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APPLICATION NUMBER:

203108Orig1s000

PHARMACOLOGY REVIEW(S)

Secondary Pharmacology and Toxicology Review for NDA 203-108 Complete Response

TO: NDA 203-108 (Boehringer Ingelheim Pharmaceuticals, Inc.)

FROM: Marcie Wood, Ph.D.
Supervisory Pharmacologist
Division of Pulmonary, Allergy, and Rheumatology Drug Products

DATE: July 18, 2014

NDA 203-108 was originally submitted on May 14, 2012, and proposed to register STRIVERDI RESPIMAT (olodaterol, a NME long-acting β_2 -adrenergic receptor agonist) for the treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. At the time of the original submission, Dr. Carol (Rivera-Lopez) Galvis reviewed the full nonclinical program for olodaterol and found that the program was complete and adequate to support the safety of olodaterol at the proposed clinical dose of 5 $\mu\text{g}/\text{day}$ (refer to the nonclinical review by Dr. Galvis dated January 17, 2013), and secondary and tertiary reviews agreed with Dr. Galvis's recommendation of approval from the nonclinical perspective (refer to reviews by Drs. Wood and Brown dated January 24, 2013, and February 26, 2013; respectively). A complete response, however, was issued on March 14, 2013, due to CMC deficiencies.

Boehringer Ingelheim submitted a complete response to NDA 203-108 on June 2, 2014. No new nonclinical studies were included in this complete response submission. The original nonclinical recommendation on the approvability, therefore, has not changed.

Labeling: Changes to Section 8.1 (Pregnancy Category C), Section 8.3 (Nursing Mothers), Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, and Impairment of Fertility) were proposed in Dr. Galvis's review dated January 17, 2013, and recommended labeling text was communicated to the sponsor in the complete response letter of March 14, 2013. Recommended labeling changes were incorporated into the proposed label submitted with the complete response, therefore, no additional nonclinical labeling recommendations were necessary.

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

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/s/

MARCIE L WOOD
07/18/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 203-108
Supporting document/s: 38
Applicant's letter date: 6/2/2014
CDER stamp date: 6/2/2014
Product: STRIVERDI RESPIMAT (Olodaterol) Inhalation
Spray
Indication: 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 Products (DPARP)
Reviewer: Carol M. Galvis, Ph.D.
Supervisor/Team Leader: Marcie Wood, Ph.D.
Division Director: Badrul A. Chowdhury, M.D., Ph.D.
Project Manager: Christine Chung, R.Ph.

Template Version: September 1, 2010

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application is for descriptive purposes only and is not relied upon for approval of NDA 203-108.

Pharmacology/Toxicology Review of Complete Response

This pharmacology/toxicology review evaluates a complete response submitted by Boehringer Ingelheim Pharmaceuticals, Inc. under NDA 203-108 on June 2, 2014. NDA 203-108 was originally submitted on May 14, 2012 to propose STRIVERDI RESPIMAT (olodaterol) inhalation spray for the treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including bronchitis and emphysema. Although no nonclinical issues were identified, a complete response letter was issued on March 14, 2013 due a CMC deficiency identified. Boehringer Ingelheim submitted a complete response on June 2, 2014 that included a safety update and final draft labeling.

Olodaterol is a new long-acting β_2 -adrenergic receptor agonist (LABA). The proposed clinical dose is two actuations once daily at the same time of the day. Each actuation contains 2.7 μg olodaterol hydrochloride (2.5 μg olodaterol free base), for a total maximum daily dose of 5 μg olodaterol.

Boehringer Ingelheim conducted a full nonclinical program including pharmacology (primary and secondary pharmacology, safety pharmacology, and pharmacokinetics), general toxicology, genetic toxicology, carcinogenicity, and reproductive and developmental toxicology studies. These studies were previously reviewed when the original NDA was submitted and no nonclinical issues were identified. The nonclinical program was considered complete and adequate to support safety of the proposed olodaterol clinical dose (5 $\mu\text{g}/\text{day}$). However, a complete response was issued on March 14, 2013 due to CMC issues (cGMP deficiencies identified during an inspection). Refer to Pharmacology/Toxicology integrated review dated January 17, 2013 for details on the nonclinical program. Refer to Dr. Craig Bertha's CMC memo dated 6/23/2014 for additional information regarding the CMC deficiencies.

Recommendations

Approvability

The nonclinical safety program for olodaterol is considered complete and adequate to support safety of olodaterol at the proposed clinical dose of 5 $\mu\text{g}/\text{day}$. NDA 203-108 is recommended for approval from the nonclinical pharmacology/toxicology perspective.

Labeling

The labeling was previously reviewed (refer to pharmacology/toxicology review dated January 17, 2013) and recommended labeling text was communicated to the sponsor in the complete response letter dated March 14, 2013. No additional labeling recommendations are included in this review.

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/s/

CAROL M GALVIS
07/11/2014

MARCIE L WOOD
07/11/2014

Secondary Pharmacology and Toxicology Review for NDA 203-108

TO: NDA 203-108 (Boehringer Ingelheim Pharmaceuticals, Inc.)

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

DATE: January 24, 2013

Overview: I concur with the recommendation of Dr. Carol Rivera-Lopez (detailed in a nonclinical review dated January 17, 2013) that the pharmacology and toxicology of STRIVERDI RESPIMAT (olodaterol) have been adequately studied and the drug product should be approved from a nonclinical perspective.

Background: Olodaterol (Code name: BI 1744 CL) is a NME long-acting β_2 -adrenergic receptor agonist (LABA). It is indicated for chronic, once-daily, oral inhalation treatment of airflow obstruction in patients with Chronic Obstructive Pulmonary Disease (COPD), including chronic bronchitis and emphysema. The proposed clinical dose is 5 $\mu\text{g}/\text{day}$ (delivered as 2 actuations of 2.5 μg each).

Pharmacology: The pharmacodynamic effects of olodaterol were investigated both *in vitro* and *in vivo*. *In vitro* studies identified olodaterol as a β_2 -adrenergic receptor partial agonist ($K_i = 0.72 \text{ nM}$), although olodaterol also acts as a partial agonist at β_1 - and β_3 -adrenergic receptors. *In vivo* studies demonstrated the bronchoprotective effects of olodaterol against acetylcholine-induced bronchospasms in anesthetized guinea pigs and dogs. In both species, bronchoprotection was observed within 10 minutes post-dose and was maintained for up to 24 hrs.

Safety Pharmacology: In cardiovascular safety pharmacology studies, olodaterol produced a dose-dependent increase in force of contraction in guinea pig papillary muscle *in vitro*, as well as a dose-dependent decrease in systolic, diastolic, and mean arterial pressure and a dose-dependent increase in heart rate *in vivo* in dogs that received a single oral olodaterol dose of 1.6 $\mu\text{g}/\text{kg}$ or greater. In a renal study in rats, olodaterol administration resulted in a reduction of urine volume and electrolyte excretion after single inhalation doses greater than 0.3 mg/mL delivered over 1 minute, suggesting an anti-diuretic effect. In a gastrointestinal study in rats, olodaterol produced a decrease in gastric emptying and gastrointestinal transit, as well as a reduction in gastric secretion after single inhalation doses greater than 1 mg/mL delivered over 1 minute. No CNS or respiratory effects were observed in rats that received single inhalation doses of olodaterol up to approximately 485 $\mu\text{g}/\text{kg}$.

Toxicology: General inhalation toxicology studies were conducted in the mouse, rat, and dog up to 3-months, 6-months, and 12-months, respectively. For rats and dogs, findings from chronic studies only are summarized below, as similar findings were also observed in shorter duration studies. The respiratory tract was a common target organ for all species and histopathological findings were observed as follows: mouse - nasal cavity, larynx, and lung; rat - nasal cavity, larynx, and trachea; and dog - nasal cavity and trachea), although nasal cavity findings were considered specific to the rodent and dog routes of administration (nose-only and face mask, respectively) and are not relevant for clinical administration (oral inhalation). In addition, larynx findings were considered a rodent-specific adaptive response to inhalation exposure. In rats and dogs, cardiac findings [increased heart weight and palpable heart beat (rat), ECG and cardiac enzyme changes (dog) and heart histopathological findings (rat and

dog)] were considered to be due to activation of β_1 - and β_2 -adrenoreceptors in cardiac tissue. Liver histopathology findings were observed in mouse (hepatocyte vacuolation) and dog (increased glycogen storage). Female reproductive tract findings were observed in mouse (numerous corpora lutea in ovary and cystic glands and myometrial hypertrophy in uterus) and rat (cysts in ovary and oviduct) and correlated with findings observed in 2-year carcinogenicity studies in mouse and rat (discussed below). Skeletal muscle-related findings included increased skeletal muscle mass in the mouse and skeletal muscle hypertrophy and single-cell necrosis in the rat. Findings observed in the heart, liver (enhanced glycogen storage), skeletal muscle, and female reproductive tract have been reported with other drugs of the same class (LABAs). Additional target organs (as identified by results of histopathological examination) included salivary glands and thymus (mouse), pancreas (rat), and kidney (dog). Chronic rat and dog studies provided adequate systemic and local safety margins for olodaterol. See Dr. Rivera-Lopez's review for further details.

Genotoxicity: Olodaterol was negative in the *in vitro* bacterial mutagenicity test (Ames assay) and in the *in vitro* mouse lymphoma assay. However, in the *in vivo* micronucleus assay, olodaterol produced an increase in percentages of polychromatic erythrocytes (PCEs) and a dose-dependent, statistically significant increase in the frequencies of micronucleated polychromatic erythrocytes (MNEs). Evaluation of additional data found that the observed increases in frequencies of MNEs most likely occurred as a result of compensatory erythropoiesis and not as a result of DNA damage. This mechanism is likely not relevant at clinical exposures.

Carcinogenicity: Two 2-year carcinogenicity bioassays were conducted with olodaterol in rats and mice at inhalation doses up to 270 and 255 $\mu\text{g}/\text{kg}/\text{day}$, respectively. In female rats, statistically significant increases in leiomyomas of the mesovarian tissue were observed at 25.8 and 270 $\mu\text{g}/\text{kg}/\text{day}$ olodaterol. In female mice, a statistically significant increase in combined benign uterine leiomyomas and malignant leiomyosarcomas was observed at doses $\geq 76.9 \mu\text{g}/\text{kg}/\text{day}$. The tumor findings in the female rodent reproductive tract are considered to be a class effect of β_2 -adrenergic receptor agonists and have been observed with other approved drugs of the same class. Currently, there is no clinical evidence to suggest that these tumor findings are relevant to humans. However, the relevance of these findings to human use is still unknown.

Reproductive and Developmental Toxicology: Reproductive and developmental toxicity studies of olodaterol were completed in rats and rabbits via the inhalation route of administration. These studies evaluated the effects of olodaterol on fertility in rats, teratogenicity in rats and rabbits, and pre- and post-natal development in rats. In rats, olodaterol had no effects on male or female fertility, was not teratogenic, and had no effects on pre- or post-natal development. However, in rabbits, olodaterol resulted in teratogenic effects [enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum] at 2,489 $\mu\text{g}/\text{kg}/\text{day}$.

Labeling: Section 8.1 (Pregnancy Category C), Section 8.3 (Nursing Mothers), Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, and Impairment of Fertility) have been revised to incorporate nonclinical findings discussed above. See Dr. Rivera-Lopez's review for complete product labeling details.

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

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/s/

MARCIE L WOOD
01/24/2013

Tertiary Pharmacology Review

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

NDA: 203108

Submission date: 5/14/2012

Drug: olodaterol

Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.

Indication: chronic obstructive pulmonary disease

Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Background Comments:

The pharmacology/toxicology reviewer and team leader in the Division of Pulmonary, Allergy and Rheumatology Products reviewed the nonclinical information for olodaterol and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

Discussion:

Carcinogenicity

Olodaterol was assessed in 2-year carcinogenicity studies in rats and mice. The Executive Carcinogenicity Assessment Committee found these studies to be acceptable. The Committee concluded that there were olodaterol-induced leiomyomas of the mesovarian tissue in female rats and benign uterine leiomyomas and malignant leiomyosarcomas combined in female mice.

Developmental and Reproductive Toxicity

Olodaterol was not teratogenic in rats but did produce some teratogenic findings in rabbits at very high multiples of the human exposure. These results are similar to other beta-2 adrenergic agonists.

Conclusions:

I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. No additional nonclinical studies are recommended. Use of a pregnancy category of C seems warranted given the findings in rabbits even though these occurred at a high multiple of the human exposure. I have provided other comments on labeling to the division separately.

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/s/

PAUL C BROWN
02/26/2013

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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 203-108
Supporting document/s: SDN 1
Applicant's letter date: May 14, 2012
CDER stamp date: May 14, 2012
Product: STRIVERDI RESPIMAT (Olodaterol) Inhalation
Spray
Indication: Treatment of airflow obstruction in patients with
Chronic Obstructive Pulmonary Disease (COPD),
including chronic bronchitis and emphysema.
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division: Division of Pulmonary, Allergy, and
Rheumatology Products (DPARP)
Reviewer: Carol M. Rivera-López, Ph.D.
Team Leader: Marcie Wood, Ph.D.
Division Director: Badrul A. Chowdhury, M.D., Ph.D.
Project Manager: Christine Chung, R.Ph.

Template Version: September 1, 2010

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List of Abbreviations

AC	Air control group
ADME	Absorption, Distribution, Metabolism, Excretion
ALD	Approximate lethal dose
ALT	Alanine transferase
APA	Action potential amplitude
APD	Action potential duration
APTT	Activated partial thromboplastin time
AUC	Area under the curve
bpm	Beats per minute
BW	Body weight
cAMP	Cyclic adenosine monophosphate
cDNA	complementary DNA
CHO	Chinese Hamster Ovary
CK	Creatine kinase
CL	Clearance
C _{max}	Maximal drug concentration in plasma
CMC	Chemistry, Manufacturing, and Controls
CNS	Central nervous system
COPD	Chronic Obstructive Pulmonary Disease
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC ₅₀	Effective concentration
ECAC	Executive Carcinogenicity Assessment Committee (FDA)
ECG	Electrocardiogram
EDR	Electronic Document Room
EDTA	Ethylenediaminetetraacetic acid
EFD	Embryo-fetal development
F	Female
F (%)	Bioavailability
FED	Fully effective dose
FOC	Force of contraction
g	Grams
GLP	Good Laboratory Practice
GSD	Geometric standard deviation
HD	High-dose
HEK	Human embryonic kidney cells
hERG	Human ether-a-go-go related gene
HPLC	High-performance Liquid Chromatography
hr	Hour
IA	Intrinsic activity
ICH	International Conference of Harmonization
<i>i.e.</i>	<i>Id est</i> (That is)
IH	Inhalation administration
IND	Investigational New Drug

IT	Intratracheal administration
IV	Intravenous
kg	Kilogram
K_i	Dissociation constant
L	Liter
LABA	Long-acting β_2 -adrenergic receptor agonist
LD	Low-dose
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantitation
m	Meter
M	Male
MAP	Mean arterial pressure
MD	Mid-dose
mg	Milligram
min	Minute(s)
mL or ml	Milliliter
MMAD	Mass median aerodynamic diameter
MNEs	Micronucleated polychromatic erythrocytes
MS	Mass spectrometry
MTD	Maximum tolerated dose
N (or no.)	Number
NDA	New Drug Application
ng	Nanogram
nm	Nanometer
NME	New Molecular Entity
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OS	Overshoot
PCEs	Polychromatic erythrocytes
PD	Pharmacodynamics
PDD	Pulmonary deposited dose
PK	Pharmacokinetics
pmol	Picomole
PO	Oral administration
PT	Prothrombin time
QA	Quality assurance
QTcB	corrected QT interval (Bazett's equation)
QTcF	corrected QT interval (Fridericia's equation)
QTcV	corrected QT interval (Van de Water's equation)
RMP	Resting membrane potential
SD	Standard deviation
SEM	Standard error of the mean
$t_{1/2}$	Half-life
TK	Toxicokinetics
T_{max}	Time to maximal drug concentration in plasma
UV	Ultraviolet

VC	Vehicle control group
V-max	Maximal velocity of phase 0 upstroke
vs.	Versus
$V_{(ss)}$	Volume of distribution
μg	Microgram

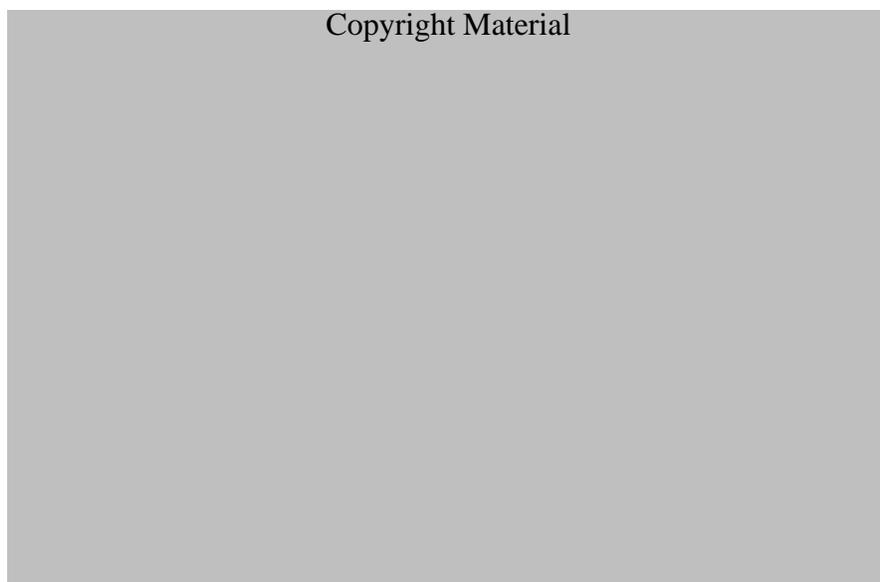
1 Executive Summary

1.1 Introduction

This review evaluates nonclinical pharmacology and toxicology data to support safety of olodaterol hydrochloride (company code: BI 1744 CL). Boehringer Ingelheim Pharmaceuticals, Inc. submitted a 505(b)(1) New Drug Application (NDA) on May 14, 2012 for STRIVERDI Respimat (olodaterol) inhalation spray, a New Molecular Entity (NME). The proposed indication is for the treatment of airflow obstruction in patients with Chronic Obstructive Pulmonary Disease (COPD), including chronic bronchitis and emphysema. The proposed human clinical dose is two actuations once daily at the same time of the day. Each actuation contains 2.7 µg olodaterol hydrochloride (2.5 µg olodaterol, free base), for a total maximum daily dose of 5 µg olodaterol.

Olodaterol is a new long-acting β_2 -adrenergic receptor agonist (LABA). β_2 -adrenergic receptors are located on airway smooth muscle, among other tissues. Stimulation of these receptors on the membrane of airway smooth muscle cells results in a downstream pathway that ultimately leads to smooth muscle relaxation (refer to Figure 1 below). The role of LABAs as bronchodilators in the management of COPD is well established.

Figure 1: Biological effects of β_2 -adrenergic receptor agonists on smooth muscle cells¹.



STRIVERDI Respimat inhalation spray contains a sterile aqueous solution of olodaterol hydrochloride in a specifically designed cartridge for delivery by the Respimat inhaler (a

¹ Figure taken from: Malerba M, Radaeli A, and Morjaria JB. (2012) Therapeutic potential for novel ultra long-acting β_2 -agonists in the management of COPD: biological and pharmacological aspects. Drug Discovery Today 17 (9/10); 496-504.

pocket-sized, propellant-free device for nebulization of inhalation solutions that has been used in other approved products). The drug product formulation does not contain novel excipients. Also, all the impurities in the drug substance and drug product are below the International Conference on Harmonisation (ICH) Guidance threshold or are considered qualified at the proposed specification. For the complete chemistry details, refer to Dr. Craig Bertha's review dated July 31, 2012.

The clinical program for olodaterol consisted of twelve Phase 1 clinical trials: nine in healthy volunteers, one in COPD patients, one in renal impairment patients, and one in hepatic impairment patients; three Phase 2 clinical trials in COPD patients; four Phase 2 clinical trials in asthma patients; and ten Phase 3 clinical trials in COPD patients. Although four Phase 2 clinical trials were conducted in asthma patients, the applicant states that there are no plans to conduct a Phase 3 program in this patient population. In the Phase 3 confirmatory trials, two doses of olodaterol were studied: 5 µg once daily and 10 µg once daily. These doses were selected based on the efficacy and dose-response relationship of olodaterol observed in the dose range finding studies. The pharmacokinetics of olodaterol were also assessed in these clinical trials. For complete details on the clinical program, refer to Dr. Robert Lim's clinical review.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical program for olodaterol included pharmacology (including primary and secondary pharmacology, safety pharmacology, and pharmacokinetics), general toxicology, genetic toxicology, carcinogenicity, and reproductive and developmental toxicology studies. A brief discussion of noteworthy findings follows.

Olodaterol's activity was investigated *in vitro* and *in vivo*. Olodaterol was identified as a β_2 -adrenergic receptor partial agonist ($K_i = 0.72$ nM), although olodaterol also acts as a partial agonist at the β_1 - and β_3 -adrenergic receptors ($K_i = 47$ nM and 5 µM, respectively). Secondary pharmacology studies found that olodaterol acts as an antagonist at the α_1 -adrenergic, and 5-HT_{2A} serotonin receptors.

Olodaterol's potential bronchodilatory effect was assessed using a model of acetylcholine-induced bronchospasm in anesthetized guinea pigs and dogs. In these studies, olodaterol showed a fast onset and long duration of action (up to 24 hours post-dose), which supports the use of this drug as a long acting beta agonist. This is also supported by the biphasic elimination of olodaterol (fast in the first 2-3 hours and then slower for up to 24 hours).

During olodaterol's development, the applicant investigated olodaterol's effects on the cardiovascular, respiratory, neurologic, renal, and gastrointestinal systems. Olodaterol did not affect the respiratory and neurologic (central nervous) systems. However, on the cardiovascular system, olodaterol induced a dose-dependent increase in force of contraction *in vitro*, produced a dose-dependent decrease in systolic, diastolic, and mean arterial pressure, and induced a dose-dependent increase in heart rate. These findings correlated with increased heart rate observed in rats and dogs in the toxicology studies. These positive chronotropic and inotropic effects of olodaterol resulted in

fibrosis/fibroplasia observed microscopically over the long term. For the histopathology findings in the heart, a NOAEL was identified in rats (4 µg/kg; exposure margin of 63 compared to expected clinical exposure at 5 µg/day) and dogs (3.9 µg/kg; exposure margin of 21 compared to expected clinical exposure at 5 µg/day).

In the renal system, olodaterol administration resulted in a reduction of urine volume and electrolyte excretion, suggesting an anti-diuretic effect. No changes in urinalysis parameters were observed in rats or dogs. In the 52-week dog toxicity study, mononuclear infiltration was observed in the kidneys microscopically. No histopathological changes were observed in the urinary system in rats in the 26-week toxicity study.

In the gastrointestinal system, olodaterol induced a decrease in gastric emptying and gastrointestinal transit and reduces gastric secretion. These findings are consistent with effects observed with β₂-adrenergic agonists. No histopathological changes were observed in the gastrointestinal tract after up to 26 weeks of treatment in the rat and up to 52 weeks of treatment in the dog.

In the liver, an increase in glycogen storage and hemorrhage were observed in the 52-week toxicity study in the dog. These effects in the liver are considered pharmacodynamic effects of olodaterol. A NOAEL was identified for this finding and therefore, there is no concern in terms of safety.

Additional target organs of toxicity in rodents were the female reproductive tract (increase in ovarian cysts in the rat and numerous corpora lutea, cystic glands in the uterus, and myometrial hypertrophy in the uterus in mice), the pancreas in rats (lobular atrophy) and skeletal muscle in rats (hypertrophy and necrosis at all dose levels with no dose-response). The findings in skeletal muscle were not dose-related and, although they are likely related to treatment (literature reports similar findings in rodents treated with other approved LABAs), they do not represent a safety concern. For the findings in the female reproductive tract and the lobular atrophy in pancreas, a NOAEL was identified.

In addition, in the respiratory tract, the trachea was identified as a target organ of toxicity in both rats and dogs. A NOAEL was identified in both species for this tissue and provides adequate safety margin for the proposed clinical dose (NOAEL of 2.73 µg/g lung weight and a safety margin of 546 in the rat, and NOAEL of 1.33 µg/g lung weight and a safety margin of 266 in the dog).

