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol).

The sponsor has conducted three in vivo pharmacology studies evaluating the combination of tiotropium and olodaterol in dogs and guinea pigs. In each study, the combination of tiotropium plus olodaterol resulted in synergistic bronchoprotection in acetylcholine-challenged animals.

Title: Investigation of combination between BI 1744 CL and tiotropium bromide, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm for 24 hours in guinea pigs
Study number: 2006/ LU III/ Lab 5/ Report 5
Document number: U06-2153
Report location: NDA 203108 (SD #1, May 14, 2012)
Test facility: Boehringer Ingelheim, Biberach an der Riss, Germany
Report date: November 27, 2006
Non-GLP

Methods

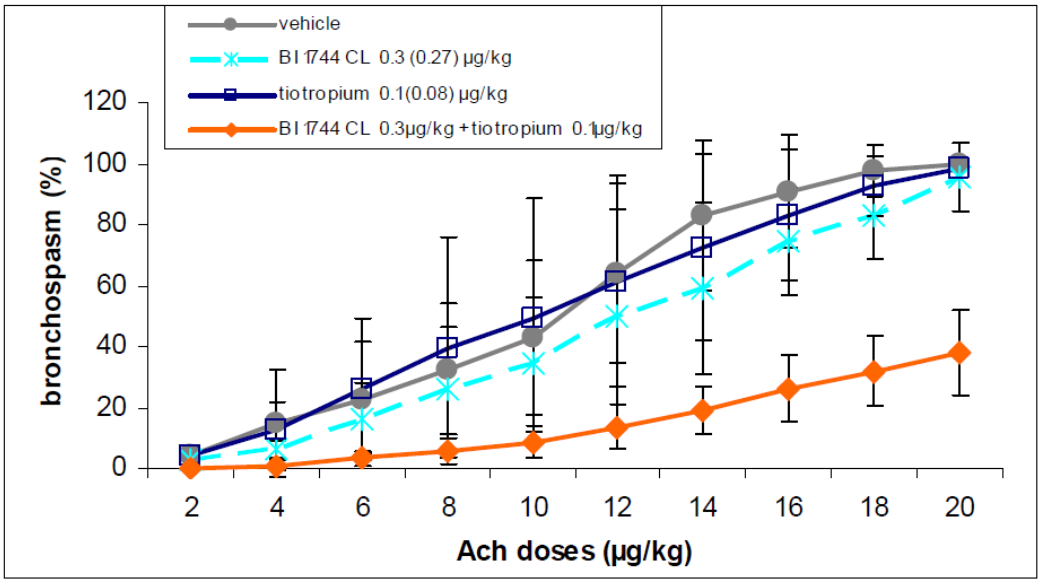
Male and female Dunkin-Harley guinea pigs (N= 4 to 6 per treatment group, body weight 400-500 g) were used in this study. Animals were allocated to receive vehicle, olodaterol (0.1, 0.3, 1 or 3 mcg/kg), tiotropium (0.1, 0.3, 1, or 3 mcg/kg) or the combination (0.1 mcg/kg tiotropium plus 0.3 or 1 mcg/kg olodaterol). The test articles were administered by tracheal instillation under slight isoflurane anesthesia. 24 hours later, animals were anesthetized by intraperitoneal injection with pentobarbital and challenged with acetylcholine (ACh). Increasing doses of ACh were tested from 2 mcg/kg up to a maximum of 20 mcg/kg (increasing every 10 minutes in 2 mcg/kg increments). The monoproducts were used at doses producing <20% bronchoprotection

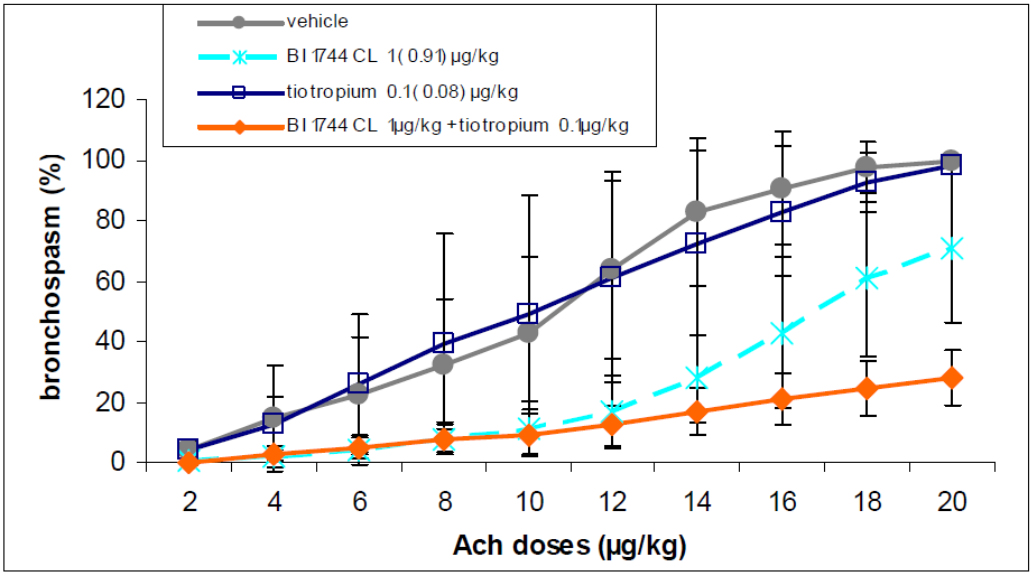
in order to be able to assess the potential synergistic effects of the tiotropium and olodaterol combination.

Results

The combination of tiotropium (0.1 mcg/kg) and olodaterol (0.3 or 1.0 mcg/kg) resulted in 70-80% bronchoprotection, which was synergistic compared to the results with the monoproducts at the same dose levels.

Figure 2. Bronchoprotection in Acetylcholine-challenged Guinea Pigs





Figures excerpted from sponsor's study report

Title: Investigation of BI 1744 CL in combination with tiotropium bromide, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm in anaesthetized dogs for 3 h

Study number: 2005/ LU III/ Lab 5/ Report 8
Document number: n00173793-03
Report location: NDA 206756 (SD #1, May 22, 2014)
Test facility: Boehringer Ingelheim, Biberach an der Riss, Germany
Report date: March 10, 2014
Experimental dates: January 2003 – June 2005
Non-GLP
Note: a previous version of this report dated December 14, 2005 was submitted as document U05-2587 to NDA 203108 (SD #1, May 14, 2012).

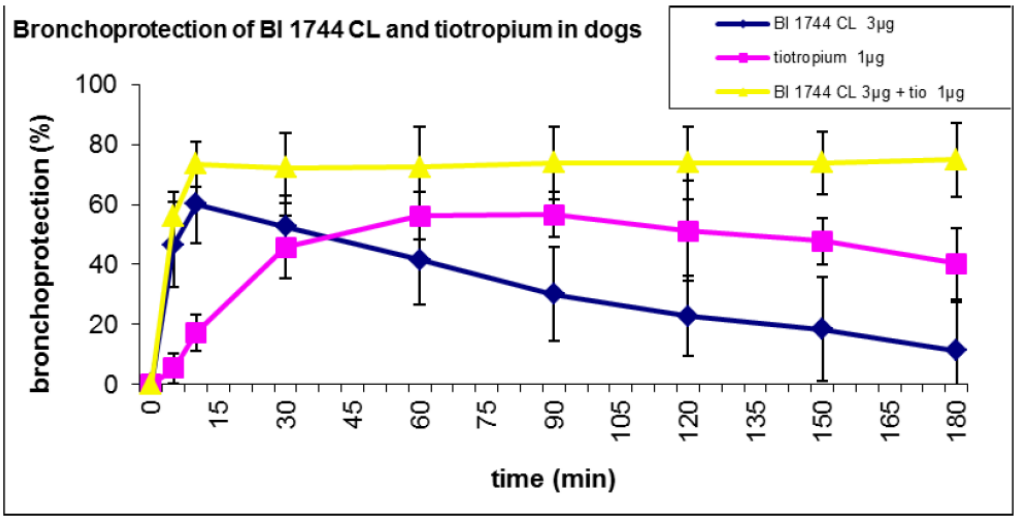
Methods

Beagle dogs (N= 4 per group, body weights 9.1-16.9 kg, ages unspecified) were anesthetized with propofol, intubated, and ventilated. After a 30-minute control period, ACh injections (10 mcg/kg intravenously [IV], expected to induce ~30% increase in bronchial resistance) were administered at -45, -30, -15, 5, 10, 30, 60, 90, 120, 150, and 180 minutes relative to treatment with the test articles. Bronchoprotection was determined based on the degree of inhibition of the ACh-induced increase in pulmonary resistance. Tiotropium (1 mcg) and olodaterol (3 mcg) were administered via inhalation at sub-optimal doses in order to allow for the detection of potential synergistic effects.

Results

The combination of tiotropium and olodaterol exhibited a rapid and synergistic bronchoprotective effect, as shown in the sponsor's figure below. 72-75% Bronchoprotection was observed consistently across all time points from 10-180 minutes. Animals receiving vehicle are not included in the figure but mean bronchoprotection scores ranged from -6 to 2% in the control group over the three-hour period. The combination also did not have any novel effects on other measured parameters such as heart rate.

Figure 3. Bronchoprotection in Acetylcholine-challenged Dogs over 3 hours



BI 1744 CL is the code name for olodaterol
Figure excerpted from sponsor's study report

Title: Bronchoprotective effects of BI 1744 CL in combination with tiotropium bromide in a model of acetylcholine-induced bronchoconstriction in anaesthetized Beagle dogs over 24 hours

Study number: 2005/ LU III/ Lab3/ Report 1
Document number: U05-2588
Report location: NDA 203108 (SD #1, May 14, 2012)
Test facility: Boehringer Ingelheim, Biberach an der Riss, Germany
Report date: January 12, 2006
Non-GLP

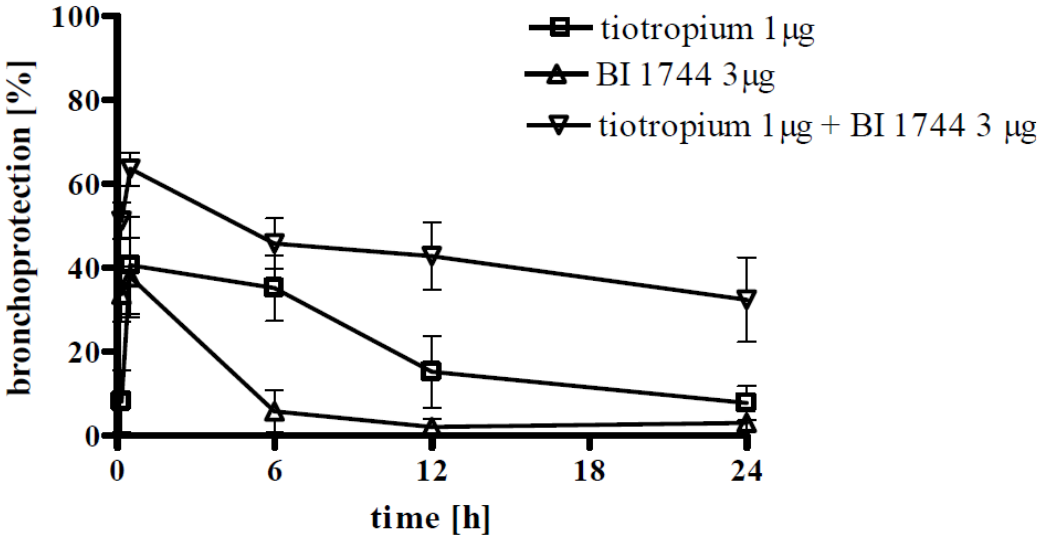
Methods

Beagle dogs (N= 3 or 4 per group, 11.1-18.2 kg, ages unspecified) were randomized to inhalation treatment with tiotropium (1 mcg), olodaterol (3 mcg), olodaterol (6 mcg), tiotropium (1 mcg) + olodaterol (3 mcg) or tiotropium (1 mcg) + olodaterol (6 mcg). Measurements for respiratory resistance, respiratory pressure, and dynamic lung compliance were obtained 10 minutes, 30 minutes, 6 hours, 12 hours, and 24 hours post-dose. Animals were anesthetized via IV propofol at each time point but were allowed to awaken between the final three time points. Bronchoconstriction was induced at each time point via two IV injections of 10 mcg/kg ACh spaced by 15 minutes. Bronchoprotection was determined based on the degree of inhibition of the ACh-induced increase in pulmonary resistance. The monoproduct doses were selected to produce 40-50% bronchoprotection in order to be able to detect a potential synergistic effect.

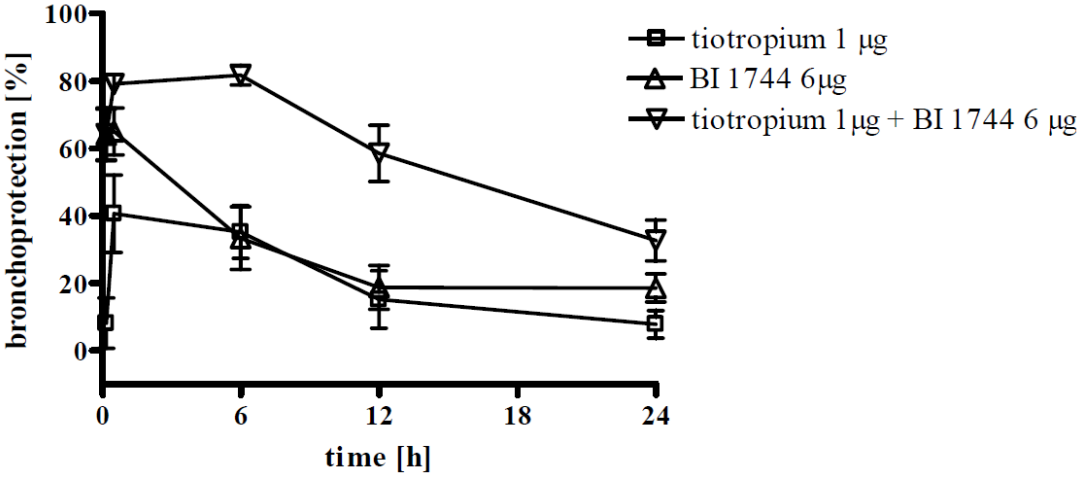
Results

A synergistic bronchoprotective effect was seen when dogs were administered 1 mcg tiotropium and 3 mcg or 6 mcg olodaterol compared to monoproduct treatment at those doses (see sponsor's figures below). Maximum bronchoprotection observed in animals receiving the combination was 64% and 82%, respectively, for the two dose levels. The combination did not have notable effects on heart rate in this study.

Figure 4. Bronchoprotection in Acetylcholine-challenged Dogs over 24 hours



Reviewer note: no measurements were taken between 30 minutes and 6 hours, so the apparent drop-off in effect compared to the 3-hour study reviewed above may be misleading.



BI 1744 CL is the code name for olodaterol. Figures excerpted from sponsor's study report

4.2 Secondary Pharmacology

The secondary pharmacology of the constituents of STIOLTO RESPIMAT have been characterized and reviewed previously under NDAs 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol).

Effects of the combination of tiotropium plus olodaterol on heart rate and plasma potassium, lactate, and glucose was assessed in dogs as part of the primary pharmacology studies reviewed above (U05-2588 and n00173793-03). There were no notable effects.

4.3 Safety Pharmacology

The safety pharmacology of the constituents of STIOLTO RESPIMAT have been characterized and reviewed previously under NDAs 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol).

The sponsor conducted a cardiovascular safety pharmacology study evaluating the combination of tiotropium plus olodaterol in dogs.

Title: BI 1744 CL and Ba 679 BR (1:1, 1:2 and 6:1 Combinations): Cardiovascular Safety Pharmacology in Telemetered Dogs Report Amendment 1
Study number: 666455 AM1
Document number: 666455 AM1
Report location: NDA 206756 (SD #1, May 22, 2014)
Test facility: (b) (4)
Report date: June 3, 2008
GLP-compliant: Yes

Methods

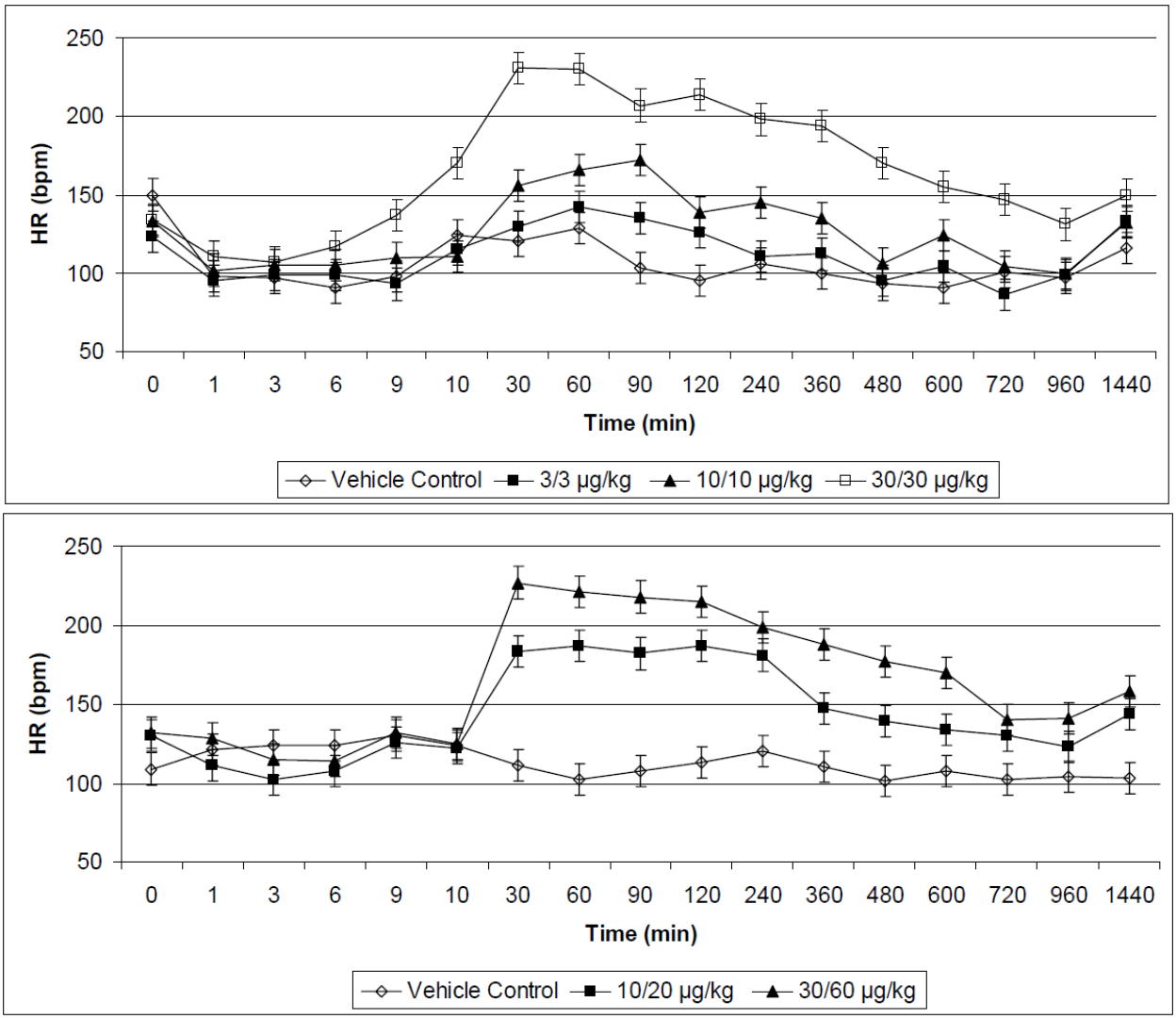
Two male and two female Beagle dogs underwent surgical implantation of telemeters. Animals received Ba 679 BR (tiotropium) and BI 1744 CL (olodaterol) at target inhalation doses of 3/3, 10/10, 30/30, 20/10, 60/30, and 5/30 mcg/kg. A minimum 7-day washout period was employed between dose administrations. Electrocardiography (ECG) recordings were obtained before dosing and for 24 hours post-dose. Additional observations included clinical signs, body weights, food consumption, and toxicokinetics.

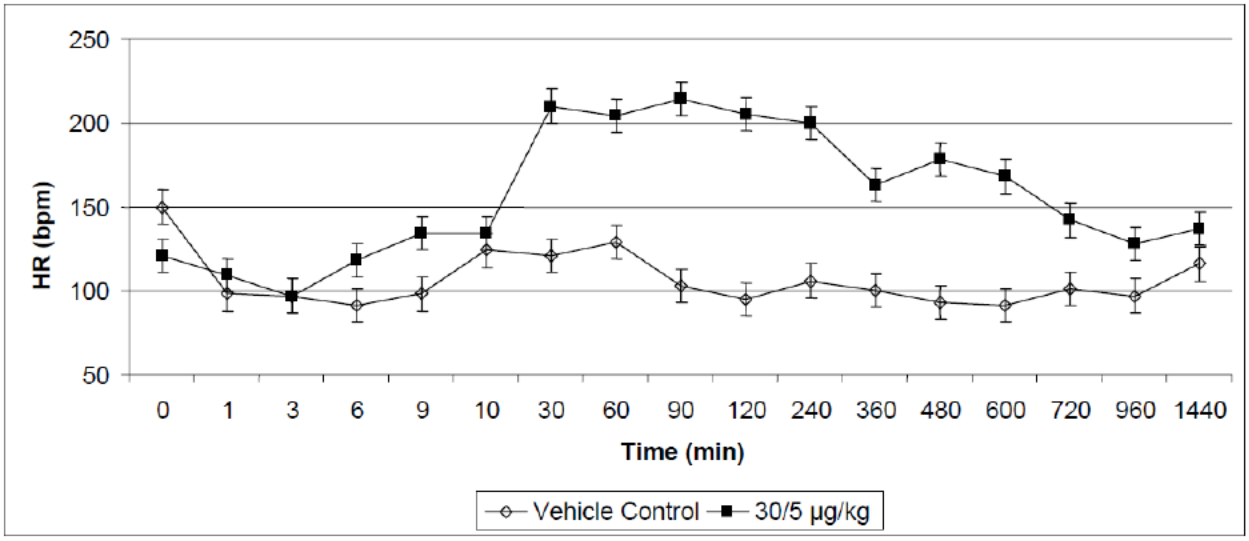
Results

There were no test article-related effects on body weights or food consumption. Achieved doses were within ~15% of the target dose levels and Mass Median Aerodynamic Diameters (MMADs) were in the respirable range for dogs (2.3-3.1 uM for each test article).

No novel cardiovascular effects were attributed to the combination. The only observed effects were consistent with past experience with olodaterol and other β_2-agonists. Specifically, statistically significant increases in heart rate (HR) were noted in all dose groups, with peak effects in the range of 0.5-4 hours post-dose. A corresponding decrease in mean arterial blood pressure of 20-30 mmHg was also observed.

Figure 5 Effect of Tiotropium plus Olodaterol on Heart Rate in Beagle Dogs





Figures excerpted from sponsor's study report.

5 Pharmacokinetics/ADME/Toxicokinetics

The pharmacokinetics and absorption, distribution, metabolism and excretion (ADME) of the constituents of STIOLTO RESPIMAT have been characterized and reviewed previously under NDAs 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol).

6 General Toxicology

6.1 Single-Dose Toxicity

The acute toxicity of the tiotropium-olodaterol combination was evaluated in a total of six studies in mice and rats, as summarized in the table below. Tiotropium and olodaterol were administered via the inhalation route of exposure at 1:1, 2:1, or 1:6 dose ratios.

In mice, the approximate lethal doses (ALD) were ≥33.8 mg/kg tiotropium plus 35.5 mg/kg olodaterol (1:1 ratio study), 41.9 mg/kg tiotropium plus 22.3 mg/kg olodaterol (2:1 ratio study), and ≥2.5 mg/kg tiotropium plus 16.4 mg/kg olodaterol (1:6 ratio study). The lowest lethal dose, observed in the 1:6 ratio study, represents tiotropium and olodaterol doses that are >2400 and >15000 times the proposed human dose on a mg/kg basis, respectively.

Table 4. Tiotropium / Olodaterol Single-dose Inhalation Toxicity Studies in Mice

Study	Methods	Findings
Document U06-1184 Study 05B099	• GLP-compliant • 8-week old Crl:NMRI mice • 1:1 (T:O) target dose ratio • 4-hour inhalation exposure followed by 14-day	• Mortality: 2/5 M, 0/5 F • Male deaths on Day 2 and Day 3 (poor condition, reduced breathing, back limb spasms) considered test article-related and attributed to exaggerated

Report Date[#] October 24, 2006	observation period • 5 animals per sex at the maximum technically feasible dose* -T dose: 33.6 mg/kg (M); 35.8 mg/kg (F) -O dose: 35.5 mg/kg (M); 37.6 mg/kg (F)	pharmacology • Clinical signs: mydriasis with or without pupillary rigidity thru Day 9 in all animals. Less frequent findings thru Day 5 included closed eyes, decreased activity, abdominal breathing, hunched posture, rough fur. • Male ALD = 33.8 mg/kg T plus 35.5 mg/kg O • Female ALD > 35.8 mg/kg T plus 37.6 mg/kg O
Document U06-1201 Study 05B194 Report Date June 27, 2006	• GLP-compliant • 9-week old Crl:NMRI mice • 2:1 (T:O) target dose ratio • 4-hour inhalation exposure followed by 14-day observation period • 5 animals per sex at the maximum technically feasible dose • T dose: 40.1 mg/kg (M); 43.7 mg/kg (F) • O dose: 21.4 mg/kg (M); 23.3 mg/kg (F)	• Mortality: 1/5 males died Day 1, 1/5 females died Day 3, attributed to exaggerated pharmacology. • Clinical signs: mydriasis / pupillary rigidity (to Day 5), closed eyes / ataxia (Day 1), dyspnea / sedation (to Day 4-5), rough coats (to Day 8) • Macroscopic finding of gaseous gastrointestinal tract in early deaths • ALD = 41.9 mg/kg T plus 22.3 mg/kg O (averaged for males and females)
Document U06-1197 Study 05B192 Report Date June 27, 2006	• GLP-compliant • 8-week old Crl:NMRI mice • 1:6 (T:O) target dose ratio • 4-hour inhalation exposure followed by 14-day observation period • 5 animals per sex per group (doses in mg/kg*) -LD: 2.5 (T) + 16.4 (O) -MD: 4.5 (T) + 29.2 (O) -HD: 10.3 (T) +66.0 (O)	• Mortality: 1/5 MDM, 1/5 HDM, 3/5 HDF died within 4 days. Remaining HD animals euthanized in moribund condition on Day 4. • Clinical signs: mydriasis, pupillary rigidity, closed eyes, sedation, rough coat, dyspnea, ataxia, peripheral hyperemia, hyperthermia, hunched posture (dose-related in incidence and severity) • Macroscopic findings in premature deaths of dilitation of the gastrointestinal tract and "pink-yellow marbled" liver • Male ALD (mg/kg) between 2.4 (T) + 15.7 (O) and 4.4 (T) + 28.2 (O) • Female ALD (mg/kg) between 2.6 (T) + 17.2 (O) and 4.7 (T) + 30.2 (O)

Table constructed by reviewer
T: tiotropium bromide; O: olodaterol hydrochloride
M: male; F: female
LD: low-dose; MD: mid-dose; HD: high-dose
ALD: Approximate lethal dose
[#]Date of study director signature. All studies performed at Boehringer Ingelheim, Birberach, Germany
*Doses expressed represent the achieved amount of tiotropium free cation and olodaterol free base, and do not reflect adjustment for pulmonary deposition factors. Dosing formulation for all studies consisted of aqueous solutions containing (b) (4)

No mortality was observed in the rat studies, therefore the ALD was >17.9 mg/kg tiotropium plus 18.8 mg/kg olodaterol (1:1 ratio study), >21.2 mg/kg tiotropium plus 11.3

mg/kg olodaterol (2:1 ratio study), and >5.2 mg/kg tiotropium plus 33.2 mg/kg olodaterol (1:6 ratio study). The doses evaluated in the 1:1 ratio study represent >34900 and >36700 times the proposed human doses of tiotropium and olodaterol on a mg/kg basis, respectively.