A full battery of genetic toxicology studies was conducted for olodaterol and submitted in support of this NDA. Olodaterol was negative in the two *in vitro* assays (*in vitro* bacterial mutagenicity test (Ames assay) and *in vitro* mouse lymphoma assay). However, in the *in vivo* micronucleus assay, olodaterol produced an increase in percentages of polychromatic erythrocytes (PCEs) and a dose-dependent, statistically significant increase in the frequencies of micronucleated polychromatic erythrocytes (MNEs). Evaluation of additional data submitted under this NDA found that the

observed increases in frequencies of MNEs most likely occurred as a result of compensatory erythropoiesis and not as a result of DNA damage. Briefly, treatment with olodaterol results in tachycardia and decrease in blood pressure, which are known to induce tissue hypoxia and erythropoietin, which in turns increases erythropoiesis and results in an increase in MNEs. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Two 2-year carcinogenicity bioassays were conducted with olodaterol in rats and mice. In female rats, olodaterol treatment resulted in leiomyomas of the mesovarian tissue at 25.8 µg/kg/day and 270 µg/kg/day [the trend and pairwise (HD vs. vehicle) assessments for this tumor finding are considered statistically significant ($p=0.0092$ for the trend and $p=0.0494$ for the pairwise high-dose assessment)]. In female mice, olodaterol induced an increase of benign uterine leiomyomas at all dose levels compared to the vehicle control (statistical significance was achieved at 255 µg/kg/day, $p=0.0038$). In addition, the incidence of malignant leiomyosarcomas was increased in olodaterol-treated females, although statistical significance was only achieved at 76.9 µg/kg/day ($p=0.0092$). Further, the incidences of combined benign uterine leiomyomas and malignant leiomyosarcomas were higher at all dose levels when compared to the vehicle control group [statistical significance achieved at doses ≥ 76.9 µg/kg/day ($p=0.0003$ and 0.0018 for the mid-dose and high-dose groups, respectively)]. The tumor findings in the female rodent reproductive tract are considered to be a class effect of β_2 -adrenergic receptors and have been observed with approved drugs of the same class. Currently, there is no clinical evidence to suggest that these tumor findings are relevant to humans. However, the relevance of these findings to human use is still unknown.

A complete battery of reproductive and developmental toxicology studies was submitted in support of this application. These studies were conducted using the inhalation route of administration and evaluated the effects of olodaterol on fertility in rats, teratogenicity in rats and rabbits, and prenatal and postnatal development in rats. Olodaterol did not affect male or female fertility in rats, teratogenicity in rats, or prenatal and post-natal development in rats. However, in the rabbit, olodaterol resulted in teratogenic effects [enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum] at 2,489 µg/kg/day (exposure margin of 1,353 compared to clinical exposure at 5 µg/day).

1.3 Recommendations

1.3.1 Approvability

The nonclinical safety program for olodaterol is considered complete and adequate to support safety of the proposed clinical dose of 5 µg/day. From the nonclinical pharmacology/toxicology perspective, NDA 203-108 is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C.

There are no adequate and well-controlled studies with (b) (4) STRIVERDI RESPIMAT in pregnant women. (b) (4) STRIVERDI RESPIMAT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

(b) (4) STRIVERDI RESPIMAT was not teratogenic in rat (b) (4) approximately (b) (4) times the maximum recommended human daily inhalation dose (MRHDID) in adults on an AUC basis (at a rat maternal inhalation dose of 1,054 mcg/kg/day). Placental transfer of STRIVERDI RESPIMAT was observed in pregnant rats.

STRIVERDI RESPIMAT has been shown to be teratogenic in New Zealand rabbits at inhalation doses approximately 7,130 times the MRHDID in adults on an AUC basis (at a rabbit maternal inhalation dose of 2,489 mcg/kg/day). STRIVERDI RESPIMAT exhibited the following fetal toxicities: enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum. No significant effects occurred at an inhalation dose approximately 1,353 times the MRHDID in adults on an AUC basis (at a rabbit maternal inhalation dose of 974 mcg/kg/day).

8.3 Nursing Mothers

Olodaterol, the active component of STRIVERDI RESPIMAT, is excreted into the milk of lactating rats. Excretion of olodaterol into human milk is probable. There are no human studies that have investigated the effects of STRIVERDI RESPIMAT on nursing infants.

(b) (4) Caution should be exercised when (b) (4) STRIVERDI RESPIMAT is administered to nursing women.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Olodaterol is a long-acting beta₂-adrenergic agonist (LABA). (b) (4) *In vitro* studies have shown that olodaterol has more than (b) (4) 241-fold greater agonist activity at beta₂-adrenoceptors compared to beta₁-adrenoceptors and (b) (4) 2299-fold greater agonist activity compared to beta₃-adrenoceptors. (b) (4)

s.

[Redacted] (b) (4)

Beta-adrenoceptors are divided into three subtypes: beta₁-adrenoceptors predominantly expressed on cardiac smooth 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

[Redacted] (b) (4)

Two-year inhalation studies were conducted in rats and mice to assess the carcinogenic potential of olodaterol. Lifetime treatment of female rats induced [Redacted] (b) (4) leiomyomas of the mesovarium at doses of 25.8 and 270 mcg/kg/day (approximately 18-and 198-fold, respectively, the MRHDID in adults on an AUC basis)

[Redacted] (b) (4) (u) (+)

No tumor findings were observed in male mice at doses up to 255 mcg/kg/day (approximately 455-fold the MRHDID in adults on an AUC basis). Increases in leiomyomas and leiomyosarcomas of the female rodent reproductive tract have been similarly demonstrated with other β₂-adrenergic agonist drugs. The relevance of these findings to human use is unknown. [Redacted] (b) (4)

[Redacted]

[Redacted] (b) (4)

(b) (4)

(b) (4)

Olodaterol was not mutagenic in the *in vitro* (Ames test; or in the *in vitro* mouse lymphoma assay)

(b) (4)

Olodaterol produced increased frequency of micronuclei in rats after intravenous doses. The increased frequency of micronuclei

(b) (4)

was likely related to drug enhanced (compensatory) erythropoiesis. This 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 3,068 mcg/kg/day (approximately 2,322 times the MRHDID in adults on an AUC basis).

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 869477-96-3

Generic Name: olodaterol hydrochloride

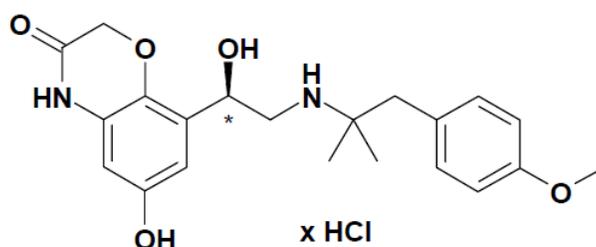
Code Name: BI 1744 CL

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: C₂₁H₂₆N₂O₅ x HCl or C₂₁H₂₇N₂O₅Cl / 386.45 g/mol (free base) or 422.91 g/mol (hydrochloride)

Structure or Biochemical Description:

Figure 2: Olodaterol Hydrochloride Structure²



Pharmacologic Class: Long-acting β_2 -adrenergic receptor agonist (LABA)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 76,362 for BI 1744 CL (sponsored by Boehringer Ingelheim Pharmaceuticals, Inc.)

2.3 Drug Formulation

STRIVERDI Respimat (olodaterol) inhalation spray consists of a sterile, aqueous, multi-dose solution of olodaterol hydrochloride in a specifically designed cartridge for delivery by the Respimat inhaler, which provides an aerosolized mist of appropriate particle size for delivery to the lung. The Respimat inhaler is a novel, pocket-sized, propellant-free device for the nebulization of inhalation solutions. This same device has been used in other approved NDAs from Boehringer Ingelheim Pharmaceuticals, Inc. Table 1 below includes the qualitative and quantitative composition of STRIVERDI Respimat.

² Figure taken from Applicant's submission

Table 1: Qualitative and quantitative composition of STRIVERDI Respimat (olodaterol) inhalation spray³

Name of ingredient	Per actuation ⁴⁾ [mg]	Percentage formula [g/100mL]	Per cartridge 4.5 mL ⁵⁾ [mg]	Function	Reference to standards
Olodaterol hydrochloride (corresponds to Olodaterol ¹⁾)	0.0027 (0.0025)	(b) (4)	(b) (4)	Drug substance	Company standard
Benzalkonium chloride (b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	NF
Edetate disodium					USP
Anhydrous citric acid					USP
Water for Injection					USP
Total mass					-

(b) (4)

- 1) 1 g olodaterol corresponds to 1.0945 g olodaterol hydrochloride.
- 2) (b) (4)
- 3) (b) (4)
- 4) One dose consists of two actuations.
- 5) This quantitative composition statement refers to a nominal filling volume of 4.5 mL per cartridge. (b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product or excipient levels above the levels in currently approved inhalation products.

2.5 Comments on Impurities/Degradants of Concern

All the impurities are below the ICH threshold or are considered qualified at the proposed specification. Impurities and/or degradants of concern are addressed in a separate review to NDA 203-108 (CMC consult review dated June 27, 2012). Refer to this review for complete details.

³ Table taken from Applicant's submission

2.6 Proposed Clinical Population and Dosing Regimen

STRIVERDI Respimat is proposed for the long-term, once-daily maintenance bronchodilator treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. The recommended dosage is two inhalations once daily at the same time of the day. Each actuation delivers 2.5 µg olodaterol for a total of 5 µg/day.

2.7 Regulatory Background

The initial IND 76,362 was submitted on January 26, 2007. Subsequently, at the sponsor's request, an end-of-phase-2 meeting was held on July 17, 2008. A pre-NDA meeting request was received in DPARP on July 29, 2011 and preliminary comments were sent to the sponsor on September 28, 2011. NDA 203-108 was submitted on May 14th, 2012.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study Number
Pharmacology	
In vitro pharmacological characterization of BI 1744 BS: Determination of agonistic potency and efficacy for the human beta 1 and beta 2 adrenoreceptors using the accumulation of cAMP in CHO-K1 cells expressing the respective receptors as functional readout.	U04-1454
In vitro pharmacological characterization of BI 1744: Determination of agonistic potency and efficacy for the human beta 3 adrenoreceptor in CHO-K1 cells.	U08-1336
In vitro pharmacological characterization of BI 1744.	U10-2584
Investigation of the onset of action of BI 1744 CL, administered by inhalation, against acetylcholine-induced bronchospasm for 1 hour in guinea pigs.	U05-2386
Investigations of BI 1744 CL, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm for 5 hours in guinea pigs.	U04-1553
Investigation of BI 1744 CL, administered by inhalation at 0.3, 1 and 3 µg/kg, for antagonistic effects against acetylcholine-induced bronchospasm for 24 hours in guinea pigs.	U06-1786
Investigation of BI 1744 CL, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm in anaesthetized dogs for 3 hours.	U04-1554
Investigation of BI 1744 CL, administered by inhalation at 0.3 and 0.6 µg/kg, for antagonistic effects against acetylcholine-induced bronchospasm in anaesthetized dogs over 24 hours.	U05-2586
In vitro pharmacology – Study of BI00001744	U03-1760
In vitro pharmacology: alpha 1 and 5-HT _{2A} receptor bioassays – Study of BI00001744	U03-1852
In vitro pharmacology: beta 1 adrenergic receptor bioassay – Study of BI00011329 and BI00001744.	U04-1602
Safety Pharmacology	
BI 1744 CL: Modified IRWIN study in rats including body temperature assessment following exposure by inhalation.	U05-2401
Influence of BI 1744 on hERG-mediated potassium current in HEK293 cells and on action potential configuration in isolated guinea pig papillary muscle.	U04-1115
BI 1744 CL: Telemetric evaluation of cardiovascular effects in the conscious dog (oral [gavage] administration).	U09-2001
BI 1744 CL: Evaluation of respiratory parameters in the conscious restrained rat during and	U05-2398

following exposure by inhalation.	
Effects of BI 1744 CL (0.3, 1, 3 mg/mL i.h.) on renal function in conscious rats in comparison with formoterol.	U04-1436
Effects of BI 1744 CL (0.3, 1, 3 mg/mL i.h.) on gastric secretion in rats in comparison with formoterol.	U04-1430
Effects of BI 1744 CL (0.3, 1, 3 mg/mL i.h.) on gastric emptying and gastrointestinal transit in rats in comparison with formoterol.	U04-1432
Pharmacokinetics	
BI 1744: Supportive non-GLP pharmacokinetics evaluation after inhalation with data derived from a 26-week inhalation toxicity study in rats with a 4-week recovery period.	U11-1351
BI 1744: Supportive non-GLP pharmacokinetics evaluation after inhalation with data derived from a 52-week inhalation toxicity study in Beagle dogs with a 6-week recovery period.	U11-1592
[¹⁴ C]BI 1744 CL: Binding to proteins in mouse and rabbit plasma in vitro.	U06-1533
Determination of the in vitro binding of [¹⁴ C]BI 1744 CL to the plasma proteins and blood cells of rat, dog, and man using equilibrium dialysis.	U04-1983
Determination of in vitro plasma protein binding of [³ H]BI 1744 BS, in plasma of rat, dog, and human.	U08-2233
Whole body autoradiography after intravenous, oral or intratracheal administration of [¹⁴ C]BI 1744 CL in male albino rats and after intravenous administration in male pigmented rats.	U05-1974
Tissue distribution and excretion after multiple intratracheal administration of [¹⁴ C]BI 1744 in the male rat.	U10-1602
Quantitative whole-body autoradiography after single intratracheal instillation of [¹⁴ C]BI 1744 to pregnant albino rat dams during embryonic and fetal stage of gestation.	U10-4128
Metabolism of BI 1744 in mice.	U06-1516
Metabolism of BI 1744 in rats.	U06-1514
Metabolite pattern in plasma following multiple intratracheal administration of BI 1744 to rats.	U10-1391
Metabolism of BI 1744 CL in female rabbits.	U08-1317
Metabolism of [¹⁴ C]BI 1744 CL in dogs.	U08-1057
In vivo metabolism of BI 1744CL: Enantioselective analysis of the parent compound in samples of humans and animal species.	U07-2170
[¹⁴ C]BI 1744: Excretion balance and sample generation for metabolism in the mouse.	U10-2458
Absorption, distribution and excretion of [¹⁴ C]BI 1744 CL in the rat.	U05-1007
Transfer of BI 1744 BS to milk after intravenous administration of [¹⁴ C]BI 1744 to lactating rats.	U10-1214
Excretion balance, C _c /C _p and Pharmacokinetics of radioactivity and parent compound after intravenous, oral, or intraduodenal administration of 0.3 mg/kg of [¹⁴ C]BI 1744 CL to female rabbits.	U08-1337
Absorption, distribution, excretion and metabolite pattern of [¹⁴ C]BI 1744 CL in the dog.	U06-1739
Toxicology	
BI 1744 CL: Acute toxicity – Acute toxic class method – in mice by inhalation.	U05-1101
BI 1744 CL: Single intravenous dose toxicity study in mice.	U05-1113
BI 1744 CL: Single oral (gavage) dose toxicity study in mice.	U05-1118
BI 1744 CL: Single dose inhalation toxicokinetic study in mice.	U08-1975
BI 1744 CL: Single intravenous dose toxicity study in rats.	U05-1112
BI 1744 CL: Single oral (gavage) dose toxicity study in rats.	U05-1065
BI 1744 CL: Acute toxicity – Acute toxic class method – in rats by inhalation.	U05-1100

BI 1744 CL: 2-week inhalation dose range finding study in rats.	U05-2143
BI 1744 CL: 26-week inhalation toxicity study in rats with a 4-week recovery period.	U08-1691
BI 1744 CL: 2-week inhalation dose range finding study in dogs.	U05-2134
Local tolerance	
BI 1744 CL: Acute dermal irritation/corrosion study in rabbits.	U05-1116
BI 1744 CL: Acute eye irritation/corrosion study in rabbits.	U04-2189

The following studies were previously reviewed under IND 76,362. See below under Section 3.3 “Previous Reviews Referenced”:

Toxicology	
BI 1744 CL: 13-week inhalation MTD study in mice.	U07-1341
BI 1744 CL: 4-week inhalation toxicity study in rats with a 4-week recovery period.	(b) (4)
BI 1744 CL: 13-week inhalation MTD study in rats with a 4-week recovery period.	U07-1342
BI 1744 CL: 26-week inhalation toxicity study in rats with a 4-week recovery period.	U08-1691
BI 1744 CL: 4-week inhalation toxicity study in dogs with a 4-week recovery.	U05-2146
BI 1744 CL: 13-week inhalation toxicity study in beagle dogs with a 6-week recovery period.	U07-1317
BI 1744 CL: 52-week inhalation toxicity study in beagle dogs with a 6-week recovery period.	U08-1740
BI 1744 CL: Mutagenicity study using the <i>Salmonella typhimurium</i> /mammalian-microsome assay (Ames test).	U05-1046
BI 1744 CL: Mutagenicity study using the mouse lymphoma (L5178Y) assay.	U04-2188
BI 1744 CL: P Intravenous mutagenicity study using micronucleus analysis of rat bone marrow.	U08-1834
BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration.	U09-1845
BI 1744 CL: 24-month inhalation carcinogenicity study in mice.	U12-1065
BI 1744 CL: 24-month inhalation carcinogenicity study in rats.	U11-2661
BI 1744 CL: Study for Effects on Fertility and Embryonic Development to Implantation in Rats (Inhalation).	U08-2297
BI 1744 CL: Study of Effects on Embryo-Fetal Development in CD Rats by Inhalation Administration.	U05-2534
BI 1744 CL: Pilot and Preliminary Embryo-Fetal Study in the Rabbit by Inhalation Administration.	U05-2535
BI 1744 CL: Study of the Effects on Embryo-Fetal Toxicity in the Rabbit by Inhalation Administration.	U06-1019

3.2 Studies Not Reviewed

The following studies were not reviewed because they were not relevant for the safety evaluation of this application or they do not provide pivotal information in the nonclinical safety evaluation of olodaterol under this application.

Pharmacology	
In vitro pharmacological characterization of CD 992 AC and (b) (4): Determination of agonistic potency and efficacy for the human beta 1 and beta 2 adrenoreceptors using the accumulation of cAMP in CHO-K1 cells expressing the respective receptors as	U06-2150

functional readout.	
In vitro pharmacological characterization of (b) (4), CD 992 XX, CD 10915 SE, CD 11249 BS, SOM 1522 BS, (b) (4).	U10-1323
In vitro pharmacological characterization of CD 12656.	U10-2582
In vitro pharmacological characterization of (b) (4): Determination of agonistic potency and efficacy for the human beta 1 and beta 2 adrenoreceptors using the accumulation of cAMP in CHO-K1 cells expressing the respective receptors as functional readout.	U06-1680
Investigation of BI 1744 CL and (b) (4), administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm for 5 hours in guinea pigs.	U06-2108
Investigation of BI 1744 CL and CD 992 AC, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm for 5 hours in guinea pigs.	U07-2475
Effects of intraduodenal administration of BI 1744 BS on hemodynamic parameters in anesthetized dogs.	U04-1712
Influence of BI00001744 CL (2) and formoterol (0.6, 1.2, and 2.4 µg/kg IH) on hemodynamic and electrocardiographic parameters in conscious dogs (using a purpose-bred labrador mix dog species).	U04-1052
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.	U06-2153
Investigation of the triple combination BI 1744 with tiotropium bromide and BI 54903 for antagonistic effects against ovalbumin-induced bronchospasm in guinea pigs.	U11-1148
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 hours.	U05-2587
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.	U05-2588
Safety Pharmacology	
Effects of BI 1744 BS as compared to formoterol on behaviour assessed by observation in a modified IRWIN-test and on nocturnal activity in mice after subcutaneous administration of 0.001, 0.003 and 0.01 mg/kg.	U04-1815
BI 1744 CL: Telemetric evaluation of cardiovascular effects in the conscious dog (single administration by inhalation).	U06-1058
Effects of BI 1744 CL as compared to formoterol on vital physiological functions in conscious rats using a telemetry/plethysmography system after inhalative administration of 0.3, 1 and 3 mg/mL.	U04-1819
Pharmacokinetics	
BI 1744 CL: A HPLC-MS/MS method for the quantification of BI 1744 BS in mouse plasma using solid phase extraction: method validation and stability data.	U06-1852
BI 1744 CL: A HPLC-MS/MS method for the quantification of BI 1744 BS in Han Wistar rat plasma using automated and manual solid phase extraction: method validation and stability data.	U04-1874
BI 1744 CL: A HPLC-MS/MS method for the quantification of BI 1744 BS in Sprague Dawley rat plasma using solid phase extraction: method validation and stability data.	U04-1960
BI 1744 CL: A HPLC-MS/MS method for the quantification of BI 1744 BS in rabbit plasma using automated solid phase extraction: method validation and stability data.	U04-1888
BI 1744 CL: A HPLC-MS/MS method for the quantification of BI 1744 BS in Beagle dog plasma using automated and manual solid phase extraction: method validation and stability data.	U04-1855
A HPLC-MS/MS method for the quantification of BI 1744 BS in Beagle dog urine using solid phase extraction: partial method validation and stability data.	U11-1647
BI 1744 CL: A HPLC-MS/MS method for the quantification of BI 1744 BS in mouse plasma using solid phase extraction: method validation and stability data.	U06-1852
Synthesis of [benzoxazine-2- ¹⁴ C]BI 1744 CL	U04-2145

Pharmacokinetics of BI 1744 CL in male mice after intravenous or oral administration.	U07-1105
Pharmacokinetics of BI 1744 CL after intratracheal, oral or intravenous administration in the rat.	U04-2124
P-glycoprotein-dependent limitation of oral absorption and bioavailability of BI 1744 CL in the rat.	U08-2038
Pharmacokinetics of BI 1744 CL after oral or intravenous administration in the dog.	U05-2163
Investigations on the contribution of P-glycoprotein on the brain penetration of BI 1744 CL and salmeterol xinafoate in mice and rats.	U09-1723
BI 1744 CL + tiotropium bromide (Ba 679 BR): Pharmacokinetic interaction potential after single dose exposition by inhalation in rats.	U05-2470
BI 1744 CL + tiotropium bromide (Ba 679 BR): Pharmacokinetic interaction potential after single dose exposition by inhalation in Beagle dogs.	U05-2446
Toxicology	
BI 1744 CL: Toxicity study by intravenous (slow bolus) administration to Han Wistar rats for 2 weeks.	U07-1375
BI 1744 CL: 2-week oral toxicity study in rats.	U07-2334
BI 1744 CL: Escalating dose toxicity study in the beagle dog after oral administration.	U08-1139
BI 1744: Exploratory toxicity study in the beagle dog after intravenous injection	U11-1625
BI 1744 CL: 2-week intravenous toxicity study in beagle dogs	U07-1051
BI 1744 CL: 2-week oral (gavage) toxicity study in beagle dogs	U07-2169
BI 1744 CL/tiotropium bromide (Ba 679 BR): 13-week inhalation toxicity study in beagle dogs with a 6-week recovery period.	U09-2249
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.	U10-1388
BI 1744 CL: Preliminary Inhalation Feasibility Study in Juvenile Beagle Dogs.	U08-1442
BI 1744 CL: 13-week Inhalation Toxicity Study in Juvenile Beagle Dogs with a 4-week Recovery Period.	U12-1257
Local Tolerance	
BI 1744 CL: Single-dose intravenous and intramuscular tolerance study in rabbits.	U06-2144
BI 1744 CL: Single-dose intra-arterial tolerance study in rabbits.	U06-2164
BI 1744 CL: Single-dose paravenous tolerance study in rats.	U06-2178
Impurities	
(b) (4) (b) (4) BI 1744 CL): Acute dermal irritation/corrosion study in rabbits.	U05-2529
BI 1744 (b) (4) Single oral (gavage) dose toxicity study in rats.	U06-1136
Mutagenicity study with (b) (4) (b) (4) using the S. <i>typhimurium</i> /mammalian microsome assay (Ames II) (non-GLP).	U04-1884
(b) (4) (impurity in BI 1744 CL): Mutagenicity study using the S. <i>typhimurium</i> /mammalian microsome assay (Ames test).	U06-1482
(b) (4) Single oral (gavage) dose toxicity study in rats.	U10-2305
Other studies	
Hemolysis test with an injectable solution of BI 1744 CL (0.01 mg/mL, calculated as base) and placebo.	U06-2118
Development of methodologies for the analysis of BI 1744 CL, in oral (gavage) dosing formulations.	U08-1347
Development of methodologies for the analysis of BI 1744 CL, degradation product (b) (4), benzalkonium chloride and disodium EDTA in inhalation dosing solutions.	U08-1524

BI 1744 CL: Development of methodologies for the analysis of BI 1744 CL in inhalation toxicology studies	U05-2430
BI 1744 CL: Development of methodologies for the formulation and analysis of BI 1744 CL in intravenous and oral gavage dosing formulations.	U06-2165
Two-week inhalation (nose/mouth-only) range-finding toxicity study in the rat on albuterol (salbutamol) (b) (4) formulation.	U98-2224
Two-week inhalation (mouth-only) range-finding toxicity study in the dog on albuterol (salbutamol) (b) (4) formulation.	U97-2076
Thirteen-week inhalation (mouth-only) toxicity study in the dog on albuterol (b) (4) formulation.	U97-2570
Ba 679 BR: 24-month inhalation carcinogenicity study in Wistar rats.	U98-2727
(Disodium EDTA) or (Benzalkonium chloride) or (Disodium EDTA + Benzalkonium chloride) in acidified demineralised water: 13-week inhalation toxicity study of selected vehicle formulations in rats.	U06-1186

3.3 Previous Reviews Referenced

Application	Relevant studies	Reviewer	Date
IND 76,362	Original submission (Studies U05-1046, U04-2188, (b) (4), U05-2146)	Molly Shea, Ph.D.	2/28/2007
IND 76,362	Carcinogenicity SPA Request (Studies U07-1341, U07-1342)	Molly Shea, Ph.D.	3/2/2007
IND 76,362	Request for ECAC Feedback	Molly Shea, Ph.D.	2/17/2009
IND 76,362	Safety Pharmacology and Genetic Toxicology (Studies U05-1046, U04-2188, U08-1834, U09-1845)	Timothy Robison, Ph.D., D.A.B.T	5/10/2010
IND 76,362	Reproductive Toxicology (Studies U08-2297, U05-2534, U05-2535, U06-1019, U08-1971)	Lawrence S. Leshin, D.V.M	10/26/2011
IND 76,362	Chronic Toxicology Studies (Studies U08-1691, U08-1740)	Hans Rosenfeldt, Ph.D.	10/28/2011
IND 76,362	Carcinogenicity Studies (Studies U12-1065, U11-2661)	Hans Rosenfeldt, Ph.D.	5/11/2011

4 Pharmacology

4.1 Primary Pharmacology

Brief Summary

The selectivity, potency, and potential efficacy of olodaterol (BI 1744) were investigated in a number of *in vitro* and *in vivo* studies described below. *In vitro* studies in CHO-K1 cells expressing the cDNA of the human β_1 -, β_2 -, and β_3 -adrenoreceptors show that BI 1744 has a high affinity for the human β_2 -adrenoreceptor ($K_i = 0.72$ nM). The affinity of BI 1744 at the β_1 - and β_3 -adrenoreceptors was also investigated and the data show that BI 1744 is less selective for these receptors (see K_i values in Table 2 below). The potency of BI 1744 was investigated at the human β_1 -, β_2 -, and β_3 -adrenoreceptors. EC_{50} values are also presented in Table 2. As shown, BI 1744 is more potent at the β_2 -adrenoreceptor ($EC_{50} = 0.1$ nM) compared to the other two receptors. Finally, the

intrinsic activity (IA, percentage of isoprenaline-induced maximal response) was also determined for BI 1744 at the β_1 -, β_2 -, and β_3 -adrenoreceptors. As shown in Table 2, BI 1744 presents the characteristics of a partial agonist, with an IA of 88% at the β_2 -adrenoreceptor. The results show that BI 1744 is also active at the human β_1 - and β_3 -adrenoreceptors, with an IA of 52% and 81%, respectively.

Table 2: Summary of BI 1744 affinity, potency, and intrinsic activity at the three human β -adrenoreceptor subtypes.

	K_i (nM)	EC_{50} (nM)	IA
hβ_1-adrenoreceptor	47	28	52%
hβ_2-adrenoreceptor	0.72	0.11	88%
hβ_3-adrenoreceptor	5500	269	81%

K_i = dissociation constant

EC_{50} = effective concentration

IA = intrinsic activity (% compared to isoprenaline, a non-selective full agonist)

In vivo pharmacology studies were conducted to assess the activity (bronchoprotection) of BI 1744 against acetylcholine-induced bronchospasms in anesthetized guinea pigs and dogs. The onset of action and the duration of action were determined. These studies demonstrate that BI 1744 has a fast onset of action (bronchoprotection observed within 10 minutes post-dose). In the guinea pig model, complete bronchoprotection (100%) was observed within 10 minutes at the fully effective dose (FED) of 3 $\mu\text{g}/\text{kg}$. In the dog model, maximum bronchoprotection (62%) was observed within 10 minutes at the FED of 0.6 $\mu\text{g}/\text{kg}$. BI 1744 also showed sustained bronchoprotection for up to 24 hours in guinea pigs and dogs at the FED (19% bronchoprotection was still present 24 hours after a single dose). These data support the applicant's proposal for a once-a-day dose in humans. In addition, as shown below, tachycardia was observed in dogs at a dose 2-fold above the FED (*i.e.*, at 1.2 $\mu\text{g}/\text{kg}$) but not at the FED.

In vitro pharmacology

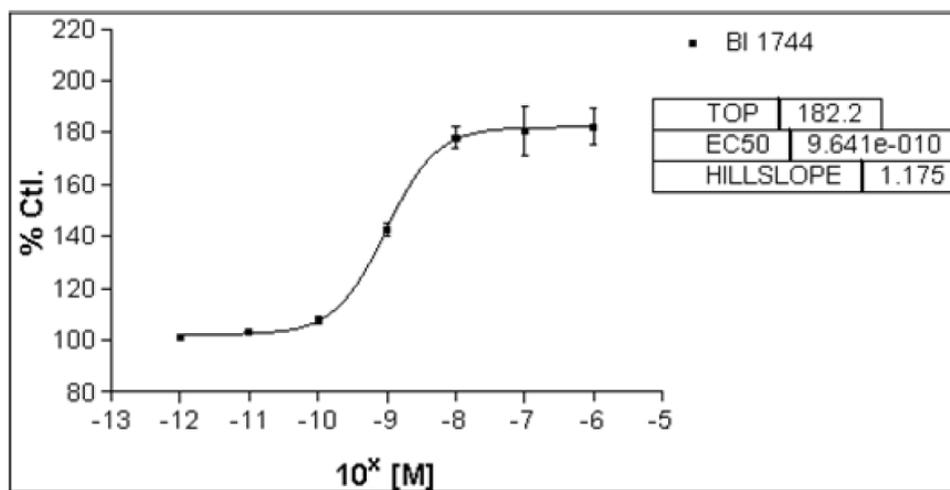
Study no. U04-1454: *In vitro* pharmacological characterization of BI 1744 BS: Determination of agonistic potency and efficacy for the human beta 1 and beta 2 adrenoreceptors using the accumulation of cAMP in CHO-K1 cells expressing the respective receptors as functional readout. The beta agonistic function of olodaterol (BI 1744) was determined in Chinese Hamster Ovarian (CHO)-K1 cells expressing the cDNA of the human β_1 - and β_2 -adrenoreceptors. The accumulation of cAMP (a downstream effector of receptor activation) was measured as a functional readout to determine the potency of olodaterol. Isoprenaline (a non-selective β_1 - and β_2 -adrenoreceptors full agonist) was used as a standard to determine olodaterol's intrinsic activity (IA) *in vitro*. Also, the results obtained with olodaterol were compared to formoterol and salmeterol (other drugs of the same class). The figures and table presented below were excerpted from the sponsor's submission (Study U04-1454 report).

Four independent experiments were conducted to assess olodaterol's function at the human β_2 -adrenoreceptor. From the four experiments, the mean EC_{50} and IA values were determined and are presented in Table 3 below. Olodaterol has an EC_{50} of 1 ± 0.4 nM for intracellular cAMP production and an IA of $68\% \pm 19\%$ compared to isoprenaline at the human β_2 -adrenoreceptor. Figure 3 below is a representative dose-response curve for cAMP accumulation induced by olodaterol in CHO-K1 cells expressing the human β_2 -adrenoreceptor.

Five independent experiments were conducted to assess olodaterol's activity at the human β_1 -adrenoreceptor. Mean EC_{50} and IA values were determined and are presented in Table 3 below. As shown, olodaterol has an EC_{50} of 60 ± 24 nM at the human β_1 -adrenoreceptor (measured by intracellular cAMP production) and an IA of $16\% \pm 3\%$ compared to isoprenaline at the human β_1 -adrenoreceptor. Figure 4 below is a representative dose-response curve for cAMP accumulation induced by olodaterol in CHO-K1 cells expressing the human β_1 -adrenoreceptor.

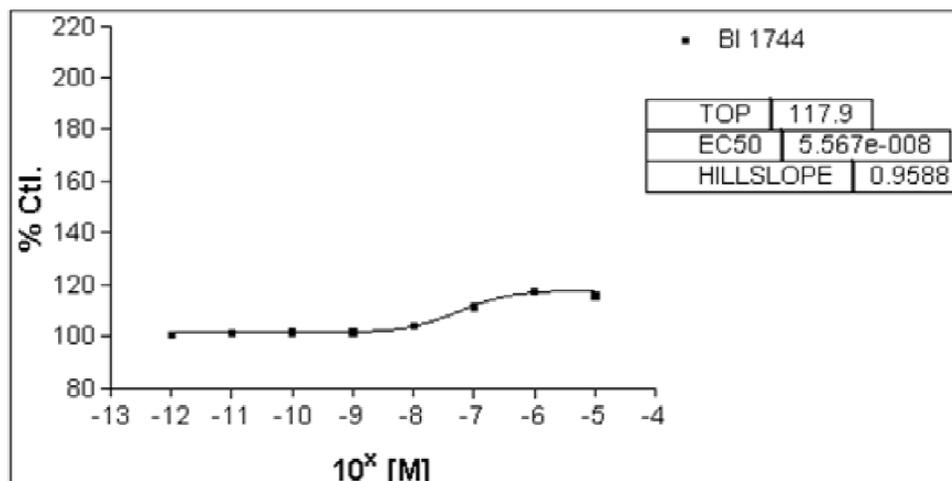
These results suggest that olodaterol is a potent and partial agonist (compared to isoprenaline) of the human β_2 -adrenoreceptor. Also, the results show that olodaterol exerts similar potency when compared to formoterol and salmeterol at the human β_2 -adrenoreceptor and is a weak partial agonist of the human β_1 -adrenoreceptor (Table 3).

Figure 3: Olodaterol-induced cAMP accumulation in CHO-K1 cells expressing the human β_2 -adrenoreceptor.



% Ctl = % of control (100% = no intrinsic activity; 200% = stimulation comparable to 10 μ M isoprenaline = full agonist = intrinsic activity of 100% compared to isoprenaline)
 Top = Maximum cAMP accumulation compared to 10 μ M isoprenaline

Figure 4: Olodaterol-induced cAMP accumulation in CHO-K1 cells expressing the human β_1 -adrenoreceptor.



% Ctl. = % of control (100% = no intrinsic activity; 200% = stimulation comparable to 10 μ M isoprenaline = full agonist = intrinsic activity of 100% compared to isoprenaline)
 Top = Maximum cAMP accumulation compared to 10 μ M isoprenaline

Table 3: Summary of results obtained for olodaterol, formoterol, and salmeterol at the human β_1 - and β_2 -adrenoreceptors.

Compound	EC ₅₀ [M]	SD	IA [% Iso]	SD	Experiments [#]
hβ2AR					
BI 1744	1,0E-09	4,1E-10	68	19	4
Salmeterol	1,3E-09	1,6E-10	27	5	2
Formoterol	1,0E-09	2,0E-10	118	9	4
hβ1AR					
BI 1744	6,0E-08	2,4E-08	16	3	5
Salmeterol	1,3E-06	4,3E-07	9	3	2
Formoterol	9,5E-08	3,0E-08	81	6	4

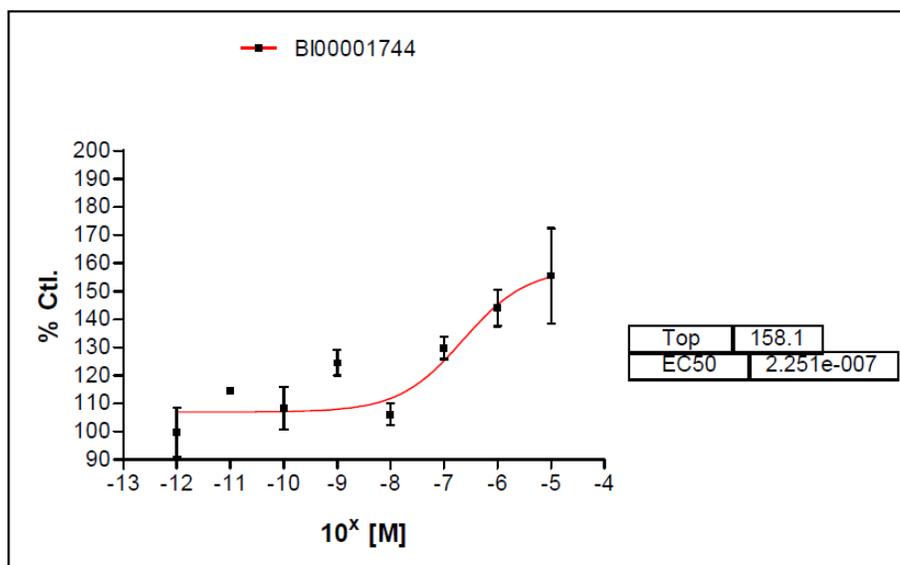
EC₅₀ = effective concentration
 SD = standard deviation
 IA = intrinsic activity (% compared to isoprenaline)

Study no. U08-1336: *In vitro* pharmacological characterization of BI 1744 BS: Determination of agonistic potency and efficacy for the human beta 3 adrenoreceptor in CHO-K1 cells. The beta agonistic function of olodaterol (BI 1744) was determined in CHO-K1 cells expressing the cDNA of the human β_3 -adrenoreceptor. The accumulation of cAMP was measured as a functional readout to determine the potency of olodaterol at the human β_3 -adrenoreceptor. Isoprenaline was used as a standard to determine olodaterol's IA.

Six individual experiments were conducted to assess olodaterol's function at the human β_3 -adrenoreceptor. EC₅₀ values and the IA compared to isoprenaline were calculated for each individual experiment and then the mean EC₅₀ and IA values were determined. Olodaterol has an EC₅₀ of 170 \pm 80 nM for intracellular cAMP production and an IA of 52% \pm 24.5% compared to isoprenaline at the human β_3 -adrenoreceptor. Figure 5

below [excerpted from the sponsor's submission (Study U08-1336 report)] is a representative dose-response curve for cAMP accumulation induced by olodaterol in CHO-K1 cells expressing the human β_3 -adrenoreceptor. The results of this study suggest that olodaterol is a weak partial (compared to isoprenaline) agonist of the human β_3 -adrenoreceptor.

Figure 5: Olodaterol-induced cAMP accumulation in CHO-K1 cells expressing the human β_3 -adrenoreceptor.



% Ctl = % of control (100% = no intrinsic activity; 200% = stimulation comparable to 10 μ M isoprenaline = full agonist = intrinsic activity of 100% compared to isoprenaline)
 Top = Maximum cAMP accumulation compared to 10 μ M isoprenaline

Study no. U10-2584: *In vitro* pharmacological characterization of BI 1744. The objective of this study was to determine olodaterol's affinity towards the human β_1 -, β_2 -, and β_3 -adrenoreceptors and to determine the potency and IA of olodaterol in activation of human β_1 -, β_2 -, and β_3 -adrenoreceptors. The activity was measured through changes in cAMP levels. According to the sponsor, this additional study included improved sensitivity of current methodologies for the cAMP assay and therefore, the EC₅₀ and IA values are different than the values reported in studies U04-1454 and U08-1336 (discussed above). Isoprenaline and formoterol were used for comparison but the data are not shown.

Three independent binding assays were conducted to determine the affinity (K_i values) of olodaterol for the human β_1 -, β_2 -, and β_3 -adrenoreceptors. The mean binding affinities (pK_i values) for olodaterol and isoprenaline are presented in Table 4 below. Olodaterol shows high affinity for the human β_2 -adrenoreceptor with a $K_i = 0.72$ nM.

Table 4: Binding affinities of different β_2 -adrenoreceptor agonists against the three human β -adrenoreceptor subtypes.

Agonist	pK_i $h\beta_1$	pK_i $h\beta_2$	pK_i $h\beta_3$
Isoprenaline	6.49 ± 0.01	6.54 ± 0.03	5.57 ± 0.07
BI 1744 CL	7.33 ± 0.05	9.14 ± 0.04	5.26 ± 0.14

pK_i = the negative logarithm to base 10 of the equilibrium dissociation constant (K_i)

Three independent experiments were conducted to determine olodaterol's potency (expressed as pEC_{50} , the negative logarithm to base 10 of the EC_{50}) and IA (percentage of isoprenaline-induced maximal response). pEC_{50} and IA values were calculated for each individual experiment and mean values were determined. Olodaterol has an EC_{50} of 0.1 nM and an IA of 88% compared to isoprenaline for the human β_2 -adrenoreceptor (Table 5). Olodaterol shows 241-fold and 2,299-fold more selectivity at the human β_2 -adrenoreceptor compared to the human β_1 - and human β_3 - adrenoreceptors, respectively.

Table 5: Functional properties of different β_2 -adrenoreceptor agonists against the three human β -adrenoreceptor subtypes.

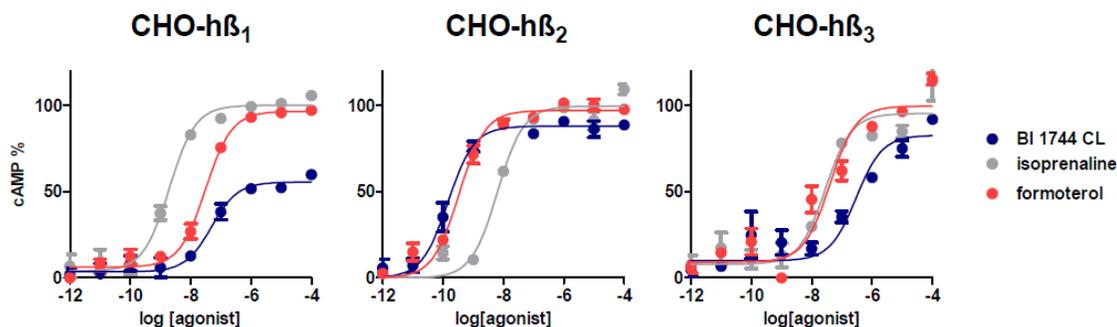
	$h\beta_1$		$h\beta_2$		$h\beta_3$	
	pEC_{50}	IA	pEC_{50}	IA	pEC_{50}	IA
Isoprenaline	9.27 ± 0.08	100%	8.58 ± 0.08	100%	7.86 ± 0.07	100%
BI 1744 CL	7.55 ± 0.08	$52 \pm 8\%$ *	9.93 ± 0.07	$88 \pm 2\%$	6.57 ± 0.08	$81 \pm 2\%$ *

* $p < 0.05$

IA = intrinsic activity (compared to isoprenaline, considered a full agonist)

Figure 6 below [excerpted from the sponsor's submission (Study U10-2584 report)] is a representative curve for cAMP accumulation induced by olodaterol (and compared to isoprenaline and formoterol) in CHO-K1 cells expressing the human β_1 -, β_2 -, or β_3 -adrenoreceptors. Data are presented as percentage of maximal isoprenaline-induced cAMP accumulation and the mean of three independent values (\pm S.E.M.) is shown.

Figure 6: Olodaterol (BI 1744)-induced cAMP accumulation in CHO-K1 cells expressing the human β_1 -, β_2 -, or β_3 -adrenoreceptors.



The results of this study show that olodaterol has high affinity and potency for the human β_2 -adrenoreceptor. In contrast to the previous study (Study U04-1454) where the IA of olodaterol at the β_2 -adrenoreceptor was only 68%, this study shows that olodaterol has the profile of an almost full agonist with an IA of 88%. Further, this study shows that olodaterol has a weak partial agonist activity at the human β_1 -adrenoreceptor with an IA of 52%. The potency of BI 1744 at the β_3 -adrenoreceptor is much less.

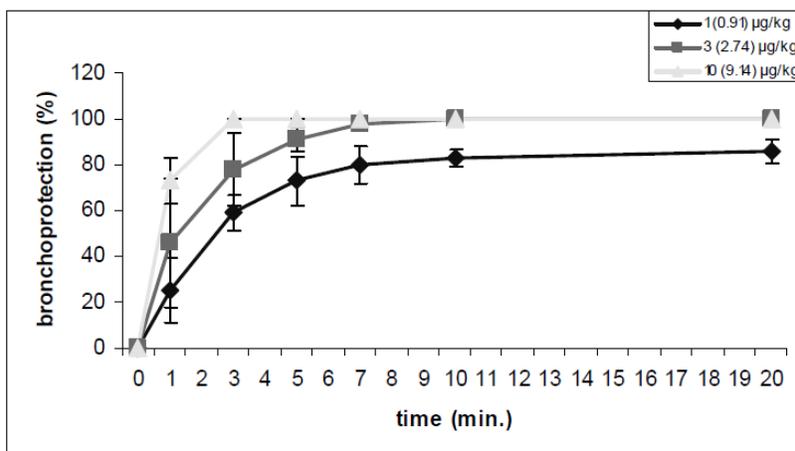
In vivo pharmacology

Study no. U05-2386: Investigation of the onset of action of BI 1744 CL, administered by inhalation, against acetylcholine-induced bronchospasm for 1 hr in guinea pigs. Bronchodilator effects (including onset of action) of BI 1744 were compared with other β_2 -adrenoreceptor agonists (salmeterol, formoterol, and the investigational drug QAB 149 MA) using an *in vivo* model of acetylcholine-induced bronchospasm in anesthetized Dunkin-Harley guinea pigs. The Konzett-Röblier model for evaluation of a drug's bronchodilatory effect was used for this study. Bronchospasm was induced by intravenous injections of acetylcholine at 1, 3, 5, 7, 10, 20, 30, 40, 50, and 60 minutes after inhalation of three doses of the β_2 -adrenoreceptor agonists: 1/3 of the fully effective dose (FED), the FED (*i.e.*, the first dose which achieves the full bronchoprotection), and three fold above the FED. In the case of BI 1744, the following doses were tested: 1, 3, and 10 $\mu\text{g}/\text{kg}$. Bronchoprotection of the drug (*i.e.*, inhibition of acetylcholine bronchoconstrictor response) was recorded for 1 hour and is expressed as a percentage of inhibition of the increase in overflow induced by acetylcholine preceding drug inhalation. Onset of action of the bronchoprotection corresponds to time (in minutes) where the activity achieves a steady state or the full (100%) bronchoprotection.

BI 1744 induced a dose-related inhibition of acetylcholine-induced bronchospasm with a dose-dependent onset of action. 1 $\mu\text{g}/\text{kg}$ inhaled BI 1744 induced 80% bronchoprotection at 7 minutes post-dose and reached a stable activity of 86% within 20 minutes post-dose. 3 $\mu\text{g}/\text{kg}$ inhaled BI 1744 exerted full bronchoprotection (100%) at 10 minutes post-dose and 10 $\mu\text{g}/\text{kg}$ inhaled BI 1744 exerted full bronchoprotection at 3

minutes post-dose (Figure 7 and Table 6, excerpted from the sponsor's submission, study U05-2386 report). Based on these results, BI 1744 is likely to have a fast onset of action.

Figure 7: Onset of action of 1, 3, and 10 µg/kg BI 1744 CL on acetylcholine-induced bronchoconstriction in anesthetized guinea pigs.



Each value represents the mean ± SD

Table 6: Bronchoprotection of BI 1744 CL (1, 3, and 10 µg/kg inhaled) against acetylcholine-induced bronchospasm.

time (min.)	Bronchoprotection (%) (1 µg/kg i.h.)	Bronchoprotection (%) (3 µg/kg i.h.)	Bronchoprotection (%) (10 µg/kg i.h.)
0	0 ± 0	0 ± 0	0 ± 0
1	25 ± 14	46 ± 28	73 ± 10
3	59 ± 8	78 ± 16	100 ± 0
5	73 ± 11	91 ± 5	100 ± 0
7	80 ± 8	98 ± 3	100 ± 0
10	83 ± 4	100 ± 0	100 ± 0
20	86 ± 5	100 ± 0	100 ± 0
30	86 ± 5	100 ± 0	100 ± 0
40	86 ± 5	100 ± 0	100 ± 0
50	86 ± 5	100 ± 0	100 ± 0
60	86 ± 5	100 ± 0	100 ± 0

Formoterol was tested at 0.3, 1, and 3 µg/kg inhaled doses. These doses induced a dose-related inhibition of acetylcholine-induced bronchospasm with a dose-dependent onset of action. 0.3 µg/kg inhaled formoterol (1/3 the FED) induced 81% bronchoprotection at 7 minutes post-dose and a stable activity of 87% at 10 minutes post-dose. 1 µg/kg formoterol reached 100% bronchoprotection at 7 minutes post-dose and 3 µg/kg reached full bronchoprotection at 3 minutes post-dose (data reviewed but not shown in this review).

Salmeterol was tested at inhalation doses of 3, 10, and 30 µg/kg. These doses showed a dose-dependent inhibition of acetylcholine-induced bronchoconstriction. Salmeterol showed a long and dose-dependent onset of action. At 3 µg/kg, 75% bronchoprotection was observed 20 minutes post-dose and a stable activity of 79% was observed 30

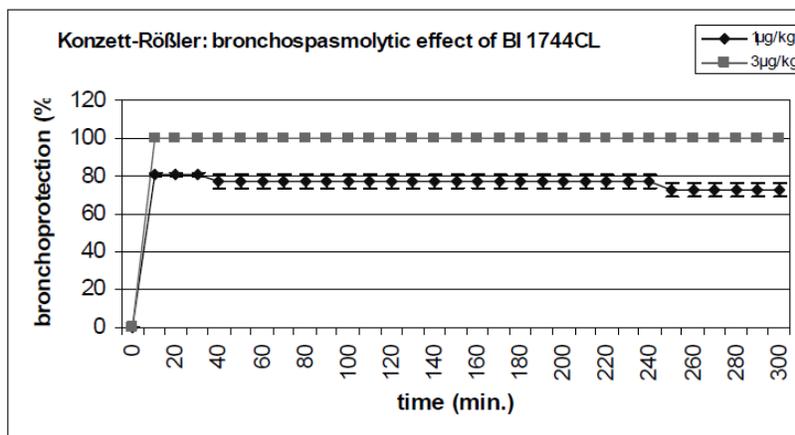
minutes post-dose. At 10 µg/kg inhaled salmeterol, 94% bronchoprotection was observed 7 minutes post-dose and full bronchoprotection was achieved 20 minutes post-dose. At 30 µg/kg, full bronchoprotection was observed within 5 minutes post-dose (data reviewed but not shown in this review).