Table 5. Tiotropium / Olodaterol Single-dose Inhalation Toxicity Studies in Rats

Study	Methods	Findings
Document U06-1182 Study 05B098 Report Date[#] October 24, 2006	• GLP-compliant • 10-week old Crl:WI(Han) rats • 1:1 (T:O) target dose ratio • 4-hour inhalation exposure followed by 14-day observation period • 5 animals per sex at the maximum technically feasible dose* -T dose: 16.7 mg/kg (M); 19.1 mg/kg (F) -O dose: 17.5 mg/kg (M); 20.1 mg/kg (F)	• Mortality: none • Clinical signs: mydriasis with or without pupillary rigidity thru Day 9. Decreased locomotor activity, abdominal breathing, repetitive nose grooming, pale or red colored skin, reddish encrustation at nose and mouth, hunched posture and rough coat thru Day 2. • ALD > 17.9 mg/kg T plus 18.8 mg/kg O (average of M and F)
Document U06-1199 Study 05B193 Report Date June 27, 2006	• GLP-compliant • 11-week old Crl:WI(Han) rats • 2:1 (T:O) target dose ratio • 4-hour inhalation exposure followed by 14-day observation period • 5 animals per sex at the maximum technically feasible dose • T dose: 19.7 mg/kg (M); 22.7 mg/kg (F) • O dose: 10.5 mg/kg (M); 12.1 mg/kg (F)	• Mortality: none • Clinical signs: mydriasis and pupillary rigidity in all animals thru Day 4. Disturbance of bedding, sedation, increased mastication, reduced locomotion, increased grooming movements, dyspnea (Day 1) and rough coat (thru Day 2). • ALD > 21.2 mg/kg T plus 11.3 mg/kg O (average of M and F)
Document U06-1185 Study 05B191 Report Date June 27, 2006	• GLP-compliant • 11-week old Crl:WI(Han) rats • 1:6 (T:O) target dose ratio • 4-hour inhalation exposure followed by 14-day observation period • 5 animals per sex at the maximum technically feasible dose -T dose: 4.8 mg/kg (M); 5.5 mg/kg (F) -O dose: 30.8 mg/kg (M); 35.5 mg/kg (F)	• Mortality: none • Clinical signs: dry mucosa, mydriasis, pupillary rigidity and rough coat thru Day 4. Disturbance of bedding, increased mastication, sedation, reduced locomotion, increased grooming movements, and dyspnea on Day 1. • ALD > 5.2 mg/kg T plus 33.2 mg/kg O (average of M and F)

Table constructed by reviewer
T: tiotropium bromide; O: olodaterol hydrochloride
M: male; F: female
ALD: Approximate lethal dose
[#]Date of study director signature. All studies performed at Boehringer Ingelheim, Birberach, Germany

*Doses expressed represent the achieved amount of tiotropium free cation and olodaterol free base, and do not reflect adjustment for pulmonary deposition factors. Dosing formulation for all studies consisted of aqueous solutions containing (b) (4)

6.2 Repeat-Dose Toxicity

The general toxicology of tiotropium and olodaterol, the monoproduct constituents of STIOLTO RESPIMAT, has been characterized and reviewed previously under NDA 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol).

The sponsor evaluated the toxicity of the combination of tiotropium and olodaterol in six GLP-compliant toxicology studies rats and dogs that have been reviewed previously under IND 76397. Four-week studies in both rats and dogs were conducted at dose ratios (tiotropium: olodaterol) of 1:1, 2:1, and 1:6 (refer to memo dated April 25, 2008 by Dr. Molly Shea). Thirteen-week studies in dogs were conducted at dose ratios of 1:1, 2.5:1 and 1:2 (refer to memo dated June 24, 2010 by Dr. Lawrence Sancilio).

The results of the combination toxicology studies are discussed below in Section **11.3**. No new toxicities were observed compared to the established toxicological profiles of the monoproduct constituents. As an addendum to the reviews cited above, the tables below present histopathological findings from the 4- and 13-week combination toxicology studies.

Table 6. Summary of Histopathological Findings in Tiotropium-Olodaterol Combination Toxicology Studies in Rats and Dogs

4-week dog inhalation study (#665933; 1:1 ratio)
Achieved doses (ug/kg/day): LD (6.0 T, 5.7 O), MD (16.8 T, 16.1 O), HD (75 T, 70 O)

	V		LD		MD		HD	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***3***	***3***	***3***	***3***	***3***	***3***	***3***	***3***
Eyes								
Keratitis, focal, bilateral	0	0	0	0	0	0	1	0
Conjunctivitis, unilateral	0	0	0	0	0	0	1	0
Vascularization, corneal, unilateral	0	0	0	0	0	0	1	0
Fibrosis, corneal, unilateral	0	0	0	0	0	0	1	0
Lacrimal gland								
Inflammatory cell infiltration	0	0	1	0	0	1	0	1
Larynx								
Exudate, inflammatory	0	0	0	0	1	0	1	0
Inflammatory cell infiltration	0	1	1	0	2	1	2	0
Laryngitis	0	1	1	0	2	2	2	0
Liver								
Glycogen reduced, centrilobular	0	0	3	2	3	3	3	3
Lung								
Squamous metaplasia, bronchiolar, focal	0	0	0	0	0	0	1	0
Nasal Cavity								
Exudate, intraluminal	0	0	0	0	0	1	0	2
Inflammatory cell infiltration	0	0	1	0	1	0	0	2
Prostate								
Inflammatory cell infiltration	1	-	2	-	1	-	3	-
Testes								
Segmental hypoplasia	0	-	0	-	1	-	2	0
Urinary bladder								
Congestion	0	0	0	0	0	1	0	2

4-week dog inhalation study (#665949; 1:6 and 2:1 ratios)
Achieved doses (ug/kg/day): 1:6 LD (T 2.7, O 16.4), 1:6 HD (T 19.8, O 124), 2:1 LD (T 28.8, O 16.5), 2:1 HD (T 385, O 191)

	V		1:6 LD		1:6 HD		2:1 LD		2:1 HD	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***
Eyes										
Keratitis, bilateral	0	0	0	0	0	0	0	0	1	0
Vascularization, corneal, focal	0	0	0	0	0	0	0	0	1	0
Heart										
Myocardial fibrosis, papillary muscle	0	0	0	0	0	0	0	0	1	0
Larynx										
Neutrophil infiltration	0	0	1	0	0	0	0	0	3	0
Larynx (REC, N=2 per group)										
Neutrophil infiltration	0	0	-	-	1	0	0	0	-	-
Liver										
Glycogen, periportal	0	0	2	1	3	3	3	1	3	3
Fibrosis, capsular	0	0	0	0	1	1	0	0	0	0
Lung										
Agonal congestion/hemorrhage	0	1	1	0	1	0	0	2	1	2
Alveolar macrophage accumulation	0	0	0	0	0	1	0	0	1	1
Lung (REC, N=2 per group)										
Alveolar macrophage accumulation	0	0	-	-	0	1	0	1	-	
Lymph node, bronchial										
Congestion	0	0	0	0	0	0	0	1	0	1
LN, bronchial (REC, N=2 per group)										
Congestion	0	0	-	-	0	1	0	1	-	-
Testes										
Seminiferous epithelial degeneration, bilateral	0	-	0	-	1	-	0	-	0	-

4-week rat inhalation study (#665912; 1:1 ratio)
Achieved doses (ug/kg/day): LD (85.2 T, 79.6 O), MD (577 T, 555 O), HD (2266 T, 2174 O)

	V		LD		MD		HD	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***10***	***10***	***10***	***10***	***10***	***10***	***10***	***10***
Larynx								
Squamous metaplasia, arytenoid cartilage	0	0	0	0	0	2	3	3
Necrosis, U-shape cartilage	6	6	5	6	5	8	7	6
Larynx (REC, N=6 per group)								
Necrosis, U-shape cartilage	0	0	-	-	-	-	4	6
Lung								
Congestion	2	4	10	9	5	8	9	7
Alveolar macrophage accumulation	2	4	1	5	3	5	4	6
Lung (REC, N=6 per group)								
Congestion	1	5	-	-	-	-	4	1
Alveolar macrophage accumulation	1	0	-	-	-	-	2	3
Lymph node, mandubular								
Plasmacytosis	0	0	-	-	-	-	3	1
Prostate								
Inflammatory cell infiltration	1	-	2	-	1	-	3	-
Testes								
Segmental hypoplasia	0	-	0	-	1	-	2	0
Urinary bladder								
Congestion	0	0	0	0	0	1	0	2

4-week rat inhalation study (#665928; 1:6 and 2:1 ratios)
Achieved doses (ug/kg/day): 1:6 LD (T 13, O 75), 1:6 HD (T 393, O 2032), 2:1 LD (T 143, O 73), 2:1 HD (T 3263, O 1747)

	V		1:6 LD		1:6 HD		2:1 LD		2:1 HD	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***10***	***10***	***10***	***10***	***10***	***10***	***10***	***10***	***10***	***10***
Larynx										
Necrosis, U-shape cartilage	5	7	7	8	8	9	8	8	7	9
Minimal	3	6	6	5	7	2	3	2	2	0
Mild	2	1	1	2	1	5	5	6	5	8
Squamous metaplasia, base of epiglottis	5	7	9	9	8	8	9	10	8	9
Minimal	4	6	4	7	6	3	3	4	1	1
Mild	1	1	5	2	2	5	6	6	7	8
Squamous metaplasia, arytenoid cartilage	0	0	0	1	4	5	1	0	5	8
Minimal	0	0	0	0	0	0	0	0	1	1
Mild	0	0	0	1	4	2	1	0	2	4
Moderate	0	0	0	0	0	3	0	0	2	3
Larynx (REC, N=6 per group)										
Necrosis, U-shape cartilage	2	5	-	-	2	5	-	-	4	3
Minimal	2	5	-	-	2	3	-	-	2	1
Mild	0	0	-	-	0	2	-	-	2	2
Squamous metaplasia, base of epiglottis	0	0	-	-	0	5	-	-	2	2
Minimal	0	0	-	-	0	5	-	-	2	1
Mild	0	0	-	-	0	0	-	-	0	1
Squamous metaplasia, arytenoid cartilage (min)	0	0	-	-	0	1	-	-	1	0
Liver										
Increased hematopoiesis, scattered	0	0	-	-	0	0	-	-	0	2
Lung										
Congestion/hemorrhage	5	0	8	6	9	6	7	8	6	4
Minimal	5	0	6	3	4	6	1	1	1	2
Mild	0	0	2	3	5	0	6	7	5	2
Alveolar macrophage accumulation	1	1	7	3	4	5	4	5	3	4
Alveolar foamy macrophage accumulation	0	0	0	1	0	0	1	3	4	0
Lung (REC, N=6 per group)										
Alveolar macrophage accumulation	0	2	-	-	1	3	-	-	2	2

13-week dog inhalation study (#07B183; 2.5:1 and 1:2 ratios)
Achieved doses (ug/kg/day): 2.5:1 LD (9 T, 4 O), 2.5:1 HD (75.5 T, 31.5 O), HD (36 T, 75 O), TIO (76), OLO (75)

	V		2.5:1 LD		2.5:1 HD		1:2 HD		OLO		TIO	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***
Heart												
Cyst	0	0	0	0	0	0	1	0	0	0	0	0
Single cell necrosis	0	0	0	0	0	0	2	0	2	0	0	0
Fibrosis/fibroplasia	0	0	0	0	0	1	2	2	2	2	0	0
Heart (REC, N=2 per group)												
Fibrosis/fibroplasia	0	0	-	-	1	0	2	0	2	0	-	-
Liver												
Glycogen depletion (centrilobular)	0	0	0	0	3	4	4	4	4	4	0	0
Glycogen increased (peripheral)	0	0	1	3	4	4	4	4	4	4	0	0
Lacrimal gland												
Dilatation of ducts	0	1	3	2	4	4	4	2	1	1	4	3
Atrophy, focal	0	0	1	0	0	0	2	0	0	0	0	0
Pharynx												
Debris, inflammatory cells, lumen	1	0	3	2	4	4	3	3	0	0	4	3
Atrophy, lobular	0	0	0	0	0	0	1	0	0	1	0	0
Concretion of secretory fluid	0	0	1	0	0	2	1	0	2	0	1	1
Dilatation of ducts	0	0	2	0	1	2	2	0	1	0	1	0
Infiltration, inflammatory	1	0	1	1	2	2	2	2	0	0	1	3
Necrosis, focal	0	0	0	0	0	0	0	1	0	0	1	0
Pharynx (REC, N=2 per group)												
Atrophy, lobular	0	0	-	-	1	0	0	0	0	0	-	

	V		2.5:1 LD		2.5:1 HD		1:2 HD		OLO		TIO	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***
Duodenum												
Dilatation of glands	0	0	0	0	0	0	1	1	0	1	0	1
Larynx												
Atrophy, glandular	0	0	1	1	1	1	1	1	0	0	0	1
Enlarged follicles	0	1	1	1	1	2	2	1	0	0	2	1
Optic Nerve												
Infiltration	0	0	0	0	0	0	0	1	0	0	1	0
Peripheral Nerve												
Infiltration	0	0	0	0	1	0	0	0	0	0	0	0
Skeletal muscle												
Infiltration	0	0	0	0	0	0	1	0	1	0	2	0
Skeletal muscles (REC, N=2 per group)												
Infiltration	-	-	-	-	0	0	0	1	0	0	-	
Spleen												
Fibrosis/adhesion, capsular	0	0	0	0	0	0	0	1	0	0	0	0
Stomach												
Necrosis, focal	0	0	0	0	0	0	1	0	0	0	0	0
Tongue												
Necrosis, single cell	0	0	2	0	0	0	2	0	1	2	0	0

13-week dog inhalation study (#07B077; 1:1 ratio)
Achieved doses (ug/kg/day): LD (4 T, 3.5 O), MD (15.5 T, 14.3 O), HD (75 T, 70 O), TIO (75), OLO (72.5)

	V		LD		MD		HD		OLO		TIO	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***
Heart												
Fibrosis/fibroplasia	0	0	0	0	0	3	3	1	2	0	0	0
Mineralization	0	0	0	0	0	0	0	1	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	1	0	0	0
Heart (Rec N=2 per group)												
Fibrosis/fibroplasia	0	0	-	-	-	-	1	1	1	0	0	0
Mineralization	0	0	-	-	-	-	1	0	0	0	0	0
Lung												
Fibrosis/fibroplasia	0	0	2	0	0	1	1	0	1	0	0	0
Hemorrhage	0	0	0	0	0	0	1	0	0	0	0	0
Infiltration, inflammatory	0	0	1	0	0	1	0	0	0	1	0	0
Lung (Rec, N=2 per group)												
Inflitration, inflammatory	0	0	-	-	-	-	0	0	0	0	1	0
Liver												
Glycogen depletion	0	0	0	0	2	0	2	3	1	1	0	0
Glycogen storage increased	0	1	0	1	0	4	1	4	0	2	0	0
Kidneys												
Basophilic tubules	0	0	0	0	1	0	0	1	1	0	0	1
Cyst	0	0	0	0	0	0	1	0	0	0	0	0
Infiltration	0	1	0	0	1	0	1	1	0	0	0	1
Mineralization, tubular	0	2	1	3	0	0	1	2	1	3	1	2

	V		LD		MD		HD		OLO		TIO	
Finding	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***	***4***
Esophagus												
Inflitration, inflammatory	0	0	0	0	0	0	1	0	0	0	0	0
Palate												
Dilatation of ducts	0	0	0	0	1	1	2	0	0	0	2	0
Hemorrhage	0	0	0	0	0	0	1	0	0	0	0	0
Salivary gland, sublingual												
Dilatation of ducts	0	0	0	0	0	0	1	0	0	0	0	0
Salivary gland, parotid												
Dilatation of ducts	0	0	0	0	0	0	1	0	0	0	0	0

7 Genetic Toxicology

The genotoxicity of tiotropium and olodaterol, the monoproduct constituents of STIOLTO RESPIMAT, has been characterized and reviewed previously under NDAs 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol). According to the *Guidance for Industry: Nonclinical Safety Evaluation of Drug or Biologic Combinations* (March 2006), combination genotoxicity studies are not needed for this product.

The sponsor incorporated bone marrow micronucleus assays into the 4-week combination rat studies 665912 and 665928. These assessments were not considered pivotal to the evaluation of the nonclinical safety of STIOLTO RESPIMAT and were not reviewed.

8 Carcinogenicity

Consistent with the *Guidance for Industry: Nonclinical Safety Evaluation of Drug or Biologic Combinations* (March 2006), no carcinogenicity studies were performed with the tiotropium-olodaterol combination. Two-year carcinogenicity studies were completed in mice and rats for each of the monoproducts and have been reviewed previously under NDAs 21395 and 203188. Results of these studies are summarized in Sections **11.1** and **11.2** below and are reflected in the FDA-approved product labeling.

9 Reproductive and Developmental Toxicology

The developmental and reproductive toxicology of tiotropium and olodaterol, the monoproduct constituents of STIOLTO RESPIMAT, has been characterized and reviewed previously under NDAs 21395 and 21936 (tiotropium) and NDA 203108 (olodaterol). . Results of these studies are summarized in Sections **11.1** and **11.2** below and are reflected in the FDA-approved product labeling.

Consistent with guidance provided under IND 76397 at the pre-IND meeting (minutes dated December 31, 2007) and in pre-NDA written responses (dated September 9, 2013), the division agreed that no combination embryo-fetal development study was required.

10 Special Toxicology Studies

Not applicable.

11 Integrated Summary and Safety Evaluation

STIOLTO RESPIMAT is a fixed-dose combination product consisting of an aqueous solution of tiotropium and olodaterol delivered via the inhalation route of administration using the Respimat (b) (4) inhaler device. The product's proposed indication is for the

treatment of COPD at a recommended daily dose of 5 mcg tiotropium and 5 mcg olodaterol, delivered as two actuations administered once daily.

The sponsor completed full nonclinical programs evaluating the monoproduct constituents, tiotropium and olodaterol, including pharmacology, safety pharmacology, ADME, general toxicology, genetic toxicology, carcinogenicity, and reproductive and developmental toxicology. The pharmacological and toxicological profiles of tiotropium and olodaterol were considered to be adequately characterized upon review under NDA 21395 (SPIRIVA HANDIHALER; tiotropium dry powder for inhalation), NDA 21936 (SPIRIVA RESPIMAT; tiotropium inhalation spray), and NDA 203188 (STRIVERDI RESPIMAT; olodaterol inhalation spray).

Brief summaries of the pharmacology and toxicology of tiotropium and olodaterol are presented in sections **11.1** and **11.2**, respectively. The pharmacology and toxicology of the tiotropium-olodaterol combination is reviewed in section **11.3**. As noted in section **11.4**, there are no outstanding nonclinical issues at this time and the application is recommended for approval from the nonclinical perspective. The reviewer's evaluation of the proposed labeling and recommended edits are provided in section **11.5**.

11.1 Tiotropium

Refer to the nonclinical primary reviews filed by Dr. Luqi Pei to NDA 21395 (September 20, 2002) and NDA 21936 (July 28, 2008) for a complete review of the pharmacology and toxicology studies conducted with tiotropium.

Tiotropium is a LAMA with higher affinity for the m_3, m_4 and m_5 muscarinic acetylcholine receptors (K_d = 9 pM) compared to the m_1 and m_2 receptors (K_d >32 pM). Tiotropium acts by binding to airway cholinergic receptors, leading to abrogation of acetylcholine-mediated bronchoconstriction and causing bronchodilation. Secondary pharmacological effects typical of muscarinic antagonists such as inhibition of salivation / lacrimation, increased heart rate, and decreased gastrointestinal motility have been observed with tiotropium.

Tiotropium exhibits high bioavailability by the inhalation route of administration and rapidly achieves C_{max}. The drug is readily distributed to blood-rich organs including the small intestine, kidneys and liver. Plasma protein binding was 66% in humans compared to 16-22% in animals. In vitro and in vivo studies demonstrated that tiotropium is metabolized in the liver by cytochrome P450 (CYP) enzymes 3A4 and 2D6 and is largely excreted in the urine.

Pivotal 52-week general toxicology studies were conducted in via the inhalation route of administration in Wistar rats and Beagle dogs, respectively. Achieved inhaled doses in the rat study were 13, 96 and 641 mcg/kg/day, corresponding to pulmonary deposited doses (PDDs) of 1.3, 9.6, and 64 mcg/kg/day. Achieved inhaled doses in the dog study were 5.2, 45, and 448 mcg/kg/day, corresponding to PDDs of 1.3, 11.2, and 112

mcg/kg/day.[1] Target organs of toxicity were identified as the eyes, gastrointestinal tract, respiratory tract, liver, urinary bladder, salivary glands and heart. Adverse findings were generally attributable to exaggerated pharmacological effects as listed above. The NOAELs (PDD) after chronic inhalation exposure to tiotropium were identified at the low-dose of 1.3 mcg/kg/day in both rats and dogs.[2]

Tiotropium was negative for genotoxic potential in bacterial reverse mutation, V79 Chinese hamster cell mutagenesis, in vitro chromosomal aberration, rat hepatocyte unscheduled DNA synthesis and in vivo micronucleus assays.

Tiotropium was evaluated in a two-year inhalation carcinogenicity studies in Wistar rats and CD-1 mice. A second two-year inhalation carcinogenicity study was conducted in male CD-1 mice at lower doses due to excessive mortality in the initial study. Tiotropium was not carcinogenic in rats or mice. Refer to the nonclinical review filed to NDA 21395 by Dr. Luqi Pei on September 20, 2002 and Executive Carcinogenicity Assessment Committee (ECAC) meeting minutes dated July 8, 2012. The tiotropium carcinogenicity studies, as well as exposure multiple calculations on a body surface area basis for labeling purposes, are summarized in the table below.

Table 7. Summary of Tiotropium Carcinogenicity Studies and Safety Margins

Species	Maximum Achieved Dose[1]		Multiple of RHDID[2]	
	(mcg/kg/day)	(mcg/m^2)	Raw	Rounded
Rat (M+F)	59	354	31.9	30
Mouse (Male)	2	435	0.54	0.5
Mouse (Female)	145	6	39.2	40
Human (5 ug/day)	*0.3*[3]	*1.1*		

Table constructed by reviewer
[1]These achieved doses have been adjusted by the salt correction factor of 1.25 to be expressed in terms of the free base and match those listed in the NDA 21395 labeling review dated December 23, 2003 as well as the proposed STIOLTO RESPIMAT product labeling. The male mouse dose conservatively reflects the lowered dose level administered from Week 63-101.
[2]RHDID: Recommended human daily inhalation dose on a mg/m^2 basis (based on 18 mcg/day HANDIHALER dose not 5 mcg/day RESPIMAT dose for reasons discussed in section **11.5**.
[3]Note calculation has been updated to use 60 kg human weight instead of 50 kg in prior tiotropium NDAs.

[1] Dr. Pei's review under NDA 21395 estimated the pulmonary deposited doses (PDD) from the sponsor's total achieved dose levels as 45 mcg/kg/day in both rats and dogs based on 7% and 10% deposition factors, respectively. The current practice of applying 10% and 25% deposition factors results in the PDD estimates listed in the text of this review.
[2] Reported as <7 mcg/kg/day in rats and 0.4 mcg/kg/day in dogs in Dr. Pei's NDA 21395 review based on 7% and 10% deposition factors.

Tiotropium was evaluated in four inhalation reproductive and developmental toxicology studies, as summarized in the table below. There was no evidence of teratogenicity but tiotropium induced embryo-fetal toxicity in rats and rabbits. Refer to the nonclinical review filed to NDA 21395 by Dr. Luqi Pei on September 20, 2002.

Table 8. Summary of Tiotropium Reproductive and Developmental Toxicology Studies and Safety Margins

Species (Dosing Period)	Achieved Dose (mcg/kg/day)[1]	Test article-related Findings	Multiple of RHDID[2]
Rat Fertility (9/2 weeks pre-mating to PP 21)	8.8	-	4.0
	77.6	▼corpora lutea, ▼ implants, ▼ live births, delayed sexual maturation	35
	1584	MD effects plus ▲ post-implantation loss	713
Rat Embryo-Fetal (GD 6-17)	8.8	Delayed sexual maturation	4.0
	80	Delayed sexual maturation	36
	1840	Delayed sexual maturation	830
Rat Peri/Postnatal (GD 17 – weaning)	8.0	Delayed sexual maturation	3.6
	80	LD effects plus ▼ fetal weight, ▲ total litter loss	36
	1470	LD effects plus ▼ fetal weight, ▲ total litter loss	660
Rabbit Embryo-Fetal (GD 6-18)	7.2	-	6.3
	88	-	79
	400	▲ Post-implantation loss	360

Table constructed by reviewer
PP: post partum day; GD: gestation day; LD: low-dose; MD: mid-dose
[1]These achieved doses have been adjusted by the salt correction factor of 1.25 to be expressed in terms of the free base and approximate those listed in the NDA 21395 labeling review dated December 23, 2003 as well as the proposed STIOLTO RESPIMAT product labeling.
[2]RHDID: Recommended human daily inhalation dose on a mg/m^2 basis, but based on 18 mcg/day HANDIHALER dose not 5 mcg/day RESPIMAT dose for reasons discussed in section **11.5**.

11.2 Olodaterol

Refer to the nonclinical primary review filed by Dr. Carol Rivera-Lopez (Galvis) to NDA 203108 (January 17, 2013) for a complete review of the pharmacology and toxicology studies conducted with olodaterol.