(b) (4)

Study no. U04-1553: Investigations of BI 1744 CL, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm for 5 hr in guinea pigs. The duration of action of the bronchodilating activity of BI 1744 was assessed *in vivo* after acetylcholine-induced bronchospasms in anesthetized Dunkin-Harley guinea pigs. Briefly, bronchospasms were induced by acetylcholine IV injections every 10 minutes in order to get a reliable bronchospasm. After 3 stable bronchospasms, the test article was administered via inhalation and then the activity (bronchoprotection) was recorded for 5 hours. Two doses of BI 1744, 1 and 3 µg/kg, were administered. A vehicle control group (40/60 water/ethanol solution) and a positive control group (1 µg/kg formoterol) were included for comparison. As in the previous study, bronchoprotection is expressed as a percentage of inhibition of the increase in overflow induced by acetylcholine preceding drug inhalation.

At 1 and 3 µg/kg, BI 1744 induced a dose-related inhibition of acetylcholine-induced bronchospasm. 1 µg/kg BI 1744 produced about 80% bronchoprotection and was maintained at approximately 70-80% for the entire recording period of 5 hours. 3 µg/kg BI 1744 produced a full (100%) bronchoprotection that lasted the entire 5 hours (Figure 8, excerpted from the sponsor's submission, study U04-1553 report). As in the previous study, BI 1744 showed a short onset of action, with a maximal effect observed 10 minutes post-dose. Also, bronchoprotection was sustained for 5 hours, demonstrating the long duration of BI 1744's activity.

Figure 8: Effect of BI 1744 CL on acetylcholine-induced bronchoconstriction in anesthetized guinea pigs, 5 hour recording period.



Each value represents the mean \pm SD

BI 1744's activity was compared to salbutamol and fenoterol (short acting compounds), and three long acting compounds (salmeterol, formoterol, and the investigational drug (b) (4)). The results of this study confirmed that BI 1744 is a long acting β -adrenoreceptor agonist.

Study no. U06-1786: Investigation of BI 1744 CL, administered by inhalation at 0.3, 1 and 3 μ g/kg, for antagonistic effects against acetylcholine-induced bronchospasm for 24 hr in guinea pigs. Another study was conducted to evaluate the potential efficacy and the duration of action of BI 1744 over a 24 hour recording period using anesthetized Dunkin-Harley guinea pigs. The animals were first slightly anesthetized using isoflurane in order to instill the test article directly into the trachea. The instillation was performed using an endoscope. The animals were then deeply anesthetized with pentobarbital (IP and then IV infusion) 2 hours prior to the acetylcholine-induced bronchospasm (at 3, 6, 12, or 24 hours after drug instillation using IV acetylcholine at increasing doses from 2 to 20 μ g/kg). 20 μ g/kg acetylcholine produces full (100%) bronchospasm and is thus considered the fully effective dose. The following treatment groups were evaluated: a vehicle control group (40/60 water-ethanol solution), 3 μ g/kg formoterol, 3 μ g/kg investigational drug (b) (4) and 0.3-1 and 3 μ g/kg intratracheal BI 1744. At the end of the study, animals were sacrificed using an overdose of pentobarbital. All the figures and the table shown below were excerpted from the sponsor's submission (Study U06-1786 report).

BI 1744 was first tested at the fully effective dose of 3 μ g/kg and the duration of action was determined at 3, 6, 12, and 24 hours post-dose. At 3 and 6 hours, BI 1744 showed full bronchoprotection up to 18 μ g/kg IV acetylcholine. At 20 μ g/kg acetylcholine, the concentration that is expected to produce a full bronchospasm, 3 μ g/kg BI 1744 protected by 93% at 3 hours and 81% at 6 hours (Figure 9 and Table 7 below). At 12 and 24 hours, BI 1744 showed full bronchoprotection up to approximately 12 μ g/kg acetylcholine. At 20 μ g/kg acetylcholine, 3 μ g/kg BI 1744 still produced 50% bronchoprotection after 12 hours and 18% bronchoprotection after 24 hours (Figure 9 and Table 7). The effect of BI 1744 was then assessed at 0.3 and 1 μ g/kg intratracheal BI 1744. At these lower doses, BI 1744 still showed a dose-dependent bronchoprotection at low doses of acetylcholine (up to 10-12 μ g/kg IV) up to 24 hours post-dose (data not shown).

Figure 9: Effect of 3 µg/kg BI 1744 CL on acetylcholine-induced bronchoconstriction in anesthetized guinea pigs, 24 hour recording period.

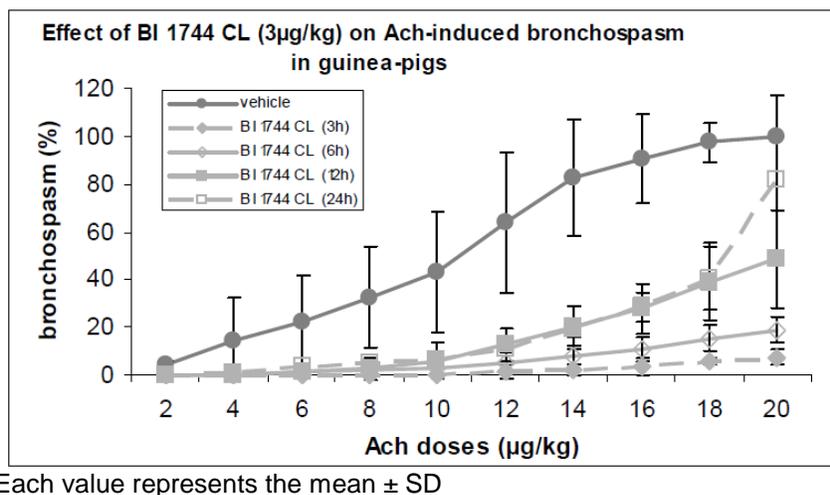


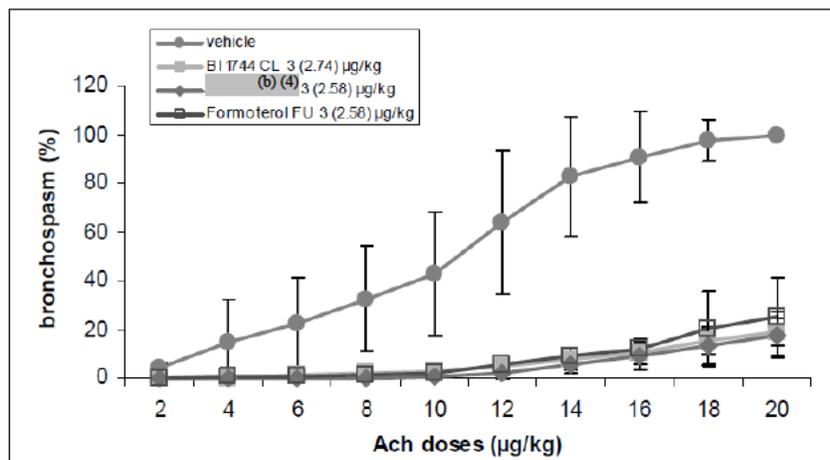
Table 7: Effect of 3 µg/kg BI 1744 CL on acetylcholine-induced bronchospasm at 3, 6, 12, and 24 hours after dose.

Acetylcholine (µg/kg i.v.)	Broncho.(%) at 3 h (n = 4)	Broncho.(%) at 6 h (n = 6)	Broncho.(%) at 12h (n = 6)	Broncho.(%) at 24 h (n = 5)
2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
4	0 ± 0	0 ± 0	0 ± 0	1 ± 1
6	0 ± 0	1 ± 1	1 ± 2	4 ± 1
8	0 ± 0	2 ± 2	3 ± 5	5 ± 1
10	0 ± 0	3 ± 3	6 ± 7	7 ± 1
12	1 ± 3	5 ± 3	13 ± 7	11 ± 1
14	2 ± 2	8 ± 5	20 ± 9	19 ± 3
16	4 ± 3	11 ± 5	28 ± 10	28 ± 6
18	6 ± 1	15 ± 5	39 ± 16	40 ± 13
20	7 ± 3	19 ± 6	49 ± 21	82 ± 36

Broncho.: Bronchospasm (%) (n = 4 - 6 animals per group)

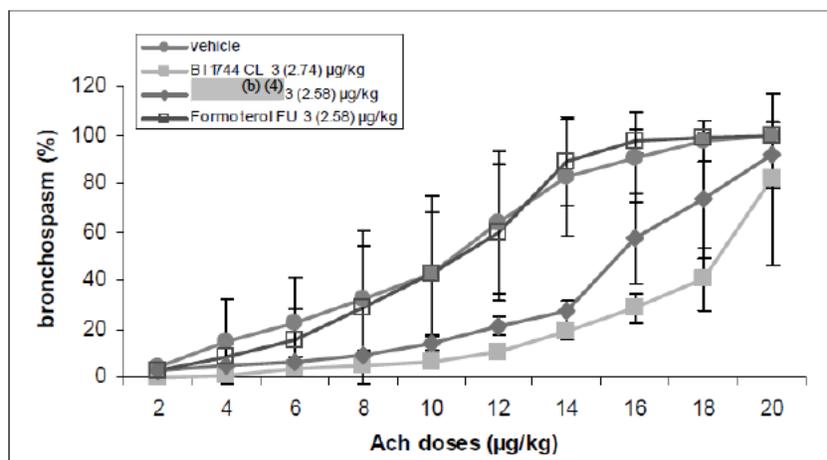
The duration of action of BI 1744-induced bronchoprotection at 3 µg/kg was compared to formoterol (3 µg/kg) and the investigational drug ^{(b) (4)} (3 µg/kg). As shown in Figure 10 below, the three compounds induced similar bronchoprotective effects at 6 hours post-dose. However, at 24 hours post-dose, the effect was still maintained with BI 1744 and ^{(b) (4)} but not with formoterol (Figure 11).

Figure 10: Effect of 3 µg/kg formoterol, (b) (4) and BI 1744 CL on acetylcholine-induced bronchoconstriction 6 hours post-dose.



Each value represents the mean ± SD

Figure 11: Effect of 3 µg/kg formoterol, (b) (4) and BI 1744 CL on acetylcholine-induced bronchoconstriction 24 hours post-dose.



Each value represents the mean ± SD

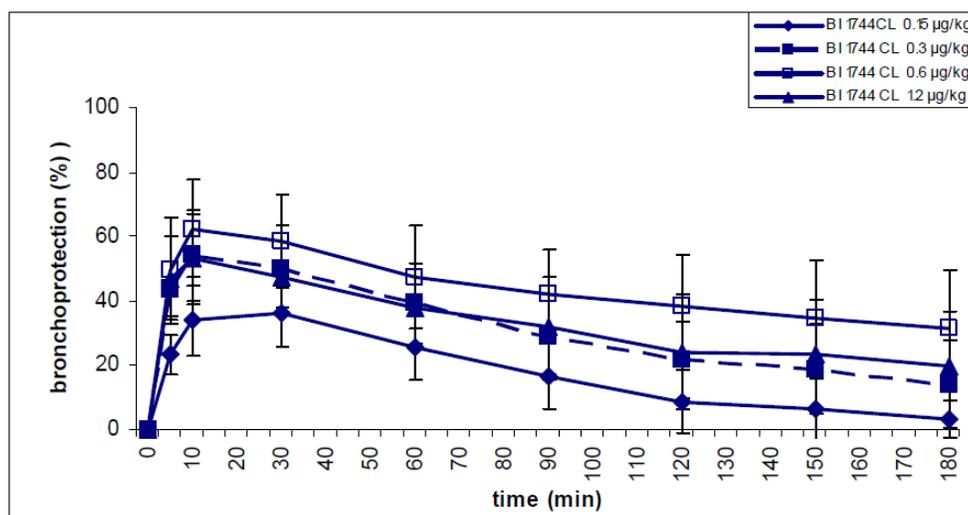
These results suggest that BI 1744 is a bronchodilator with a long duration of action (up to 24 hours).

Study no. U04-1554: Investigation of BI 1744 CL, administered by inhalation, for antagonistic effects against acetylcholine-induced bronchospasm in anaesthetized dogs for 3 hours. The bronchodilating activity, the duration of action (up to 3 hours) and the potential side effects of BI 1744 were evaluated in anesthetized male and female Beagle dogs over a recording period of 3 hours. In this study, anesthesia was induced by IV bolus injection of 10 mg/kg propofol followed by an IV infusion of 30 mg/kg propofol into the cephalic vein. Acetylcholine was injected IV at a dose of 10 µg/kg to induce a transient bronchospasm (approximately 30% increase in bronchial resistance) at the following time points pre-dose (negative values) and post-dose (positive values): -45, -30, -15, 5, 10, 30, 60, 90, 120, 150, and 180 minutes.

Bronchoprotection was expressed as a percentage of inhibition of the increase in pulmonary resistance induced by 10 µg/kg acetylcholine preceding drug administration. The following treatments (administered by intra-tracheal inhalation) were evaluated in this study: vehicle control (40/60 water/ethanol solution), or 0.15, 0.3, 0.6 (FED), and 1.2 µg/kg BI 1744. BI 1744's activity was compared to two other drugs of the same class: formoterol and (b) (4) (another investigational drug). Cardiovascular parameters were evaluated at the same times immediately before induction of bronchospasm. Increase in heart rate was expressed as a percentage of heart rate pre-dose (in beats/min). In addition, metabolic parameters (levels of potassium, lactate, and glucose) were determined 10 minutes and 30 minutes after drug administration and then every hour for 3 hours in order to further evaluate potential systemic side effects. All the figures below were excerpted from the sponsor's submission (Study U04-1554 report).

The FED was identified as 0.6 µg/kg BI 1744 under the conditions of this study, with a maximum bronchoprotection of 62% at 10 minutes after drug administration. The bronchodilatory effect of 0.6 µg/kg BI 1744 was maintained at approximately 31% at 3 hours post-dose (Figure 12).

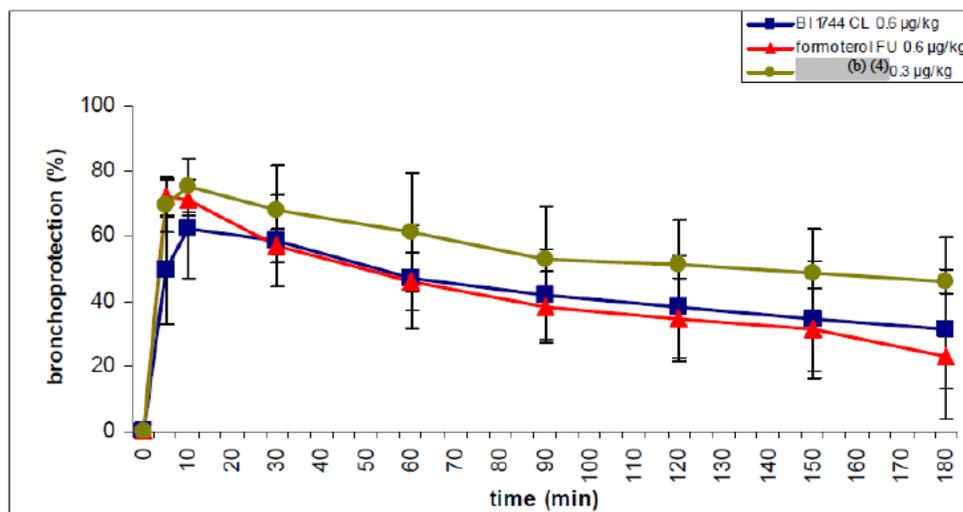
Figure 12: Effect of BI 1744 CL on acetylcholine-induced bronchoconstriction in anesthetized dogs.



Each value represents the mean ± SD

Figure 13 below shows a comparison of bronchoprotection induced by administration of 0.6 µg/kg BI 1744, 0.6 µg/kg formoterol, or 0.3 µg/kg (b) (4). As shown, BI 1744 is comparable to the other two drugs used in this study. The effect produced was maintained for 3 hours under the conditions of this study.

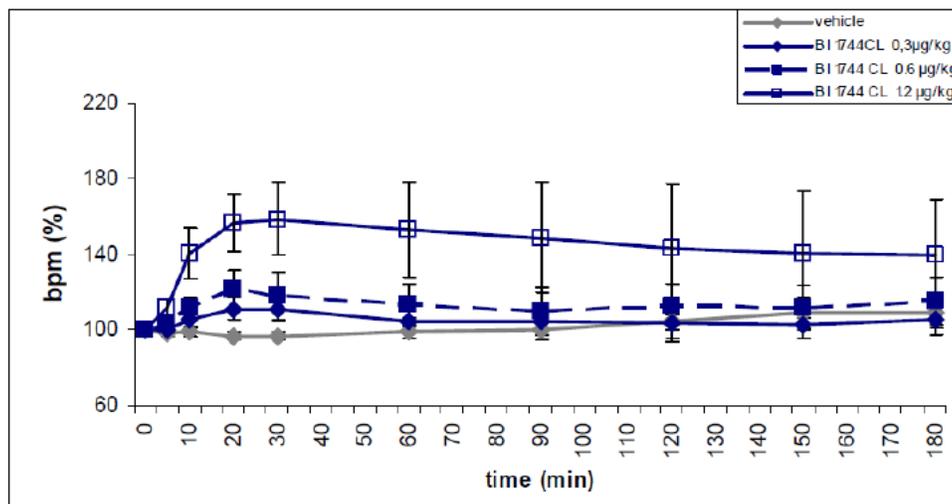
Figure 13: Effect of BI 1744 CL, compared to formoterol and (b) (4) on acetylcholine-induced bronchoconstriction in anesthetized dogs.



Each value represents the mean ± SD

The heart rate was measured after administration of BI 1744 and other drugs of the same class (data not shown). Figure 14 below shows the heart rates (expressed as a percentage of heart rate pre-dose, in beats/minute, bpm) after three doses of BI 1744. As shown, the heart rates were increased (approximately 60%) within 30 minutes after administration of 1.2 µg/kg BI 1744. This drug-induced tachycardia decreased gradually over time. However, it was still slightly increased (~39%) at 3 hours post-dose.

Figure 14: Effect of three doses of BI 1744 CL on heart rate in anesthetized dogs.



Each value represents the mean ± SD

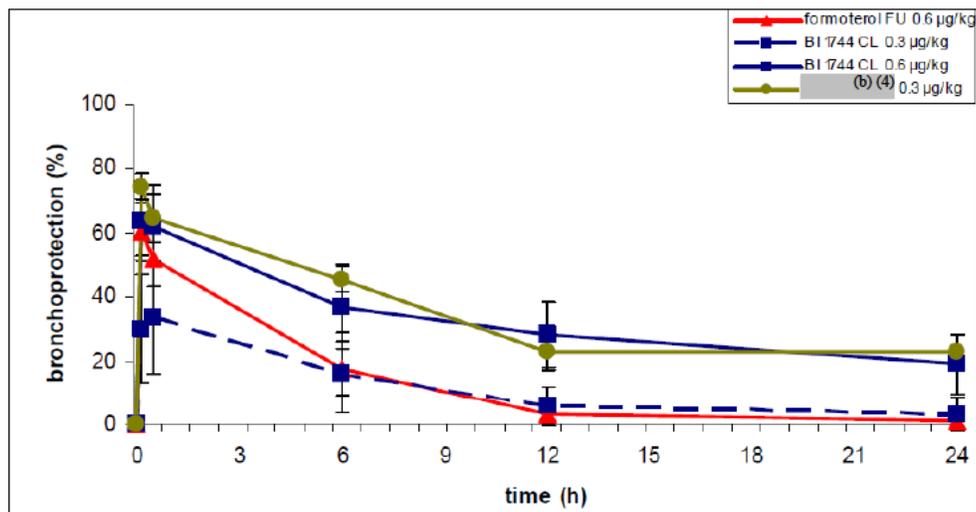
No BI 1744-related changes were observed in potassium, lactate, or glucose levels at any dose (data not shown).

The results of this study further suggest that BI 1744 is a potent bronchodilator and that it has the potential to induce tachycardia at high doses.

Study no. U05-2586: Investigation of BI 1744 CL, administered by inhalation at 0.3 and 0.6 µg/kg, for antagonistic effects against acetylcholine-induced bronchospasm in anaesthetized dogs over 24 hours. The bronchodilating activity and the duration of action of BI 1744 (at 0.3 and 0.6 µg/kg) were investigated in this study over a recording period of 24 hours in anesthetized Beagle dogs. Anesthesia was induced by IV bolus injection of 10 mg/kg propofol followed by an IV infusion of 30 mg/kg propofol into the cephalic vein. Acetylcholine was injected into the contralateral cephalic vein at a dose of 10 µg/kg for the induction of transient bronchospasm at -45, -30, and -15 minutes (pre-dose) and at 10 min, 30 minutes, 6 hours, 12 hours, and 24 hours post-dose. Bronchoprotection was expressed as a percentage of inhibition of the increase in pulmonary resistance induced by acetylcholine preceding drug administration. In addition, cardiovascular parameters (heart rate) were evaluated at the same times immediately before induction of bronchospasm. Also, metabolic parameters (potassium, lactate and glucose levels) were determined 4 times (every 6 hours) for the 24 hour recording period to evaluate potential systemic side effects. The figures presented below were excerpted from the sponsor's submission (Study U05-2586 report).

BI 1744 at 0.3 µg/kg induced 30% bronchoprotection with a fast onset of action (*i.e.*, 10 minutes post-dose) and then decreased to 16% at 6 hours post-dose. The bronchoprotection was not sustained with 0.3 µg/kg BI 1744, as the effect was almost gone by 12 hours. 0.6 µg/kg BI 1744 (the FED) also induced bronchoprotection within a short timeframe. The maximum bronchoprotection (approximately 64%) was observed within 10 minutes post-dose. After 6 hours, the activity of BI 1744 was decreased to 37%, and it continued to decrease but was still present 24 hours post-dose (19% bronchoprotection) (Figure 15 and Table 8 below). Formoterol also showed a fast onset of action, with 60% bronchoprotection observed within 10 minutes post-dose. Formoterol's bronchoprotection decreased rapidly and was almost gone after 12 hours. (b) (4) showed a rapid onset of action (75% bronchoprotection achieved within 10 minutes post-dose), and the effect was maintained for the 24-hour recording period (23% bronchoprotection at 24 hours post-dose) (Figure 15 and Table 8).

Figure 15: Effect of BI 1744 CL, formoterol, and (b) (4) on acetylcholine-induced bronchoconstriction in anesthetized dogs, 24 hour recording period.



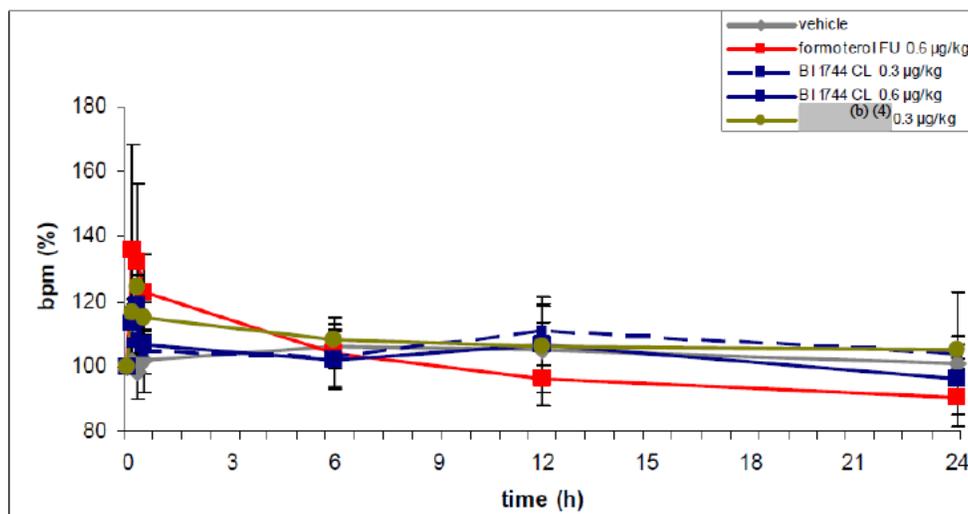
Each value represents the mean ± SD

Table 8: Effect of BI 1744 CL on acetylcholine-induced bronchospasm at 0.1, 0.5, 6, 12, and 24 hours post-dose.

Time (hours)	Vehicle % bronchoprotection	BI 1744 CL (0.3 µg/kg i.h.) % bronchoprotection	BI 1744 CL (0.6 µg/kg i.h.) % bronchoprotection
0	0 ± 0	0 ± 0	0 ± 0
0.1	-8 ± 11	30 ± 17	64 ± 11
0.5	-15 ± 7	34 ± 18	62 ± 13
6	1 ± 13	16 ± 13	37 ± 13
12	-6 ± 8	6 ± 6	28 ± 10
24	-1 ± 9	3 ± 5	19 ± 9

Figure 16 and Table 9 below show the effects of BI 1744, formoterol, and (b) (4) on heart rate over the 24-hour recording period. As observed, formoterol, 0.6 µg/kg BI 1744, and (b) (4) induced an increase in heart rate (approximately 36%, 19%, and 25%, respectively) during the first 30 minutes post-dose.

Figure 16: Effect of BI 1744 CL, formoterol, and (b) (4) on heart rate in anesthetized dogs, 24 hour recording period.