Olodaterol is a LABA with greater affinity for the β_2-adrenoreceptor (K_i = 0.72 nM) compared to the β_1- and β_3-adrenorectors (K_i = 47 nM and 5 uM, respectively). In vivo, olodaterol protected against acetylcholine-induced bronchospasm with a rapid onset of action (~10 minutes) and a long duration of effect (~24 hours). As with other members of the β_2-agonist class, olodaterol induces increases in heart rate (tachycardia), decreases in mean arterial blood pressure, as well as decreases in gastric emptying and gastrointestinal transit.

When administered via the inhalation route, olodaterol is rapidly absorbed and achieves C_{max} within 30 minutes. Olodaterol distributes to the lungs, pancreas, gastrointestinal

tract and eyes after inhalation and is moderately plasma protein-bound (50-70% across all species). Olodaterol is metabolized in the liver by CYP 2C8 and 2C9; two disproportionate human phase II metabolites were identified but are present at very low levels in human plasma. The primary route of excretion for olodaterol is via the feces, with the urine as a secondary route.

The sponsor conducted pivotal general toxicology studies with olodaterol in Wistar rats and Beagle dogs via the inhalation route of administration. Achieved doses in the 26-week rat study were 0, 49, 200 and 3400 mcg/kg/day (PDD of 0, 4.9, 20 and 340 mcg/kg/day). Test article-related effects included anabolic (increased body weights, skeletal muscle hypertrophy / single cell necrosis), cardiac (tachycardia, increased organ weights, congestion, scar formation), and ovarian (cyst formation; see also the tumors noted below under carcinogenicity) findings that have also been observed with other members of the LABA class. In addition, dose-limiting tracheal squamous cell metaplasia and pancreatic lobular atrophy were observed. The NOAEL for chronic dosing in the rat was determined as an achieved dose of 49 mcg/kg/day, representing a 63-fold systemic safety margin on an AUC basis and a 546-fold local safety margin on a mg/g lung weight basis compared to the proposed human dose of 5 mcg/day.

In the 52-week dog studies, achieved olodaterol inhalation doses were 0, 15, 60 and 330 mcg/kg/day (PDD of 0, 3.8, 15 and 83 mcg/kg/day). In line with the rat study results and other members of the LABA class, anabolic and cardiac effects were also noted in the chronic dog study. Olodaterol treatment resulted in increased heart force and heart rate, increased serum creatine kinase and cardiac-specific troponin 1, and ECG findings of ventricular premature beats and tachycardia. Histopathological findings in the liver of increased glycogen storage was also considered to be a class effect of β_2-agonists and not dose-limiting. Dose-limiting histopathological findings were noted in the kidneys (mononuclear cell infiltration), liver (hemorrhage), and trachea (epithelial atrophy, infiltration and mineralization). The NOAEL for chronic dosing in the dog was determined as an achieved dose of 15 mcg/kg/day, representing a 21-fold systemic safety margin on an AUC basis and a 266-fold local safety margin on a mg/g lung weight basis compared to the proposed human dose of 5 mcg/day.

Olodaterol was negative for genotoxicity in a bacterial reverse mutation assay and an in vitro mouse lymphoma assay. However, olodaterol increased the percentage of polychromatic erythrocytes and micronucleated polychromatic erythrocytes in an in vivo rat micronucleus via the intravenous route of administration. Based on this finding, additional cardiovascular safety pharmacology and mechanistic studies were conducted. The review conclusion was that the observations in the rat study were attributable to compensatory erythropoiesis and were unlikely to be relevant at clinical exposures.

Olodaterol was evaluated in two-year inhalation carcinogenicity studies in Wistar rats and CD-1 mice. In female rats, a statistically significant increase in mesovarian leiomyomas was observed. In female mice, a statistically significant increase in uterine leiomyomas / leiomyosarcomas was observed. Refer to the nonclinical review filed to

IND 76362 by Dr. Hans Rosenfeld on May 11, 2012 and ECAC meeting minutes dated July 11, 2012. Female reproductive tract tumors have been observed with other approved members of the class, but the relevance of these findings to patients is still unknown.

Table 9. Summary of Olodaterol Carcinogenicity Studies and Safety Margins

Species	Achieved Dose (mcg/kg/day)	Test article-related Tumor Findings[1]	AUC Multiple vs. RHDID[2]
Rat	25.8	Mesovarian tissue leiomyoma (1/55 F)	18
	75.9	-	64
	270	Mesovarian tissue leiomyoma (4/55 F)	198
Mouse	26.1	-	42
	76.9	Uterus leiomyoma / leiomyosarcoma	106
	255	Uterus leiomyoma / leiomyosarcoma	387

Table constructed by reviewer
[1]Findings listed reflect the conclusions of the ECAC and are described in product labeling.
[2]RHDID: Recommended human daily inhalation dose on an AUC basis

Olodaterol had no effects on male or female fertility, teratogenicity or pre/postnatal development in rats via the inhalation route of administration. The NOAELs in the rat studies were identified as the highest evaluated dose, corresponding to greater than 2000-fold safety margins compared to the clinical dose on an AUC basis. In an inhalation embryo-fetal development (EFD) study in rabbits, olodaterol was teratogenic at the highest achieved dose of 2489 mcg/kg/day. The NOAEL in the rabbit EFD study was the mid-dose of 974 mcg/kg/day, representing a 1353-fold safety margin compared to the clinical dose on an AUC basis.

11.3 Tiotropium-Olodaterol Combination

In addition to the complete monoproduct nonclinical programs with tiotropium and olodaterol outlined above, Boehringer-Ingelheim conducted certain pharmacology and toxicology studies with the drug combination. These studies have been reviewed under IND 76397 and/or the present NDA 206756 review.

The sponsor has submitted three pharmacology studies investigating the in vivo effects of the combination of tiotropium and olodaterol (see detailed review in Section **4.1** above). In guinea pig and dog models of acetylcholine-induced bronchospasm, the combination of tiotropium plus olodaterol resulted in synergistic bronchoprotection compared to either monoproduct alone. A cardiovascular safety pharmacology study in dogs revealed no novel effects attributable to the combined test articles.

The acute toxicity of the tiotropium-olodaterol combination was evaluated in a total of six inhalation studies in mice and rats at 1:1, 2:1, or 1:6 dose ratios. The lowest lethal dose in mice (≥2.5 mg/kg tiotropium plus 16.4 mg/kg olodaterol in the 1:6 ratio study), represents tiotropium and olodaterol doses that are >2400 and >15000 times the proposed human dose on a mg/kg basis, respectively. In rats, no mortality was observed. The doses evaluated in the 1:1 ratio rat study represent >34900 and >36700

times the proposed human doses of tiotropium and olodaterol on a mg/kg basis, respectively.

The tiotropium-olodaterol combination was evaluated in two 4-week inhalation studies in Wistar rats and two 4-week inhalation studies in Beagle dogs, with tiotropium: olodaterol dose ratios of 1:1, 2:1 and 1:6. In addition, two 13-week inhalation studies in dogs were conducted at tiotropium: olodaterol dose ratios of 1:1, 2.5:1, and 1:2.

Table 10. Summary of Tiotropium-Olodaterol Combination Toxicology Studies

Study (#)	Dose Ratio	Total Achieved Dose Levels (mcg/kg/day)*
4-week rat (665912)	1:1	85.2+79.6, 577+555, 2266+2174
4-week rat (665928)	1:6	13+75, 393+2032
	2:1	143+73, 3263+1747
4-week dog (665933)	1:1	6.07+5.71, 16.8+16.1, 157+152
4-week dog (665949)	1:6	2.66+16.4, 19.8+124
	2:1	28.8+16.5, 385+191
13-week dog (07B077)	1:1	14+16, 57+62, 290+310
13-week dog (07B183)	1:2	140+300
	2.5:1	36+16, 300+130

Table constructed by reviewer
*Pulmonary deposited doses (PDD) are calculated as 10% and 25% of the achieved doses for rats and dogs, respectively.

In the two 4-week rat studies, test article-related deaths were noted at the mid- and high-dose levels and were attributed to food asphyxiation and/or lung congestion / hemorrhage. Target organs were identified as the larynx (necrosis of U-shaped cartilage), lungs (congestion and alveolar macrophage accumulation), liver (increased hematopoiesis) and mandibular lymph nodes (plasmacytosis). In the two 4-week dog studies, there were no premature deaths. Clinical observations of mydriasis, dry mucosa, and increased heart rate (with evidence of adaptation) were consistent with the established pharmacological effects of tiotropium and olodaterol. Target organs of toxicity were identified as the larynx, lungs, eyes, heart, liver, lymph nodes, testes and thymus.

No NOAELs were identified in the 4-week studies due to the histopathological findings in the lungs (congestion) and liver (portal and capsular fibrosis) at all doses in rats and dogs, respectively. Periportal glycogen accumulation in the liver, a class effect of β_2-agonists, was also observed. However, no new test article-related findings were observed compared to tiotropium and olodaterol monoproduct toxicology studies conducted at similar dose levels (IND 76397 safety evaluation by Dr. Molly Shea, April 25, 2008).

The sponsor subsequently conducted two 13-week dog studies (with 1:1 dose ratio and 2.5:1 / 1:2 dose ratios) via the inhalation route of administration as the pivotal general

toxicology studies for the tiotropium-olodaterol combination program. In each study, clinical signs consistent with the β_2-agonist and antimuscarinic activity of the test articles were observed, including tachycardia, mydriasis, and dry mouth. A synergistic increase in heart rates was observed in both studies, but this finding is considered to be monitorable in the clinical setting. Target organs of toxicity included the heart (decreased organ weights, gross discoloration, and fibrosis / necrosis / mineralization upon microscopic examination) and liver (glycogen depletion / increased storage). There were no novel histopathological findings attributed to the combination and the heart and liver observations were not considered to be dose-limiting. Gross and microscopic changes in the heart were attributed to the increased heart rate, and the changes in liver glycogen are considered a class effect for β_2-agonists such as olodaterol. The prior review by Dr. Sancilio (IND 76397, June 24, 2010) did not make a NOAEL determination. However, based on a lack of adverse test article-related histopathological findings in the heart, liver or other organs, the lowest achieved dose of 14 mcg/kg/day tiotropium plus 16 mcg/kg/day olodaterol in the 1:1 dose ratio study can be considered as a NOAEL for 13-week exposure to tiotropium plus olodaterol in the dog.

In summary, toxicology studies conducted in rats and dogs with the tiotropium-olodaterol combination did not reveal any novel toxicities of clinical concern. There was no evidence of any additive or synergistic toxicity between tiotropium and olodaterol beyond observed increases of heart rate. Further, there was no evidence of any interactions with respect to toxicokinetics. The completed nonclinical program adequately supports the proposed clinical use of the STIOLTO RESPIMAT product consisting of 5 mcg per day tiotropium and 5 mcg per day olodaterol. Safety margins between the pivotal nonclinical study and the proposed clinical dose level are summarized in the table below.

Table 11. Summary of STIOLTO RESPIMAT Safety Margins

Proposed Clinical Dosing		13-week Dog Study		Safety Margin (Systemic AUC)
Daily Dose	AUC	Achieved Dose	AUC	
Tiotropium (5 mcg per day)	83.3 pM*h[a]	14 mcg/kg/day	3165 pM*h[b]	38.0
Olodaterol (5 mcg per day)	134.5 pM*h[c]	16 mcg/kg/day	2380 pM*h[b]	17.7
Proposed Clinical Dosing		**13-week Dog Study**		**Safety Margin (mcg/g lung weight)**
Daily Dose	**PDD**	**Achieved Dose**	**PDD**	
Tiotropium (5 mcg per day)	0.005 mcg/g LW[d]	14 mcg/kg/day	1.33 mcg/g LW[e]	266
Olodaterol (5 mcg per day)	0.005 mcg/g LW[d]	16 mcg/kg/day	1.52 mcg/g LW[e]	304

Table constructed by reviewer
LW: lung weight; PDD: Pulmonary deposited dose (25% deposition factor)
[a] From PK study 1237.3 report Table 11.5.2:2, page 92. AUC reported as 32.7 pg*h/mL (free base) was converted to 40.86 pg*h/mL tiotropium salt and then to pM with tiotropium bromide formula weight of 490.3 g/mol
[b] Day 86 $AUC_{0\text{-}24h}$, average of males and females
[c] From Carol Rivera-Lopez NDA 203188 nonclinical review dated January 17, 2013

[d] Calculated based on 1000 g human lung weight
[e] Calculated based on observed terminal body weights and lung organ weights in 13-week dog study

11.4 Recommendations

NDA 206756 is recommended for approval from the nonclinical perspective. The program of pharmacology and toxicology studies evaluating tiotropium, olodaterol, and the tiotropium-olodaterol combination are considered complete and adequate to support the safety of the proposed clinical dose of 5 mcg tiotropium and 5 mcg olodaterol per day. There are no outstanding nonclinical issues and no further nonclinical studies are recommended.

The following section contains an evaluation of the sponsor's proposed labeling, the reviewer's recommended changes, and related discussion.

11.5 Labeling Evaluation

The following labeling recommendations are based on the sponsor's proposed Prescribing Information as submitted to NDA 206756 on May 22, 2014. Consideration was also given to the current in-use labeling for the FDA-approved SPIRIVA HANDIHALER, SPIRIVA RESPIMAT, and STRIVERDI RESPIMAT products. The reviewer's proposed insertions and deletions are indicated in red font and ~~red strikethrough~~ text, respectively. The reviewer's proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

INDICATION AND USAGE

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C.
There are no adequate and well-controlled studies with STIOLTO RESPIMAT or its individual components, tiotropium bromide and olodaterol, in pregnant women. STIOLTO RESPIMAT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Tiotropium
No evidence of structural alterations was observed in rats and rabbits at (b) (4) (b) (4) approximately (b) (4) times the recommended human daily inhalation dose (RHDID~~;~~) on a mcg/m^2 basis at maternal inhalation doses of 1471 and 7 mcg/kg/day in rats and rabbits, respectively). However, in rats, tiotropium caused fetal resorption, litter loss, decreases in the number of live pups at birth and the mean pup weights, and a delay in pup sexual maturation at (b) (4) approximately (b) (4) times the RHDID (on a mcg/m^2 basis at a maternal inhalation dose of 78 mcg/kg/day). In rabbits, tiotropium caused an increase in post-implantation loss at (b) (4) approximately (b) (4) times the RHDID (on a mcg/m^2

basis at a maternal inhalation dose of 400 mcg/kg/day). Such effects were not observed at [redacted] approximately [redacted] times the RHDID (on a mcg/m^2 basis at maternal inhalation doses of 9 and 88 mcg/kg/day in rats and rabbits, respectively). [redacted]

Olodaterol

Olodaterol was not teratogenic in rats at [redacted] approximately 2731 times the [redacted] RHDID (on an AUC basis at a [redacted] maternal inhalation dose of 1054 mcg/kg/day). Placental transfer of olodaterol was observed in pregnant rats.

Olodaterol has been shown to be teratogenic in New Zealand rabbits at [redacted] approximately 7130 times the [redacted] RHDID in adults (on an AUC basis at a [redacted] maternal inhalation dose of 2489 mcg/kg/day). Olodaterol exhibited the following fetal toxicities: enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum. No significant effects occurred at [redacted] approximately 1353 times the [redacted] RHDID in adults (on an AUC basis at a [redacted] maternal inhalation dose of 974 mcg/kg/day).

Reviewer's comments:

The summary risk statement has been adapted from the two monoproduct labels and appears appropriate.

Throughout the labeling, the reviewer recommends adjustments to the sponsor's stated multiples comparing human vs. animal doses of tiotropium. This issue was addressed in detail by Dr. Luqi Pei during review of NDA 21396 for SPIRIVA RESPIMAT (see memo filed August 26, 2014). In brief, while there is a nominal dose difference between the SPIRIVA HANDIHALER (18 ug/day) and SPIRIVA RESPIMAT (5 ug/day) products, the actual clinical exposure to tiotropium was similar. Therefore, the [redacted] The revised dose multiples are consistent with the SPIRIVA RESPIMAT product labeling.

The maternal dose levels for tiotropium studies have been inserted to maintain consistency with the SPIRIVA RESPIMAT labeling as well as the olodaterol sections of this label.

[redacted]

[redacted] *for olodaterol has been replaced by "RHDID" based on there being only a single proposed dose level for the STIOLTO RESPIMAT product.*

8.3 Nursing Mothers

Clinical data from nursing women or infants exposed to STIOLTO RESPIMAT or its individual active components are not available. Tiotropium, olodaterol, and metabolites of olodaterol are excreted into the milk of lactating rats.

It is not known whether these compounds are excreted in human milk, but because many drugs are excreted in human milk and given these findings in rats, caution should be exercised if STILTO RESPIMAT is administered to a nursing woman.

Reviewer's comments:

The sponsor's proposed labeling is considered acceptable.

10 OVERDOSAGE

(b) (4)

STIOLTO RESPIMAT contains both tiotropium bromide and olodaterol; therefore, the risks associated with overdosage for the individual components described below apply to STIOLTO RESPIMAT.

Tiotropium
High doses of tiotropium may lead to anticholinergic signs and symptoms. However, there were no systemic anticholinergic adverse effects following a single inhaled dose of up to 282 mcg tiotropium in 6 healthy volunteers. In a study of 12 healthy volunteers, bilateral conjunctivitis and dry mouth were seen following repeated once-daily inhalation of 141 mcg of tiotropium.

(b) (4)

(b) (4)

Olodaterol
The expected signs and symptoms with overdosage of olodaterol are those of excessive beta-adrenergic stimulation and occurrence or exaggeration of any of the signs and symptoms, e.g., myocardial ischemia, angina pectoris, hypertension or hypotension, tachycardia, arrhythmias, palpitations, dizziness, nervousness, insomnia, anxiety, headache, tremor, dry mouth, muscle spasms, nausea, fatigue, malaise, hypokalemia, hyperglycemia, and metabolic acidosis. As with all inhaled sympathomimetic medications, cardiac arrest and even death may be associated with an overdose of olodaterol.

Treatment of overdosage consists of discontinuation of STIOLTO RESPIMAT together with institution of appropriate symptomatic and supportive therapy. The judicious use of a cardioselective beta-receptor blocker may be considered, bearing in mind that such medication can produce bronchospasm. There is insufficient evidence to determine if dialysis is beneficial for overdosage of STIOLTO RESPIMAT. Cardiac monitoring is recommended in cases of overdosage.

Reviewer's Comments:

The language related to human overdoses has been modified slightly compared to the monoproducts. The reviewer defers to the Medical Officer for evaluation of these changes.

(b) (4)

Animal data is not presented in Section 10 of the monoproduct labels and is likewise considered unnecessary for the STIOLTO RESPIMAT product.

12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action

STIOLTO RESPIMAT
STIOLTO RESPIMAT contains both tiotropium and olodaterol. The (b) (4) ~~properties~~ described below for the individual components apply to STIOLTO

RESPIMAT. These drugs represent 2 different classes of medication (an anticholinergic [redacted] and a [redacted]) that have different effects on clinical and physiological indices. [redacted]

Tiotropium
Tiotropium is a long-acting, muscarinic antagonist which is often referred to as an [redacted] anticholinergic. It has similar affinity to the subtypes of muscarinic receptors, M_1 to M_5. In the airways, it exhibits pharmacological effects through inhibition of M_3-receptors 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-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The bronchodilation following inhalation of tiotropium is predominantly a site-specific effect.

Olodaterol
Olodaterol is a long-acting $beta_2$-adrenergic agonist (LABA). The compound exerts its pharmacological effects by binding and activation of beta2-adrenoceptors after topical administration by inhalation. Activation of these receptors in the airways results in a stimulation of intracellular adenyl cyclase, an enzyme that mediates the synthesis of cyclic-3', 5' adenosine monophosphate (cAMP). Elevated levels of cAMP induce bronchodilation by relaxation of airway smooth muscle cells. *In vitro* studies have shown that olodaterol has 241-fold greater agonist activity at $beta_2$-adrenoceptors compared to $beta_1$-adrenoceptors and 2299-fold greater agonist activity compared to $beta_3$-adrenoceptors. The clinical significance of these findings is unknown.

Beta-adrenoceptors are divided into three subtypes: $beta_1$-adrenoceptors predominantly expressed on cardiac muscle, $beta_2$-adrenoceptors predominantly expressed on airway smooth muscle, and $beta_3$-adrenoceptors predominantly expressed on adipose tissue. $Beta_2$-agonists cause bronchodilation. Although the $beta_2$-adrenoceptor is the predominant adrenergic receptor in the airway smooth muscle, it is also present on the surface of a variety of other cells, including lung epithelial and endothelial cells and in the heart. The precise function of $beta_2$-receptors in the heart is not known, but their presence raises the possibility that even highly selective $beta_2$-agonists may have cardiac effects.

Reviewer's Comments:

The summary paragraph at the beginning of Section 12.1 describes the combination product and is therefore not present in the monoproduct labels. The sponsor's language has been modified to be consistent with the ANORO ELLIPTA label. The clinical pharmacology reviewer and Medical Officer may have additional comments. Minor edits have been made to the first sentence of the tiotropium section compared to the SPIRIVA products, which the reviewer has removed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

STIOLTO RESPIMAT

No studies of the carcinogenicity, *in vitro* mutagenicity, or impairment of fertility were conducted with STIOLTO RESPIMAT, however, studies are available for the individual components, tiotropium and olodaterol.

(b) (4)

Tiotropium

No evidence of tumorigenicity was observed in a 104-week inhalation study in rats at tiotropium doses up to (b) (4) 59 mcg/kg/day, in an 83-week inhalation study in female mice at doses up to (b) (4) 145 mcg/kg/day, and in a 101-week inhalation study in male mice at doses up to (b) (4) 2 mcg/kg/day. These doses correspond to approximately 30 (b) (4), 40 (b) (4), and 0.5 (b) (4) times the recommended human daily inhalation dose (RHDID) on a mcg/m^2 basis, respectively. (b) (4)

Tiotropium bromide demonstrated no evidence of mutagenicity or clastogenicity in the following assays: the bacterial gene mutation assay, the V79 Chinese hamster cell mutagenesis assay, the chromosomal aberration assay (b) (4) in human lymphocytes *in vitro*, (b) (4) the mouse micronucleus assay (b) (4) *in vivo*, and the unscheduled DNA synthesis assay in primary rat hepatocytes *in vitro* (b) (4).

In rats, decreases in the number of corpora lutea and the percentage of implants were noted at inhalation tiotropium doses of (b) (4) 78 mcg/kg/day or greater (approximately 35 (b) (4) times the RHDID on a mcg/m^2 basis). No such effects were observed at (b) (4) 9 mcg/kg/day (approximately 4 (b) (4) times than the RHDID on a mcg/m^2 basis). The fertility index; however, was not affected at inhalation doses up to 1-689 mcg/kg/day (approximately 760 (b) (4) times the RHDID on a mcg/m^2 basis). (b) (4).

Olodaterol

Two-year inhalation studies were conducted in rats and mice to assess the carcinogenic potential of olodaterol. Lifetime treatment of female rats induced leiomyomas of the mesovarium at doses of 25.8 and 270 mcg/kg/day (approximately 18- and 198-fold, respectively, the (b) (4) RHDID on an AUC basis). No tumor findings were observed in male rats at doses up to 270 mcg/kg/day (approximately 230-fold the (b) (4) RHDID on an AUC basis). Lifetime treatment of female mice induced leiomyomas and leiomyosarcomas of the uterus at doses ≥76.9 mcg/kg/day (approximately 106-fold the (b) (4) RHDID on an AUC basis). No tumor findings were observed in male mice at doses up to 255 mcg/kg/day (approximately 455-fold the (b) (4) RHDID on an AUC basis). Increases in leiomyomas and

leiomyosarcomas of the female rodent reproductive tract have been similarly demonstrated with other beta$_2$-adrenergic agonist drugs. The relevance of these findings to human use is unknown.

Olodaterol was not mutagenic in the *in vitro* Ames test or in the *in vitro* mouse lymphoma assay. Olodaterol produced increased frequency of micronuclei in rats after intravenous doses. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. The mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Olodaterol did not impair male or female fertility in rats at inhalation doses up to 3068 mcg/kg/day (approximately 2322 times the (b) (4) RHDID on an AUC basis).

(b) (4)

(b) (4)

Reviewer's Comments:

(b) (4)

(b) (4)

(b) (4)

12 Appendix/Attachments

1. IND 76397 Nonclinical Review, Dr. Molly Shea, April 25, 2008.
2. IND 76397 Nonclinical Review, Dr. Lawrence Sancilio, June 24, 2010.

DIVISION OF PULMONARY AND ALLERGY PRODUCTS
PRELIMINARY PHARMACOLOGY SAFETY REVIEW

IND: 76,397
Drug: Fixed Dose Combination of BI 1744 Cl plus Tiotropium
Drug Category: Long acting β_2 agonist and anticholinergic
Review completion date: April 25, 2008

Boehringer Ingelheim Pharmaceuticals, Inc. (BI) proposed two Phase 2 clinical studies (study nos. 1237.4 and 1237.9) to open this IND. For both studies, the efficacy and safety of inhaled BI 1744 CL in fixed dose combination (FDC) with tiotropium will be assessed in chronic obstructive pulmonary disease (COPD) adult patients (males and females ≥ 40 years of age; females will be using 2 adequate methods of birth control). Study no. 1237.4 proposes to treat ~320 COPD patients with 2 µg/5 µg, 5 µg/5 µg or 10 µg/5 µg of BI 1744 Cl/tiotropium bromide and compare it to the active control, 5 µg tiotropium, once daily for 4 weeks using the Respimat inhaler. Study no. 1237.9 proposes to treat ~ 96 COPD patients once daily for 4 weeks duration at 2 µg/5 µg or 5 µg/5 µg of BI 1744 Cl/tiotropium using the Respimat inhaler.

Prior to submitting this IND, the sponsor has conducted 2 clinical trials using BI 1744 Cl in combination with tiotropium bromide. Study no. 1237.1 assessed the safety and tolerability of single inhalation doses of 2.5, 5, 10, 20 and 40 µg of BI 1744 in free combination with 5 and 10 µg of tiotropium bromide in healthy males. Study no. 1237.1 dosed healthy males for 14 days with 2 µg/5 µg, 10 µg/5 µg and 40 µg/5 µg as FDC of BI 1744/tiotropium. Adverse events observed in these studies included headache, migraine, tachycardia, dry mouth and/or throat, throat irritation, nasopharyngitis restlessness and diarrhea.

On January 29, 2007, BI submitted IND 76,362 for BI 1744 Cl monoproduct for the treatment of COPD. Review of the relevant repeat dose inhalation toxicology studies in the rat (4-week and 13-week) and dog (4-week) resulted in a systemic NOAEL of 133.5 µg/kg and a local NOAEL of 6.17 µg/kg in the rat and a systemic NOAEL of 0.55 µg/kg and local toxicity NOAEL of 3.43 µg/kg in the dog as pulmonary deposited doses. The 4-week rat study dosed animals with 0 (vehicle control: 0.01% benzalkonium chloride, 0.01% edetate sodium and citric acid to adjust pH), 7.71 (LD), 26.2 (MD) and 133.5 µg/kg (HD) of BI 1744 as pulmonary deposited doses. Histopathology data showed minimal heart cardiomyopathy in the HD male group (2/10 males) and hemapoiesis of the spleen that increased in incidence and severity with dose in males (2/10 males, 4/10 males, 8/10 males and 9/10 males for the VC, LD, MD and HD, respectively). Local toxicity was observed in the larynx (squamous metaplasia in HD males, necrosis of U-shaped cartilage in MD and HD males and HD females, and ventral pouch inflammation in HD males and females) and the lungs (alveolar macrophage accumulation at all doses in males and females). Based on an evaluation of this study alone, no systemic NOAEL could be defined due to the absence of histology data from the LD and MD male groups for cardiomyopathy and due to the splenic findings in the LD group for this study. Additionally, no NOAEL for local toxicity could be defined due to the findings in the lung at the LD group. However, the sponsor submitted a 13-week repeat dose inhalation

rat study on December 27, 2006 in support of their proposed 2-year rat carcinogenicity study. This 13-week study exposed rats to 0 (air control), 0 (VC), 6.17, 23.9, 97.1 and 283.3 µg/kg pulmonary deposited doses of BI 1744 Cl. The systemic histopathology findings identified in the 4-week rat study were not observed in the 13-week rat study up to 283.3 µg/kg doses of BI 1744 Cl indicating that the findings in the 4-week study were not drug-related. Additionally, rats dosed up to 283.3 µg/kg of BI 1744 Cl for 13-weeks did not show the alveolar macrophage accumulation in the lungs observed from the 4-week rat study. Therefore, the systemic NOAEL for the 4-week study was considered 133.5 µg/kg, which coincided with an AUC of 148,500 pMol*h/L, and the local toxicity NOAEL was considered 7.71 µg/kg of BI 1744 Cl due to the laryngeal cartilage necrosis observed at higher doses.

In the 4-week repeat dose inhalation dog study, animals were exposed to 0 (VC), 0.55 (LD), 3.43 (MD) and 31.8 µg/kg (HD) of BI 1744 Cl as pulmonary deposited doses. The MD and HD groups showed increased heart contractile force. On Day 2 of dosing, LD, MD and HD males (+24%, +64% and +60%, respectively) and females (+41%, +72% and 100%, respectively) showed an increase in heart rate. On Day 27, HD males showed an increase of QTcB by 12.5%. This was not observed in females. Due to findings in the liver at the MD and HD, the systemic NOAEL was considered the LD (0.55 µg/kg) which had an AUC of 309 pMol*h/L. The local toxicity NOAEL was defined as the MD (3.43 µg/kg) due to laryngitis of the larynx and focal pleural fibrosis of the lung at the HD. Based on the calculated safety margins using systemic exposures (AUCs) and lung burden ratios, the proposed clinical trial for IND (b) (4) was supported. Adequate systemic safety ratios based on AUC levels in rat (594) and dog (1.24) and adequate local safety ratios in rat (46) and dog (11.14) were achieved.

BI 1744 Cl did not induce genetic toxicity in the microbial mutagenesis or the *in vitro* mouse lymphoma assay. The *in vivo* rat micronucleus assay was conducted as part of the 4-week repeat dose inhalation study at BI 1744 Cl pulmonary deposited doses up to 133.5 µg/kg/day. The test produced negative results but did not reach a maximum tolerated or acceptable limit dose for this assay and the sponsor will need to repeat an evaluation of this endpoint under appropriate test conditions. Given that BI submitted this IND for COPD, this deficiency was not a clinical hold issue. The sponsor was informed of this deficiency for BI 1744 Cl monoproduct for IND 76,362 and was notified of this deficiency for the current IND 76,397 at the time of the pre-IND. They indicated at the pre-IND meeting that the follow-up assay would be conducted in the first quarter of 2008.

Spiriva (tiotropium 18 µg; NDA 21-395) was approved for marketing in the U.S. for the treatment of bronchospasm and dyspnea associated with COPD on January 30, 2004. The dose of tiotropium for this IND (76,397) is 5 µg. Reference is made to the nonclinical review of NDA 21-395 for tiotropium bromide monohydrate completed by Dr. Luqi Pei on September 17, 2002. Briefly, pharmacological studies showed that tiotropium acts as an acetylcholine antagonist. The blockade of acetylcholine activity at the m_3 receptor in the respiratory tree results in bronchodilation. Toxicological studies performed with tiotropium showed no evidence of genetic toxicity and carcinogenicity.

Additionally, there was no evidence of teratogenicity in rats or rabbits treated with tiotropium. However, embryo/fetal death in rats and rabbits may result after exposure to tiotropium during pregnancy. Spiriva is classified as a Pregnancy Category C agent due to the observations of fetal absorption, litter loss, decreases in the number of live pups at birth and mean pup weights and a delay in pup sexual maturation observed in rats. The primary target organs of toxicity were gastrointestinal tract (decreased GI motility) and secretory glands (decreased tear and saliva production). Secondary target organs included eye, respiratory tract, heart and urinary bladder. BI has submitted a new NDA (NDA 21-936) for tiotropium bromide (5 and 10 µg) to be administered as a soft mist aerosol using the Respimat inhaler. This NDA is currently under review within the Division of Pulmonary and Allergy Products; no additional nonclinical studies to support the Respimat formulation.

In support of the two clinical trials proposed for this IND (76,397), the sponsor submitted four 4-week repeat dose inhalation studies (2 rat and 2 dog studies). One rat and one dog study examined 6:1 and 1:2 dose ratios of BI 1744 Cl/tiotropium bromide and the other rat and dog studies examined 1:1 dose ratios BI 1744 Cl/tiotropium bromide, covering the dose ratios proposed in their clinical protocols. In rats, the 6:1 and 1:2 ratios of the FDC BI 1744 Cl/tiotropium bromide were administered as 0 (VC-containing benzalkonium chloride), 7.5/1.3, 203.2/39.3, 7.3/14.3 and 174.7/326.3 µg/kg/day as the pulmonary deposited doses. Two males in the 203.2/39.3 µg/kg/day group died (1 due to food asphyxiation on Day 1 and 1 due to diffuse moderate lung congestion/hemorrhage on Day 23) and two males in 174.7/326.3 µg/kg/day (deaths due to moderate lung congestion/hemorrhage). Two females in 174.7/326.3 µg/kg/day group died (1 due to food asphyxiation and the second death due to an unknown cause). Ophthalmic examination showed 1 female treated with 203.2/39.3 µg/kg/day and 1 female treated with 174.7/326.3 µg/kg/day having rough surfaces on their corneas by Day 23 of dosing. After cessation of treatment, the cornea returned to normal after 4-weeks. The potential target organs of toxicity are the larynx (necrosis of U-shaped cartilage), lungs (congestion and alveolar macrophage accumulation) and liver (increased hematopoesis).

In the second rat study, rats were exposed to 1:1 ratios of the FDC BI 1744 Cl/tiotropium bromide at 0 (VC), 77.86/8.52 (LD), 55.5/57.7 (MD) and 217.4/226.6 µg/kg/day (HD) as the pulmonary deposited doses for 4 weeks. Three males died (1 MD and 1 HD male on due to asphyxiation by food, and 1 HD male due to lung abscess associated with pleuritis). No females died prior to scheduled sacrifice. The potential target organs of toxicity relevant to human dosing were the larynx (necrosis of U-shaped cartilage), lung (congestion and alveolar macrophage accumulation and abscess in lung of 1 rodent that died) and lymph node (plasmacytosis of mandibular lymph node).

Dogs were exposed to 6:1 and 1:2 ratios of BI 1744 Cl/tiotropium bromide for 4 weeks at pulmonary deposited doses of 0 (VC), 4.1/0.67, 31/4.95, 4.1/7.2 and 47.8/96.3 µg/kg/day of BI1744/tiotropium. No dogs died prior to scheduled sacrifice. There were statistically significant increases in heart rates in BI 1744 Cl/tiotropium treated males and females compared to the VC animals on both Day 2 (males Day 2 +69% to +125%, and females +32% to 110%) and Day 27 (males +31% to +68% and females +31% to 71%) through 4 hours post-dose. By Day 27, this increase of HR was blunted compared to the Day 2

measurements, suggesting an adaptive response. QT interval was slightly decreased in males on Day 2 immediately post-dose in males from Groups 3 (-14%), 4 (-14%) and 5 (-24%) and 4 hours post-dose in males from Groups 3 (-24%) and 5 (-24%). On Day 27, QT-interval was slightly decreased (-15%) in male Groups 3 and 5 immediately post-dose but did not reach statistical significant at any of the time point measured. QT-interval was unchanged in females on both Days 2 and 27 of measurement. When QT was corrected for HR, no statistically significant change compared to the VC group was observed in males or females throughout the study. Target organs of toxicity were the larynx (neutrophil infiltration), lung (agonal congestion/hemorrhage and alveolar macrophage accumulation), eyes (bilateral keratitis and vascularization), heart (papillary muscle focal myocardial fibrosis), liver (periportal glycogen with portal and capsular fibrosis), lymph node (congestion), testes (bilateral seminiferous epithelial degeneration) and thymus (involution).

In the second dog study, dogs were exposed to 1:1 FDC of BI 1744/tiotropium at pulmonary deposited doses of 0 (VC), 1.43/1.52 (LD), 4.03/4.2 (MD) and 38/39.3 (HD) µg/kg/day for 4 weeks. No dogs died prior to scheduled sacrifice. The target organs of toxicity were the eyes (keratitis, conjunctivitis and vascularization), lacrimal gland (inflammatory cell infiltration), larynx (inflammatory exudate), lung (bronchiolar focal squamous metaplasia), liver (centrilobular glycogen reduced), urinary bladder (congestion), prostate (minimal focal inflammatory cell infiltration), testes (segmental hypoplasia) and thymus (involution).

No NOAEL was determined in any of these studies due to findings in the lung at all doses in the rat and findings in the liver at all doses in the dog. Although no NOAELs were determined for these studies, no new toxicities were observed in these studies compared to observations made in rats and dogs administered inhalation doses of BI 1744 or tiotropium as monoproducts at comparable doses. Additionally, the doses of BI 1744 Cl used in the FDC formulations went up to substantially higher doses than those explored with BI 1744 Cl monoproduct. At comparable doses and in combination with tiotropium, rats and dogs did not show evidence of a drug interaction in terms of an increase in incidence or severity of the toxicities previously identified with the monoproduct.

Recommendation: The toxicity profiles for the monoproducts (tiotropium for chronic administration and BI 1744 up to 4 weeks administration) have been adequately characterized. After repeated daily dosing for 4 weeks in rats and dogs with the BI 1744 Cl/tiotropium FDC, no new toxicities were identified. The sponsor has proposed maximal inhalation doses of BI 1744 Cl (10 µg) that are less than the previously supported maximum dose in clinical trials (20 µg). The proposed use of tiotropium (5 µg) is substantially below the currently approved tiotropium level allowed for inhalation administration (18 µg/day). Additionally, the sponsor has had previous clinical experience with the FDC product at levels substantially higher than what they have proposed for this IND with no serious adverse events observed up to 14 days in healthy male volunteers. Taking these data as a whole, the proposed studies are considered safe to proceed from the nonclinical perspective.

External Comments: None.

Molly E. Shea, Ph.D.

Linked Applications	Sponsor Name	Drug Name
IND 76397	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	BI 1744 CL AND TIOTROPIUM BROMIDE

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/s/

MOLLY E SHEA
04/25/2008

TIMOTHY J MCGOVERN
04/25/2008

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:	IND76397
Supporting document/s:	SDN 24
Sponsor's letter date:	11/13/09
CDER stamp date:	11/16/09
Product:	Combination of BI 1744 CL and tiotropium bromide monohydrate
Indication:	Treatment of COPD
Sponsor:	. Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division:	Pulmonary, Allergy and Rheumatology Products
Reviewer:	Lawrence F. Sancilio, Ph.D.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Eunice H. Chung

Template Version: December 7, 2009

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1 Executive Summary

1.1 Recommendations

1.1.1

In two 13-week combination inhalation toxicity study in dogs, at dose ratios of 1:1, 2:1 and 1:2.5 of BI 1744 CL: tiotropium bromide, the results in the 1:1 ratio [(4:3.5 µg/kg (LD), 15.5:14.25 µg/kg (MD) and 75:70 µg/kg (HD)) and in the 2:1 and 1:2.5 studies [(4:9 µg/kg (LD 1:2.5), 31.5:75.5 µg/kg (HD 1:2.5), and 74.5: 36.3 µg/kg (2:1)], no new toxicities related to BI 1744 CL and tiotropium bromide or drug interactions were observed. From a nonclinical standpoint, It is recommended that clinical studies with a 4:1 and 8:1 dose ratios may be conducted without any additional 13-week inhalation toxicity studies in dogs.

Q. Boehringer Ingelheim believes that submission of 13-week inhalation toxicity studies in dogs at dose ratios of 1:1, 2:1 and 1:2.5 of BI 1744 CL: tiotropium bromide allows them to conduct additional doses ratios in Phase 3 (and potentially within a marketed product) without conducting additional 13-week combination toxicity study in dogs with a 4:1 and 8:1 doses ratios. Does the Agency agree that these ratios are covered by the submitted 13-week inhalation toxicity studies in dogs?

Response

The 13-week inhalation toxicity studies in dogs at dose ratios of 1:1, 2:1 and 1:2.5 of BI 1744 CL: tiotropium bromide was reviewed. No new toxicities related to BI 1744 CL and tiotropium bromide or drug interactions of clinical concern were observed. Considering these data and the previously submitted 4-week dog toxicity studies using dose ratios of 6:1 and 1:2, clinical studies with a 4:1 and 8:1 dose ratios may be conducted without any additional 13-week inhalation toxicity studies in dogs. We remind you that the final reports of these studies need to be submitted and any differences between the draft and final reports should be presented with the final reports.

1.2 Brief Discussion of Nonclinical Findings

Two 13-week combination inhalation toxicity studies were conducted in dogs with a 6-week recovery period. In the first study, the ratio of increasing doses of BI 1744 CL to tiotropium bromide was 1:1. The deposited doses (µg/kg) were: 4:3.5 (LD), 15.5:14.25 (MD) and 75:70 (HD) along with 72.5 µg/kg of BI 1744 CL and 75 µg/kg of tiotropium bromide as monotherapy arms. Dogs were exposed for 10 min to the aerosol in both studies. There were no treatment related deaths and the clinical signs were manifestations of the pharmacological action of both compounds, i.e. mydriasis, dry mouth, rapid heart rate and dry nose. There was no weight loss. In males, body weight gain in the BI 1744 CL group was greater than that of the control group; body weight gain that was less than the control occurred in males dosed with the combination. In females, body weight gain in the BI 1744 CL groups was greater (≥ 0.2 kg) than the

control group; body weight gained that was comparable or less than the control group was in the MD and HD combination groups and the tiotropium bromide groups. In the recovery group, males did not show an increase that was greater than the control group. In females, only the tiotropium bromide group showed an increase that was greater than that observed with the control group (C, +1.3 kg vs. T, +1.6 kg). Heart rate was increased immediately following administration of the combination on days 22 and 85. At the HD, there was a decrease in the hematocrit (males, -10%) and hemoglobin (males,-10% and females, -9%) similar to that seen in males with BI 1744 CL (-9%) alone; the decrease in the hematocrit and hemoglobin in males was still evident at the end of the 6-week recovery period. On day 3, cTnI levels were increased at the MD and HD combination groups and at the BI 1744 CL alone group. Although there was a low incidence of the CK-MB mass in the HD and in the BI 1744 CL groups and none in the MD group, the increase in the cTnI levels, a sensitive biomarker for cardiac toxicity, indicates some cardiac damage occurred in all 3 groups. Troponin is present in cardiac cells, and when cardiac damage occurs, the troponin is released in the circulation. The lack of cardiac histopathology in the MD group was due to healing of the lesion so that it was not detectable at necropsy while at the higher dose combination and BI 1744 CL alone, healing did not readily occur and fibrosis/fibroplasia was observed. This lack of cardiac damage observed at necropsy is common with LABAs where there was an increase in troponin levels at an earlier period. The AUCs on the combinations were not affected. Organ weights were statistically changed in several organs. Based on absolute weight, there was a decreased in the males in the BI 1744 CL group of adrenals (-25%) and thyroid glands (-32%) and in females of the heart (LD, -17%, MD, -17%, HD, -21% and tiotropium bromide, -17%). Relative to body weight, the changes occurred in males were: heart (LD, -17%, MD, -14%, HD, -20% and BI 1744 CL,-11%), testes (tiotropium bromide, -17%), thyroid (HD, -38% and BI 1744 CL, -33%) and adrenals (BI 1744 CL, -33%). In females, the following changes occurred: heart (LD, -20%, MD, -15%, HD, -16%). Relative to brain weight, the following changes occurred in males: adrenals (HD, -31% and BI 1744 CL, -35%) and in females: the heart (LD, -20%, MD, -12%, HD, -20% and tiotropium bromide, -17%), liver (MD, +15%, BI 1744 CL, +20%) and kidneys, BI 1744 CL (+19%). However, there was no correlation with the heart and other organ changes with the histopathology. The severity of the histopathological changes was mainly in the minimal to slight range. Targeted organs for BI 1744 CL in males were: heart (fibrosis/fibroplasia, cyst and necrosis), liver (glycogen depletion), kidney (glomerulopathy), trachea (infiltration), duodenum (debris, inflammatory cells, luminal and dilatation), epididymides (fibrosis/fibroplasia) and rectum (edema and infiltration). For females, the organs targeted were: heart (cyst), lung (infiltration, inflammatory), liver (glycogen depletion and increased storage), kidney (hyperplasia, epithelium), duodenum (degeneration and infiltration), thyroid (atrophy, focal) and mesenteric lymph node (blood resorption). For tiotropium bromide, the targeted organs in males were: tongue (infiltration, inflammatory), prostate (fibrosis/fibroplasia), skeletal muscle (infiltration), skin (inflammation) and mesenteric lymph node (blood resorption). For tiotropium bromide, the targeted organs in females were: kidney (basophilic tubules, focal and scar formation), stomach (infiltration), thyroid (fatty growth), mesenteric lymph node (blood resorption), uterus (hemorrhage), and urinary bladder (infiltration).
In the combination groups, increased incidence occurred in the heart (HD, males, necrosis, fibrosis/fibroplasia; MD and HD females, fibrosis/fibroplasia) and liver (males:

MD and HD, glycogen depletion and HD, increase glycogen storage; females: liver (HD, glycogen depletion; MD and HD, increase glycogen storage). There were no new toxicities or drug interactions as a result of combining the two compounds. The kidney showed dilatation of tubules in one male at the MD and HD. This was not considered a drug related effect since there was no dose relationship and in both groups, and the severity was minimal.

In the second study, the ratio of increasing doses of BI 1744 CL to tiotropium bromide was LD (1:2.5), HD (1:2.5) and HD (2:1). The deposited doses (µg/kg) were: 4: 9 (LD (1:2.5)), 31.5:75.5 (HD (1:.2.5)) and 75:36.3 (HD (2:1)) along with 74.8 µg/kg of BI 1744 CL and 76 µg/kg of tiotropium bromide monotherapies. There were no treatment related deaths, and the clinical signs were related to the pharmacological actions of the two compounds, i.e., dry mouth, rapid heart rate, mydriasis and dry nose. There was no weight loss. In males, body weight gain (≥ 0.2kg) over the control group occurred in the LD (1: 2.5) and BI 1744 CL groups; body weight gain that was equal to the control was the HD (2:1) and less than the control group was the HD (1:2.5) and tiotropium bromide groups. In females, only the body weight gain over control was the BI 1744 CL group: body weight gain that was equal to the control was the LD (1:2.5) group and less than the control group was the HD (1:2.5), HD (2:1) and tiotropium bromide groups. In the recovery group, the body weight gain in the males did not achieve that of the control group. In females, only the tiotropium bromide group showed an increase that was greater than that of the control group. There was a slight decrease (9%) in food consumption at the LD (1:2.5) which was completely reversible at the end of the recovery period. There was a drug interaction on the heart rate. On day 22, the percent increase in heart rate over baseline after the administration of BI 1744 CL and tiotropium bromide was 7% and 15%, respectively. When the combination groups were administered, the increase in heart rate over baseline was 19% for the LD 1.25; 40% for the HD (1:25) and 49% for the HD 2:1 showing a drug interaction.This effect was also observed on day 88. The increase in heart rate was accompanied by a shortening of the PR interval (BI 1744 CL -5%, tiotropium bromide, -5%, LD, -9% MD, -15%; HD -14% and an increase in QTc (Friderecia) interval (BI 1744 CL +6 %, tiotropium bromide, +3%, LD, 0, MD, +10%, HD +5%) intervals related to the tachycardia. This drug interaction occurred whereby tiotropium bromide by its anticholinergic effect blocks the vagal effect (the vagus has a pronounced inhibitory effect on the heart rate in dogs) and accompanied by a direct stimulating cardiac effect by the β_2 agonist results in a significant increase in heart rate. There was an initial effect on the blood pressure on day 22 following administration, the HD (2:1) showed an increase of 22 mm Hg while this was not seen with either BI 1744 CL or tiotropium bromide. However, this change was not seen on day 85. The diastolic pressure was not affected by the administration of the combination. There was a drug interaction with the hematological parameters in males as the changes over control [white blood cells (HD 1:2.5, +26% HD 2:1, +36%), neutrophils (HD 1:2.5, +37% HD 2:1, +50%), hemoglobin (HD (2:1), -11%), hematocrit (HD (2:1), -10%] seen in the combination groups that were not seen with BI 1744 CL or tiotropium bromide alone. In females, there was a drug interaction at the HD (1:2.5) where there was a decrease in the basophils (HD, 2:1, -36%) and white blood cells (+32%) and inorganic phosphorous levels (HD 2:1, +15%) which was not seen with either BI 1744 CL and tiotropium bromide. On day 3, cTnI levels were increased at the MD and HD and at the BI 1744 CL alone group. The incidence of increased cTnI levels

was: C, 0/12; LD (1:2.5) 0/8; HD (1:2.5), 8/12; HD (2:1), 11/12; BI 1744 CL, 10/12 and tiotropium bromide, 0/8. This increase is indicative of cardiac damage which was not seen histologically at necropsy since healing took place. This effect is characteristic of β2 agonism. Creatine kinase another biomarker of tissue damage was also increased in the HD (2:1) and BI 1744 CL but not at the time the cTnl levels were increased. Creatinine levels were increased at all combination doses and BI 1744 CL in males. However, in the combination female groups, there was a decrease creatinine levels. The increase in the creatinine levels in males was attributed to the anabolic effect of β_2 agonism on the skeletal muscle which was reflected by an increase in body weight gained (C, +1.5 kg; T, +0.5kg).

The toxicokinetics of BI 1744 CL and tiotropium bromide were not altered. Organ weights were changed based on absolute weight and relative to body and brain weights. The only correlation was with the HD 2:1 female spleen where there was fibrosis/adhesion capsular that correlated with an increase in weight based on absolute (+54%) and relative to body (+36%) and brain (65%) weights. In males, the target organs seen with BI 1744 CL alone were: heart (fibrosis/fibroplasia, infiltration, mineralization, pseudocysts, and necrosis), lacrimal gland (atrophy, lobular and dilatation of ducts), liver (glycogen depletion and increased glycogen storage), and kidney (mineralization, cortical). lymph node bifurcation (blood resorption), pharynx (dilatation of ducts), pituitary gland (distension, luminal), trachea (atrophy, epithelial), lung (granuloma), thyroid gland (hypertrophy, c-cell), tongue (necrosis, single cell), skeletal muscle (infiltration), skin (regeneration), spleen (congestion, acute), stomach (hyperplasia, lymphoid) and nasal cavity (goblet cell and lymphoid, hyperplasia).

In females the target organs for BI 1744 CL were: duodenum (dilatation of glands), eye (infiltration, inflammatory), heart (fibrosis/fibroplasia), lacrimal gland (dilatation of ducts), larynx (infiltration), liver (fibrosis/adhesion, capsular, infiltration, glycogen depletion and increased glycogen storage), lung (infiltration, inflammatory), LN mesenteric (blood resorption), parotid salivary gland (atrophy, lobular and glandular), Peyers patch (hyaline droplets), pharynx (atrophy, lobular and inflammation), thymus (involution), skin (infiltration, inflammatory), stomach (infiltration, inflammatory) and tongue (necrosis, single cell). In males, the target organs with tiotropium bromide were: heart (mineralization), lacrimal gland (dilatation of ducts), kidney (mineralization, cortical) optic nerve (infiltration), pharynx (debris, inflammatory cells, luminal, dilatation of ducts and necrosis, focal), trachea (infiltration), thyroid (hypertrophy, C-cell), skeletal muscle (infiltration), submandibular salivary gland (infiltration), skin (regeneration), spleen (congestion, acute), sublingual salivary gland (infiltration) and nasal cavity (atrophy, epithelial, goblet cell and lymphoid hyperplasia and infiltration, inflammatory). In females, the target organs with tiotropium bromide were: duodenum (dilatation of glands), esophagus (infiltration), lacrimal gland (dilatation of ducts), larynx (infiltration), lung (infiltration), lymph node mesenteric (blood resorption), pancreas (infiltration, inflammatory), parotid salivary gland (atrophy, lobular, and mucoid change), pharynx (concretion of secretory fluids, debris, inflammatory cells, luminal and infiltration, inflammatory), pituitary gland (cysts), thymus (involution),trachea (infiltration, inflammatory), skin (infiltration, inflammatory), stomach (infiltration, inflammatory) and nasal cavity (dilatation of glands).