Each value represents the mean \pm SD

Table 9: Effect of BI 1744 CL on heart rate in anesthetized dogs, 24 hour recording period.

Time (hours)	Vehicle % bpm	BI 1744 CL (0.3 µg/kg i.h.) % bpm	BI 1744 CL (0.6 µg/kg i.h.) % bpm
0	100 \pm 0	100 \pm 0	100 \pm 0
0.1	101 \pm 21	107 \pm 6	114 \pm 7
0.2	98 \pm 8	109 \pm 8	119 \pm 8
0.5	102 \pm 9	104 \pm 6	107 \pm 5
6	106 \pm 7	103 \pm 10	102 \pm 9
12	105 \pm 13	111 \pm 10	107 \pm 13
24	101 \pm 5	104 \pm 19	96 \pm 6

bpm = beats per minute

4.2 Secondary Pharmacology

Secondary pharmacology studies were performed to investigate potential off-target effects of BI 1744. Initially, an *in vitro* screen assay was conducted (Study no. U03-1760) to identify potential targets of BI 1744 at 1 µM. The results of this assay identified the following human receptors as targets: β_2 -adrenergic, β_1 -adrenergic, α_1 -adrenergic, and 5-HT_{2A} serotonin receptor.

The activity at α_1 -adrenergic and 5-HT_{2A} receptors was further investigated in an *ex vivo* model using rabbit thoracic aorta rings (Study no. U03-1852). In this study, BI 1744 (0.1 to 10 µM) showed a dose-dependent inhibition of phenylephrine-induced contraction at the α_1 -adrenergic receptor and a dose-dependent inhibition (although much less) of serotonin-induced contraction at the 5-HT_{2A} receptor. These data suggest that BI 1744

has some antagonist activity at the α_1 -adrenergic and 5-HT_{2A} receptors, but no agonist activity at either receptor.

A second study to further investigate BI 1744's activity at the human β_1 -adrenergic receptor found that this drug exerts partial agonist activity at this receptor subtype (Study no. U04-1602). This was confirmed with additional studies that are discussed above under "Primary Pharmacology", section 4.1. The activity of olodaterol at the human β_3 -adrenergic receptor is also discussed in section 4.1.

4.3 Safety Pharmacology

The effects of olodaterol on the cardiovascular, pulmonary, neurological, renal, and gastrointestinal systems were investigated. Below is a detailed review of the safety pharmacology studies submitted to the IND and with this NDA. As discussed below, BI 1744-related effects were observed on the cardiovascular, renal, and gastrointestinal systems.

The effects on the cardiovascular system were investigated *in vitro* and *in vivo*. In the *in vitro* study, BI 1744 induced a dose-dependent increase in force of contraction in guinea pig papillary muscle. *In vivo*, BI 1744 produced a dose-dependent decrease in systolic, diastolic, and mean arterial blood pressure (MAP) as early as 30 minutes after drug administration. The decrease in MAP was observed up to approximately 18 hours post-dose but was recovered by 24 hours. The decrease in MAP was associated with an increase in heart rate observed 30 minutes (16 and 50 $\mu\text{g}/\text{kg}$) or 1-2 hours (1.6 and 5 $\mu\text{g}/\text{kg}$) after drug administration. The increase in heart rate was dose-dependent and persisted for up to 24 hours at the highest dose. BI 1744 also induced a dose-dependent shortening of the corrected QT interval (QTc) at 16 and 50 $\mu\text{g}/\text{kg}$. This effect was observed at 30 minutes (16 $\mu\text{g}/\text{kg}$) to 1 hour (50 $\mu\text{g}/\text{kg}$) post-dose and persisted for approximately 4-5 hours. These effects on the cardiovascular system are considered to be class effects for β_2 -adrenergic agonists.

The effects on the respiratory system were investigated in conscious rats following a single inhalation dose of BI 1744. No significant effects were observed in respiratory rate, tidal volume, or minute volume after treatment with BI 1744.

To study the effects of BI 1744 on the neurological system, the sponsor conducted a study using a single inhalation dose in rats. The modified Irwin test was used for assessment of neurological effects. The results of this study show that BI 1744 does not affect behavior or the physiological state of rats.

The sponsor conducted another study to assess the effects of BI 1744 on renal function in rats after a single administration. The results of this study demonstrate that BI 1744 induces a mild antidiuretic effect (reduced urine volume and reduced urine electrolyte excretion).

Finally, two studies were conducted in rats to determine the effects of BI 1744 on gastric function and secretion. The results demonstrate that BI 1744 induces a decrease in gastric emptying and gastrointestinal transit and reduces gastric secretion.

Cardiovascular effects

Study no. U04-1115: Influence of BI 1744 on hERG-mediated potassium current in HEK293 cells and on action potential configuration in isolated guinea pig papillary muscle. The objective of this study was to determine the effects of BI 1744 on human ether-a-go-go (hERG)-mediated potassium current in human embryonic kidney (HEK) 293 cells stably expressing hERG cDNA. Whole-cell patch clamp experiments were conducted using BI 1744 in concentrations of 0.1, 1, 3, 10, and 30 μM (in triplicate) to measure membrane currents. Control experiments were conducted every day to confirm the stability of the HEK cell preparation and to correct for the current run-down of the test system (*i.e.*, the spontaneous loss of current over time without treatment).

In addition, the effects of BI 1744 (0.3, 0.3, 1, 3, and 10 μM) on myocardial action potential configuration were determined using the isolated guinea pig papillary muscle test system. Measurements were recorded when the muscles were stimulated at a frequency of 0.33 Hz (20 cycles/min). The action potential duration to 10%, 30%, and 90% repolarization (APD10, APD30, and APD90, respectively), resting membrane potential (RMP), maximal velocity of phase 0 upstroke (V-max), action potential overshoot (OS), action potential amplitude (APA), and the force of contraction (FOC) were measured. Dimethyl sulfoxide (DMSO) was used as vehicle and as a negative control and formoterol was used as a reference product for comparison. The figures and tables below were excerpted from the sponsor's submission (Study U04-1115 final report).

BI 1744 did not affect the hERG current at doses up to 30 μM (Table 10 below).

Table 10: Effect of BI 1744 on hERG-mediated current. Fraction of hERG current (I/I_0) at five different concentrations of BI 1744.

Concentration	individual experiment			mean	SD
0.1 μM	0.70	0.80	0.87	0.79	0.08
1 μM	0.69	0.71	0.75	0.71	0.03
3 μM	0.85	0.73	0.74	0.77	0.07
10 μM	0.62	0.61	0.84	0.69	0.13
30 μM	0.70	0.76	0.59	0.69	0.09

Results of the action potential configuration show that BI 1744 induces a dose-dependent slight shortening of APD90 and APD30 and a slight lengthening of APD10 (Figure 17 and Table 11 below). Formoterol induced similar effects on action potential duration (data not shown).

Figure 17: An example of action potentials before (control) and after 10 µM BI 1744.

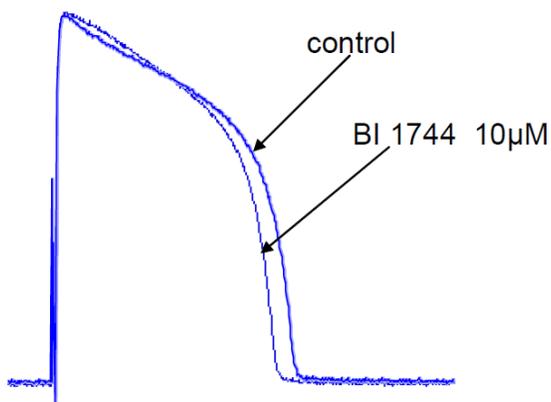


Table 11: Effects of BI 1744 on action potential duration (APD) versus control.

Concentration	APD90 [%]						sign.
	Control			BI 1744			
	mean	SD	N	mean	SD	N	
Predrug	100.5	0.7	5	100.0	0.9	5	
0.1 µM	101.1	0.9	5	101.2	0.4	5	
0.3 µM	102.0	1.6	5	101.4	1.5	5	
1.0 µM	102.8	2.5	5	98.7	1.6	5	*
3.0 µM	102.2	2.4	5	96.5	2.0	5	**
10.0 µM	102.2	2.0	5	95.6	2.1	5	**

Concentration	APD30 [%]						sign.
	Control			BI 1744			
	mean	SD	N	mean	SD	N	
Predrug	100.2	1.9	5	99.8	1.2	5	
0.1 µM	98.9	2.8	5	101.8	1.1	5	
0.3 µM	102.8	2.3	5	101.8	1.3	5	
1.0 µM	103.8	3.9	5	98.8	1.4	5	*
3.0 µM	102.0	6.1	5	95.9	2.4	5	
10.0 µM	101.3	3.6	5	94.9	2.5	5	*

Concentration	APD10 [%]						sign.
	Control			BI 1744			
	mean	SD	N	mean	SD	N	
Predrug	96.7	8.5	5	99.7	3.5	5	
0.1 µM	98.4	4.8	5	107.9	8.3	5	
0.3 µM	101.3	4.1	5	115.9	7.5	5	**
1.0 µM	102.3	4.0	5	115.7	7.9	5	**
3.0 µM	102.9	4.0	5	116.4	11.7	5	*
10.0 µM	97.8	9.3	5	113.7	10.7	5	*

* p < 0.05 vs. control

** p < 0.01 vs. control

In addition, BI 1744 induced a dose-dependent increase in force of contraction, or FOC (Figure 18 and Table 12 below). Formoterol induced similar effects on FOC (data not shown).

Figure 18: Effect of BI 1744 on force of contraction (FOC) in guinea pig papillary muscles.

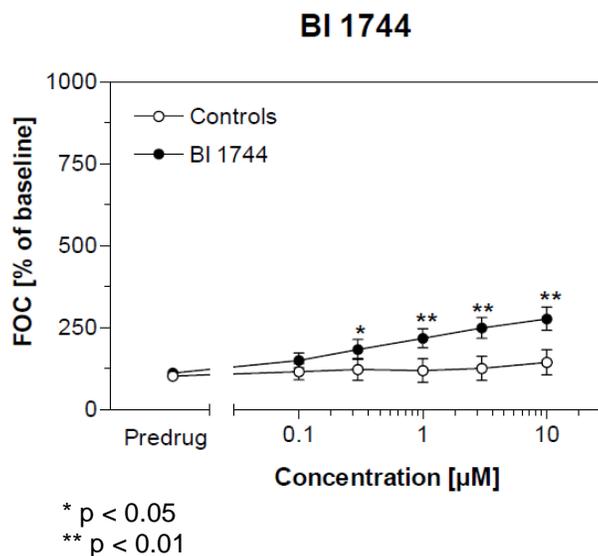


Table 12: Effect of BI 1744 on force of contraction (FOC) versus a control group.

Concentration	FOC [%]						sign.
	Control			BI 1744			
	mean	SD	N	mean	SD	N	
Predrug	103.1	12.1	5	112.3	6.6	5	
0.1 µM	116.1	24.9	5	150.5	22.6	5	
0.3 µM	122.8	33.1	5	184.0	30.3	5	*
1.0 µM	119.8	36.0	5	217.9	29.2	5	**
3.0 µM	126.1	36.5	5	249.7	31.7	5	**
10.0 µM	144.7	38.5	5	277.3	35.2	5	**

* p < 0.05
** p < 0.01

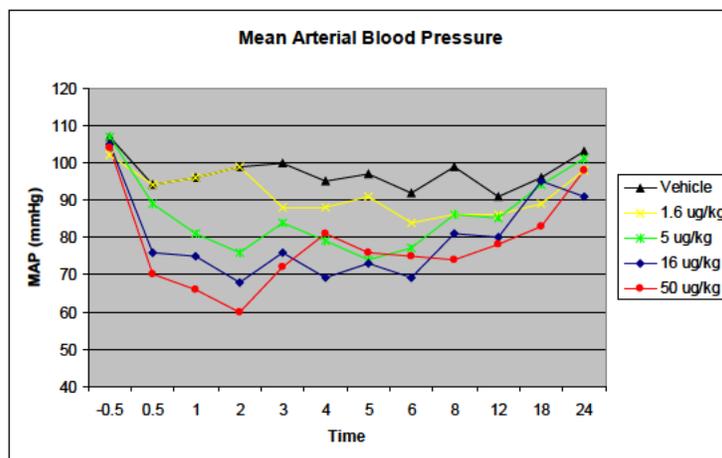
Study no. U09-2001: BI 1744 CL: Telemetric Evaluation of Cardiovascular Effects in the Conscious Dog (oral [gavage] administration). This GLP study was conducted to assess the effects of BI 1744 (administered as a single oral gavage dose) on the cardiovascular system. Another study was conducted by the sponsor (Study no. U06-1058) using a single inhalation dose of BI 1744 at 1, 3, or 10 µg/kg (0.91, 2.74, and 9.14 µg/kg achieved doses) but the systemic exposure was very low at these doses. Therefore, Study U09-2001 was used to characterize the cardiovascular effects of BI 1744. The following parameters were measured: blood pressure, heart rate, body temperature, and electrocardiography (ECG). Beagle dogs (3 males and 3 females approximately 19-34 months, previously implanted with radio telemetry devices) received a single oral gavage dose of BI 1744 using an escalating dose design at dose

intervals of 7 days. In this design, animals received vehicle (acidified demineralized water adjusted to pH of 4 with 0.1N NaCl) on study week 1, and then four escalating doses of BI 1744 (at 1.6, 5, 16 or 50 µg/kg). Telemetry recordings were initiated at least 90 minutes prior to administration of treatment and continued for at least 24 hours post-dose (timepoints of -60, -55, -50, -45, -40, -35, and -30 minutes pre-dose and then at 30 minutes, and 1, 2, 3, 4, 5, 6, 8, 12, 18, and 24 hours post-dose). In addition, the animals were observed for behavioral changes and clinical signs of toxicity. Blood samples were collected from all the animals from the jugular vein into tubes containing K₂ EDTA as anticoagulant at 1 and 2 hours post-dose. The concentration of BI 1744 in plasma was then determined by solid-phase extraction followed by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS).

BI 1744 induced an increased incidence of body tremors (all doses), underactivity (at ≥ 5 µg/kg), inappetence (at ≥ 5 µg/kg), and rapid/strong pulse (at ≥ 16 µg/kg). Most findings were observed from 1 to 4 hours post-dose. No significant changes were observed in body temperature after treatment with BI 1744. Although the sponsor reports a temperature increase in treated animals, the change was only 0.6 to 1.1 °C and temperatures were still within the normal range for dogs. Therefore, there is no concern for this effect.

BI 1744 produced a dose-dependent decrease in systolic, diastolic, and MAP immediately after drug administration. The effect was more pronounced at 2 hours post-dose (around the T_{max} after oral administration in dogs) and persisted for approximately 18 hours post-dose. The decrease in MAP was recovered at 24 hours post-dose (Figure 19 below shows group mean data).

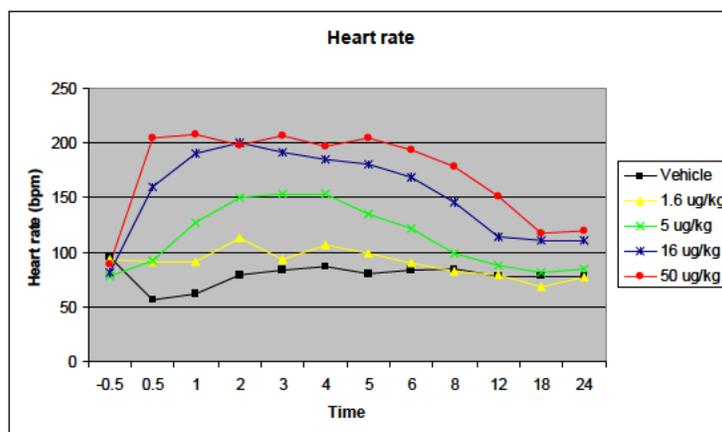
Figure 19: Effects of BI 1744 on mean arterial pressure (MAP) following oral administration of BI 1744 or vehicle (group mean values)



As expected, BI 1744 also induced a dose-dependent increase in heart rate that was observed as early as 30 minutes post-dose. At lower doses (1.6 and 5 µg/kg), this effect was observed at 1-2 hours post-dose and was more pronounced at approximately 2 hours post-dose. The effect persisted from one hour (1.6 µg/kg) to approximately 8

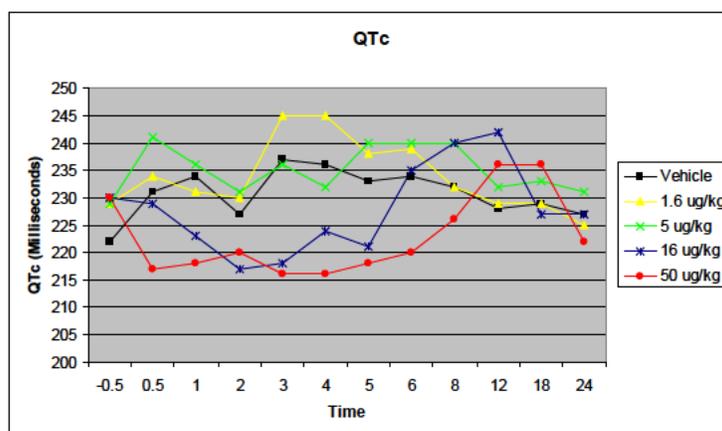
hours (5 µg/kg). At higher doses (16 and 50 µg/kg), the effect was observed immediately after drug administration and persisted for approximately 12-18 hours post-dose. The increase in heart rate was still observed at 24 hours post-dose for the two higher dose groups, although the change was less prominent (Figure 20 below shows group mean data).

Figure 20: Effects of BI 1744 on heart rate following oral administration of BI 1744 or vehicle (group mean values)



The effects of BI 1744 on ECG were also assessed in this study. BI 1744 induced a dose-dependent shortening of the PR and QT intervals that coincided with the increases in heart rate (data not shown). The effects were observed as early as 30 minutes post-dose and were maximal at approximately 2 hours post-dose. Following correction of the QT interval for heart rate (QTc), no effects were observed at 1.6 or 5 µg/kg. However, a transient shortening of the QTc interval was observed with 16 and 50 µg/kg BI 1744 (Figure 21 below). This effect was observed at 30 minutes (16 µg/kg) to 1 hour (50 µg/kg) post-dose and persisted for approximately 4-5 hours.

Figure 21: Effects of BI 1744 on QTc following oral administration of BI 1744 or vehicle (group mean values)



Analysis of BI 1744 levels in plasma shows evidence of systemic exposure. Plasma BI 1744 concentrations (C) increased in a dose-dependent manner across all doses. In general, the increase was dose proportional (Table 13 below).

Table 13: Mean BI 1744 concentrations (C) at 1 and 2 hours after drug administration.

BI 1744 (µg/kg)	C (1 hr) pmol/L	C (2 hr) pmol/L
1.6	22.6	36.8
5	125	135
16	598	516
50	1690	1070

Respiratory effects

Study no. U05-2398: BI 1744 CL: Evaluation of Respiratory Parameters in the Conscious Restrained Rat During and Following Exposure by Inhalation. This GLP study was conducted to assess the effects of BI 1744 (a single inhalation dose) on respiratory parameters (respiration rate, tidal volume, and minute volume) in conscious rats. Wistar Han rats (8 males per group) received 17.2, 64.3, or 485 µg/kg BI 1744 (free base equivalent) as a single inhalation dose for 30 minutes. Two additional groups (8 male rats/group) were included: one group received a placebo inhalation solution containing 0.01% benzalkonium chloride and (b) (4) % disodium edetate, and the second group received 30 minutes exposure to an atmosphere with increased carbon dioxide concentration (as positive control, 8% CO₂/21% O₂/71% N₂). The rats were placed in plethysmographs to record respiration rate, tidal volume and minute volume prior to dosing, during exposure, and continuously for approximately 5 hours following cessation of exposure. Respiratory parameters were averaged for each animal over each recording period.

BI 1744 did not change any of the respiratory parameters tested in this study.

Neurological effects

Study no. U05-2401: BI 1744 CL: Modified Irwin Study in Rats Including Body Temperature Assessment Following Exposure by Inhalation. This GLP study was conducted to assess the effects of a single BI 1744 inhalation dose in the central nervous system (CNS) in rats using a modified Irwin model. Wistar Han rats (4 males/group) received 17.1, 63.4, or 483 µg/kg BI 1744 as a single inhalation dose for approximately 30 minutes duration. An additional group of 4 male rats was included as a vehicle control group and received a placebo inhalation solution (0.01% benzalkonium chloride and (b) (4) % disodium edetate). Also, an additional 4 male rats per group was included for analysis of BI 1744 exposure (satellite group). Subjective evaluations (modified Irwin test observations to assess any changes in general behavior or physiological state) were performed pre-dose and at 30, 90, 150, and 300 minutes post-dose. The rectal temperature of each animal was obtained pre-dose and at 30, 90, 150, and 300 minutes post-dose. The animals were inspected daily from study days 2-7 for

mortalities and for clinical signs of toxicity. Blood samples were collected from the tail vein of satellite animals immediately following exposure (30 minutes) and at 2 and 5.5 hours post-exposure.

No mortalities or signs of toxicity were observed in any of the groups. BI 1744 did not produce any changes in general behavior or in the physiological state of rats under the conditions of this study. Although the sponsor reports a statistically significant decrease in body temperature in rats treated with 63.4 and 483 µg/kg BI 1744, a review of the data found that this change was only 2-3% (0.8-1.2°C) and that the temperatures were still within the normal range for rats. Therefore, this change in body temperature is not considered of biological significance. Results of the exposure analysis found a dose-dependent increase in exposure in BI 1744-treated animals. However, the increase was more than proportional with dose.

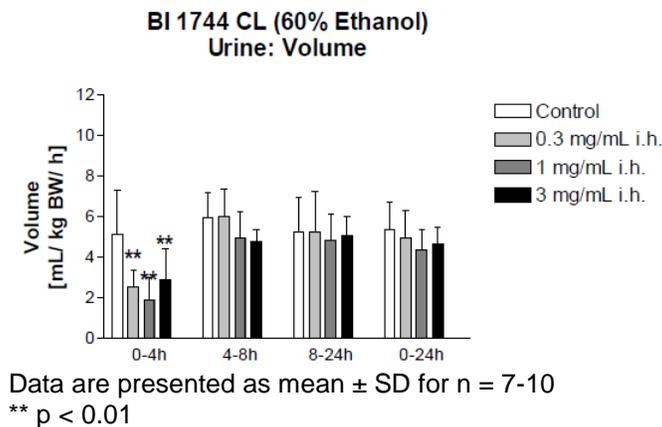
Renal effects

Study no. U04-1436: Effects of BI 1744 CL (0.3, 1, 3 mg/mL by inhalation) on renal function in conscious rats in comparison with formoterol. Renal function was investigated in conscious rats after a single administration of BI 1744 via inhalation. Formoterol (another LABA) was used for comparison. Wistar rats (10/sex/group) received vehicle (60% ethanol solution), 0.3, 1, or 3 mg/mL BI 1744 solution (or 0.3, 1, or 3 mg/mL formoterol solution) by inhalation over 1 minute. Urine was collected in half of the animals for each group (5/sex) and blood samples were collected in the other half (5 animals/sex). The rats used for urine collection were housed in metabolic cages and received a tap water load of 2 mL/100 g of body weight before drug (or vehicle) administration, and 4 and 8 hours post-dose. Urine was collected at 4, 8, and 24 hours post-dose and a full battery of urinalysis and clearance parameters was evaluated (urine volume, osmolality, pH; concentrations of sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, glucose, blood urea nitrogen (BUN), albumin, protein as well as the levels of the following enzymes: aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, lactate dehydrogenase, alkaline phosphatase, and N-acetyl-β-D-glucosaminidase). Blood was collected at 4, 8, and 24 hours post-dose and the following parameters were evaluated: osmolality, pH, concentrations of sodium, potassium, chloride, calcium (free and total), magnesium, phosphate; the metabolites creatinine, glucose, protein, BUN, cholesterol, free fatty acids, triglycerides, total and conjugated bilirubin; and liver enzymes. Although formoterol was used for comparison, only the results for BI 1744 are discussed below.

BI 1744 induced a transient reduction in urine volume (during the first 4 hours, refer to Figure 22 below excerpted from the sponsor's submission, study U04-1436 report) and decreased excretion of sodium, potassium, and chloride at all the doses evaluated. These results suggest a transient antidiuretic effect of BI 1744. However, the effects observed were not dose-dependent. In addition, the urine pH was decreased and excretion of calcium and magnesium was increased only at the highest dose (*i.e.*, 3 mg/mL). Also at the highest dose, levels of gamma-glutamyl transferase and alkaline phosphatase were increased in urine during the first 4 hours and then returned to

normal, suggesting a possible transient effect in the liver. In the serum, only a slight increase in pH was observed. Other serum parameters were not significantly affected by BI 1744.

Figure 22: Effect of BI 1744 CL on urine volume in rats.