Drug interaction occurred in females whereby the incidence of skin infiltration, inflammatory was decreased in the LD (1:2.5) and HD (1:2.5) groups or abolished (HD (2:1) from the incidence observed with BI 1744 CL and tiotropium bromide alone. There were histopathological changes that were not seen with either BI 1744 CL or tiotropium bromide alone. In males, the organs affected were: peripheral nerve HD (1:2.5), infiltration, inflammatory), lacrimal gland (HD (2:1), atrophy and mineralization), urinary bladder (LD (1:2.5) and HD (1:2.5), infiltration, inflammatory), lung (HD (1:2.5), foam cell accumulation), nasal cavity (HD (1:2.5), dilatation of glands and polyp), duodenum (HD (2:1),dilatation of glands) , pharynx (HD 2:1) atrophy, lobular) and stomach (HD (2:1), necrosis). In females, the organs affected were: duodenum (HD (1:2.5), hyperplasia, lymphoid), pharynx (HD, (1:2.5), dilatation of ducts), optic nerve (HD (2:1), infiltration), trachea (HD (1:2.5), deformation and metaplasia, squamous cell) and spleen (HD (2:1), fibrosis/adhesion, capsular). These were not attributed to the combination of BI 1744 CL and tiotropium bromide, since they were not observed in the 4-week inhalation toxicity in dogs where the ratios were 6:1 and 1:2. The deposited doses (BI 1744 CL/tiotropium bromide) were: 3.75/0.625 mcg/kg, 37.5/6.25 mcg/kg, 3.75/7.5 mcg/kg and 37.5/75 mcg/kg (BI 1744 CL and Tiotropium bromide (6:1) combination and 1:2 combination): 4-Week Inhalation Toxicity in dogs with a 4-Week Recovery Period, Document /Study No.: U06-1895).The findings were in the minimal to slight category. At the end of the recovery period some of the toxicity was still present. From the literature, these findings were not spontaneous and from in-house INDs and NDAs, they were not characteristic of antimuscarinic (aclidinium) and β_2 agonist (formoterol) compounds and are not combination related.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number: BI 1744 CL, NA

Tiotropium bromide monohydrate, 411207-31-3

2.1.2 Generic Name: BI 1744 CL, NA

Tiotropium bromide monohydrate

2.1.3 Code Name: BI 1744 CL ; Tiotropium bromide monohydrate (Ba 679 BR)

2.1.4 Chemical Name: BI 1744 CL

2H -1 ,4-Benzoxazin-3(4H)-one, 6-hydroxy-8-[(1R)-1-hydroxy-2-[[2-(4-methoxyphenyl)-1, 1-dimethylethyl] amino] ethyl]-, monohydrochloride

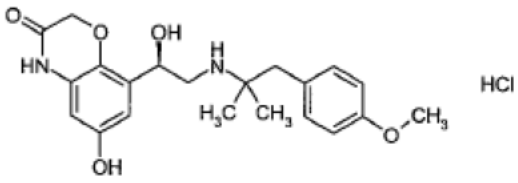
Tiotropium bromide monohydrate

3-Oxa-9-azoniatricyclo [3.3.1.$0^{2,4}$]nonane, 7-[(hydroxydi-2-thienylacetyl) oxy]- 9, 9-dimethyl-, bromide, monohydrate, (1α, 2β, 4β, 5α, 7β)-

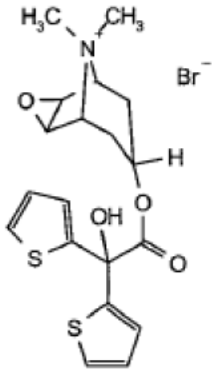
2.1.5 Molecular Formula/Molecular Weight:

BI 1744 Cl: $C_{21}H_{26}N_2O_5 \cdot HCl$/422.9 (salt) and 386.5(free base)
Tiotropium $C_{19}H_{22}NO_4S_2Br$: 472.4

2.1.6 Structure: BI 1744 CL



Tiotropium bromide monohydrate



2.1.7 Pharmacologic class: BI 1744 CL, β2 agonist
Tiotropium bromide, long- acting anti muscarinic agent

2.2 Relevant IND/s, NDA/s, and DMF/s: IND [(b) (4)] (BI 1744 CL Respimat for COPD and asthma); IND [(b) (4)] (tiotropium/salmeterol for COPD); IND [(b) (4)]); IND 65,127 (tiotropium bromide respimat); NDA 21-937 (Spiriva Respimat); NDA 21-395(tiotropium); DMF [(b) (4)] (tiotropium bromide monohydrate)

2.3 Clinical Formulation: Refer to the Dr. Molly Shea's May 6, 2008 review of IND [(b) (4)] submission date, 3/28/10).

2.3.1 Drug Formulation: [(b) (4)]% benzalkonium chloride, [(b) (4)]% disodium EDTA.H_2O [(b) (4)] HCl added [(b) (4)]

2.3.2 Comments on Novel Excipients: None are novel.

2.3.3 Comments on Impurities/Degradants of Concern: NA

2.5 Regulatory Background: This is a combination product of tiotropium bromide, an antimuscarinic agent, and BI 1744 CL, a β_2 agonist. Tiotropium bromide was approved for marketing as Spiriva for the treatment of COPD (1/3/04). To support their proposed clinical trial in the opening IND with this combination, two 4-week inhalation toxicity

studies in dogs. One study examined 1:1 dose ratios of BI 1744 CL /tiotropium bromide covering their dose ratios in their originally proposed clinical trial. In the second dog inhalation toxicity study, the ratios were 6:1 and 1:2 (see the May 6, 2008 review of the March 28 submission of IND 76,397 by Molly Shea, Ph.D). The results of these studies and those inhalation toxicity studies in rats (4- and 13- weeks) and dogs (4-weeks) with BI 1744 CL alone, supported the 4-week clinical inhalation studies of 2 µg/5 µg, 5 µg/5 µg and 10 µg/5 µg of BI 1744 CL/tiotropium bromide combination and 2 µg/5 µg, 5 µg/5 µg of BI 1744 CL/tiotropium bromide combination using the Respimat inhaler. To support longer term studies, two combination 13- week inhalation toxicity studies with 6-week recovery periods in dogs (combinations of 1:1, 1:2.5 and 2:1) were proposed and accepted (see the May 6, 2008 review of IND76,397 of M. Shea, submission date, 3/28/08) and submitted for review in this submission.

2.5.1 Previous Clinical Experience: Four-week clinical inhalation studies of 2 µg/5 µg, 5 µg/5 µg and 10 µg/5 µg of BI 1744 CL/tiotropium bromide combination and 2 µg/5 µg, 5 µg/5 µg of BI 1744 CL/tiotropium bromide combination using the Respimat inhaler.

History of Regulatory Submission: In the May 6, 2008 review of IND76,397 of M. Shea, submission date, 3/28/08, the minutes of the pre-IND meeting held on November 27, 2007 regarding the development of a combination product of tiotropium bromide and BI 1744 CL were summarized. Their proposed 1:1 combination ratio was adequate to support an NDA application, assuming that the complete development program for the mono-product BI 1744 CL is conducted. However, if Boehringer Ingelheim, wished to pursue additional dose ratios in addition to their 4-week 1:1, 6:1 and 1:2 inhalation toxicity studies (2 rat and 2 dog), their ongoing 1:1 13-week repeat dose inhalation dog toxicity studies should be expanded to include higher ratios and a high dose of the BI 1744 CL and tiotropium bromide alone to determine toxic and no adverse dose levels. At this point, the sponsor proposed a 13-week inhalation dog toxicity study using rations of 1:2.5 and 2:1 ratios (tiotropium bromide: BI 1744 CL). Based on the results of two combination dog inhalation toxicity studies involving ratios of1:1 dose ratios in the first study and 1:1, 6:1 and 1:2 in the second study, the proposed 1:2.5 and 2:1 13-week inhalation toxicity study with a 6-week recovery period in dogs was acceptable.

Original submission was made on March 28, 2008 and review completed on May 6, 2008.

3 Studies Submitted

3.1 Studies Reviewed

Toxicology

Multidose

13-Week combination inhalation toxicity study of BI 1744 CL and tiotropium bromide in Beagle dogs with a 6-week recovery period, No. 07B077, vol. 1

13-Week combination inhalation toxicity study of BI 1744 CL and tiotropium bromide in Beagle dogs with a 6-week recovery period, No. 07B183, vol. 5

3.2 Studies Not Reviewed: None.

3.3 Previous Review Referenced: Dr. Molly Shea's May 6, 2008 review of IND 76,397, submission date Mar. 28, 2008.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: 13-Week combination inhalation toxicity study of BI 1744 CL and tiotropium bromide in Beagle dogs with a 6-week recovery period (DRAFT)

Study no.:	07B077
Study report location:	Vol. 1
Conducting laboratory and location:	Dept. of Non-Clinical Drug Safety of Boehringer Ingelheim Pharma GmbH & Co. Germany
Date of study initiation:	8/7/07
GLP compliance:	No. No signature. The study was conducted under GLP conditions
QA statement:	No. No signature
Drug, lot #, and % purity:	BI 1744 CL ,06147, 99.4% Tiotropium bromide, 1026724, 99.7%

Key Study Findings

- The dose groups were: C, LD (BI 1744 CL 4 µg/kg + 3.5 µg/kg of tiotropium bromide), MD (BI 1744 CL 15.5 µg/kg + 14.25 µg/kg of tiotropium bromide) and HD (BI 1744 CL 75 µg/kg + 70 µg/kg of tiotropium bromide), BI 1744 CL (72.5 µg/kg) and tiotropium bromide (75 µg/kg)
- Clinical signs were characteristic of a B2 agonist (tolerance to increased heart rate) and an antimuscarinic agent (tachycardia, mydriasis and dry mouth).
- The combination (HD) group increased the heart rate that was higher than that seen with either BI 1744 CL or tiotropium bromide alone.
- In the combination groups, increased incidence of toxicity occurred at the MD and HD in the heart (fibrosis/fibroplasia) and liver (glycogen depletion and increased glycogen storage) in both sexes. Both are typical effects of LABAs.
- In the combination groups, there was no new toxicity.

Methods

Doses:	See table below. Doses are those of the free base
Frequency of dosing:	Daily
Route of administration:	Oral inhalation, face mask with oropharyngeal tube MMAD 1.95-2.22 µm; GSD, 2.71-3.42
Exposure to aerosol:	10 min
Formulation/Vehicle:	(b) (4) % benzalkonium chloride (b) (4) % disodium EDTA.H_2O (b) (4) HCl added (b) (4)
Species/Strain:	Beagle dog
Number/Sex/Group:	Main: 4; Recovery: Control, 2; HD, 2; BI 1744 CL and tiotropium bromide alone, 2
Age:	ca 7 months
Weight:	Males, 7.3-10.4 kg; females, 5.6-8.7 kg
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	Related to food administration and failure to visually inspect the palate. There was no impact on the validity of this study.

Justification of dose levels:

The doses were selected based on the previously conducted 4-week inhalation studies in dogs and the proposed clinical doses

Doses

Achieved doses (AD) were determined from the following formula:

$$AD = \frac{C \times RMV \times t}{BW}$$

Where C= aerosol concentration (µg/L), RMV= measured respiratory minute volume (L/min), t= exposure time (min), BW= body weight

Compound	Achieved Dose, µg/kg	Deposited Dose, µg/kg 25%
Control, Vehicle		
LD BI 1744 CL + Tiotropium bromide	 16 + 14	 4 + 3.5
MD BI 1744 CL + Tiotropium bromide	 62 + 57	 15.5 + 14.25
HD BI 1744 CL + Tiotropium bromide	 300 + 280	 75 + 70
BI 1744 CL	290	72.5
Tiotropium bromide	300	75

Observations and Results

Mortality: Daily
Results: None.

Clinical Signs: Daily
Results: A qualitative and semi quantitative description is presented in the following table excerpted from the submission. In the combination groups, there was a dose related increase in increased heart force, rapid heart beat, dry mouth mucosa, dry nose and mydriasis. The BI 1744 CL alone treated animals showed tolerance to the increased heart force and rapid heart rate. There were intermittent days of dry mouth mucosa. The tiotropium bromide alone treated animals showed rapid heart beat, dry mouth mucosa, mydriasis and dry nose; some of the animals showed intermittent increased heart force, dry eyes, swollen eyelids and reddened conjunctiva. These signs were due to the pharmacological activity of the compounds.
Recovery period (day 134):
Males: C, 0/2; HD, dry mouth, 1/2; BI 1744 CL , 0/2; tiotropium bromide, reddened ears, 1/2.
Females: Complete recovery.

Table 3.2.2: 1 Overview on treatment related clinical signs in dogs exposed to a BI 1744 CL / tiotropium FDC (1:1) and the mono compounds

Clinical sign	Vehicle-Control	Low-Dose	Mid-Dose	High-Dose	Mono-1744	Mono-Tio
Increased heart force	One female on one day	Individual animals intermittently on individual days	Individual animals intermittently on individual days	Most animals on first two days of study, individual animals on individual days towards end of treatment	Most animals on most days at beginning of study, decrease over time	Some animals intermittently on most days of the study
Rapid heart rate	One male on one day	Individual animals intermittently on individual days	Many animals on many days at beginning of study, decrease over time	Most animals on nearly all days of main study	Many animals on many days	Most animals on nearly all days of the main study
Dry mouth mucosa	Individual animals in-termittently on indivi-dual days	Many animals on most days	Many animals on most days	Most animals on nearly all days of main study	Individual animals intermittently on individual days	Most animals on nearly all days of main study
Dry nose	Not observed	Many animals on many days	Most animals on nearly all days	Nearly all animals on nearly all days of main study	Some animals on first 6 days of study	Nearly all animals on nearly all days of main study
Mydriasis	Not observed	Individual animals intermittently on individual days	Many animals on most days	All animals on practically all days of main study	Practically not observed	All animals on practically all days of main study
Dry eyes, swollen eyelids, reddened conjunc-tivae	Not observed	Not observed	Practically not observed	Some animals intermittently on individual days	Not observed	Some animals intermittently on individual days

Study no. 07B077: BI 1744 CL / tio (1:1) – 13-wk tox dog ih

Body Weights Gained: Prior to testing and weekly thereafter.
Results: Weight gained was determined by comparing the difference prior to dosing and at termination between the treated and control groups. The results are presented in the following table. Increased body weight gained occurred in both sexes at all doses. In males, the increase (≥0.2 kg) was greater than controls in the BI 1744 CL group and equal to or less than controls in the MD, HD and tiotropium bromide groups. In females, increases over control was observed in the BI 1744 CL group and equal to or less than the control group occurred in the LD, MD, HD and tiotropium bromide groups. In the recovery group, data for the LD and tiotropium bromide groups in both sexes were not reported. In males, the increase in body weight gained was less than that in the control

group. In the HD group there was no gain but a loss. In females, there was no gain in the control group. Increases occurred in the BI 1744 CL groups. Weight loss occurred in the MD group.

Sex/Group	Body Weight Gained, kg (Day 87-Day -1)	Recovery Period Body Weight Gained, kg (Day 133-Day 91
Male		
C	+1.5	+1.2
LD	+2.0	NR
MD	+0.7	NR
HD	+1.6	-1.0
BI 1744 CL	+2.0	-0.6
Tiotropium bromide	+0.9	+0.1
Female		
C	+1.3	+0.6
LD	+1.4	NR
MD	+1.0	NR
HD	+0.7	+0.1
BI 1744 CL	+1.6	+0.4
Tiotropium bromide	+0.7	1.4

NR, Data were not reported.

At the end of the recovery period (day 134), relative to controls, there was a decrease in body weight in the HD, BI 1744 CL and tiotropium bromide males and in females, a decrease in the HD and an increase in the BI 1744 CL group that was less than control and the tiotropium bromide group which was higher than the control group (>0.2kg).

Feed Consumption: Daily.
Results: As seen in the following two graphs excerpted from the submission, both males and females showed a similar effect in the MD combination and HD combination groups. Initially the food was pellet food which was changed to tinned food. There was initially a decrease in food intake which peaked between days 29 and 36 and then was similar to the control groups throughout the dosing and recovery periods. This was a transient effect which may have been attributed to the type of food given to the dogs.

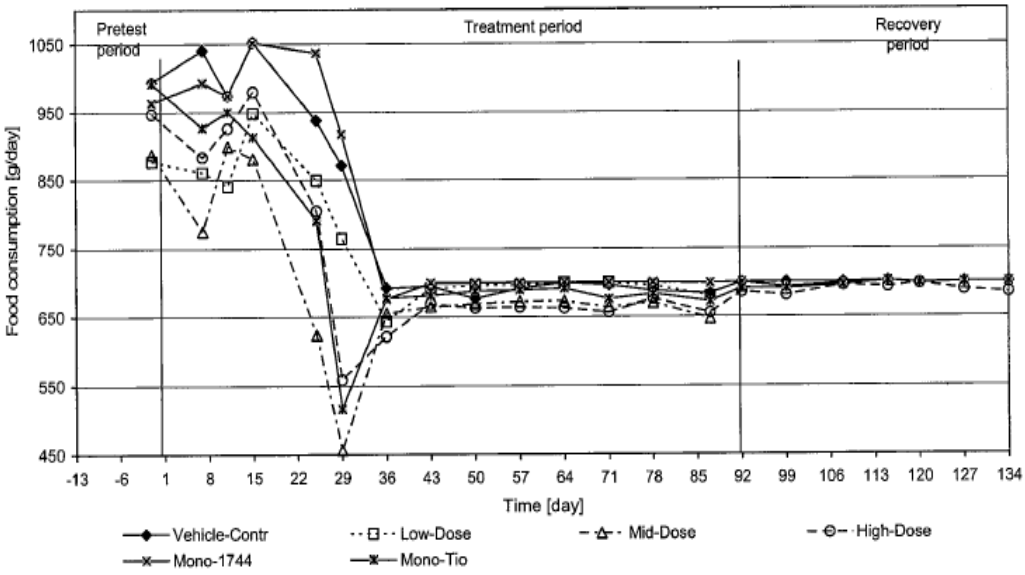


Figure 11.2: 1 Food consumption males - absolute

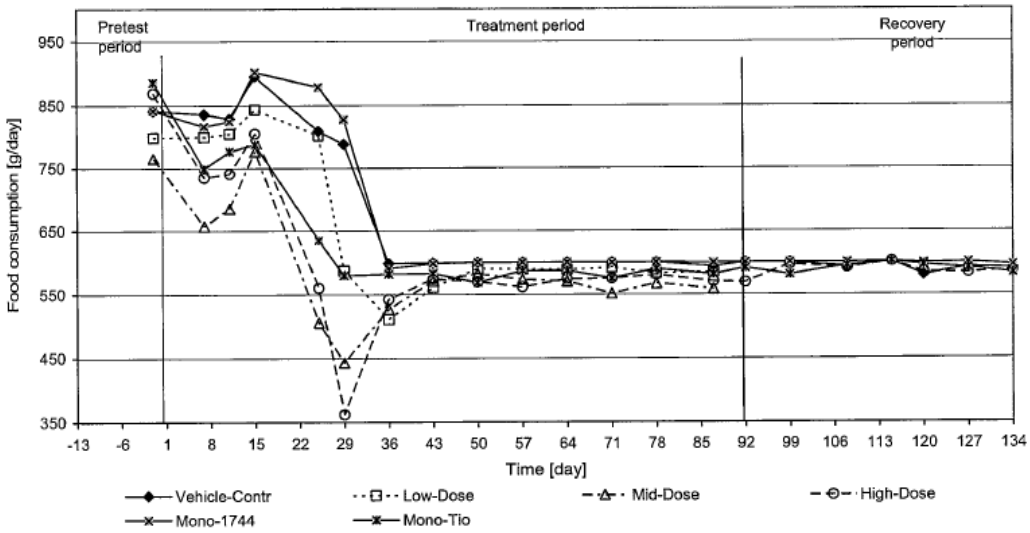


Figure 11.2: 2 Food consumption females - absolute

Ophthalmoscopy: Prior to initiation of dosing and on days 28 and 77 and on day 128 of the recovery period (HD combination, and mono dosed BI 1744 CL and tiotropium bromide).
Results: Day 77: Grey discharge tear liquid monoocular:
Males, C, 0/6; HD combination, 1/6
Females, C, 0/6; HD combination, 3/6
Males, tiotropium bromide alone (monocular and binocular, 1/6
Discharge slimy tear liquid monoocular:

Males, C, 0/6; HD combination, 1/6
Females, C, 0/6; HD combination, 3/6
Males, tiotropium bromide alone (monocular and binocular), 1/6
Females, tiotropium bromide alone (monocular), 1/6
Day 128, Recovery period, complete recovery from grey discharge and slimy tear discharge.

ECG and Hemodynamic Measurements: Prior to initiation of dosing (day - 5) and on days 22 and 85 prior to and immediately after exposure and on day 126 of the recovery period (C, HD combination, and mono dosed BI 1744 CL and tiotropium bromide). Results: The results are summarized in the following figures excerpted from the submission. The BI 1744 CL, tiotropium bromide and MD and HD combination groups showed immediately after dosing on days 22 and 85: Day 22, an increased heart rate (BI 1744 CL +12%, tiotropium bromide, +19%, LD, +11%, MD, +36%; HD +31%) accompanied by decreased PR interval (BI 1744 CL -5%, tiotropium bromide, -5%, LD, -9% MD, -15%; HD -14% and an increase in QTc (Friderecia) interval (BI 1744 CL +6 %, tiotropium bromide, +3%, LD, 0, MD, +10%, HD +5%). There was a drug interaction reflected by the direct effect of the β_2 agonist and an indirect effect by the antimuscarinic, tiotropium bromide, (blockade of the vagus) resulting in an increase in heart rate that was greater than either BI 1744 CL and tiotropium bromide alone. The changes occurring at day 85 were less than those seen on day 22. At the end of the recovery period, these changes returned to baseline.

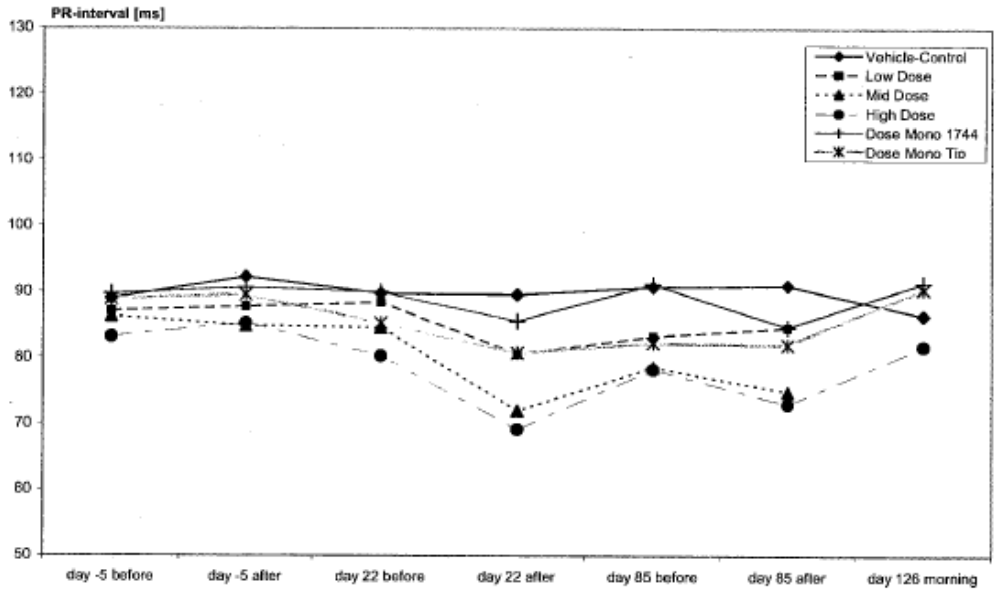


Figure 9: 1 BI 1744 CL / tiotropium bromide (Ba 679 BR): 13-week inhalation toxicity study in Beagle dogs with a 6-week recovery period.

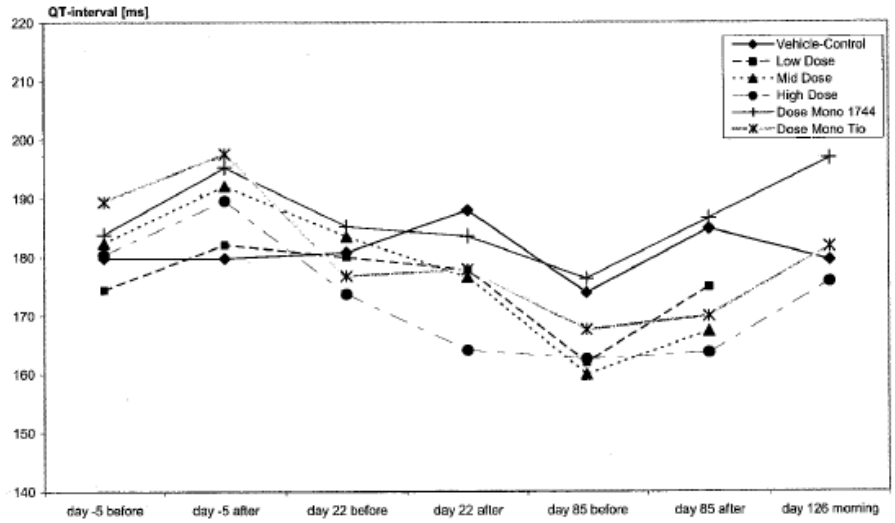


Figure 9: 3 BI 1744 CL / tiotropium bromide (Ba 679 BR): 13-week inhalation toxicity study in Beagle dogs

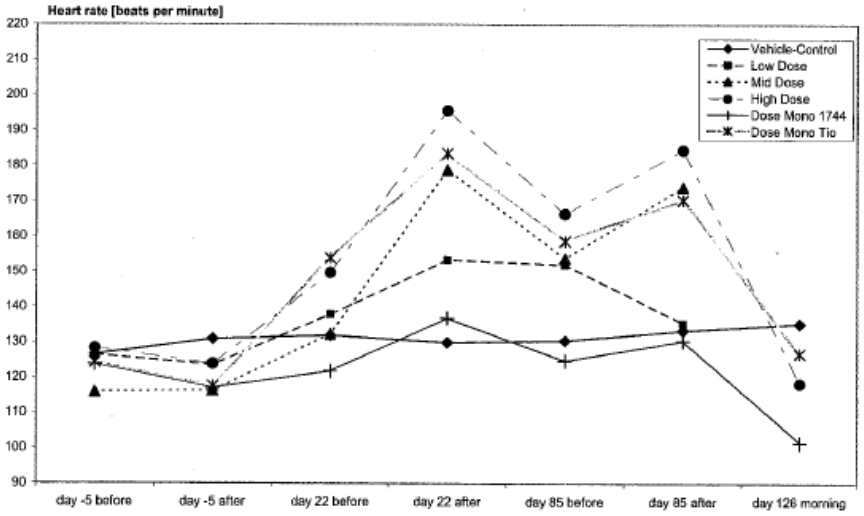


Figure 9: 4 BI 1744 CL / tiotropium bromide (Ba 679 BR): 13-week inhalation toxicity study in Beagle dogs with a 6-week recovery period.

Blood pressure was measured before and after the compound administration. There was no remarkable effect on the systolic and diastolic blood pressure with the combined doses and the individual doses of BI 1744 CL and tiotropium bromide indicating no drug interaction.