Gastrointestinal effects

Study no. U04-1432: Effects of BI 1744 CL (0.3, 1, 3 mg/mL by inhalation) on gastric emptying and gastrointestinal transit in rats in comparison with formoterol. The gastric emptying and gastrointestinal transit were investigated in rats after administration of BI 1744 (or formoterol, for comparison) via inhalation. Five rats of each sex received vehicle (60% ethanol solution), 0.3, 1, or 3 mg/mL BI 1744 solution or 0.1, 0.3, 1, 3, or 10 mg/mL formoterol solution by inhalation over 1 minute. The animals were fasted for 17 hours prior to the study and only allowed free access to water during this period. Fifteen minutes after drug administration, a test meal of BaSO₄ (a suspension of 7.5 g Neobar[®] (Barium Sulfate) in 10 mL salt-free water) was given orally by gavage at 2 mL/100 g body weight. Thirty minutes after administration of the test meal, animals were euthanized with an overdose of ethylchloride. The stomach and the intestines were then exposed by laparotomy and removed for analysis. The stomach was weighed, incised and the contents removed, and then was weighed again. The gastric content was calculated from the weight difference between the filled and empty stomach and was normalized to 100 g of body weight. Thus, an increase in weight difference indicated inhibition of gastric emptying, whereas a decrease in weight difference indicated enhanced gastric emptying. Gastrointestinal transit was determined as the percentage movement of barium sulfate in the intestine in relation to the whole length of the gut as determined by visual inspection of the test meal passage in the intestine. Only the results of BI 1744 are presented and discussed in this review.

The results are presented in Table 14 below (excerpted from the sponsor's submission, study U04-1432 report). BI 1744 induced a decrease in gastric emptying of 16 and 27% at 1 and 3 mg/mL, respectively. Also, BI 1744 induced a dose-dependent decrease in gastrointestinal transit at 1 and 3 mg/mL (20-35%). No statistically significant effects

were observed at 0.3 mg/mL BI 1744. Formoterol also showed similar results (data not shown).

Table 14: Effects of BI 1744 CL, administered by inhalation, on gastric emptying and gastrointestinal transit in conscious rats.

BI 1744 CL	Control	0.3 mg/mL	1.0 mg/mL	3.0 mg/mL
Gastric emptying [mg/100g BW]	2709±204	2897±380	3137±231	3433±158
Diff. [%]		+7	+16	+27
Gastrointestinal transit [%]	55±5	52±8	44±7	36±14
Diff. [%]		-6	-20	-35

Data are presented as mean ± SD for n=8-20.

* p < 0.05, ** p < 0.01, and *** p < 0.0001 vs. vehicle controls

Study no. U04-1430: Effects of BI 1744 CL (0.3, 1, 3 mg/mL by inhalation) on gastric secretion in rats in comparison with formoterol. The gastric secretion was then investigated in rats after administration of BI 1744 (or formoterol, for comparison) via inhalation. Eight male rats received vehicle (60% ethanol solution), 0.3, 1, or 3 mg/mL BI 1744 solution or 0.1, 0.3, 1, 3, or 10 mg/mL formoterol solution by inhalation over 1 minute. Immediately after drug administration, the rats were anesthetized with isoflurane. A midline abdominal incision along the “linea alba” was made and the pylorus was ligated. Immediately after closure of the abdominal wall the loss of peritoneal fluid was replaced with 3 mL 0.9 NaCl per 100 g body weight IP. The animals were placed in cages provided with a special bottom to prevent coprophagy. Two hours later, the rats were sacrificed by an overdose of isoflurane, and the gastric contents were collected and analyzed for volume and acidity. Total acidity was determined by automatic titration of the gastric juice with 0.1 N NaOH to pH 7 and pH was measured with a pH-meter. Acid output was calculated by multiplication of total acidity and volume of gastric juice and normalized to the period of one hour and 100 g body weight.

The results (only for BI 1744) are presented below in Table 15 (excerpted from the sponsor’s submission, study U04-1430 report). As shown, BI 1744 reduced all the gastric secretion parameters in a dose-dependent manner. The effects on volume and acid output were statistically significant at doses ≥ 1 mg/mL, and the effects on pH and total acidity were statistically significant only at 3 mg/mL. Formoterol also showed similar dose-dependent results (data with formoterol not shown).

Table 15: Effects of BI 1744 CL, administered by inhalation, on gastric secretion in rats.

BI 1744 CL	Control	0.3 mg/mL	1 mg/mL	3 mg/mL
pH	1.17±0.13	1.11±0.08	1.48±0.25	3.25±2.14 **
Diff. [%]		-5	+27	+178
Volume [mL/100 g BW/h]	1.30±0.34	1.22±0.37	0.51±0.28 **	0.24±0.29 ***
Diff. [%]		-6	-61	-81
Total acidity [mmol/L]	96±14	100±18	78±17	43±28 **
Diff. [%]		+4	-18	-56
Acid output [µmol/h]	207±65	212±98	73±41 **	29±26 **
Diff. [%]		+2	-65	-86

Data are presented as mean ± SD for n=4-16

** p < 0.01, and *** p < 0.0001 vs. vehicle controls

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Brief Summary

The pharmacokinetics and Absorption, Distribution, Metabolism, Excretion (ADME) of olodaterol were investigated in mice, rats, dogs and rabbits using different routes of administration. The evaluations included plasma/blood concentration-time profiles, drug-related radioactivity, whole body autoradiography, quantitative tissue distribution, plasma protein binding, ADME studies, as well as investigations of metabolism.

Olodaterol is rapidly absorbed after inhalation administration in rats and dogs and the maximum plasma concentrations (C_{max}) were reached within the first 30 minutes. After oral administration, C_{max} were reached at around 0.5-2 hours. After inhalation administration, the mouse showed the highest bioavailability (54.4%), followed by the rat (23.1%) and the dog (9.2%). Olodaterol is widely distributed into most tissues except the central nervous system. The highest distribution was found in the lungs (after inhalation administration), the pancreas, the gastrointestinal tract, and the melanin-containing portions of the eye. In addition, the results show extensive distribution of the drug from plasma to blood cells. Protein binding data show moderate binding of olodaterol to plasma proteins (50-70%) in all species tested. No difference was observed after single-dose compared to repeat-dose.

Olodaterol can easily cross the placenta at both the embryonic and fetal stages of development. Distribution data in fetuses show that olodaterol is distributed into the liver, lung, blood, and brain.

Two main metabolites of olodaterol were identified: CD992 (a glucuronide) and SOM1522 (formed after demethylation of BI 1744). A number of other glucuronide metabolites were identified and all of them were classified as pharmacologically inactive.

Olodaterol shows a biphasic elimination (fast in the first 2-3 hours post-dose and slower for up to 24 hours post-dose). It is excreted primarily via feces in most species, with urine as the secondary route of excretion. Also, olodaterol is rapidly transferred to milk of lactating rats after a single IV administration. The concentration of olodaterol in milk was approximately 6-fold higher than the concentration in plasma.

Absorption

Olodaterol levels in plasma samples were analyzed using a validated high performance liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS) method with a lower limit of quantitation (LLOQ) of 25 pmol/L for rodent plasma and 20 pmol/L for dog and rabbit plasma. The summary below includes data from multiple study reports.

Olodaterol is rapidly absorbed in mouse, rat and dog after inhalation or intratracheal administration. However, after oral administration, olodaterol showed poor or incomplete absorption. Bioavailability (F) was highly dependent on the route of administration and species. After inhalation administration, the mouse showed the highest bioavailability (54.4%), followed by the rat (23.1%) and the dog (9.2%). However, after oral administration, the dog showed the highest bioavailability (although still low compared to other routes, 13.1%), followed by the mouse (4%), the rat (1.74%), and the rabbit (0.03%). This low bioavailability was considered due to incomplete absorption and to a significant first pass effect to the absorbed drug.

Maximum plasma concentrations were reached within 0.583 hours in rodents and 0.333 hours in dogs (the earliest time point collected) after inhalation administration. After oral administration, C_{max} was reached within 1 hour in rodents, 0.75 hours in the rabbit, and 0.5-2 hours in the dog. Table 16 below presents a summary of species comparison of different PK parameters after a single inhalation, intratracheal, intravenous, or oral dose.

Table 16: Species comparison of dose-normalized pharmacokinetic parameters of olodaterol after single inhalation (IH), intratracheal (IT), intravenous (IV), or oral (PO) administration*.

Route	Parameter (unit)	Mouse (m)	Rat (m/f)	Dog (m/f)	Rabbit (f)
IH (IT)	Dose ($\mu\text{g}/\text{kg}$)	200	200	60	-
	inhalation time (min)	35	35	10	-
	C_{max} [$\text{pM}/(\mu\text{g}/\text{kg})$]	109	70.5	37.8	-
	T_{max} (hr)	0.583	0.583	0.333	-

	AUC _{0-∞} [pM·hr/(μg/kg)]	334	235	136	-
	t _{1/2} (hr)	5.69	6.6	9.43	-
	F (%)	54.4	23.1	9.2	-
IV	Dose (μg/kg)	155	155	30	301
	AUC _{0-∞} [pM·hr/(μg/kg)]	665	1026	1720	2206
	t _{1/2} (hr)	13.2	5.81	17.5	14.5
	CL [(mL/min)/kg]	64.5	43.3	26.2	19.7
	V _(ss) (L/kg)	14.7	10.7	17.6	9.12
PO	Dose (μg/kg)	155	155	100	301
	C _{max} [pM/(μg/kg)]	3.97	2.77	22.8	0.233
	T _{max} (hr)	1	1.25	0.5	0.75
	AUC _{0-∞} [pM·hr/(μg/kg)]	26.5	17.9	230	0.543
	t _{1/2} (hr)	1.84	4.41	17.6	-
	F (%)	4	1.74	13.1	0.03

* = Data gathered from studies U11-1351, U11-1592, U05-1007, U06-1739

m = male

f = female

(-) = not evaluated

Distribution

Distribution studies were conducted using radiolabeled [¹⁴C] BI 1744 (Studies U05-1974, U10-1602, and U10-4128). Autoradiography (for visualization) and radioluminography (for tissue quantification) were used to determine tissue distribution of [¹⁴C] BI 1744. In addition, plasma protein binding in mouse, rat, dog, rabbit, and human was determined using equilibrium dialysis. The summary below contains data from multiple study reports.

Olodaterol showed a high volume of distribution at steady state (V_(ss)) in all species examined (9.12-17.6 L/kg, refer to Table 16 above), indicating a wide tissue distribution. After IV and IT administration, radioactivity was rapidly distributed into most tissues except the central nervous system (CNS). Table 17 below (excerpted from the sponsor's study U10-1602 report) presents a summary of concentrations of drug-related radioactivity in tissues after multiple IT instillation in male Wistar rats. As presented, the drug is highly distributed to the lungs (site of drug administration), the pancreas, and the gastrointestinal tract. Tissue distribution was similar after single or multiple dose administration and no significant gender difference was observed.

Table 17: Average concentrations of drug-related radioactivity in tissue after multiple intratracheal instillation of 0.8 µmol/kg [¹⁴C]BI 1744 to male rats.

Sample	24 h	96 h	168 h	240 h	336 h §	Accumulation- Factor # R _A
	(Day 2)	(Day 5)	(Day 8)	(Day 11)	(Day 15)	
	[nmol/L]					
LIVER	176	356	420	452	549	3.1
EPIDIDYMIS	101	143	174	200	186	1.8
GASTROINTESTINAL TRACT	1860	827	1100	909	1030	0.6
LYMPH NODE	283	305	318	452	458	1.6
ADRENAL GLAND	266	287	398	439	428	1.6
BONE MARROW	149	166	183	218	207	1.4
THYROID	153	191	247	344	392	2.6
SUBMANDIBULAR GLANDS	208	346	291	491	407	2.0
SKIN (non-pigmented)	85.5	103	112	100	137	1.6
PITUITARY GLAND	986	965	1000	1230	1617*	1.6
BRAIN	10.2	16.7	18.9	26.0	31.9	3.1
WHITE FAT	55	49.2	60	52.9	64.5	1.2
BROWN FAT	297	229	339	322	431	1.5
HEART	39.7	58.8	79.7	90.2	124	3.1
LUNGS	1590	4590	4410	6750	4560	2.9
SPLEEN	323	270	397	413	492	1.5
KIDNEYS	131	157	201	228	261	2.0
THYMUS	104	114	134	155	206	2.0
TESTES	30.3	95.8	146	202	319	10.5
PANCREAS	2980	2250	2990	3690	3640	1.2
MUSCLE	45.3	51.4	72.5	79.9	109	2.4
BLOOD	16.7	25.7	33.2	37.0	49.5	3.0
PLASMA	12.2	23.2	25.1	26.9	33.1	2.7
PLASMA (PARENT DRUG)	0.986	0.502	0.508	0.869	0.748	0.8

§ mean calculated from animals 502 - 507

concentration at 336 h after the first dose divided by concentration at 24 h after the first dose

* no data from animals 501 and 504, mean calculated with data from animals 502, 503, 505, 506 and 507

In addition, a study in pigmented rats administered IV [¹⁴C] BI 1744 showed high affinity to the melanin containing parts of the eye. The highest concentration in retina was found at 24 hours post-dose (data not shown).

The partitioning of [¹⁴C] BI 1744 between blood cells and plasma (C_C/C_P) was also evaluated in different animal species and after different routes of administration. The results show extensive distribution of drug-related radioactivity from plasma to blood cells after IV or IT administration. However, after oral administration distribution into plasma prevails, suggesting a different metabolic pathway compared to IV and IT (possibly a first pass effect).

Protein binding was also investigated in mouse, rat, rabbit (female), dog, and human [Studies U06-1533, U04-1983, and U08-2233]. Results show moderate binding of olodaterol to plasma proteins in all species tested. The % binding was comparable in all species, except for the mouse, in which protein binding was slightly higher (refer to Table 18 below). No gender difference was observed.

Table 18: Mean *in vitro* protein binding of [¹⁴C]BI 1744 to mouse, rat, dog, rabbit, and human.

	Mouse	Rat	Dog	Rabbit	Human
% Bound [¹⁴ C] BI 1744	69.8%	50-51%	57-64%	59%	57-65%

Olodaterol ([¹⁴C]BI 1744) tissue distribution was also investigated in pregnant albino rat dams during embryonic and fetal gestational stages (Study no. U10-4128). In this study, maternal tissue distribution was comparable to distribution observed in male albino rats. Also, the data show that placental transfer is unrestricted at both the embryonic and fetal stages (gestation days 12-15 and 17-20, respectively). According to the study report, quantification of tissue distribution was not possible in embryos and therefore, the total radioactivity is provided. In fetuses, olodaterol is distributed to the liver, lung, blood, and brain (refer to Table 19 below excerpted from the sponsor's submission, study U10-4128 report). There was a trend towards accumulation in fetal tissues.

Table 19: Concentrations of radioactivity in embryos (total) and fetal tissues after single intratracheal instillation of 2 µmol/kg [¹⁴C]BI 1744 to the dams on gestation days 12 and 17.

		Time	8 hours	48 hours	72 hours
embryo	total		37.2	35.9	33.4
fetus	digestive apparatus				
	tongue		ND	30.8	ND
	liver		178	59.2	40.2
	respiratory apparatus				
	lung		122	37.5	29.4
	urogenital apparatus				
	kidney (total)		ND	ND	33.9
	cardiovascular system				
	myocardium		ND	41.3	32.0
	thymus		ND	ND	35.0
central nervous system					
brain (total)		24.7	31.9	26.1	
blood					
heart blood (RLG)		87.6	38.2	31.9	

ND not detected

Metabolism

The metabolism of olodaterol was investigated in different species (mouse, rat, dog, and rabbit) and after different routes of administration (IV, IT, and oral) using a validated HPLC/MS/MS method [Studies U06-1516, U06-1514, U10-1391, U08-1317, U08-1057, and U07-2170]. Several metabolites were identified and are presented in Figure 23 (excerpted from the sponsor's Nonclinical Overview, Figure 3.3.2:1) and Table 20 below.

The main metabolites identified were CD992 (glucuronide metabolite) and SOM1522 (formed by demethylation). Subsequent glucuronidation of SOM1522 resulted in M551(1) [or CD11249]. Other glucuronide metabolites identified were: M565(2), M583 (only in mouse and rabbit), and CD10915 (only in human, mouse, and rabbit). In addition, CD12656 was found only in humans and is a result of sulfation of SOM1522. CD10915 and CD12656 were identified as disproportionate human metabolites. CD10915 was also found in mouse and rabbit. However, CD12656 was not found in nonclinical species. This metabolite was found at very low levels in human after IV or oral administration. All of these metabolites were classified as pharmacologically inactive, except for SOM1522, which exhibits some pharmacological activity on the β_2 -receptor. In addition, a number of highly hydrophilic metabolites of unknown structure were identified (not extractable radioactivity).

Figure 23: Metabolism pathways of olodaterol (rectangle) *in vivo* in humans (H) and different nonclinical species: mouse (M), rat (R), dog (D), and rabbit (Rab).



Table 20: Metabolite pattern in plasma of different nonclinical species 4 hours (3 hours in rat and 6 hours in rabbit) after single IV, IT, or oral administration of [¹⁴C]BI 1744 (% of sample radioactivity).

Route	Metabolite	Mouse	Rat	Dog	Rabbit (female)
IH (IT)	CD992	28.5	16.3	-	-
	SOM1522	ND	ND	-	-
	M551(1)	23.6	ND	-	-
	CD10915	ND	ND	-	-
	M565(2)	1.2	6.4	-	-
	M583	ND	ND	-	-
	m6	27.6	19.9	-	-
	m4	ND	ND	-	-
	m13	ND	ND	-	-
	m9	ND	1.9	-	-
m10	ND	ND	-	-	
IV	CD992	16.2	11.5	5.6	79.6
	SOM1522	1.2	ND	2.3	ND

	M551(1)	15.5	ND	ND	6.2
	CD10915	ND	ND	ND	8.6
	M565(2)	ND	4.5	1.1	ND
	M583	ND	ND	ND	0.6
	m6	44	23.9	5.8	ND
	m4	ND	ND	ND	ND
	m13	ND	ND	1.1	ND
	m9	ND	ND	1.4	ND
	m10	ND	ND	3.2	ND
PO	CD992	27.4	61.6	23.6	-
	SOM1522	2.3	ND	1.9	-
	M551(1)	22.3	4	2.5	-
	CD10915	ND	ND	ND	-
	M565(2)	2.2	ND	2	-
	M583	ND	ND	ND	-
	m6	22.8	12.2	31.5	-
	m4	ND	ND	ND	-
	m13	ND	ND	ND	-
	m9	ND	ND	1.1	-
	m10	ND	ND	0.4	-

ND = not detected

(-) = not measured

Excretion

The plasma clearance of olodaterol after IV administration decreased in the following order: mouse > rat > dog > female rabbit (refer to Table 16 above, under "Absorption"). Olodaterol shows a biphasic elimination (*i.e.*, fast in the first 2-3 hours and then slower up to 24 hours) and is excreted primarily via feces in most species tested (refer to Table 21 below). The secondary route of excretion is via urine. The percentages in feces and urine were similar between the mouse, rat, and dog. Only the female rabbit shows a preference for urine excretion after IV administration, but not after oral administration. Of note, the route of excretion did not change with single vs. multiple doses of olodaterol.

Table 21: Species comparison of excretion data (% of dose) after dosing (intra-tracheal, intravenous, or oral) of [¹⁴C]BI1744*.

Route	Matrix	Mouse	Rat	Dog	Rabbit (female)
IH (IT)	Feces	70%	79.2%	-	-
	Urine	13.6%	15.3%	-	-
IV	Feces	68.4%	69.1%	66.3%	42.1%
	Urine	25.8%	23.6%	16.7%	54.8%
PO	Feces	89.2%	98.5%	87.2%	56.9%
	Urine	5.4%	1.72%	5.7%	23.1%

* = Data gathered from studies U10-2458, U08-1337, U05-1007, and U06-1739.

(-) = not measured

Study no. U10-1214 was conducted to investigate BI 1744's transfer to milk after a single IV administration to lactating rats. Data from this study show that the concentration of BI 1744 in milk was approximately 6-fold higher than in plasma, indicating a rapid transfer to milk. At 48 hours post-dose, the concentration in milk and plasma were similar. Assessment of the PK parameters in milk and plasma show that the AUC in milk was approximately 7-fold the AUC in plasma (refer to Table 22 below). Also, data from this study show that the amount of radioactivity secreted in milk at 24 hours post-dose was 2.8-4.7% of the administered dose.

Table 22: Pharmacokinetic parameters (mean values) of BI 1744 in milk and plasma after a single intravenous administration of 0.4 $\mu\text{mol/kg}$ [^{14}C]BI1744 to lactating rats.

Parameter (Unit)	Milk	Plasma
No. of animals	5	5
C_{max} (nmol/L)	169	26.5
$t_{1/2}$ (hr)	7.25	22.2
$\text{AUC}_{0-\text{inf}}$ (nmol·hr/L)	2970	523
AUC_{0-24} (nmol·hr/L)	2590	291
AUC_{0-48} (nmol·hr/L)	2940	413

5.2 Toxicokinetics

Toxicokinetics (TK) of olodaterol are discussed briefly under "General Toxicology", section 6.2. The TK parameters of olodaterol in rats and dogs are presented below in Tables 23 and 24 [excerpted from the sponsor's submission, studies U11-1351 (rat) and U11-1592 (dog)].

In the rat, plasma olodaterol concentrations increased rapidly and the maximum plasma concentration (C_{max}) was achieved at 0.583 hours (the first time point after drug administration). Plasma concentrations presented a rapid decrease and then a slower decrease, suggesting a biphasic excretion. Absorption and excretion were not affected by repeat dosing. The half-life of the drug ($t_{1/2}$) was identified at 6.47 hours on day 1 and 7.24-9.84 hours at steady state. In general, males presented a slightly higher C_{max} and systemic exposure compared to females. This may be due to a slightly higher clearance (CL/F) in females compared to males.

Table 23: Mean (%CV) data for pharmacokinetic parameters of olodaterol in the rat*.

Parameter	Unit	Day	Group 2 males	Group 2 females	Group 3 males	Group 3 females	Group 4 males	Group 4 females
C(max)/ Dose	[(pmol/L)/ (µg/kg)]	1	55 (21)	46 (18)	77 (31)	64 (26)	91 (22)	75 (15)
		177	79 (22)	67 (26)	86 (25)	66 (16)	130 (22)	87 (13)
AUC(t0-tn)/ Dose	[(pmol·h)/L/ (µg/kg)]	1	240 (24)	170 (27)	270 (30)	200 (38)	260 (35)	210 (26)
		177	210 (17)	160 (18)	370 (13)	180 (16)	320 (30)	220 (21)
CL/F	[(mL/min)/ kg]	1	190 (26)	270 (23)	170 (26)	270 (57)	180 (26)	220 (24)
		177	210 (16)	280 (16)	160 (14)	250 (17)	150 (38)	210 (25)
Vss/F	[L/kg]	1	120 (43)	120(29)	74 (20)	100 (36)	57 (35)	68 (19)
		177	120 (33)	150 (44)	110 (24)	140 (57)	92 (31)	90 (24)
t(1/2)	[h]	1	8.5 (19)	6.6 (22)	6.7 (18)	6.4 (28)	5.0 (22)	5.4 (15)
		177	8.8 (31)	8.7 (32)	10 (23)	8.9 (38)	10 (21)	7.5 (26)
MRT(tot)	[h]	1	10 (22)	7.2 (23)	7.3 (19)	6.9 (28)	5.3 (13)	5.4 (14)
		177	9.3 (35)	8.7 (35)	12 (29)	9.1 (42)	11 (27)	7.4 (26)
F	[%]	1	25.4	15.2	28.4	17.8	28.0	19.2
		177	NC	NC	NC	NC	NC	NC
MAT	[h]	1	5.68	2.73	2.86	2.47	0.89	0.89
		177	NC	NC	NC	NC	NC	NC

NC = not calculated

* = C_{max} and AUC values are dose-normalized.

In the dog, plasma olodaterol concentrations also increased rapidly. C_{max} was achieved at 0.250-0.333 hours (the first time point after drug administration). Plasma concentrations in the dog also presented a biphasic excretion, with a rapid decrease within the first 3 hours and then a slower decrease up to 24 hours post-dose. Absorption and excretion were not affected by repeat dose. The half-life of the drug (t_{1/2}) was approximately 9-10 hours on day 1 and 10-13 hours at steady state. No significant gender differences were observed in the dog.

Table 24: Mean (%CV) data for pharmacokinetic parameters of olodaterol in the dog*.

Parameter	Unit	Day	Group 2 males	Group 2 females	Group 3 males	Group 3 females	Group 4 males	Group 4 females
C(max)/ Dose	[(pmol/L)/ (µg/kg)]	1	13.8 (32.0)	26.6 (18.6)	30.6 (26.6)	44.9 (98.7)	40.8 (33.8)	34.9 (43.0)
		72	21.1 (37.5)	23.2 (14.8)	24.7 (76.8)	17.4 (33.8)	21.6 (50.4)	39.0 (27.9)
		358	24.3 (25.2)	27.3 (38.4)	24.4 (44.4)	20.8 (45.9)	28.2 (25.7)	28.1 (29.0)
AUC(0-24h)/ Dose	[(pmol·h)/L/ (µg/kg)]	1	84.1 (33.4)	143 (18.1)	121 (27.0)	150 (30.2)	156 (40.7)	139 (35.7)
		72	150 (16.5)	201 (24.3)	142 (35.1)	135 (19.3)	113 (17.7)	139 (30.6)
		358	137 (5.9)	230 (41.7)	120 (29.8)	139 (26.2)	144 (10.9)	123 (23.6)
CL/F	[(mL/min)/ kg]	1	484 (37.3)	252 (14.1)	321 (24.8)	263 (28.3)	272 (40.3)	279 (32.8)
		72	295 (18.8)	234 (20.7)	340 (43.4)	328 (17.2)	394 (17.8)	334 (28.1)
		358	315 (6.0)	207 (29.8)	387 (32.0)	324 (21.5)	302 (10.9)	365 (19.7)
V(ss)/F	[L/kg]	1	324 (32.3)	197 (30.9)	211 (35.1)	174 (41.2)	163 (32.5)	211 (36.0)
		72	286 (33.2)	190 (39.9)	278 (49.4)	305 (37.9)	290 (31.7)	203 (42.5)
		358	321 (17.2)	210 (40.4)	331 (38.4)	276 (19.5)	208 (17.3)	245 (18.6)
t(1/2)	[h]	1	8.91 (11.4)	10.4 (17.6)	9.64 (14.6)	9.22 (9.0)	10.0 (22.9)	10.7 (18.0)
		72	12.4 (18.4)	10.6 (28.2)	11.0 (22.8)	11.8 (19.6)	10.2 (16.0)	9.26 (16.0)
		358	13.7 (18.9)	12.7 (17.7)	11.9 (16.3)	11.6 (16.2)	10.7 (8.8)	10.3 (4.3)
MRT(tot)	[h]	1	11.4 (11.7)	12.9 (21.9)	10.8 (15.2)	10.7 (16.0)	10.7 (32.6)	12.7 (27.5)
		72	15.9 (18.4)	13.7 (30.9)	13.5 (30.7)	15.2 (24.7)	12.1 (18.4)	9.94 (15.6)
		358	17.0 (14.8)	16.4 (20.5)	14.4 (21.3)	14.4 (14.2)	11.4 (10.7)	11.2 (6.9)
F	[%]	1	5.8	10.1	8.2	10.1	10.9	9.9
MAT	[h]	1	0.12	1.62	-0.48	-0.58	-0.58	1.42

* = C_{max} and AUC values are dose-normalized.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies were conducted in CD-1 mice and Wistar Han rats following inhalation exposure, oral gavage, and intravenous administration of olodaterol. The main purpose of these studies was to evaluate the acute toxicity of olodaterol following single administration by the inhalation, oral, or IV routes. For each study, the maximum non-lethal dose and the approximate lethal dose (ALD) were identified. Clinical signs of toxicity that were common to all routes of administration in both mice and rats included decreased motor activity, increased breathing frequency, and piloerection. In addition, closed eyes were observed in mice and rats after inhalation exposure but not after oral or IV administration. This observation is considered to be related to the anabolic effect of olodaterol.

Mortality was observed in mice and rats after oral and IV administration but not after inhalation exposure. This may be due to the fact that the highest technically feasible dose for the inhalation route was lower compared to the doses used for oral and IV

administration (formulations were different). Therefore, the maximum non-lethal dose in both mice and rats after inhalation exposure was identified as the highest dose level tested (*i.e.*, 54.4 mg/kg in mice and 27.1 mg/kg in rats). The maximum non-lethal dose after oral administration was higher compared to IV administration in both mice and rats (1000 mg/kg oral vs. 20 mg/kg IV in mice, and 316 mg/kg oral vs. 40 mg/kg IV in rats). These dose levels are much higher than the doses used in the repeat-dose toxicology studies discussed below and also compared to the proposed clinical dose. A summary of all the single-dose studies with noteworthy findings is presented below in Table 25.

Table 25: Summary of single-dose studies with olodaterol.

Study No.	Study Design and Objective(s)	Noteworthy findings
Mouse		
U05-1101	5 mice/sex/group Route: inhalation Highest technically feasible achieved dose: 54.4 mg/kg (52.2 mg/kg in males and 56.36 mg/kg in females) Objective: to determine the approximate lethal dose (ALD) of olodaterol following single inhalation exposure in mice.	<ul style="list-style-type: none"> • No mortality • Clinical signs at day 1: decreased locomotor activity, ataxia, tremors, closed eyes, abdominal breathing, piloerection • No necropsy findings • Maximum non-lethal dose is 52.2 mg/kg in males and 56.6 mg/kg in females • ALD is > 54.4 mg/kg
U08-1975	10 mice/sex/group Route: inhalation Achieved doses: 0 [air or vehicle (0.01% benzalkonium chloride, (b) (4)% disodium edetate) controls], 69, 196, 930, and 3033 µg/kg Objective: assess toxicokinetics in CD-1 mice after a single-dose inhalation exposure (35 min).	<ul style="list-style-type: none"> • Variability of olodaterol plasma concentrations was moderate • Systemic exposure increased almost proportional with dose and no gender effect was observed.
U05-1118	3 mice/sex/group Route: oral (gavage) Doses: 100, 316, 1000 and 2000 mg/kg (vehicle: 0.5% aqueous hydroxyethylcellulose) Objective: to evaluate the acute toxicity of olodaterol following single oral administration in mice.	<ul style="list-style-type: none"> • Mortality observed at 2000 mg/kg • Clinical signs at day 1: reduced motor activity, increased breathing rate, piloerection • Necropsy findings at 2000 mg/kg: stomach filled with drug and enlarged adrenals • Maximum non-lethal dose (oral) is 1000 mg/kg • ALD > 1000 mg/kg
U05-1113	3 mice/sex/group Route: intravenous Doses: 10, 20, and 40 mg/kg (vehicle: 0.9% NaCl, isotonic saline). Injection rate = 1 mL/min Objective: to evaluate the acute toxicity of olodaterol following single intravenous administration in mice.	<ul style="list-style-type: none"> • Mortality observed at 40 mg/kg • Clinical signs at day 1: reduced motor activity, ventral recumbency, increased breathing frequency • Necropsy findings at 40 mg/kg: discoloration of lungs, stasis in various organs • Maximum non-lethal dose (IV) is 20 mg/kg • ALD > 20 mg/kg, < 40 mg/kg
Rat		
U05-1100	5 rats/sex/group Route: inhalation Highest technically feasible achieved dose: 27.1	<ul style="list-style-type: none"> • No mortality • Clinical signs at day 1: decreased locomotor activity, closed eyes, abdominal

	mg/kg (25 mg/kg in males and 29.1 mg/kg in females) Objective: to determine the ALD of olodaterol following single inhalation exposure of rats.	breathing, repetitive nose grooming, increased chewing • No necropsy findings • Maximum non-lethal dose is 25 mg/kg in males and 29.1 mg/kg in females • ALD is > 27.1 mg/kg
U05-1065	3 rats/sex/group Route: oral (gavage) Doses: 100, 316, and 1000 mg/kg (vehicle: 0.5% aqueous hydroxyethylcellulose) Objective: to evaluate the acute toxicity of olodaterol following single oral dose administration in rats.	• Mortality observed at 1000 mg/kg • Clinical signs at day 1: transient increased locomotor activity and then decreased, increased breathing frequency, piloerection • Necropsy findings at 1000 mg/kg: stasis of various organs (heart, liver, and kidney), discoloration of lungs, stomach enlarged • Maximum non-lethal dose (oral) is 316 mg/kg • ALD is > 316 mg/kg
U05-1112	3 rats/sex/group Route: intravenous Doses: 10, 20, 40 and 80 mg/kg (vehicle: 0.9% NaCl, isotonic saline). Injection rate = 1 mL/min Objective: to evaluate the acute toxicity of olodaterol following single intravenous administration in rats.	• Mortality observed at 80 mg/kg • Clinical signs at day 1: reduced motor activity, ventral recumbency, increased breathing rate, piloerection • Necropsy findings at 80 mg/kg: blood stasis in lungs, liver, and kidneys • Maximum non-lethal dose (IV) is 40 mg/kg • ALD is > 40 mg/kg

6.2 Repeat-Dose Toxicity

Repeat-dose toxicity studies were conducted in CD-1 mice (13 weeks of dosing), Wistar Han rats (4, 13, and 26 weeks of dosing), and Beagle dogs (4, 13, and 52 weeks of dosing) following inhalation exposure. A summary of the toxicology findings is presented below.

Mouse

A repeat-dose (13-week) toxicology study was conducted in CD-1 mice using the inhalation route of administration. This study was previously reviewed and only a summary of the study design and noteworthy findings is included below.

Study no. U07-1341, BI 1744 CL: 13-week inhalation MTD study in mice. CD-1 mice (15/sex/group) were administered 0 [air or vehicle (0.01% benzalkonium chloride, and (b) (4) % disodium EDTA and citric acid) controls], 63 (LD), 211 (MD1), 900 (MD2), or 3258 (HD) µg/kg/day olodaterol (achieved doses) via snout-only inhalation (35 minutes/day) for at least 13 weeks. The pulmonary deposited doses were estimated using a deposition factor of 0.1 (10% lung deposition): 6.3, 21.1, 90, and 325.8 µg/kg/day olodaterol. The 3258 µg/kg/day dose level was the maximum technically feasible dose using the 35-min daily dosing duration. An additional 20 mice/sex/group were included for toxicokinetic analysis. At the end of the dosing period, main study animals were sacrificed and necropsies were performed. Assessment of toxicity was based on evaluation of olodaterol-related mortality, clinical signs, body weights, food

and water consumption, ophthalmic examinations, and clinical and anatomic pathology observations.

Survival was not affected by olodaterol. Excessive salivation was observed in all treated male and female mice and also in vehicle control males, compared to the air controls. Olodaterol-related increases in body weights were also observed in males (40-166%) and females (20-75%) relative to the vehicle and air controls. This is related to the anabolic effect of olodaterol as an agonist to the β_2 -adrenergic receptor.

Olodaterol-related necropsy findings include increased skeletal muscle mass and thickened uterus. Both of these changes are also considered related to the anabolic effect of olodaterol. Olodaterol-related microscopic lesions were observed in the liver (hepatocyte vacuolation, associated with an increase in bilirubin, at all doses but with increased severity compared to controls only at the MD2 and HD), ovary (numerous corpora lutea at HD), uterus (cystic glands and myometrial hypertrophy at HD), thymus (lymphoid hyperplasia at all doses but also in the vehicle control), salivary glands (inflammatory cell foci at HD), lung (inflammatory cell foci at $\geq 211 \mu\text{g}/\text{kg}$), nasal cavity (hyaline droplets and regeneration at all doses and $\geq 900 \mu\text{g}/\text{kg}$, respectively), and larynx (squamous metaplasia at $\geq 211 \mu\text{g}/\text{kg}$). Refer to Pharm/Tox review under IND 76,362 dated March 2, 2007 for additional details and for the 13-week mouse histopathology table.

Olodaterol systemic exposure increased with dose but less than proportional. There was a 3-4 fold higher exposure on study day 1 compared to study day 87 (end of dosing period). No significant gender differences were observed in systemic exposure. No NOAEL could be determined for systemic toxicity because of lack of examination of the LD, MD1, and MD2 groups. The NOAEL for local (respiratory tract) toxicities was identified as the LD of $63 \mu\text{g}/\text{kg}/\text{day}$ based on findings in the larynx and lungs. The maximum tolerated dose (MTD) in females is considered the HD ($3258 \mu\text{g}/\text{kg}/\text{day}$) due to hepatocyte vacuolation and increase in bilirubin levels. A MTD was not identified in males because of a lack of significant toxicity findings.

Rat

Repeat-dose toxicology studies (4-week, 13-week, and 26-week) were conducted in Wistar Han rats using the inhalation route of administration. All these studies were previously reviewed and only summaries of study designs and noteworthy findings are presented below.

(b) (4)

(b) (4)

(b) (4)

Study no. U07-1342, BI 1744 CL: 13-week inhalation MTD study in rats with a 4-week recovery period. Wistar Han rats (10/sex/group) received 0 [air or vehicle (0.01% benzalkonium chloride, and (b) (4) % disodium EDTA and citric acid) controls],

61.7 (LD), 239 (MD1), 971 (MD2), or 2833 (HD) µg/kg/day olodaterol (achieved doses) via inhalation (snout-only, 35 minutes/day) for at least 91 consecutive days. The pulmonary deposited doses were estimated as 6.17, 23.9, 97.1, and 283.3 µg/kg/day olodaterol (10% of the achieved dose). An additional 8 rats/sex/group were included for a 4-week recovery period and toxicokinetic analysis. At the end of the dosing period, main study animals were sacrificed and necropsies were performed to assess toxicity. Assessment of toxicity was based on evaluation of olodaterol-related mortality, clinical signs, body weights, food consumption, ophthalmic examinations, and clinical and anatomic pathology observations.

There was one premature death during the dosing period (animal no. 411M, from the MD1 toxicokinetic group). This death was not considered related to treatment as the animal was sacrificed following an accidental fracture to the left tibia. There were no necropsy findings in this animal and no other animals died during the study. A dose-related increased incidence of excessive salivation was observed. In male rats, the onset of excessive salivation was observed earlier with increased olodaterol dose. A slight increase in body weight gain was observed in all olodaterol-treated animals (2-14% in males and 3-30% in females) compared to the controls and is associated with the anabolic effect of olodaterol. No associated changes were observed in food consumption.

Olodaterol-related histopathology findings were observed in the cervical and mandibular lymph nodes (lymphoid depletion and hemorrhage at the MD1, MD2, and HD groups), uterus (oestrus dilatation at the HD), pancreas (acinar cell atrophy at the HD), and larynx (squamous metaplasia and necrosis of the U shaped cartilage at all doses, and also in the vehicle control). Refer to Pharm/Tox review under IND 76,362 dated March 2, 2007 for additional details and for the 13-week rat histopathology table.

Systemic exposure (AUC) in this study increased more than proportional to dose. There was no significant gender difference in exposure and no change was observed after repeat dosing (no drug accumulation). No systemic NOAEL could be determined in this study because of the lack of examination of the LD, MD1 and MD2 groups. The local (respiratory tract) NOAEL was identified as the LD of 61.7 µg/kg (6.17 µg/kg PDD) based on the finding of cartilage necrosis in the larynx. The 239 µg/kg/day dose level was considered the MTD in the rat (males and females).

Study no. U08-1691, BI 1744 CL: 26-week inhalation toxicity study in rats with a 4-week recovery period. Crl:WI(Han) rats (20/sex/group) received 0 (vehicle control: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA, and 0.003% citric acid), 49, 200, or 3,400 µg/kg/day olodaterol (achieved doses) via inhalation (nose-only, 35 minutes/day) for at least 182 days. The pulmonary deposited doses were estimated as 4.0, 16.4, and 288 µg/kg/day olodaterol (refer to page # 15 of the Pharm/Tox review under IND 76,362 dated October 28, 2011 for the complete details). An additional 5 rats/sex/group were included for toxicokinetic analysis and an additional 10 rats/sex in the vehicle control and HD groups were included for a 4-week recovery period following completion of the treatment period. At the end of the dosing period, main study animals

were euthanized and necropsies were performed. Assessment of toxicity was based on evaluation of mortality, clinical signs, body weights, food consumption, ophthalmic examinations, and clinical and anatomic pathology observations.

Olodaterol did not affect survival in rats. Olodaterol treatment resulted in increased food consumption and increased body weights in both sexes at all dose levels. Ophthalmic examinations found increases in monocular and binocular lens opacities in olodaterol-treated animals (males and females, but no dose-response). In addition, olodaterol-related effects were observed in skeletal muscle (hypertrophy and single cell necrosis at all dose levels with no dose-response) and were associated with a decrease in white adipose tissue. Skeletal muscle necrosis was also observed in a few animals in the vehicle control group (5 males and 5 females). Literature reports note similar findings of skeletal muscle hypertrophy and necrosis in rodents treated with other β -adrenergic agonists⁴. Because there is not a dose-response for these skeletal muscle findings and because these are reported class effects for LABAs, they are not considered dose-limiting.

In the heart, microscopic findings of increased congestion at doses ≥ 200 $\mu\text{g}/\text{kg}$ and left ventricular scar formation in HD males were observed. Also, palpable heart beat was detected directly after dosing in both sexes in rats treated at ≥ 200 $\mu\text{g}/\text{kg}$ olodaterol and increased heart weights were observed in all dose groups. In the pancreas, lobular atrophy was observed in a few HD males and females. In females, there was increased incidence and severity of cysts in the ovary and oviduct at 3,400 $\mu\text{g}/\text{kg}$. Clinical pathology evaluations found decreases in creatine kinase and glucose in both sexes (not dose-related) and also decreases in triglycerides in males. Non-dose related changes in electrolytes were also observed and included decreases in magnesium and increases in potassium and inorganic phosphate. Most findings were recovered by the end of the dosing period, except for findings of reduced adipose tissue and skeletal muscle hypertrophy, which were only partially recovered.

In the eye, epithelial atrophy was observed in the cornea at all dose levels in a dose-dependent manner. This finding was not included in the October 28, 2011 IND review and therefore, a summary of incidence and severity is presented below in Table 26.

⁴ Owen K, Beck SL, and Damment SJP. (2010) The preclinical toxicology of salmeterol hydroxynaphthoate. *Human and Experimental Toxicology* 29(5); 393-407.

Yang YT, and McElligott MA. (1989) Multiple actions of β -adrenergic agonists on skeletal muscle and adipose tissue. *Biochemistry Journal* 261; 1-10.

Suzuki J, Gao M, Xie Z, and Koyama T. (1997) Effects of the β_2 -adrenergic agonist clenbuterol on capillary geometry in cardiac and skeletal muscles in young and middle-aged rats. *Acta Physiologica Scandinavica* 161; 317-326.

Table 26: Incidence and severity of epithelial atrophy of the ocular cornea.

	Treatment period								Recovery period			
	0		50		200		4000		0		4000	
Target daily dose of olodaterol (µg/kg)	0		50		200		4000		0		4000	
Achieved daily dose of olodaterol (µg/kg)	0		49		200		3400		0		3400	
Gender	M	F	M	F	M	F	M	F	M	F	M	F
No. of animals	20	20	20	20	20	20	20	20	10	10	10	10
Eye, cornea												
Epithelial atrophy												
Minimal	2	0	10	3	14	13	12	7	0	0	0	1
Slight	0	0	0	0	0	0	5	4	0	0	0	0

An information request was sent to the applicant on November 2, 2012 to ask for an assessment of the toxicological significance of this finding. The applicant submitted a response to the NDA on November 13, 2012 and provided justification and supporting data to conclude that this finding is not of concern. According to the applicant, the epithelial atrophy observed in the cornea was considered to be an effect of the long term mechanical irritation in custom-made glass inhalation restriction tubes that increase the risk of mechanical contact between the ocular surface and the inner tube wall in cases of exophthalmos (bulging of the eye). Olodaterol-treated animals developed dose-dependent exophthalmos at all dose levels due to hypertrophy of the skeletal muscles surrounding the orbita (known anabolic effect of LABAs). This resulted in increased mechanical contact with the glass restriction tubes used in this study and caused atrophy of the corneal epithelium. Other rat toxicity studies (4- and 13-week studies and the rat carcinogenicity study) were conducted using regular plastic restriction tubes and corneal findings were not observed. To further support their rationale, the applicant submitted a report of another study using salmeterol (an approved LABA) with the same custom-made glass restriction tubes. In this study, epithelial atrophy was also observed in the cornea of salmeterol-treated animals in a dose-dependent manner. The applicant proposes the same mechanism for this finding (increased mechanical contact due to exophthalmos in salmeterol-treated rats) and, therefore, the applicant concludes that this finding has no human relevance.

Review of the applicant's response and the additional data alleviated the concern that this eye finding is related to long-term exposure with olodaterol. Because the finding is not specific to olodaterol, this reviewer agrees that it is not of concern and is not dose-limiting. Also, this finding is likely not of human relevance.

In terms of local (respiratory tract) toxicities, a number of findings were observed in the larynx: squamous cell hyperplasia/metaplasia in HD males and females, epithelial atrophy in HD females, degeneration in both sexes at all doses, and regeneration in males and females at ≥ 16.4 µg/kg (PDD). These findings in the larynx are considered rat-specific⁵ and not dose-limiting. Also, findings of epithelial atrophy in LD and HD,

⁵ Lewis DJ. (1991) Morphological Assessment of Pathological Changes within the Rat Larynx. *Toxicologic Pathology* 19; 352-357.

inflammation in HD, luminal cellular debris in HD males, and squamous cell metaplasia in HD males and females were observed in the nasal cavity. These findings in the nasal cavity are considered route specific and are not relevant to clinical use (nose-only inhalation in the rat vs. oral inhalation in humans). In addition, reversible squamous cell metaplasia was observed in the trachea of HD males and females. Although this finding could be considered rat-specific (an adaptive response), because it was observed lower in the respiratory tract, it was considered dose-limiting. Although technically there is no NOAEL for local toxicities, findings in the larynx and nasal cavity are not considered dose-limiting. Therefore, the NOAEL for local toxicities in this study was identified as the mid-dose of 16.4 µg/kg (PDD). Refer to pages # 27-30 of the October 28, 2011 Pharm/Tox review under IND 76,362 for the histopathology table.

The toxicokinetic analysis shows that systemic exposure increased almost more than proportional to dose, that there is no drug accumulation, and that there is no difference in exposure in males compared to females. Based on the aforementioned findings in the heart and pancreas, the systemic NOAEL was identified at the low-dose level of 49 µg/kg, with an associated AUC_{0-24 hr} (at Day 177) of 9,010 pmol·hr/L in males and 7,880 pmol·hr/L in females (8,445 pmol·hr/L combined, refer to Table 27 below excerpted from study U08-1691 final report). Refer to Pharm/Tox review under IND 76,362 dated October 28, 2011 for the complete details.

Table 27: Mean toxicokinetic parameters in the rat.

parameter	day	gender	target dose [µg/kg]		
			group 2 50	group 3 200	group 4 4000
C(max) [pmol/L]	1	m	3480	17100	314000
	177	m	3450	13500	341000
	1	f	3240	15700	292000
	177	f	3310	12000	270000
AUC(0-24h) [pmol·h/L]	1	m	13400	54600	881000
	177	m	9010	43000	878000
	1	f	11000	44900	785000
	177	f	7880	32400	668000

Dog

Repeat-dose toxicology studies (4-week, 13-week, and 52-week) were conducted in Beagle dogs using the inhalation route of administration. The 4-week and 52-week

Osimitz TG, Droege W, and Finch JM. (2007) Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: A critical review. *Toxicology and Applied Pharmacology* 225; 229-237.

Kaufmann W, Bader R, Ernst H, Harada T, Hardisty J, Kittel B, Kolling A, Pino M, Renne R, Rittinghausen S, Schulte A, Wohrmann T, and Rosenbruch M. (2009) 1st International ESTP Expert Workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. *Experimental and Toxicologic Pathology* 61; 591-603.

studies were previously reviewed and summaries of study designs and noteworthy findings are presented below. A detailed review of the 13-week toxicity study in dogs is presented below.

Study no. U05-2146, BI 1744 CL: 4-week inhalation toxicity study in dogs with 4-week recovery. Beagle dogs (3/sex/group) received 0 (vehicle control: 0.01% benzalkonium chloride, and (b) (4) % disodium EDTA), 2.2 (LD), 13.7 (MD), or 127 (HD) µg/kg/day olodaterol (achieved doses) via inhalation (face mask with a mouth tube, 11 minutes/day) for 28 consecutive days. The pulmonary deposited doses were estimated as 0.55, 3.43, and 31.8 82.6 µg/kg/day olodaterol (25% of the achieved dose). An additional 2 dogs/sex were included in the vehicle and 127 µg/kg groups for a 4-week recovery period. At the end of the dosing period, main study animals were sacrificed and necropsies were performed. Assessment of toxicity was based on evaluation of mortality, clinical signs, body weights, food consumption, ECG and ophthalmic examinations, and clinical and anatomic pathology observations.

The dose levels in this study were selected based on the results obtained in study U05-2134 (non-GLP), *BI 1744 CL: 2-week inhalation dose range finding study in dogs*. In this study, Beagle dogs were treated with 0, 1.28/1.4, 9.68/9.4, 29.8/33.8, or 112 /120 µg/kg/day olodaterol (male/female values) for 14 consecutive days (4 minutes daily). Olodaterol-related findings included increased body weights at ≥ 9.68/9.4 µg/kg, increased heart rate and heart force at ≥ 29.8/33.8 µg/kg, and minimal-mild periportal glycogen in the liver of dogs at ≥ 29.8/33.8 µg/kg. There was no evidence of respiratory tract irritation in this study. For study U05-2146 (4-week study), the highest exposure concentration was reduced and the corresponding exposure duration was extended.

In the 4-week study U05-2146, olodaterol treatment did not affect survival. Body weights were increased in olodaterol-treated animals relative to controls at the MD (100% in males and females) and HD (130% in males and 80% in females). The MD and HD animals showed clinical signs of increased salivation. Also, increased heart contractile force was observed in MD and HD animals. This correlates with increased heart rate observed at all doses (24-64% in males and 41-100% in females) on study day 2 and with increased levels of creatine kinase (89%) on study day 28 in HD males. No histopathology findings were observed in the heart. Olodaterol-related microscopic findings were observed in the kidney (chronic/focal inflammation in one HD male), liver (glycogen, periportal in MD and HD males and females), gallbladder (epithelial vacuolation in one HD male), larynx (laryngitis in two HD males), and lungs (pleural fibrosis in one HD female). In addition, increased potassium and phosphate levels were observed in HD males and females on study day 3 and HD males on study day 28. Refer to Pharm/Tox review under IND 76,362 dated February 28, 2007 for additional details and for the histopathology table.