Hematology: A complete analysis was conducted. Prior to initiation of dosing (day -10) and on days 32, 88 and 129.
Results: The results on the final day of treatment (day 88) are summarized in the following table. In males, decrease in the blood parameters occurred at the HD and BI 1744 CL groups. This was attributed to the BI 1744 CL since there was no change with tiotropium bromide alone. Increase white blood cells occurred at the MD. Similar increase in the platelet count and in the APTT occurred in the combination groups which was attributed to BI 1744 CL. Tiotropium bromide induced no change in males.
In females, there was an equal decrease in the hemoglobin in the HD, BI 1744 CL and tiotropium bromide. As seen in males, there was an increase in the WBCs and neutrophils at the MD. However, tiotropium bromide also induced an increase in the WBCs and neutrophils which were not seen in males. These results indicate that there was no drug interaction between BI 1744 CL and tiotropium bromide. Further, there was no dose related effect.

Sex/Parameter	**Percent Change from Control (Day 88), P<0.05**				
	LD	**MD**	**HD**	**BI 1744 CL**	**Tiotropium bromide**
Male					
Hemoglobin			-10	-9	
Hematocrit			-10		
MCH			-5	-4	
MCV			-4	-4	
WBC		+87			
Neutrophils		+136			
Platelets	+25	+26	+31	+27	
APTT		+8	+6	+7	
Female					
Hemoglobin			-9	-11	-9
RBC				-9	-10
Hematocrit				-11	-9
MCH			-7		
MCV			-5	-3	
WBC		+39			+36
Neutrophil		+61			+54
Basophils (%)		-23	-49		
Platelets			+31		+40

In the recovery group (day 129, C, HD, BI 1744 CL and tiotropium bromide alone), the HD male group showed a significant 17% and 14% decrease in the hemoglobin and hematocrit levels, respectively. There was partial or complete recovery in the remaining male groups and in all the female groups.

Clinical Chemistry: Prior to initiation of dosing (day -10) and on days 4, 32, 88 and 129.
Results: The results observed on day 88 are presented in the following table. Increased creatine kinase (CK) is indicative of tissue damage. At day 88, the levels from CK were increased in males and decreased in females. This may be attributed to the histological presence of cardiac damage in males and not in females. The increase in the creatinine levels was attributed to the anabolic effect on skeletal muscle by the β_2 agonist, BI 1744

CL. In males, the changes were not dose related. In females, there was a dose related in crease in urea levels.

Parameter	% Change from Control (Day 88), P<0.05				
	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Male					
Creatine kinase		+30		+39	
Glucose		-30		-16	
Total cholesterol		+28			
Tryglycerides		+49			+78
Urea	+64			+37	+43
Creatinine	+33	+27	+42	+51	
Calcium	+21	+14	+29	+16	+17
Magnesium		+26	+13	+23	+24
Inorganic phosphorous	+16	+26	+21		+21
Female					
Creatine kinase				-69	
AST					-25
ALT					-45
Total bilirubin		-22	-26		-16
Glucose	-34	-29	-21	-24	
Urea	+42	+56	+71	+44	+32
Creatinine	+26	+30	+27	+41	
Magnesium	+14		+15		
Inorganic phosphorous	+30	+25	+23		+21

In the recovery groups (day 129, C, HD, BI 1744 CL and tiotropium bromide alone), there was partial or complete recovery.

Urinalysis: On days 91-95 (main study animals) and on days 135-136 (recovery animals). Specific gravity and components of the urine were reported and not urinary volume. In the methods section urine volume was one parameter that was cited to be measured.
Results: No treatment related change.

Biomarkers for myocardial damage: Troponin I, (CTn1), creatine kinase –MB (CK) determined on days -11, 3, 29, 84 and 127.
Results: The cut-off level for troponin 1 (cTn1) was 0.1 mcg/L and 3.2 mcg/L for CK-MB (mass). The following table excerpted from the submission lists the results seen on day 3. In the MD, HD and BI 1744 CL, the levels of cTn1 were increased above the cut-off in 6/8 at the MD, 10/12 at the HD and BI 1744 CL and 1/12 at tiotropium bromide. The levels return to normal by day 29 indicating a transient effect. CK-(mass) levels exceeded the cut-off levels in 1/12 in the HD and 2/12 in the BI 1744 CL group and none in the tiotropium bromide group. The increased incidence in the cTnI levels indicate that the increase incidence cTn1 levels were attributed to cardiac lesions which is characteristic of B2 agonism. CK-MB is another indicator of cardiac damage. In this study, the incidence was low as compared to the high incidence seen with cTn1.This may be attributed to the difference in duration whereby CK-MB lasts 24-48 hours as

compared to 5-10 days with cTnI (Widmann's Clinical Interpretation of Laboratory Tests, 11th ed, 1991). There was no evidence of a drug interaction.

Table 3.4.4: 1 Individual values of cTnI and CK-MB (mass): cTnI and CK-MB concentrations on Day 3 [µg/L]

	Target daily dose of BI 1744 BS / tiotropium [µg/kg]																	
	0.0 / 0.0 (Vehicle-Control)			15 / 15 (Low-Dose)			60 / 60 (Mid-Dose)			300 / 300 (High-Dose)			300 / 0.0 (Mono-1744)			0.0 / 300 (Mono-Tio)		
Group	1			2			3			4			5			6		
Gender	Animal No.	cTnI	CK-MB (mass)	Animal No..	cTnI	CK-MB (mass)	Animal No.	cTnI	CK-MB (mass)	Animal No.	cTnI	CK-MB (mass)	Animal No.	cTnI	CK-MB (mass)	Animal No.	cTnI	CK-MB (mass)
M	101	0.00	1.80	201	0.00	1.10	301	↑0.54	1.60	401	↑1.26	1.00	501	↑0.24	↑3.30	601	0.09	1.20
	102	0.00	1.40	202	0.05	1.60	302	0.06	1.50	402	↑0.18	1.30	507	↑1.08	1.70	602	↑0.13	1.30
	103	0.00	1.30	203	0.01	1.10	303	↑0.29	1.20	403	↑0.66	1.00	503	↑0.45	2.00	603	0.08	2.00
	104	0.01	2.70	204	0.01	2.50	304	↑0.62	0.80	404	↑11.7	2.50	504	↑0.69	2.00	604	0.02	1.00
	105	0.01	1.80	---	---	---	---	---	---	405	↑0.13	0.90	505	↑4.76	↑4.60	605	0.02	1.70
	106	0.01	1.10	---	---	---	---	---	---	406	↑0.93	1.70	506	↑0.60	2.00	606	0.03	1.40
F	151	0.01	1.20	251	0.01	1.00	351	↑0.76	0.90	451	0.05	1.10	551	0.07	2.30	451	0.07	1.70
	152	0.01	1.50	252	0.00	1.70	352	↑0.31	1.50	452	↑0.12	2.20	552	↑0.47	1.70	452	0.02	1.00
	153	0.01	1.30	253	0.01	1.40	353	↑0.86	1.60	453	↑0.71	1.90	553	0.07	1.40	453	#0.10	1.70
	154	0.01	1.40	254	0.02	1.70	354	0.00	1.20	454	↑0.74	1.40	554	↑0.12	2.90	454	#0.10	1.20
	155	0.00	1.20	---	---	---	---	---	---	455	0.05	1.40	555	↑0.18	2.10	455	0.01	0.60
	156	0.02	1.80	---	---	---	---	---	---	456	↑3.43	↑3.30	556	↑0.53	2.20	406	0.05	0.80

Study no. 07B077: BI 1744 CL / tio (1:1) – 13-wk tox dog ih
M: males; F: females
↑ concentration exceeds cut-off: 0.1 µg/L for cTnI and 3.2 µg/L for CK-MB (mass)
borderline concentration related to cut-off

Toxicokinetics: Day -1, predose; days 1, 30 and 86, 0.167, 0.333 1, 3, 8 and 24 h after finishing dosing. Assay was HPLC coupled to tandem mass spectroscopy. The LOQ was 20 pmol/l for BI 1744 CL and 25 pmol/l for tiotropium bromide.
Results: The results are summarized in the following tables excerpted from the submission. In males and females, the Cmax of BI 1744 CL alone was only affected on day 86; it decreased from day 1 by 62% and 53%, respectively, when compared to day 1. However, in both sexes, there was no change in the AUCs for BI 1744 CL alone and in combination with tiotropium bromide indicating no drug interaction. However, in the combination doses, the AUC of tiotropium bromide with the LD increased 2.3 fold when comparing day 86 with day 1. This did not occur with the MD and HD combinations indicating that this is not considered a true effect.

Table 3.5: 1 Mean toxicokinetic parameters of BI 1744 BS

Parameter	Day	Gender	Low-Dose (15/15)*	Mid-Dose (60/60)*	High-Dose (300/300)*	Mono 1744 (300/0)*
C(max) [pmol/L]	1	m	371	1610	15800	19000
	30	m	326	1810	7300	7410
	86	m	231	1550	9540	7300
	1	f	341	1920	14500	16100
	30	f	485	2070	16400	9080
	86	f	409	1540	15500	7520
AUC(0-24h) [pmol·h/L]	1	m	1960	7340	47000	45100
	30	m	2400	9490	50700	30900
	86	m	1850	8140	43400	30600
	1	f	1870	6890	46600	44600
	30	f	3370	13000	55000	43500
	86	f	2910	7370	51000	40600

Study no. 07B077: BI 1744 / tio (1:1) – 13-wk tox dog ih
* µg/kg BI 1744 BS / tiotropium; target doses

Table 3.5: 2 Mean toxicokinetic parameters of Ba 679

Parameter	Day	Gender	Low-Dose (15/15)*	Mid-Dose (60/60)*	High-Dose (300/300)*	Mono Tio (0/300)*
C(max) [pmol/L]	1	m	1570	6570	34800	35900
	30	m	1620	11300	30200	37800
	86	m	1500	6140	45800	16700
	1	f	1370	7550	49300	34900
	30	f	2810	14200	55200	25200
	86	f	2910	7970	78300	25700
AUC(0-24h) [pmol·h/L]	1	m	1830	9120	54300	57900
	30	m	2680	14700	50900	49300
	86	m	2040	9310	51000	19800
	1	f	1860	9500	61700	63200
	30	f	3970	20400	73200	36400
	86	f	4290	9920	69200	47600

Study no. 07B077: BI 1744 / tio (1:1) – 13-wk tox dog ih
* µg/kg BI 1744 BS / tiotropium; target doses

Gross Pathology: Complete necropsy was conducted on all groups:
Results: Heart, discoloration: Males: C, 0/4; HD, 3/4; females, C, 0/4; HD, 1/4.
Recovery group: Heart, discoloration: Complete recovery.

Organ Weights: The following organs were weighed: adrenals, heart, brain, kidneys, liver, lungs, ovaries, pituitary gland, prostate, spleen, testes and thyroid and parathyroid glands.
Results: The results are summarized in the following table. In females, the heart absolute, relative to body and brain weight was decreased in the three combination groups, and the decrease was not dose related. In males this was seen based on relative body weight. No significance is attributed to this heart weight change since there was no dose relationship and no cardiac histopathology with the LD and HD. Other organs that significantly changed were the adrenals, thyroid, lung and testes in males and the spleen, liver and kidneys in females. There was no indication of a drug interaction.

Absolute Weight

Sex/Parameter	Percent Change from Control, P<0.05 (Day 91)				
	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Male					
Adrenals				-25	
Thyroid				-32	
Female					
Heart	-17	-17	-21		-17

Relative to Body Weight

Sex/Parameter	Percent Change from Control, P<0.05 (Day 91)				
	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Male					
Heart	-17	-14	-20	-11	
Testes					-17
Adrenals				-33	
Thyroid			-38		
Female					
Heart	-20	-15	-16		

Relative to Brain Weight

Sex/Parameter	Percent Change from Control, P<0.05 (Day 91)				
	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Male					
Adrenals			-31	-35	
Female					
Heart	-20	-12	-20		-17
Liver		+15		+20	
Kidneys				+19	

Recovery period (135 days): Only the C, HD, BI 1744 CL and tiotropium bromide groups were in the recovery group. There was complete recovery in these groups.

Histopathology: The following is a list excerpted from the submission of tissues processed and examined. Tissues from all animals were examined.

Table 2.10.3: 1 Organs and tissues removed at necropsy (histological samples and levels)

Adrenal glands	Ovaries
Aorta	Oviduct
Bone (sternum)	Pancreas
Bone marrow (sternum)	Parathyroid glands (within thyroids, at least one)
Brain	Parotid salivary glands (at least unilateral)
Caecum	Peripheral (sciatic) nerve
Cervix uteri	Peyer's patches
Colon	Pharynx (soft palate and nasopharynx)
Duodenum	Pituitary gland
Epididymides	Prostate
Oesophagus	Rectum
Eyes	Skeletal muscle
Gall bladder	Skin
Heart	Spinal cord (cervical, thoracic, lumbar)
Ileum	Spleen
Jejunum	Stomach
Kidneys	Sublingual salivary glands (at least unilateral)
Knee joint	Submandibular salivary glands (at least unilateral)
Lacrimal gland (at least unilateral)	Testes
Larynx (longitudinal section)	Thymus
Liver	Thyroid glands
Lungs (7 samples)	Tongue
Lymph nodes, bifurcational	Trachea (including bifurcation)
Lymph nodes, mesenteric	Ureters
Mammary gland (only females)	Urinary bladder
Nasal cavity (level I and level II)	Uterus
Optic nerves	Vagina

Adequate Battery: Yes.
Peer Review: No.

Results

The results are summarized in the following tables. A scoring system of 1, minimal; 2, slight; 3, moderate; 4, severe was used to describe the severity of the lesion. The lesions are presented as the average severity score. The incidences were low and the severity of the lesions was predominantly in the minimal and slight category. The organs targeted with BI 1744 CL alone in males were: heart, liver, kidney, trachea, duodenum, epididymides and rectum. In females, the organs targeted were: heart, lung, liver, kidney, duodenum, thyroid and mesenteric lymph node. For tiotropium bromide alone, the targeted organs in males were: the tongue, palate, prostate, skeletal muscle, skin and mesenteric lymph node. In females, the targeted organs alone were: kidney, palate, stomach, thyroid, mesenteric lymph node, uterus, and urinary bladder.

Sex/ Organ/ Observation	Incidence, N=4 (Average Severity Score)					
	C	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Males						
Heart						
Fibrosis/fibroplasia	0	0	0	3 (2.3)	2 (1.5)	0
Cyst	0	0	0	0	1	0
Necrosis	0	0	0	1 (2.0)	0	0
Liver						
Glycogen depletion	0	0	2 (2.0)	2 (2.0)	1(1.0)	0
Glycogen storage increased	0	0	0	1(2.0)	0	0
Kidney						
Cyst	0	0	0	1	0	0
Glomerulopathy	0	0	0	0	1 (1.0)	0
Dilatation of tubules	0	0	1(1.0)	(1.0)	0	0
Esophagus						
Infiltration	0	0	0	1(2.0)	0	0
Trachea						
Infiltration	0	0	0	0	1(1.0)	0
Tongue						
Infiltration, inflammatory	0	0	0	0	0	1(3.0)
Palate						
Dilatation of ducts	0	0	1(2.0)	2 (2.0)	0	2(2.0)
Hemorrhage	0	0	0	1 (2.0)	0	0
Duodenum						
Debris, inflam, cells, luminal	0	0	0	0	1(2.0)	0
Dilatation	0	0	0	0	1(2.0)	0
Epididymides						
Fibrosis/fibroplasia	0	0	0	0	1 (2.0)	0
Prostate						
Fibrosis/fibroplasia	0	0	0	0	0	1 (2.0)
Skeletal muscle						
Infiltration	0	0	0	0	0	1(2.0)
Skin						
Inflammation	1(1.0)	1(1.0)	1 (4.0)	0	0	2 (1.0)
Rectum						
Edema	0	0	0	0	1(1.0)	0
Infiltration	0	0	0	0	1(1.0)	0
LN, mesenteric						
Blood resorption	0	0	0	0	0	1(2.0)
Parotid salivary gland						
Dilatation of ducts	0	0	0	1 (1.0)	0	0
N=3, C						
Sublingual salivary gland, N=3C						
Dilatation of ducts	0	0	0	1 (2.0)	0	0

Sex/ Organ/ Observation	Incidence, N=4 (Average Severity Score)					
	C	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Females						
Heart						
Cyst	0	0	0	0	1	0
Fibrosis/fibroplasia	0	0	3(1.3)	1(1.0)	0	0
Mineralization	0	0	0	1(2.0)	0	0
Lung						
Infiltration, inflammatory	0	0	1(1.0)	0	1 (2.0)	0
Liver						
Glycogen, depletion	0	0	0	3(1.7)	1(1.0)	0
Glycogen storage increased	1(2.0)	1(1.0)	4(1.5)	4(1.5)	2(1.5)	0
Kidney						
Hyperplasia, epithelium	0	0	0	0	1(2.0	0
Basophilic tubules, focal	0	0	0	1(1.0)	0	1 (1.0)
Scar formation, cortical	0	0	0	0	0	1(2.0)
Palate						
Infiltration, inflammatory	0	0	0	0	0	2(1.5)
Stomach						
Infiltration	0	0	0	0	0	1(2.0)
Duodenum						
Degeneration	0	0	0	0	1(1.0)	0
Infiltration	0	0	0	0	1(2.0)	0
Thyroid						
Atrophy, focal	0	0	0	0	1(2.0)	0
Fatty growth	0	0	0	0	0	1(2.0)
LN, mesenteric						
Blood, resorption	0	1(2.0)	0	1(2.0)	1(1.0)	1(2.0)
Uterus						
Hemorrhage	0	0	0	0	0	1(1.0)
Urinary bladder						
Infiltration	0	0	0	0	0	1(2.0)

For toxicity to be considered new, the histopathology should be absent in both individual compound groups and in the control group and also not observed in the monotherapy product nonclinical development programs. The following table presents the apparent new toxicities resulting from the combination of the two compounds as perceived by this criterion. The infiltration seen in the esophagus has been reported in a 4-week inhalation toxicity study with tiotropium bromide in a 4-week inhalation study in dogs (see the 9/17/02 NDA 21-395 review of L. Pei), and therefore, is not considered a new toxicity. The hemorrhage seen in the palate may be attributed to the method of inhalation (use of oropharyngeal tube that is known to irritate the palate) and, therefore, is also not considered a new toxicity directly attributed to the HD combination. The mineralization in the papillary muscle of the heart was the result of the tachycardia seen with β_2 agonism and is not considered a new toxicity as a result of the combination (see L. Pei's May 6, 2008 review of IND (b) (4)) in a 13 week inhalation toxicity study in

dogs. Dilatation of the renal tubules is associated with toxicity. The kidney (dilatation of tubules) appears to be a targeted organ. However, at the MD and HD, there was no dose related increase in incidence to be considered a drug related effect. Further, the severity was minimal. The salivary gland was a target organ of tiotropium bromide in 13- and 52- week inhalation study in dogs (See the 9/17/09 review of L. Pei of NDA 21-395). However, in a 13-week inhalation combination toxicity study in dogs with Formoterol, a β_2 agonist, and Aclidinium, an antimuscarinic compound), the salivary gland was not a target organ of Aclidinium alone. However, there were no clinical signs of an antimuscarinic effect. From the results with tiotropium bromide, the dilatation of ducts of the parotid salivary gland is not considered a toxicity from the combination of the 2 compounds.

Sex/ Organ/ Observation	Incidence, N=4 (Average Severity Score)					
	C	LD	MD	HD	BI 1744 CL	Tiotropium bromide
Males						
Esophagus						
Infiltration	0	0	0	1(2.0)	0	0
Palate						
Hemorrhage	0	0	0	1(2.0)	0	0
Parotid salivary gland						
Dilatation of ducts	0	0	0	1(1.0)	0	0
Kidneys						
Cyst	0	0	0	1	0	0
Dilatation of tubules	0	0	1(1.0)	1(1.0)	0	0
Females						
Heart						
Mineralization	0	0	0	1(2.0)	0	0

Recovery Period: Only the organs from the C, HD, BI 1744 CL and tiotropium bromide groups were examined. There was partial recovery of the cardiac fibrosis/fibroplasia at the HD (1, mean severity score, 2) in males and full recovery from the other histopathology in males and females.

Study title: 13-Week combination inhalation toxicity study of BI 1744 CL and tiotropium Bromide in Beagle dogs with a 6-week recovery period (DRAFT)

Study no.:	07B183
Study report location:	Vol. 5
Conducting laboratory and location:	Dept. of Non-Clinical Drug Safety of Boehringer Ingelheim Pharma GmbH & Co. Germany
Date of study initiation:	6/24/08
GLP compliance:	No The study was conducted under GLP conditions
QA statement:	No
Drug, lot #, and % purity:	BI 1744 CL,06147, 100.2% Tiotropium bromide, 1026724, 99.9%

Key Study Findings

- The deposited doses (µg/kg) were: 4:9 (LD (1:2.5)), 31.5:75.5 (HD (1:2.5)) and 75:70 (HD (2:1)) along with 74.8 µg/kg of BI 1744 CL and 76 µg/kg of tiotropium bromide.
- The combination product increased the already observed tachycardia observed with both monotherapies.
- Drug interaction occurred in hematology and clinical chemistry. Males: The HD (2:1) produced a decrease in the hemoglobin and hematocrit levels. At the HD (1:2.5) and HD (2:1), there was a dose related increase in white blood cells and neutrophil levels. Females: There was at the HD (2:1) an increase in white blood cells and inorganic phosphorous levels, and a decrease in basophil levels.
- Drug interaction occurred in females where the incidence of inflammatory infiltration in the skin that occurred in BI 1744 CL and tiotropium bromide alone was reduced by LD (1:2.5) and HD (1:2.5) and abolished by HD (2.1).
- No histopathology related to the either BI 1744 CL or tiotropium bromide was observed.

Methods

Doses:	See table below. Doses are those of the free base
Frequency of dosing:	Daily
Route of administration:	Oral inhalation, face mask with tube achieves 25% deposition. MMAD 1.93-2.36 µm; GSD, 2.69-3.40
Exposure to aerosol:	10 min
Formulation/Vehicle:	(b) (4) % benzalkonium chloride (b) (4) % disodium EDTA.H_2O (b) (4) HCl added (b) (4)
Species/Strain:	Beagle dog
Number/Sex/Group:	Main: 4; Recovery: Control, 2; HD, 2; BI 1744 CL and tiotropium bromide alone, 2
Age:	6- 7 months
Weight:	Males, 7.3-8.7 kg; females, 5.7-6.6 kg
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	Related to food administration and failure to visual inspect the palate. These deviations had no impact on the study.

Justification of dose ratios:

At the pre-IND meeting on Nov. 27, 2007, FDA commented on a 13-week bridging inhalation combination toxicity study in dogs involving BI 1744 CL and tiotropium bromide. In addition to the proposed 1:1 ratio of BI 1744 CL to tiotropium bromide to support clinical dosing of the 1:1 ratio, FDA recommended ratios of 1:2.5 and 2:1 to support the proposed additional clinical dose ratios. The HD in the 1:2.5 combination (120 mcg/kg of BI 1744 CL and 300 mcg/kg of tiotropium bromide) was based on the preliminary results of the 13-week inhalation toxicity study with the 1:1 combination (300 mcg/kg of BI 1744 CL and 300 mcg/kg) of tiotropium bromide showing pronounced tachycardia with histopathological changes in the myocardium. The HD of BI 1744 CL, 300 mcg/kg was the maximum dose administered in the of the 52-week inhalation toxicity study in dogs.

Doses

Achieved doses (AD) were determined from the following formula:

$$AD = \frac{C \times RMV \times t}{BW}$$

Where C= aerosol concentration (µg/L), RMV= measured respiratory minute volume (L/min), t= exposure time (min), BW= body weight

Compound	Achieved Dose, µg/kg	Deposited Dose, µg/kg 25% Deposition
Control, Vehicle		
LD (1:2.5) BI 1744 CL + Tiotropium bromide	16 + 36	4 + 9
HD (1:2.5) BI 1744 CL + Tiotropium bromide	126 + 302	31.5 + 75.5
HD (2:1) BI 1744 CL + Tiotropium bromide	298 + 145	74.5 + 36.3
BI 1744 CL	299	74.8
Tiotropium bromide	304	76

Observations and Results

Mortality: Daily

Results: None.

Clinical Signs: Daily

Results: The clinical signs in the following table excerpted from the submission were attributed to the pharmacological actions of BI 1744 CL and tiotropium bromide. The cardiac effects were related to the action of both compounds while the dry mouth mucosa, dry nose and ocular effects were related to the anticholinergic effect of tiotropium bromide.
Recovery period: All of these clinical signs were absent.

Table 3.2.2: 1 Overview on major clinical signs after exposure

Clinical sign	Group					
	Vehicle-Control	Low-Dose 1:2.5	High-Dose 1:2.5	High-Dose 2:1	Mono BI 1744	Mono Tio
Rapid heart rate	Not observed	Individual animals affected on very few individual days	Many animals affected on several days	Many animals affected on several days	Individual animals affected on very few individual days	Individual animals affected on very few individual days
Increased heart force	Not observed	Individual animals affected on very few individual days	Individual animals affected on very few individual days	Individual animals affected on very few individual days	Individual animals affected on very few individual days	Not observed
Dry mouth mucosa	Very few individual animals affected on very few individual days	Many animals affected on most days	Most animals affected on nearly all days	Most animals affected on nearly all days	Very few individual animals affected on very few individual days	Many animals affected on most days
Dry nose	Not observed	Many animals affected from Day 20	All animals affected on nearly all days	All animals affected on nearly all days	Very few individual animals affected on very few individual days	Most animals affected on nearly all days
Mydriasis	Not observed	All animals affected on many days	All animals affected on nearly all days	All animals affected on nearly all days	Not observed	Individual animals affected on very few individual days
Pupillary rigidity	Not observed	Individual animals affected on few days	All animals affected from Day 36	All animals affected from Day 36	Not observed	All animals affected from Day 36

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Body Weights Gained: Prior to testing and weekly thereafter.
Results: Weight gained was determined by comparing the difference prior to dosing and at termination. The results are presented in the following table. Increased body weight gained occurred in both sexes at all doses. In males, the increase (≥0.2kg) was greater than controls in the LD (1:2.5) and BI 1744 CL groups and less than controls in the HD (1:2.5 and 2:1) and tiotropium bromide groups. There was no change in the HD (2:1). In females, increases over control were observed in the BI 1744 CL group; in the other groups, the increase was less than the control group. The LD (1:2.5) was no different from the control. In the recovery groups, the increased body weight groups determined were less than the respective controls with the exception of the BI 1744 CL group where there was a loss. This was attributed to the recovery from the increased pharmacological (anabolic) effect of BI 1744 CL.

Sex/Group	Body Weight Gained, kg (Day 87-Day -1)	Recovery Period Body Weight Gained, kg (Day 133-Day 91
Male		
C	+1.5	+1.2
LD (1:2.5)	+2.0	NR
MD (1:2.5)	+0.7	NR
HD (2:1)	+1.6	+1.0
BI 1744 CL	+2.0	-0.6
Tiotropium bromide	+0.9	+0.1
Female		
C	+1.3	+0.6
LD(1:2.5)	+1.4	NR
MD(1:2.5)	+1.0	NR
HD (2:1)	+0.7	+0.1
BI 1744 CL	+1.6	+0.4
Tiotropium bromide	+0.7	+1.5

NR, Data were not reported.

Food consumption: Pre-test, day 3 and weekly thereafter.

Results: The results are presented in the following table. Food consumption in females was not affected. There was a slight decrease in males in the MD (2:1) and tiotropium bromide groups. In the recovery period, there was recovery in the HD (1:1.25) group. Data for the tiotropium bromide group at the end of the recovery period was not reported.

Sex/Group	% Change from Control, P<0.05	
	Treatment, Day 91	Recovery, Day 133
Male		
LD (1:2.5)	NS	Not reported
HD (1:2.5)	-8	NS
HD (2:1)	NS	NS
BI 1744 CL	NS	NS
Tiotropium bromide	-9	Not reported

NS, Not significant

Ophthalmoscopy: Prior to initiation of dosing and on days 28 and 77 and on day 128 of the recovery period.

Results: No treatment related effect. However, the sponsor did not conduct additional endpoint evaluations such as the Schirmer test to examine for dry eyes.

ECG and Hemodynamic Measurements: Prior to initiation of dosing (day - 4) and on days 22 and 85 prior to and immediately after exposure and on day 126 of the recovery period (C, HD (1:1.25) and HD (2:1) combinations, and 1744 CL).

Results: In the following figure excerpted from the submission presents the change in heart rate before and after the inhalation administration on days 22 and 88. The LD (1:1.25) and BI 1744 CL did not show any remarkable effect. The HD (1:1.25) and HD (2:1) groups showed on day 22 a rapid increase in heart rate that was dose related. This effect was reproduced on day 88. On day 22, the percent increase in heart rate after the administration of BI 1744 CL and tiotropium bromide was 7 and 15%, respectively. When the combination groups were administered, the increase in heart rate was 19% for the LD 1.25; 40% for the HD (1:25) and 49% for the HD 2:1 showing a drug interaction. The increase in heart rate was accompanied by a shortening of the PR- (LD 1:2.5, -7%; HD 1:2.5, -15%; HD 2:1, -16%) and an increase in the QTcF(LD 1:2.5, +3% HD 1:2.5, +5% HD 2:1, +3%)intervals. This drug interaction was due to tiotropium bromide by its anticholinergic effect blocking the vagal effect on the heart (the vagus has a pronounced inhibitory effect on the heart rate more so than in humans) and the direct stimulating cardiac effect by the β_2 agonist, BI 1744 CL. At the end of the recovery period, the heart rate in the BI 1744 CL group was still increased by 14% over the pretest level. All other groups returned to baseline levels. Similar changes occurred on the day 85 reading of the heart rate as presented in the following figure.

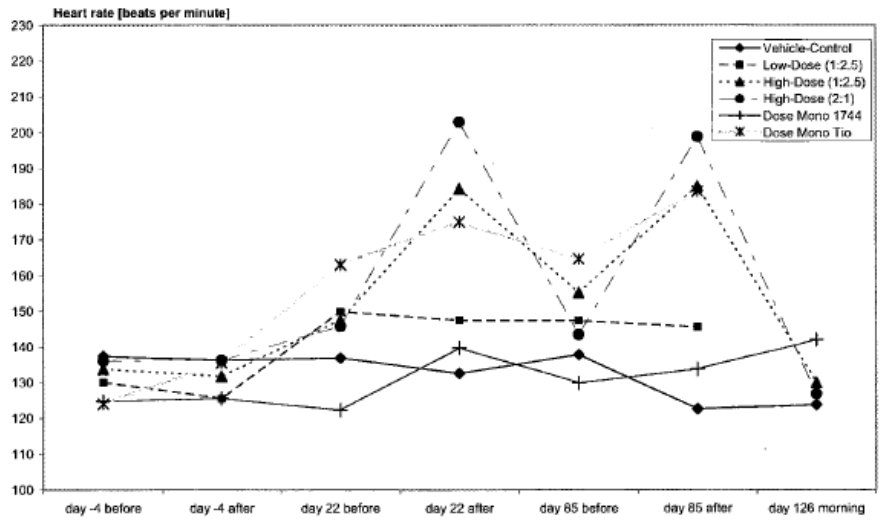

Figure 9: 4 BI 1744 CL / tiotropium bromide (Ba 679 BR) (1:2.5 and 2:1 combination): 13-week inhalation toxicity study in Beagle dogs with a 6-week recovery period.
Cardiovascular investigations: Heart rate (beats per minute), means.

The following figures excerpted from the submission presents the systolic and diastolic blood pressure. On day 22 the significant increase in systolic blood pressure following administration occurred only in the HD (2:1) group (+22.3 mmHg) while there was no significant increase with either BI 1744 CL (+7.1 mmHg) or tiotropium bromide (+2.1

mmHg) alone indicating a possible drug interaction. However, by day 85, the systolic blood pressure of HD was not increased after exposure indicating that the drug interaction was not a reality or tolerance developed.

Table 10: 5 BI 1744 CL / tiotropium bromide (Ba 679 BR) (1:2.5 and 2:1 combination): 13-week inhalation toxicity study in Beagle dogs with a 6-week recovery period.
Cardiovascular investigations: Systolic blood pressure [mmHg], mean±SD.

Dose	Systolic blood pressure [mmHg]															
	Statistics		Day -4 before		Day -4 imm. after		Day 22 before		Day 22 imm. after		Day 85 before		Day 85 imm. after		Day 126 morning	
Vehicle-Control	mean		152.2		142.5		128.1 #		127.0		145.4		132.9		130.0	
	n	SD	11	18.3	11	15.5	12	18.6	8	26.1	10	21.3	10	28.5	4	28.1
Low-Dose (1:2.5)	mean		142.4		118.1	***##	148.5 *		146.8 *		141.8		156.3 *		-	
	n	SD	8	31.7	8	26.1	8	29.2	8	19.2	5	25.7	7	27.3	-	-
High-Dose (1:2.5)	mean		136.3		137.5		147.5 *		147.2 *		152.8	#+(*)	152.0		140.3	
	n	SD	11	22.3	12	14.5	8	18.4	10	30.9	12	29.2	11	26.5	4	25.2
High-Dose (2:1)	mean		131.7	(***)	129.5		134.4		156.7	##+**	147.0	(#)	130.5	(##)	123.3	
	n	SD	10	13.0	11	15.4	8	15.6	10	17.3	12	20.5	10	14.3	4	5.4
Dose mono BI 1744	mean		137.3		134.4		129.0		136.2		138.4		158.9	##+(**)	151.5	
	n	SD	12	31.8	12	17.1	11	26.9	11	25.4	11	26.7	10	32.7	2	7.8
Dose mono Tio	mean		160.0		147.3		146.1		144.8		173.9 *		154.0	(##)	-	
	n	SD	7	20.9	8	30.8	8	9.9	8	26.9	8	25.0	7	20.9	-	-

* p<0.05 vs Control with Pre-test value before/after training as baseline
** p<0.05 vs Control with respective pre-dose value as baseline
*** p<0.05 vs Control (absolute values)
# p<0.05 vs Pre-test before training
p<0.05 vs prior to exposure/training
+ p<0.05 vs recovery (Day 126 morning)
(x) symbol in brackets trend

Table 10: 6 BI 1744 CL / tiotropium bromide (Ba 679 BR) (1:2.5 and 2:1 combination): 13-week inhalation toxicity study in Beagle dogs with a 6-week recovery period.
Cardiovascular investigations: Diastolic blood pressure [mmHg], mean±SD.

Dose	Diastolic blood pressure [mmHg]															
	Statistics		Day -4 before		Day -4 imm. after		Day 22 before		Day 22 imm. after		Day 85 before		Day 85 imm. after		Day 126 morning	
Vehicle-Control	mean		110.7		93.1 ##		84.6 #		79.6		96.6 #+		84.3		63.5 #	
	n	SD	11	18.6	11	17.1	12	12.5	8	25.1	10	25.3	10	23.5	4	18.1
Low-Dose (1:2.5)	mean		108.0		78.3	(***)##	97.8 (*)		81.5 (##)		89.6 #		89.6		-	
	n	SD	8	30.3	8	24.1	8	17.2	8	15.5	5	27.1	7	21.9	-	-
High-Dose (1:2.5)	mean		92.2 ***		79.2	***##	92.1		84.8		90.5		95.8		84.5	
	n	SD	11	12.9	12	13.6	8	9.9	10	19.6	12	18.6	11	20.4	4	19.4
High-Dose (2:1)	mean		94.5	(***)	87.5		78.5 #		89.2		88.1		84.4		70.5	
	n	SD	10	12.0	11	9.0	8	11.6	10	12.3	12	16.1	10	16.7	4	12.7
Dose mono BI 1744	mean		92.7 ***		88.3		78.5 (#)		74.2		79.9 (#)		95.3 ##		101.5 *	
	n	SD	12	23.7	12	9.7	11	23.4	11	14.4	11	17.0	10	30.4	2	10.6
Dose mono Tio	mean		90.6 ***		87.0		91.6		93.6		115.8 #*		99.4 (##)		-	
	n	SD	7	17.6	8	24.7	8	10.9	8	26.7	8	18.1	7	14.5	-	-

* p<0.05 vs Control with Pre-test value before/after training as baseline
** p<0.05 vs Control with respective pre-dose value as baseline
*** p<0.05 vs Control (absolute values)
# p<0.05 vs Pre-test before training
p<0.05 vs prior to exposure/training
+ p<0.05 vs recovery (Day 126 morning)
(x) symbol in brackets trend

Hematology and Clinical Chemistry: Hematology: A complete analysis was conducted. Blood was taken from the jugular vein prior to initiation of dosing (day -8) and on days 4, 32, 88 and 129. 129. Cardiomarker (CK) was determined from the sera collected on days -15, 3, 4, 29, 32, 84, 88, 127 and 129 and CTnI was determined on day 3.

Results:

The results for day 88 are summarized in the following tables. In the hematology evaluation, no toxicity was observed in the LD (1:1.25) group. In the combination groups, there was a dose related decrease in the hematological parameters in males. These changes were not observed with BI 1744 CL and tiotropium bromide indicating a drug interaction. In females, there was no indication of a drug interaction as changes seen in the HD (1:1.25) and HD (2:1) were seen with either BI 1744 CL or tiotropium bromide.

Sex/Parameter	Percent Change from Control, P<0.05 (Day 88)			
	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male				
Hemoglobin	NS	-11	NS	NS
Hematocrit	NS	-10	NS	NS
MCH	NS	-5	NS	NS
MCV	NS	-4	NS	NS
Reticulocyte	+48	NS	NS	NS
White Blood Cells	+26	+36	NS	NS
Neutrophils	+37	+50	NS	NS
Basophils	+30	+30	NS	NS
APTT	NS	+7	+6	NS
Female				
Hemoglobin	NS	NS	-10	NS
Hematocrit	NS	NS	-9	NS
MCH	-5	-8	-5	NS
MCHC	NS	-2	-2	-3
MCV	-4	-6	NS	NS
White Blood Cells	NS	+32	NS	+32
Neutrophils	+58	+58	+35	NS
Basophils	NS	-36	NS	NS
Platelets	+50	+50	+36	+58
APTT	+13	+13	+12	+13

.

NS Not significant

Recovery period: Only HD (1:2.5), HD (2:1) and BI 1744 CL were in the recovery group. Tiotropium bromide: No data reported.
There was full recovery.

Clinical Chemistry: One HD (1:2.5) male dog (306) on day 4 showed a transient increase in ALT (5.4xULN), AST (4.1x ULN) and elevated CK (day 3, 27.3x ULN and day 4, 8.6 x ULN). The report indicated along with the marked increase in the CTnI (C, 0.005; T, 5.86 mcg/l) indicated that this was attributed to cardiac damage. On day 29 the levels of ALT, AST and CK were normal. This animal was in the recovery group.

Upon examination of the histopathology of this animal on week 20 revealed no evidence of any cardiac damage indicating that there was complete healing of the cardiac tissue.

The results on day 88 are presented in the following table. There was no indication of a drug interaction.

	Percent Change from Control, P<0.05 (Day 88)				
	LD (1:2.5)	**HD (1:2.5)**	**HD (2:1)**	**BI 1744 CL**	**Tiotropium bromide**
Male					
AST	NS	NS	NS	+27	NS
ALP	NS	NS	-37	NS	NS
GLDH	NS	NS	-40	-42	NS
Triglycerides	NS	NS	NS	-23	NS
Creatinine	+21	+29	+28	+45	NS
Inorganic phosphorous	NS	+12	NS	-14	NS
Globulin	NS	-8	NS	NS	NS
B-Globulin%	NS	NS	-7	NS	NS
G-Globulin%	NS	+46	+28	NS	NS
A/G Ratio	NS	-15	NS	NS	NS
Female					
Glucose	NS	NS	-11	NS	-10
Total Cholesterol	NS	NS	-17	-18	NS
Creatinine	NS	NS	NS	+23	-22
Potassium	+12	+8	NS	+7	+7
Inorganic Phosphorous	NS	+15	NS	NS	NS
Total protein	NS	NS	NS	NS	-8
Albumin	NS	NS	NS	NS	-11

Recovery period (Day 129): Only C, HD (1:2.5), HD (2:1) and BI 1744 CL were in the recovery group. Tiotropium bromide: No data reported.
Males: G-Globulin, HD (1:2.5), -28%, HD (2:1) -29%.
Females: Glucose, HD (2:1), -13%.

Urinalysis: Complete analysis was conducted on days 91-95 (main study) and on days 135-136 (recovery period).
Results: There was no treatment related effect.

cTnI, Creatinine and Creatine Kinase Levels

The following tables excerpted from the submission presents the levels of cTnI on day 3 and the levels of creatinine and creatine kinase over the course of the study. The incidence of increased cTnI levels was: C, 0/12; LD (1:2.5) 0/8; HD (1:2.5), 8/12; HD (2:1), 11/12; BI 1744 CL, 10/12 and tiotropium bromide, 0/8. This increase is representative of cardiac damage. Creatine kinase levels were determined periodically during the study. Creatinine began to increase by day 32 in males and never increased in females. On day 88, the creatinine levels were significantly increased (%) in the following groups: Males, LD (1:2.5), (21%), HD (1:2.5), (29%) and HD (2:1), (28%) and BI 1744 CL, (45%). Increased creatinine levels is indicative of a skeletal muscle resulting from the anabolic effect by BI 1744 CL. Creatine kinase changes were indicative of cardiac damage were seen only in the HD (2:1) and BI 1744 CL groups. In the HD (2:1) males, increases were not seen until day 29 (77%); at day 84 there was a

102% increase in males and a 94% increase in females and by day 88, there was no significant decrease, a fast recovery. In contrast, BI 1744 CL showed an increase in males beginning on day 29 and in females on day 32. By 88, creatine kinase increased by102% in males and 56% in females. By day 129, the creatine kinase levels were still elevated in females by 74%. This was surprising since its duration is transient from the effects of cardiac damage. However, there was no evidence of cardiac damage at the end of the recovery period.

Table 3.4.3.5: 1 Individual data of cTnI on Day 3.

Sex	Day 3											
	Target daily dose of BI 1744 BS / tiotropium [µg/kg]											
	Vehicle Control 0/0		Low-Dose 1:2.5 15/37.5		High-Dose 1:2.5 120/300		High-Dose 2:1 300/150		Mono BI 1744 300		Mono Tio 300	
	No. [1]	cTnI* [µg/L]	No.	cTnI* [µg/L]	No.	cTnI* [µg/L]	No.	cTnI* [µg/L]	No.	cTnI* [µg/L]	No.	cTnI* [µg/L]
M	101	0.00	201	0.00	301	↑0.12	401	↑0.59	501	0.02	601	0.00
	102	0.03	202	0.00	302	0.00	402	↑0.48	502	↑0.54	602	0.00
	103	0.00	203	0.00	303	↑0.31	403	↑0.28	503	↑0.25	603	0.04
	104	0.00	204	0.01	304	0.02	404	↑0.76	504	0.08	604	0.03
	105	0.00			305	0.08	405	↑0.19	505	↑0.34		
	106	0.00			306	↑5.86	406	↑11.9	506	↑0.73		
F	151	0.04	251	0.01	351	0.02	451	↑0.65	551	↑0.53	651	0.00
	152	0.00	252	0.00	352	↑0.26	452	↑0.24	552	↑0.21	652	0.00
	153	0.00	253	0.01	353	↑0.41	453	↑0.70	553	↑7.07	653	0.07
	154	0.00	254	0.00	354	↑0.51	454	↑0.20	554	↑0.62	654	0.00
	155	0.00			355	↑1.88	455	↑0.19	555	↑0.40		
	156	0.00			356	↑0.12	456	0.09	556	↑0.13		

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[1] Animal Number
* Cut-off: 0.1 µg/L; values exceeding the cut-off:
M, F: male, female

Table 3.4.3.2: 1 Changes in creatinine (Crea).

Parameter [unit]	Daily target dose of BI 1744 BS / tiotropium [µg/kg]		Vehicle Control	Low-Dose 1:2.5		High-Dose 1: 2.5		High-Dose 2:1		Mono BI 1744		Mono Tio	
			0/0	15/37.5		120/300		300/150		300		300	
	Sex	Group	1	2		3		4		5		6	
		Day	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
Crea [µmol/L]	M	-8	53.5	55.1	*2.8*	57.3	*7.0*	58.3	*8.9*	56.6	*5.6*	55.6	*3.9*
		4	46.6	50.0	*7.2*	48.0	*2.9*	51.0	*9.3*	48.2	*3.3*	49.6	*6.4*
		32	48.8	↑59.6	*22.1*	57.6	*17.9*	↑61.4	*25.7*	↑65.3	*33.6*	47.5	*-2.8*
		88	57.7	↑69.6	*20.5*	↑74.4	*28.9*	↑74.2	*28.4*	↑83.5	*44.5*	51.7	*-10.5*
		129	63.4	n.a.	*n.a.*	61.2	*-3.5*	56.0	*-11.7*	66.7	*5.3*	n.a.	*n.a.*
	F	-8	65.1	60.6	*-6.8*	64.1	*-1.5*	57.2	*-12.0*	↓55.3	*-15.0*	54.8	*-15.7*
		4	57.0	58.4	*2.5*	52.0	*-8.7*	↓48.2	*-15.3*	↓44.3	*-22.2*	48.1	*-15.6*
		32	57.6	65.4	*13.4*	60.7	*5.3*	56.3	*-2.4*	66.1	*14.6*	↓ 42.7	*-26.0*
		88	65.3	75.0	*14.9*	74.8	*14.7*	69.2	*6.1*	80.1	*22.8*	↓ 50.8	*-22.2*
		129	69.2	n.a.	*n.a.*	55.7	*-19.5*	56.4	*-18.5*	62.9	*-9.0*	n.a.	*n.a.*

Study no. 07B183: BI 1744 CL/tio – 13-wk tox dog ih (1:2.5 + 2:1)
Δ% percent deviation from Control (calculated from original raw data/rounded)
↥ statistically significant increase compared with Control; p<=0.05, many to one t-test, two sided
↧ statistically significant decrease compared with Control; p<=0.05, many to one t-test, two sided
M, F: male, female

Table 3.4.3.5: 2 Creatine kinase group mean values and changes compared to Vehicle-Control.

Parameter [unit]	Target daily dose of BI 1744 BS/ tiotropium		Vehicle Control	Low-Dose 1:2.5		High-Dose 1:2.5		High-Dose 2:1		Mono BI 1744		Mono Tio	
			0/0 [µg/kg]	15/37.5 [µg/kg]		120/300 [µg/kg]		300/150 [µg/kg]		300 [µg/kg]		300 [µg/kg]	
	Day	Group	1	2		3		4		5		6	
		Gender	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
CK [U/L]	-15	M	413	307	*-25.6*	245	*-40.7*	399	*-3.4*	340	*-17.6*	410	*-0.6*
		F	271	229	*-15.8*	365	*34.5*	239	*-11.9*	266	*-1.9*	287	*5.9*
	-8	M	249	250	*0.4*	262	*5.1*	257	*3.2*	294	*17.8*	316	*26.8*
		F	230	197	*-14.5*	255	*10.9*	291	*26.9*	258	*12.3*	269	*16.9*
	3	M	263	287	*9.3*	1128	*329.5*	236	*-10.0*	259	*-1.5*	260	*-1.2*
		F	318	221	*-30.7*	261	*-18.0*	↓177	*-44.5*	↓212	*-33.3*	273	*-14.2*
	4	M	178	182	*2.2*	460	*157.5*	153	*-14.2*	144	*-19.6*	185	*3.6*
		F	185	179	*-2.9*	245	*32.4*	155	*-16.2*	152	*-17.5*	192	*4.2*
	29	M	207	299	*44.5*	230	*10.9*	↑365	*76.6*	↑569	*175.0*	244	*17.9*
		F	422	238	*-43.7*	294	*-30.2*	366	*-13.2*	650	*54.1*	195	*-53.7*
	32	M	169	200	*18.0*	164	*-3.0*	179	*5.9*	↑245	*44.9*	170	*0.2*
		F	149	176	*18.5*	159	*6.9*	156	*4.8*	↑248	*66.8*	136	*-8.9*
	84	M	258	278	*7.7*	340	*31.5*	↑522	*102.2*	↑567	*119.6*	207	*-20.0*
		F	183	198	*8.0*	269	*47.2*	↑356	*94.4*	↑445	*143.2*	201	*10.0*
	88	M	119	126	*5.8*	190	*59.9*	189	*59.3*	↑240	*101.6*	138	*16.2*
		F	132	138	*4.7*	157	*18.7*	154	*16.8*	↑205	*55.6*	129	*-2.1*
	127	M	271	n.a.	*n.a.*	177	*-34.7*	273	*1.0*	300	*10.9*	n.a.	*n.a.*
		F	161	n.a.	*n.a.*	191	*18.5*	171	*6.0*	219	*35.6*	n.a.	*n.a.*
	129	M	160	n.a.	*n.a.*	115	*-28.0*	227	*41.5*	179	*11.5*	n.a.	*n.a.*
		F	124	n.a.	*n.a.*	115	*-7.1*	149	*20.1*	↑216	***74.1***	n.a.	*n.a.*

Study no. 07B183: BI 1744 CL/tio – 13-wk tox dog ih (1:2.5 + 2:1)
Δ% percent deviation from Control (calculated from original raw data/rounded)
↥ statistically significant increase compared with Control; p<=0.05, many to one t-test, two sided
↧ statistically significant decrease compared with Control; p<=0.05, many to one t-test, two sided
n.a.: not applicable
M, F: male, female

Toxicokinetics: Blood was collected once from the jugular vein on day -1 and at 10 and 30 min and at 1, 3, 8 and 24 hr post dosing on days 1, 30 and 86. Plasma levels were determined by a validated HPLC-MS/MS assay. The LLOQ was 20 pmol/l for BI 1744 CL and 25 pmol/l for tiotropium bromide.

Results: The results are presented in the following table excerpted from the submission In females, the HD (2:1) AUC of BI 1744 CL on day 1 was more than 1/2 that (22,000 pg.h/ml vs. 49,700) of BI 1744 CL alone on day 1 and yet, by day 86, the AUCs were comparable 86 (48,800 pg.h/ml vs. 46,500). Otherwise, there was no effect on the kinetics of BI 1744 CL by the combination. The toxicokinetics of tiotropium bromide was not affected when combined with BI 1744 CL .

Table 3.5: 1 Mean toxicokinetic parameters of BI 1744 BS (target doses[a] are reported as BI 1744 BS)

Parameter	Day	Gender	Low-Dose 1:2.5 15/37.5 [µg/kg]	High-Dose 1:2.5 120/300 [µg/kg]	High-Dose 2:1 300/150 [µg/kg]	Mono BI 1744 300 [µg/kg]
C(max) [pmol/L]	1	M	742	2980	9810	9460
	30	M	215	5760	8410	10700
	86	M	474	1850	12100	9110
	1	F	437	5440	6470	16000
	30	F	320	2950	9390	13500
	86	F	293	2830	12600	12000
AUC(0-24h) [pmol·h/L]	1	M	2050	10300	25200	32200
	30	M	1790	25200	34300	45200
	86	M	2410	12300	42500	38300
	1	F	1370	16200	22000	49700
	30	F	2170	18200	39300	61900
	86	F	1890	12900	48800	46500

Study no. 07B183: BI 1744 CL/tio – 13-wk tox dog ih (1:2.5 + 2:1)
[a] target dose BI 1744 BS / tiotropium in µg/kg
M, F: male, female

Table 3.5: 2 Mean toxicokinetic parameters of tiotropium cation (target doses[a] are reported as Tio)

Parameter	Day	Gender	Low-Dose 1:2.5 15/37.5 [µg/kg]	High-Dose 1:2.5 120/300 [µg/kg]	High-Dose 2:1 300/150 [µg/kg]	Mono Tio 300 [µg/kg]
C(max) [pmol/L]	1	M	2680	19300	9090	28300
	30	M	2300	87100	22300	35600
	86	M	6730	28400	28300	45700
	1	F	2260	36300	8390	28700
	30	F	3300	30800	13400	61300
	86	F	3010	28500	18400	41600
AUC(0-24h) [pmol·h/L]	1	M	4760	25500	10600	37600
	30	M	3570	86200	25100	50000
	86	M	7560	37800	27600	51000
	1	F	4230	37800	8800	47500
	30	F	5370	50300	21800	64000
	86	F	4900	32700	24700	54100

Study no. 07B183: BI 1744 CL/tio – 13-wk tox dog ih (1:2.5 + 2:1)
[a] target dose BI 1744 BS / tiotropium in µg/kg
M, F: male, female

Organ weights: These were determined at necropsy on days 91 and 135 (recovery period).

Results: The results (absolute and relative weights) are presented in the following tables. In the HD (1:2.5) male group, the heart weight decreased in males based on absolute weight and weights relative to the body and brain weights. Decreased absolute weight of hearts occurred with tiotropium bromide in both sexes in this study and in other inhalation toxicity studies with tiotropium bromide (see L. Pei's 9/17/02 review of NDA 21-395). In the female HD (2:1) group, there was an increase in spleen weight based on absolute weight and weights relative to the body and brain weights. Histologically, at this dose there was increased fibrosis/adhesion which correlates with the increased organ weight. The sponsor discounted the change in testicular weight attributing it to the state of immaturity of these animals.

Absolute Weight

Sex/Parameter	Percent Change from Control, P<0.05 (Day 91)				
	LD (1:2.5)	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male					
Heart		-25			-16
Testes					+27
Pituitary	-23				
Female					
Heart					-25
Spleen			+54		
Brain		-15			-11
Adrenals					-19

Relative to Body Weight

Sex/Parameter	Percent Change from Control, P<0.05 (Day 91)				
	LD (1:2.5)	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male					
Heart	-8	-15	-10		
Liver				-11	
Kidneys				-18	
Testes		+26			+39
Adrenals		+30			+30
Pituitary	-25				
Thyroid				-29	
Female					
Lung					+21
Liver					+16
Kidneys					+22
Spleen			+36		
Brain					+21

Relative to Brain Weight

Sex/Parameter	Percent Change from Control, P<0.05 (Day 91)				
	LD (1:2.5)	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male					
Brain			-6		
Heart		-14			-17
Testes			+28		+22
Body Wt.				+15	
Female					
Brain		-15			-11
Spleen			+65		
Body Wt.					-18

Gross Pathology: These were made at necropsy, day 91. An in depth analysis was made as forty-eight internal organs were examined.

Results: None of the findings in the treatment and recovery animals were considered treatment related.

Heart: Discoloration: C, 0; LD (1:2.5), 0; HD (1:2.5), 0; HD (2:1), 3; BI 1744 CL., 1; tiotropium bromide, 1.

Histopathology: The following is a list excerpted from the submission of tissues processed and examined.

Histopathology: The following is a list excerpted from the submission of tissues processed and examined.

Adequate Battery: Yes.
Peer Review: No.

Table 2.10.3: 1 Organs and tissues removed at necropsy (histological samples and levels)

Adrenal glands	Ovaries
Aorta	Oviduct
Bone (sternum)	Pancreas
Bone marrow (sternum)	Parathyroid glands (within thyroids, at least one)
Brain	Parotid salivary glands (at least unilateral)
Caecum	Peripheral (sciatic) nerve
Cervix uteri	Peyer's patches
Colon	Pharynx (soft palate and nasopharynx)
Duodenum	Pituitary gland
Epididymides	Prostate
Oesophagus	Rectum
Eyes	Skeletal muscle
Gall bladder	Skin
Heart	Spinal cord (cervical, thoracic, lumbar)
Ileum	Spleen
Jejunum	Stomach
Kidneys	Sublingual salivary glands (at least unilateral)
Knee joint	Submandibular salivary glands (at least unilateral)
Lacrimal gland (at least unilateral)	Testes
Larynx (longitudinal section)	Thymus
Liver	Thyroid glands
Lungs (7 samples)	Tongue
Lymph nodes, bifurcational	Trachea (including bifurcation)
Lymph nodes, mesenteric	Ureters
Mammary gland (only females)	Urinary bladder
Nasal cavity (level I and level II)	Uterus
Optic nerves	Vagina

.

Adequate Battery: Yes.
Peer Review: No.

Results

The results are summarized in the following tables. A scoring system of 1, minimal; 2, slight; 3, moderate; 4, severe was used to describe the severity of the lesion. The lesions are presented as the average severity score. The severity of the lesions in this study was predominantly in the minimal and slight category.
In males, the target organs seen with BI 1744 CL alone were: heart (fibrosis, infiltration, mineralization, and necrosis), lacrimal gland (atrophy, lobular and dilatation of ducts), liver (glycogen depletion and increased glycogen storage), lymph node bifurcation (blood resorption), Peyers patch (hyaline droplets), pharynx (dilatation of ducts), pituitary gland (distension, luminal), trachea (atrophy, epithelial), lung (granuloma), thyroid gland (hypertrophy, c-cell), tongue (necrosis, single cell), skeletal muscle (infiltration), submandibular salivary gland (infiltration), spleen (congestion, acute), stomach (hyperplasia, lymphoid) and nasal cavity (goblet cell and lymphoid, hyperplasia).
In females the target organs for BI 1744 CL were: duodenum (dilatation of glands), eye (infiltration, inflammatory), heart (fibrosis/fibroplasia), lacrimal gland (dilatation of ducts), larynx (infiltration), liver (fibrosis/adhesion, capsular, infiltration, glycogen depletion and increased glycogen storage), lung (infiltration, inflammatory), LN mesenteric (blood resorption), parotid salivary gland (atrophy, lobular and glandular), Peyers patch

(hyaline droplets), pharynx (inflammatory cells and inflammatory), thymus (involution) and tongue (necrosis, single cell).

In males, the target organs with tiotropium bromide were: heart (pseudocysts), lacrimal gland (dilatation of ducts), liver (hemorrhage), optic nerve (infiltration), pharynx (debris, inflammatory cells, luminal, dilatation of ducts and necrosis, focal), prostate (desquamation and infiltration), trachea (infiltration), thyroid (hypertrophy, c-cell), skeletal muscle (infiltration), submandibular salivary gland (infiltration), skin (infiltration, inflammatory and regeneration), spleen (congestion, acute), sublingual salivary gland (infiltration) and nasal cavity (atrophy, epithelial, goblet cell and lymphoid hyperplasia and infiltration, inflammatory).

In females, the target organs with tiotropium bromide were: duodenum (dilatation of glands), esophagus (infiltration), heart (pseudocyst), lacrimal gland (dilatation of ducts), larynx (infiltration), lung (infiltration and infiltration, inflammatory), lymph node mesenteric (blood resorption), pancreas (infiltration, inflammatory), parotid salivary gland (atrophy, lobular, and mucoid change), pharynx (concretion of secretory fluids, debris, inflammatory cells, luminal and infiltration, inflammatory), pituitary gland (cysts), thymus (involution),trachea (infiltration, inflammatory), skin (infiltration, inflammatory), stomach (infiltration, inflammatory) and nasal cavity (dilatation of glands).

Sex/ Organ/ Observation	Incidence, N=4/Group (Average Severity Score)					
	C	LD (1:.2.5)	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male						
Duodenum						
Dilatation of glands	0	0	0	1 (1.0)	0	0
Hyperplasia, lymphoid	0	0	2 (1.5)	0	0	0
Gall bladder						
Infiltration	0	1 (1.0)	0	1 (1.0)	0	0
Heart						
Fibrosis/fibroplasia	0	0	0	2 (1.5)	2 (2.0)	0
Infiltration	1 (1.0)	1 (1.0)	2 (1.0)	2 (1.0)	2 (1.0)	0
Necrosis	0	0	0	0	1 (1.0)	0
Pseudocysts	0	0	0	2 (1.0)	2 (1.0)	0
Mineralization	0	0	0	0	1(1.0)	0
Lacrimal gland, N= 3, C						
Atrophy, lobular	0	0	0	0	1(1.0)	0
Atrophy, focal	0	1 (1.0)	0	2 (1.5)	0	0
Dilatation of ducts	0	3 (1.3)	4(1.5)	4 (1.0)	1 (1.0)	3 (1.3)
Mineralization	0	0	0	2 (1.0)	0	0
Liver						
Glycogen depletion	0	0	3 (1.0)	4 (2.0)	4 (1.5)	0
Glycogen storage inc.	0	1 (1.0)	4 (1.0)	4 (2.0)	4 (1.5)	0
Kidney						
Mineralization, cortical	0	0	0	0	2(1.0)	1(1.0)
Lymph node bifurcation			0	0	1 (2.0)	0
Blood resorption	0	0				
Optic nerve		0	0	0	0	1 (1.0)
Infiltration	0					
Peripheral Nerve		0				
Infiltration, inflammatory	0		1 (1.0)	0	0	0
Peyers patch						
Hyaline droplets	0	0	0	1 (1.0)	0	0
Pharynx						
Atrophy, lobular	0	0	0	1 (1.0)	0	0
Debris, inflam. cells luminal	1 (1.0)	1 (1.0)	4(1.0)	3 (1.3)	0	4 (1.0)
Dilatation of ducts	0	0	1 (1.0)	2 (2.0)	1(2.0)	1 (1.0)
Necrosis, focal	0	0	0	0	0	1 (1.0)
Pituitary gland						
Distension, luminal	0	0	0	0	1 (1.0)	0
Prostate						
Desquamation	0	0	0	1 (1.0)	0	1 (1.0)
Infiltration	0	0	1 (1.0)	2 (1.0)	0	1 (1.0)

Sex/ Organ/ Observation	Incidence, N=4/Group (Average Severity Score)					
	C	LD (1:1.25)	HD (1:1.25)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male, cont.						
Trachea						
Atrophy, epithelial	0	0	0	0	1 (1.0)	0
Infiltration	0	3 (1.0)	0	1 (1.0)	0	1 (1.0)
Urinary bladder						
Infiltration, inflammatory	0	1 (1.0)	2 (1.0)	0	0	0
Lung						
Foam cell accumulation	0	0	1 (1.0)	0	0	0
Granuloma	0	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	0
Thyroid gland						
Hypertrophy, C-cell	2(1.0)	0	3 (1.3)	3 (1.7)	2 (1.0)	1 (2.0)
Tongue						
Necrosis, single cell	0	2 (1.0)	0	2 (1.0)	1 (1.0)	0
Skeletal muscle						
Infiltration	0	0	0	1 (1.0)	1 (1.0)	2 (1.0)
Submandibular salivary gland						
Infiltration	0	1 (1.0)	0	0	0	2 (1.0)
Skin						
Regeneration	0	0	0	0	1(1.0)	2 (1.5)
Spleen						
Congestion, acute	2 (2.0)	3 (1.3)	3 (1.3)	4 (1.8)	1 (1.0)	1 (1.0)
Stomach						
Hyperplasia, lymphoid	3 (2.0)	2 (2.0)	1 (2.0)	4 (2.0)	2 (2.0)	0
Necrosis	0	0	0	1 (1.0)	0	0
Sublingual salivary gland						
Infiltration	0	1 (2.0)	0	0	0	1 (2.0)
Nasal cavity						
Atrophy, epithelial	0	0	0	0	0	1 (1.0)
Dilatation of glands	0	0	1 (1.0)	0	0	0
Goblet cells, hyperplasia	0	0	1 (1.0)	0	2 (1.0)	1 (1.0)
Lymphoid, hyperplasia	2 (1.5)	1 (1.0)	0	1 (1.0)	4 (1.3)	2 (1.0)
Polyp	0	0	1 (1.0)	0	0	0
Infiltration, inflammatory	2 (1.5)	1(1.0)	0	1(1.0)	0	3 (1.0)

Sex/ Organ/ Observation	Incidence, N=4/Group (Average Severity Score)					
	C	LD (1:.2.5)	HD (1:.2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Female						
Duodenum						
Dilatation of glands	0	0	0	1 (1.0)	1 (1.0)	1 (1.0)
Hyperplasia, lymphoid	0	0	2 (1.5)	0	0	0
Esophagus						
Infiltration	2 (1.0)	0	1 (1.0)	1 (1.0)	0	4 (1.0)
Eye						
Infiltration, inflammatory	0	0	0	0	1 (1.0)	0
Optic nerve						
Infiltration	0	0	0	1(1.0)	0	0
Heart						
Fibrosis/fibroplasia	0	0	1 (1.0)	2 (1.5)	2 (1.5)	0
Lacrimal gland						
Dilatation of ducts	1 (1.0)	2 (1.0)	4 (1.0)	2 (1.0)	1 (1.0)	3 (1.0)
Larynx						
Infiltration	1 (1.0)	1 (2.0)	4 (1.0)	1 (1.0)	2 (1.0)	1 (1.0)
Liver						
Fibrosis/adhesion, capsular	0	0	0	0	1 (2.0)	0
Glycogen depletion	0	0	4 (1.0)	4 (1.5)	4 (1.8)	0
Glycogen storage increase	0	3 (1.0)	4 (2.0)	4 (1.5)	4 (1.8)	0
Infiltration	0	0	0	0	1 (1.0)	0

Sex/ Organ/ Observation	Incidence, N=4/Group (Average Severity Score)					
	C	LD (1:1.25)	HD (1:1.25)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Female, cont.						
Lung						
Infiltration	1 (1.0)	1 (1.0)	3 (1.0)	2 (1.0)	1 (1.0)	2 (1.0)
Lymph node mesenteric						
Blood resorption	2 (2.0)	2 (2.0)	3 (2.3)	2 (2.0)	1 (2.0)	2 (2.0)
Pancreas						
Infiltration, inflammatory	0	1 (1.0)	0	0	0	1 (1.0)
Parotid salivary gland						
Atrophy, glandular	0	0	1 (1.0)	0	1 (1.0)	0
Atrophy, lobular	1 (1.0)	2 (1.0)	1 (1.0)	1 (2.0)	3 (1.7)	1 (1.0)
Mucoid change	0	0		0	0	1 (1.0)
Peyer's patch						
Hyaline droplets	0	1 (1.0)	0	0	1 (1.0)	0
Pharynx						

Sex/ Organ/ Observation	Incidence, N=4/Group (Average Severity Score)					
	C	LD (1:1.25)	HD (1:1.25)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Atrophy, lobular	0	0	0	0	1 (1.0)	0
Concretion of secretory fluids	0	0	2 (1.0)	0	0	1 (1.0)
Debris, inflam. cells, luminal	0	2 (1.0)	4 (1.0)	3 (1.0)	0	3 (1.0)
Dilatation of ducts	0	0	2 (1.0)	0	0	0
Infiltration, inflammatory	0	1 (1.0)	2 (1.0)	2 (1.0)	0	3(1.0)
Inflammation	0	0	0	0	1 (3.0)	0
Necrosis, focal	0	0	0	1(3.0)	0	0
Pituitary gland						
Cysts	1 (1.0)	1 (1.0)	2 (1.0)	1 (1.0)	0	2 (1.0)
Thymus						
Involution	0	1 (1.0)	2 (1.0)	2 (1.5)	1 (1.0)	1 (1.0)
Tongue						
Necrosis, single cell	0	0	0	0	2 (1.0)	0
Trachea						
Deformation	0	0	1 (2.0)	0	0	0
Infiltration, Inflammatory	0	0	0	0	0	1 (1.0)
Metaplasia, squamous cell	0	0	1 (1.0)	0	0	0
Skin						
Infiltration, inflammatory	0	1 (1.0)	1 (1.0)	0	2 (1.0)	4 (1.8)
Spleen						
Fibrosis/adhesion, capsular	0	0	0	1 (1.0)	0	0
Stomach						
Infiltration, Inflammatory	0	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)
Nasal cavity						
Dilatation of glands	0	0	0	0	0	1 (1.0)

For drug interaction, comparisons were made between the Group 6 (2:1, tiotropium bromide, 76 µg/kg) and Group 3 (1:2.5) tiotropium bromide, 75.5 µg/kg and 31.5 µg/kg of BI 1744 CL) and a comparison between Group 5 (BI 1744 CL 74.8 µg//kg) and Group 4 (2:1, BI 1744 CL, 74.8 µg/kg and 36.3 µg/kg of tiotropium bromide). An interaction would be an increase or decrease incidence of 2 between the two groups where there was an incidence in one group and, provided that there is 0 incidences in the control group. The results are presented in the following table show that there was a decrease in the incidence of skin inflammation when BI 1744 CL was combined with tiotropium bromide.

Group Comparison/ Organ/ Observation	Incidence, N=4 (Average Severity Score)					
	C	LD (1:2.5)	MD (1:2.5)	HD (1:2.1)	BI 1744 CL	Tiotropium bromide
Group 3 vs. Group 6						
Female						
Skin						
Infiltration, inflammatory	0	1 (1.0)	1(1.0)	0	2(1.0)	4(1.8)

There were histopathological changes where the combination groups showed an increase incidence and there was no incidence in either the individual compound and in the control groups. The severity of the following histopathology was in the minimal to slight category. The results are shown in the following table.

In males, the organs showing the changes were: peripheral nerve (HD (1:2.5),infiltration, inflammatory), lacrimal gland (HD (2:1), atrophy and mineralization), urinary bladder (LD (1:2.5) and HD (1:2.5), infiltration, inflammatory), lung (HD (1:2.5), foam cell accumulation), nasal cavity (HD (1:2.5), dilatation of glands and polyps), duodenum (HD (2:1),dilatation of glands) and HD (1:2.5) hyperplasia, lymphoid), stomach (HD (2:1), necrosis) and pharynx (HD (2:1), atrophy, lobular).

In females, the organs affected were: duodenum (HD (1:2.5), hyperplasia, lymphoid), pharynx (HD, (1:2.5), dilatation of ducts), optic nerve (HD (2:1),infiltration), trachea (HD (1:2.5), deformation and metaplasia, squamous cell) and spleen (HD (2:1), fibrosis/adhesion, capsular). Since the metaplasia squamous cell seen in the trachea was also seen in the control recovery group, it is no longer considered a new toxicity.

To rule out whether the Histopathological findings were spontaneous or were seen with tiotropium bromide or other antimuscarinic agents or with BI 1744 CL or other β_2 agonists, the following book, articles and reports were reviewed: Handbook of Toxicology, 2ed edition, Derelenko, M.J. and Hollinger, M. A., pp 723-740, 2002, Histopathology of Spontaneous Lesions in Beagles Used for Toxicity Studies (Yasuba et al., Japan J. Vet. Sci. 49:51-59, 1987), Lesions of Spontaneous Subclinical Disease in Beagle Dogs (Hottendorf, G.H. and Hirth, R. S., Vet. Path.11: 240-258, 1974), Histopathological Studies on Distribution of Spontaneous Lesions and Age Changes in the Beagle (Oghiso et. Al., Japan J. Vet. Sci. 44: 941-950, 1982), Tiotropium bromide, NDA 21-395, 9/17/02 review by L. Pei, Formoterol, IND (b) (4), 3/5/97 review by L. Pei, Formoterol and Mometasone, IND (b) (4), 1/12/07 review by T.W. Robison, Formoterol and an antimuscarinic agent, Aclidnium, IND (b) (4) 4/10/08 review by T.W. Robison. However, in the 4-week inhalation toxicity study in dogs (No. U06-1895), where there were high combination ratios (BI 1744 CL/tiotropium bromide) were high, i.e., 6:1 and 1:2 (deposited doses: 3.75/0.625 mcg/kg, 37.5/6.25 mcg/kg, 3.75/7.5 mcg/kg and 37.5/75 mcg/kg), none of these findings were seen suggesting that these lesions were not related to the combination.

Sex/Organ/ Observation	Incidence, N=4 (Average Severity Score)					
	C	LD (1:2.5)	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Male						
Peripheral Nerve						
Infiltration, inflammatory	0	0	1(1.0)	0	0	0
Lacrimal Gland						
Atrophy	0	1 (1.0)	0	2(1.5)	0	0
Mineralization	0	0	0	2 (1.0)	0	0
Urinary bladder						
Infiltration, inflammatory	0	1 (1.0)	2(1.0)	0	0	0
Lung						
Foam cell accumulation	0	0	1 (1.0)	0	0	0
Nasal cavity						
Dilatation of glands	0	0	1(1.0)	0	0	0
Polyp	0	0	1(1.0)	0	0	0
Duodenum						
Dilatation of glands	0	0	0	1 (1.0)	0	0
Pharynx						
Atrophy, lobular	0	0	0	1(1.0)	0	0
Stomach						
Necrosis	0	0	0	1(1.0)	0	0

Sex/Organ/ Observation	Incidence, N=4 (Average Severity Score)					
	C	LD (1:2.5)	HD (1:2.5)	HD (2:1)	BI 1744 CL	Tiotropium bromide
Female						
Duodenum						
Hyperplasia, lymphoid	0	0	2 (1.5)	0	0	0
Pharynx						
Dilatation of ducts	0	0	2 (1.0)	0	0	0
Necrosis, focal	0	0	0	1 (3.0)	0	0
Optic nerve						
Infiltration	0	0	0	1(1.0)	0	0
Trachea						
Deformation	0	0	1 (2.0)	0	0	0
Metaplasia, squamous cell	0	0	1(1.0)	0	0	0
Spleen						
Fibrosis/adhesion, capsular	0	0	0	1(1.0)	0	0

Recovery period: The control, HD (1:2.5), HD (2:1) and BI 1744 CL groups were only examined. The histopathology that was seen at the end of the recovery period is

presented in the following table. Not all the tissues recovered. The peripheral nerve was not examined.

Sex/ Organ/ Observation	Incidence, N=2/Group (Average Severity Score)			
	C	HD 1:2.5)	HD (2:1)	BI 1744 CL
Male				
Lacrimal gland				
Atrophy, focal	0 N=1	0	1 (1.0)	0
Heart				
Fibrosis/fibroplasia	0	1 (3.0)	1(2.0)	2(2.0)
Mineralization	0	1 (3.0)	0	1(1.0)
Lung				
Foam accumulation	0	0	1(1.0)	0
Female				
Lacrimal gland				
Dilatation of ducts	2 (1.0)	0	1 (1.0)	0
Larynx				
Infiltration	1(1.0)	0	2(1.0)	0
Parotid Salivary Gland				
Atrophy, Lobular	1(1.0)	0	0	2(1.0)
Trachea				
Metaplasia, squamous cell	2(1.0)	0	0	1(1.0)

11 INTEGRATED SUMMARY, CONCLUSION AND SAFETY EVALUATION

Summary

Two 13-week inhalation combination toxicity studies with a 6-week recovery period were conducted in dog with BI 1744 CL, a β_2 agonist, and tiotropium bromide, an approved antimuscarinic agent. (NDA 21-937). The object of these studies was to determine whether there was a drug interaction when both compounds were combined. If the results of these two studies showed no findings with the combination groups of clinical concern, the sponsor requests that no further studies would be required for them to conduct additional 13-week inhalation studies in dogs with a 4:1 and 8:1 dose ratios in order to conduct additional clinical dose ratios.

In the first study, the ratio of increasing doses of BI 1744 CL to tiotropium bromide was 1:1. The deposited doses (µg/kg) were: 4: 3.5 (LD), 15.5:14.25 (MD) and 75:70 (HD) along with 72.5 µg/kg of BI 1744 CL and 75 µg/kg of tiotropium bromide as monoproducts. The changes noted were characteristic of their pharmacological activities. For BI 1744 CL, a β_2 agonist, there was increased body weight gain, tachycardia resulting in the typical cardiac histopathology of fibrosis/fibroplasia, liver changes with respect to glycogen storage and depletion. For tiotropium bromide, there were the typical clinical signs of an antimuscarinic effect, dry mouth, eyes and nose,

and tachycardia. An expected drug interaction occurred as there was an increase in heart rate that was higher than seen with each compound alone due to a direct stimulating effect by BI 1744 CL and an indirect effect by vagal blockade due to the antimuscarinic effect of tiotropium bromide. There was no new toxicity resulting from the combination.
In the second study, the ratio of increasing doses of BI 1744 CL to tiotropium bromide was LD (1:2.5), HD (1:2.5) and HD (2:1). The deposited doses (µg/kg) were: 4: 9 (LD (1:2.5)), 31.5:75.5 (HD (1:2.5)) and 75:70 (HD (2:1)) along with 74.8 µg/kg of BI 1744 CL and 76 µg/kg of tiotropium bromide. The clinical signs were related to the pharmacological actions of the two compounds. In the cardiovascular system, there was the expected cardiac drug interaction as the heart rate increase over and above the increased heart rate seen with either compound. This is not a clinical concern, since the vagus in the dog has an inhibitory effect that is more profound than in humans. The increased tachycardia due to the vagal block caused by tiotropium bromide is not expected to be as severe clinically with the administration of the combination. There was a drug interaction with hematological parameters that may be monitored. With respect to organ weights, only the increased in spleen weight in the HD (2:1) females correlated with the fibrosis/adhesion, capsular. The toxicokinetics of either combination was not changed. In the histopathology finding of skin inflammation in the BI 1744 CL and tiotropium bromide groups alone, the incidence was reduced or abolished in the combination groups. Histopathological findings were observed in the combination groups that were attributed to either BI 1744 CL or tiotropium bromide groups. However, there were findings in the combination groups that were not observed with either compound alone. A literature search was conducted to determine whether these lesions were spontaneous or whether these findings were seen in other toxicity studies in dogs with tiotropium bromide (NDA 21-395), aclidinium (IND (b) (4)) another long acting antimuscarinic agent, and formoterol (IND (b) (4) and IND (b) (4)), another β_2 agonist. These findings were found to not be spontaneous and were not seen with other toxicity studies. However, in the 4-week inhalation combination toxicity study with in dogs (No. U06-1895), where there were high combination ratios (BI 1744 CL/tiotropium bromide), i.e., 6:1 and 1:2 (deposited doses: 3.75/0.625 mcg/kg, 37.5/6.25 mcg/kg, 3.75/7.5 mcg/kg and 37.5/75 mcg/kg), than those in the current study (1:2.5 and 2:1), none of these findings were seen suggesting that these lesions were not related to the combination.

Conclusion

In the two-13 week inhalation toxicity studies in dogs, where combination doses of 1:1, 1:.2.5 and 2:1, there were no toxicities unrelated to BI 1744 CL and tiotropium bromide alone and no drug interactions of clinical concern.

Q. Boehringer Ingelheim believes that submission of 13-week inhalation toxicity studies in dogs at dose ratios of 1:1, 2:1 and 1:2.5 of BI 1744 CL: tiotropium bromide allows them to conduct additional doses ratios in Phase 3 (and potentially within a marketed product) without conducting additional 13-week combination toxicity study in dogs with a

4:1 and 8:1 doses ratios. Does the Agency agree that these ratios are covered by the submitted 13-week inhalation toxicity studies in dogs?

Response

The 13-week inhalation toxicity studies in dogs at dose ratios of 1:1, 2:1 and 1:2.5 of BI 1744 CL: tiotropium bromide were reviewed. Since there were no new toxicities and there were no drug interactions of clinical concern, from a nonclinical standpoint, clinical studies with a 4:1 and 8:1 dose ratios may be conducted without any additional 13-week inhalation toxicity studies in dogs.
We remind you that final reports of these studies need to be submitted and any differences between the draft and final report should be presented with the final reports.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-76397	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	BI 1744 CL AND TIOTROPIUM BROMIDE

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/s/

LAWRENCE F SANCILIO
06/14/2010

MOLLY E SHEA
06/14/2010

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/s/

ANDREW C GOODWIN
01/23/2015

TIMOTHY W ROBISON
01/23/2015
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206756 **Applicant:** Boehringer Ingelheim **Stamp Date:** 5/22/2014

Drug Name: Tiotropium Br / Olodaterol HCl **NDA Type:** Original 505(b)(1) NDA

On **initial** overview of the NDA 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		Studies submitted in eCTD format.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
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		
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).			NA. Pivotal toxicology studies utilized the to be marketed formulation.
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, all pivotal studies were conducted with the inhalation route of administration.
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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Nonclinical section is consistent with agreement reached at IND 76397 EOP2 meeting (8/4/2011 minutes) and in pre-NDA written responses (9/9/2013).

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
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 proposed labeling is in the PLR format. Text will be reviewed and edited after review of nonclinical data.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Filing review did not identify any issues. Full review will be conducted in consultation with the reviewing chemist.
11	Has the applicant addressed any abuse potential issues in the submission?			NA
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

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.

N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

Andrew Goodwin, PhD 6/30/2014
Reviewing Pharmacologist Date

Timothy Robison, PhD 6/30/2014
Team Leader Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

ANDREW C GOODWIN
06/30/2014

TIMOTHY W ROBISON
06/30/2014
I concur