Systemic exposure increased almost proportional to the dose. Also, exposure was slightly higher at study day 28 compared to study day 1 and was slightly higher in females compared to males. Based on the findings in the liver at the MD and HD, the systemic NOAEL was identified as the LD of 2.2 µg/kg (0.55 µg/kg PDD), which is

associated with an exposure ($AUC_{0-24 \text{ hr}}$) of 205 pmol·hr/L in males and 414 pmol·hr/L in females (310 pmol·hr/L combined). Based on the findings in the larynx and lungs, the local NOAEL was identified as the MD of 13.7 µg/kg (3.43 µg/kg PDD).

Study title: BI 1744 CL: 13-week inhalation toxicity study in Beagle dogs with a 6-week recovery period

Study no.: U07-1317
Study report location: EDR (Volume 4.2.3.2)
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co. KG
Department of Non-Clinical Drug Safety
Birkendorfer Straße 65, 88397
Biberach/Riss, Germany

CV analysis performed at:

(b) (4)

Date of study initiation: January 17, 2006
GLP compliance: Yes (OECD)
QA statement: Yes
Drug, lot #, and % purity: BI 1744 CL, Lot 8460120, 98.8% pure

Key Study Findings

- Body weights were slightly increased in olodaterol-treated dogs compared to the vehicle controls.
- Heart rate was increased in MD and HD males and females. This correlates with increased creatine kinase observed in serum of olodaterol-treated dogs. However, no olodaterol-related microscopic findings were observed in the heart.
- Glycogen depletion and increased glycogen storage were observed in the liver of MD and HD dogs microscopically. In addition, increased liver enzymes were observed in serum.
- Serum creatinine and urea levels were elevated in olodaterol-treated animals relative to the vehicle controls and suggest renal damage. However, no olodaterol-related histopathology changes were observed in the kidneys.
- Olodaterol exposure (AUC) increased almost proportionally with dose. No drug accumulation was observed with repeat dosing and drug exposure was similar in males and females.
- The NOAEL for systemic and local (respiratory tract) toxicities was identified as the high-dose of 160 µg/kg/day (PDD = 40 µg/kg), which is associated with a systemic exposure ($AUC_{0-24 \text{ hr}}$) of 18,500 pmol·hr/L in males and 18,600 pmol·hr/L in females (18,550 pmol·hr/L combined).

Methods

Aerosol Concentration:	0 (vehicle control, VC; air control, AC), 0.82 (LD), 2.5 (MD), and 26 (HD) µg/L
Achieved Doses ⁶ :	Three dose levels at 4.9 (LD), 15 (MD), or 160 (HD) µg/kg/day
Pulmonary Deposited Doses ⁷ :	1.23 (LD), 3.75 (MD), or 40 (HD) µg/kg/day
Frequency of dosing:	10 minutes exposure/day
Route of administration:	Inhalation (using a face mask with a mouth tube)
Formulation/Vehicle:	Aqueous solution containing: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA x 2 H ₂ O, and (b) (4) % citric acid. An air control group was also included in the study.
Species/Strain:	Beagle dogs
Number/Sex/Group:	2/sex/group (except the vehicle control and HD, which had an additional 2/sex for recovery)
Age:	approximately 8-9 months at day 1
Weight:	Males: 5.6 to 8.5 kg Females: 4.2 to 6.7 kg
Satellite groups:	2 animals/sex in the vehicle control and HD groups were used for recovery
Unique study design:	2 animals/sex in the vehicle control and HD groups were used for recovery
Deviation from study protocol:	Protocol deviations were listed and were considered minor and did not affect the quality or integrity of the data.

Observations and Results**Mortality**

Mortality and the general health condition of the animals were monitored at least twice daily during the dosing period.

Survival was not affected by olodaterol treatment.

Clinical Signs

The overall appearance and behavior of each animal were monitored at least twice daily during the dosing period. The condition of the palate of all animals was visually inspected starting with the pre-test period once a month.

A summary of the olodaterol-related clinical observations is presented below in Table 28. Olodaterol treatment resulted in increased heart rate and more palpable (strengthened) heart beat. These effects were observed with higher incidence in males and females at the HD group and immediately after the end of exposure on study day 1

⁶ Estimated achieved doses as reported by the applicant

⁷ A deposition factor of 0.25 was used to calculate the pulmonary deposited dose

(HD group only). These heart effects disappeared prior to dosing on the next day. Also, the effects were more prominent at the beginning of the study and there was a decrease in number of animals and duration as the study progressed (e.g., all HD animals in the first few days vs. 1-3 HD animals at the end of dosing). In addition, one HD male presented cardiac arrhythmia on study day 81. All these cardiac effects were recovered immediately after the end of the dosing period.

Table 28: Summary of olodaterol (BI 1744)-related clinical observations - Dog

	Males			Females		
BI 1744 (µg/kg/dose)	4.9	15	160	4.9	15	160
PDD	1.23	3.75	40	1.23	3.75	40
No. of animals examined	4	4	6	4	4	6
Observations						
No. of animals (Days observed)						
Increased heart rate	1 (52)	1 (50,68,69,79)	1-6 (1-89)	1-2 (43,66)	1 (52,65,68)	1-6 (1-89)
Strengthened heart beat	NC	1 (33,65)	1-3 (36,50-89)	1 (36,80)	2 (35)	1-4 (36,53-89)
Cardiac arrhythmia	NC	NC	1 (81)	NC	NC	NC

VC = vehicle control

AC = air control

NC = no drug-related change observed

Further, dry oral mucosa, increased salivation, and reddened snout were observed with similar incidence in all groups (including controls) throughout the dosing period. These are considered related to the inhalation route of administration and not to the treatment.

Body Weights

The body weights of the animals were recorded weekly in the morning during pre-test, dosing, and recovery periods. During the dosing period, the body weights were recorded prior to dose administration. Body weights were also recorded before necropsy.

In general, absolute body weights were slightly ($\leq 10\%$) higher in all olodaterol-treated dogs (males and females) compared to the vehicle controls. However, the changes were not significant or related to the dose.

Feed Consumption

Each day during the study, all animals were given a measured amount of food. Remaining food was collected over the time period to the next body weight measurement and the weight was recorded.

No olodaterol-related changes were observed.

Ophthalmoscopy

Ophthalmoscopy examinations of the conjunctiva, sclera, cornea, anterior chamber of the eye, iris, lens and anterior part of the vitreous body were done using a slit lamp.

The fundus was examined using a fundus camera. Examinations were performed once pre-test and on study day 80 (vehicle control, air control, and high-dose groups). Additionally, the recovery animals were examined on study day 128. Pupillary dilatation was induced at least 30-60 minutes before examination using a mydriatic agent.

According to the final study report, no ophthalmic changes were observed in olodaterol-treated dogs compared to controls. However, the data were not provided for review, and no ophthalmologist report was attached to the final study report.

ECG

Electrocardiographic and hemodynamic measurements were performed during the pre-test and on study days 2, 23, and 85 only prior to and immediately after exposure. No additional measurements were performed. Electrocardiograms (ECGs) and arterial blood pressure were recorded from the recovery animals on study day 126. The limb leads I, II, and III were recorded with a nine-channel ECG recorder. The signals were filtered by a 25 Hz filter. Arterial blood pressure was recorded non-invasively with a tail cuff using the blood pressure analyzer and measured manually. Heart rate, heart rhythm, and waveform configuration were determined from Lead II. The QT intervals were corrected for heart rate using Van de Water's (QTcV), Bazett's (QTcB), and Fridericia's (QTcF) equations. For the purposes of this study, because literature suggest that Van de Water's correction is the best for dogs⁸, QTcV was used for analysis of the data.

On study day 2, the heart rates prior to and immediately after exposure were higher in olodaterol-treated animals [MD (27-82%) and HD (28-94%)] compared to the vehicle controls. On study day 23, the heart rates immediately after exposure were slightly higher in MD and HD males (39% and 43%, respectively) compared to the vehicle controls. On study day 85, the heart rates immediately after exposure were slightly higher in MD males (31%) and HD females (13%) only, relative to the vehicle controls. All these changes in heart rate are considered related to treatment.

These results correlate with clinical signs of increased heart rate and palpable heart beats in olodaterol-treated animals, which were more prominent early during the dosing period and decreased in number and duration as the study progressed (see Table 28 above). These results also correlate with the TK data that show higher drug concentration (C_{max}) and exposure (AUC) on study day 1 compared to study day 86 (Table 33 below).

No significant changes were observed in ECG parameters or in blood pressure in olodaterol-treated animals relative to controls.

Hematology

Blood samples were collected in the morning by puncture of the jugular vein of fasted animals without anesthesia. Blood samples were taken during the pre-test period and

⁸ Soloviev MV, Hamlin RL, Barrett RM, Chengelis CP, and Schaefer GJ. (2006) Different species require different correction factors for the QT interval. *Cardiovascular Toxicology* 06; 145-157.

on study days 4, 25 and 88. Blood samples were collected from recovery animals on study day 129. Collection tubes contained potassium EDTA (for determination of a standard battery of individual hematological parameters) or sodium citrate [for determination of activated partial thromboplastin time (APTT) and prothrombin time (PT)].

A summary of olodaterol-related changes relative to the vehicle controls is presented below in Table 29. Olodaterol treatment resulted in slight decreases in red blood cells and associated hemoglobin and hematocrit. No microscopic findings are related to these changes, and therefore are not considered of concern or dose-limiting.

Table 29: Summary of olodaterol (BI 1744)-related changes in hematology parameters relative to vehicle control - Dog

	Males			Females		
BI 1744 (µg/kg)	4.9	15	160	4.9	15	160
PDD (µg/kg)	1.23	3.75	40	1.23	3.75	40
Parameter						
Hemoglobin						
Day 25	NC	-10%	-8%	NC	NC	NC
Day 88	NC	-7%	-7%	NC	NC	NC
Red blood cells						
Day 25	NC	-9%	-7%	NC	NC	NC
Day 88	NC	-6%	-5%	NC	NC	NC
Hematocrit						
Day 25	NC	-10%	-9%	NC	NC	NC
Day 88	NC	-9%	-8%	NC	NC	NC

NC = no drug-related change observed

Clinical Chemistry

Blood samples were collected in the morning (fasted animals) by puncture of the jugular vein without anesthesia. Blood samples were collected from all animals during the pre-test period and on study days 4, 25 and 88. Blood samples were collected from recovery animals on study day 129. Individual clinical chemistry parameters were determined in serum, except for lactate dehydrogenase (LDH) and creatine kinase (CK), which were determined in plasma derived from lithium heparinized blood. A standard battery of parameters was evaluated.

A summary of olodaterol-related changes in clinical chemistry parameters is presented below in Table 30. Olodaterol treatment resulted in increased levels of some liver enzymes (aspartate and alanine aminotransferases in HD females and alkaline phosphatase in HD males, LD females, and HD females). Also, levels of creatine kinase were elevated in males and females at all dose levels with a clear dose-response in females but not in males. This correlates with findings of increased heart rate observed in olodaterol-treated animals (see above). Levels of serum creatinine and urea were increased (in males and females at all dose levels and in MD and HD males, respectively) and suggest renal damage. However, the microscopic changes observed in the kidney did not follow a clear dose-response and some findings were

observed with similar incidence and severity in the control groups (Table 32). Further, electrolyte changes (increased levels) were observed in a number of olodaterol-treated animals but did not follow a dose-response. The increased levels of lactate dehydrogenase in females (all dose levels) could be related to liver or heart damage, as different isoforms of this enzyme are present in different tissues.

Table 30: Summary of olodaterol (BI 1744)-related changes in clinical chemistry parameters relative to vehicle controls - Dog

	Males			Females		
BI 1744 (µg/kg)	4.9	15	160	4.9	15	160
PDD (µg/kg)	1.23	3.75	40	1.23	3.75	40
Parameter						
Aspartate aminotransferase						
Day 4	NC	NC	NC	NC	NC	NC
Day 25	NC	NC	NC	NC	NC	+58%
Day 88	NC	NC	NC	NC	NC	+50%
Alanine aminotransferase						
Day 4	NC	NC	NC	NC	NC	NC
Day 25	NC	NC	NC	NC	NC	+78%
Day 88	NC	NC	NC	NC	NC	+45%
Alkaline phosphatase						
Day 4	NC	NC	+50%	+44%	NC	+92%
Day 25	NC	NC	NC	+47%	NC	+31%
Day 88	NC	NC	NC	+54%	NC	+36%
Lactate dehydrogenase						
Day 4	NC	NC	NC	NC	NC	NC
Day 25	NC	NC	NC	NC	NC	NC
Day 88	NC	NC	NC	+38%	+43%	+50%
Creatine kinase						
Day 4	+38%	+50%	+24%	NC	NC	NC
Day 25	NC	NC	NC	NC	+84%	+109%
Day 88	+61%	+68%	+63%	+51%	+73%	+203%
Urea						
Day 4	NC	NC	NC	-17%	-29%	NC
Day 25	NC	+29%	+26%	NC	NC	NC
Day 88	NC	+17%	+25%	NC	NC	+14%
Creatinine						
Day 4	NC	NC	NC	NC	NC	NC
Day 25	+11%	+26%	+21%	+27%	+16%	+22%
Day 88	+19%	+32%	+35%	+21%	+22%	+39%
Sodium						
Day 4	NC	NC	NC	NC	NC	NC
Day 25	NC	+6%	+6%	+6%	+6%	+6%
Day 88	NC	NC	NC	NC	NC	NC
Potassium						
Day 4	NC	NC	NC	NC	+9%	NC
Day 25	NC	NC	NC	+16%	+15%	+19%
Day 88	+10%	+6%	+10%	NC	NC	+6%
Magnesium						
Day 4	NC	NC	NC	NC	NC	NC
Day 25	NC	+9%	+7%	+13%	+8%	NC
Day 88	NC	NC	NC	NC	NC	NC

Chloride							
Day 4	NC	NC	NC	NC	NC	NC	NC
Day 25	NC	+5%	+5%	+6%	+6%	+6%	+6%
Day 88	NC	NC	NC	NC	NC	NC	NC
Inorganic phosphate							
Day 4	NC	-18%	-9%	NC	NC	NC	NC
Day 25	+9%	+18%	NC	+12%	+11%	NC	NC
Day 88	NC	NC	NC	NC	NC	NC	NC

NC = no drug-related change observed

Urinalysis

Urine was collected at necropsy by puncture of the urinary bladder on study days 91, 92, 93, and 94 as well as on study day 134 for recovery animals. The urine was tested and the urine sediment was examined microscopically. A full battery of urinalysis parameters was evaluated.

No changes were observed in olodaterol-treated dogs compared to controls.

Gross Pathology

A complete necropsy was performed on all animals, and all gross macroscopic changes were recorded. All animals were anesthetized with an intravenous injection of sodium pentobarbital and a supplementary intramuscular injection of ketamine for analgesia. Following the cutting of all major axillar blood vessels, the animals were sacrificed by exsanguination.

No olodaterol-related gross findings were observed at necropsy.

Organ Weights

The following organs of all animals sacrificed were weighed: adrenals, brain, heart, kidneys, liver, lung (before instillation), ovaries, pituitary gland, prostate (following fixation), spleen, testes, and thyroid glands (including parathyroid glands).

Table 31 below presents a summary of olodaterol-related changes in organ weights. The absolute heart weight was slightly increased in HD females but no change was observed when normalized for body (BW) or brain weights. In males, heart weight normalized to BW was slightly decreased (14%) at all dose levels with no dose-relationship. Because the opposite effect is observed in males and females, it is difficult to conclude that these changes are related to olodaterol. Lung weights (normalized to BW) were decreased in HD females and males at all doses. When normalized to brain weight, only HD males showed a decrease in lung weight (15%). It is not clear how these changes in lung weights correlate with microscopic findings in the lungs (see Table 32 below).

Table 31: Summary of olodaterol (BI 1744)-related changes in organ weights relative to vehicle controls - Dog

	Males			Females		
BI 1744 (µg/kg)	4.9	15	160	4.9	15	160
PDD (µg/kg)	1.23	3.75	40	1.23	3.75	40

Heart	absolute	NC	NC	NC	NC	NC	+7%
	relative to BW	-14%	-14%	-14%	NC	NC	NC
	relative to brain	NC	NC	NC	NC	NC	NC
Lungs	absolute	NC	NC	NC	NC	NC	NC
	relative to BW	-17%	-12%	-17%	NC	NC	-14%
	relative to brain	NC	NC	-15%	NC	NC	NC

NC = no drug-related change observed

(-) = not examined

Histopathology

Adequate Battery

An adequate battery of tissues from all animals in all groups was examined microscopically.

Peer Review

A pathology peer review was conducted by Dr. (b) (4) and a signed peer review statement is included in the final study report to confirm that the final pathology report accurately reflects the results of the review.

Histological Findings

Olodaterol-related microscopic findings include reversible glycogen depletion and increased glycogen storage in the liver of MD and HD males and females. These findings were observed with increased severity in the HD group and are considered a pharmacological effect of β_2 -adrenoreceptor activation. Similar liver findings were observed in the 4-week dog study. In the pancreas, multifocal apoptosis was observed in one HD male dog and is considered possibly related to treatment. No other findings were observed in the pancreas in other treated or recovery dogs. Hemorrhagic resorption was observed in the lymph nodes (bifurcational) of one HD male and has been considered possibly related to treatment. This finding was not reversible as it was also observed in one HD recovery animal after the 6-week recovery period.

In the heart, slight/focal arterial hypertrophy was observed across all groups, including the vehicle-control and air-control groups, suggesting that this is a background finding. No other microscopic findings were observed in the heart. Also, in the lungs, a number of findings were observed with similar incidence in all groups, including the controls. These include foam cell accumulation, hemorrhage, histiocytosis, and inflammatory infiltration. In addition, hyperemia was observed in one MD male and pleural fibrosis was observed in one LD male. Because these lung findings do not follow a dose-response, they have been considered incidental. In the kidneys; infiltration and mineralization were observed with similar incidence across all groups and therefore have been considered incidental. Tubular protein casts were observed in one HD female, but this finding was unilateral and minimal in severity. Although clinical chemistry findings of increased creatinine and urea suggest renal damage, no olodaterol-related microscopic changes were observed in the kidneys.

In the trachea; epithelial atrophy, inflammatory infiltration, squamous cell metaplasia, and reduced number of Goblet cells were observed with similar incidence and severity across all groups, including the controls. Therefore all these findings are not considered related to treatment.

Taken all the aforementioned findings together, the NOAEL for systemic and local (respiratory tract) toxicities has been identified as the high-dose level (160 µg/kg, PDD = 40 µg/kg). This dose level is associated with a systemic exposure (AUC_{0-24 hr}) of 18,500 pmol·hr/L in males and 18,600 pmol·hr/L in females (18,550 pmol·hr/L combined).

Table 32: Summary of Histological Observations (Incidence and Severity) - Dog

	Males					Females				
	VC	AC	4.9	15	160	VC	AC	4.9	15	160
BI 1744 CL (µg/kg, achieved dose)	0	0	1.2	3.8	40	0	0	1.2	3.8	40
PDD (µg/kg)	4	4	4	4	4	4	4	4	4	4
Number of animals examined (N)	4	4	4	4	4	4	4	4	4	4
Observations – Dosing Phase										
Heart										
Arterial media hypertrophy, focal (Slight)	3	1	1	1	4	1	3	1	1	2
Liver										
Glycogen depletion, centrolubular										
Minimal	0	0	0	0	3	0	0	0	0	1
Slight	0	0	0	0	1	0	0	0	0	1
Glycogen storage increased, peripherolubular										
Minimal	0	0	0	2	2	0	0	0	3	1
Slight	0	0	0	0	2	0	0	0	1	3
Lymph node, bifurcational										
Resorption, hemorrhagic, acute (Min)	0	0	0	0	1	0	0	0	0	0
Pancreas										
Apoptosis, multifocal (Slight)	0	0	0	0	1	0	0	0	0	0
Lungs										
Foam cell accumulation (Min)	0	2	1	0	0	1	0	1	2	2
Hemorrhage, alveolus (Min)	0	1	0	1	0	0	1	0	1	0
Histiocytosis, increased (Min)	0	0	1	0	1	0	1	0	1	1
Hyperemia (Slight)	0	0	0	1	0	0	0	0	0	0
Infiltration, inflammatory										
Minimal	3	4	2	3	1	1	2	2	3	3
Slight	0	0	0	0	0	0	0	0	0	1
Pleural fibrosis/adhesion (Min)	0	0	1	0	0	0	0	0	0	0
Kidneys										
Infiltration, cortex										
Minimal, unilateral	0	0	0	1	0	2	0	1	0	0
Minimal, bilateral	0	0	1	0	0	0	1	0	0	0
Mineralization, corticomedullary (Min)	0	0	0	0	0	0	0	0	2	0
Protein cast(s), tubular, unilateral (Min)	0	0	0	0	0	0	0	0	0	1
Trachea										
Atrophy, epithelial										
Minimal	0	0	0	0	0	1	1	0	1	1
Slight	0	1	2	2	2	1	0	0	1	0
Infiltration, inflammatory (Min)	2	1	0	3	2	2	1	1	1	2

Metaplasia, squamous cell (Slight)	1	0	0	0	0	1	0	1	0	1
Goblet cell, reduced in number										
Minimal	0	0	0	0	0	0	0	0	1	1
Slight	2	1	1	0	0	0	0	0	1	0
Observations – Recovery Phase (N=2)										
Heart										
Arterial media hypertrophy, focal (Slight)	1	-	-	-	0	2	-	-	-	1
Lymph node, bifurcational										
Resorption, hemorrhagic, acute, Slight	0	-	-	-	1	0	-	-	-	0
Kidneys										
Infiltration, unilateral (Min)	1	-	-	-	0	0	-	-	-	0
Lungs										
Foam cell accumulation (Min)	1	-	-	-	0	0	-	-	-	0
Hemorrhage, alveolus (Min)	0	-	-	-	0	0	-	-	-	1
Histiocytosis (Min)	0	-	-	-	1	0	-	-	-	1
Infiltration, inflammatory										
Minimal	0	-	-	-	1	1	-	-	-	2
Slight	1	-	-	-	0	0	-	-	-	0
Trachea										
Infiltration, inflammatory										
Minimal	1	-	-	-	1	1	-	-	-	1
Slight	0	-	-	-	1	0	-	-	-	0
Metaplasia, squamous cell (Slight)	1	-	-	-	1	0	-	-	-	0
Goblet cell, reduced in number (Slight)	0	-	-	-	0	1	-	-	-	0

VC = vehicle control

AC = air control

(-) = tissue not examined

Toxicokinetics

Approximately 2 mL of blood was collected from the vena jugularis into tubes containing potassium EDTA as anticoagulant. Blood was collected pre-test and on study days 1 and 86 (end of dosing period). BI 1744 BS (free base) was quantified in plasma using a validated high performance liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS) method (LLOQ = 20.0 pmol/L) with solid phase extraction.

In general, olodaterol exposure (AUC) increased almost proportionally with dose. No drug accumulation was observed with repeat dosing (accumulation ratio = 1.22). Also, drug exposure was similar in males and females (Table 33 below).

BI 1744 was detected in two control samples, suggesting sample contamination. The measured concentrations (43.1 pmol/L and 434 pmol/L) were close to or in the range of the C_{max} for the treated animals. According to the study report, the origin of the contaminations could not be clarified. Because these two samples represent <1% of the control samples collected and analyzed during the study (total of 288 control samples analyzed), this deviation is not considered to negatively affect the data overall.

Table 33: Mean toxicokinetic parameters in males and females⁹

Parameter	Day	Gender	5 µg/kg	15 µg/kg	150 µg/kg
C(max)	1	m	170	546	6570
	86	m	195	143	2780
[pmol/L]	1	f	104	648	5090
	86	f	67.9	258	3060
AUC(0-24h)	1	m	822	2320	30300
	86	m	616	1540	18500
[pmol·h/L]	1	f	844	2410	24900
	86	f	689	2080	18600

Dosing Solution Analysis

Samples of the dosing formulations were analyzed at the beginning of the inhalation period for concentration of BI 1744 and for pH. Additionally, a sample was taken from each of the dilutions at the time of preparation and the time of last use. These samples were analyzed for concentration of BI 1744 and for pH. The analyses were performed by the Department of Analytical Sciences using a validated HPLC-UV method (UV detection = 225 nm).

A summary of the results is presented in Table 34 below. Temperature and relative humidity were acceptable for a liquid aerosol. The mass median aerodynamic diameters (MMAD) and the geometric standard deviation (GSD) were similar between groups and within the respirable range for dogs.

Table 34: Test atmosphere parameters and achieved doses of BI 1744¹⁰

Parameter		Treatment Group				
		Vehicle-Control	Low-Dose	Mid-Dose	High-Dose	Air-Control
Temperature	[°C]	20.7 - 24.1	18.7 - 22.8	17.6 - 22.4	19.1 - 22.4	20.4 - 24.3
Relative humidity	(%)	60 - 98	51 - 100	84 - 100	50 - 100	21 - 83
Target aerosol conc.	[µg/L]	0.0	0.88	2.6	26	0.0
Analysed aerosol conc.	[µg/L]	0.0	0.82	2.5	26	0.0
Target daily dose	[µg/kg]	0.0	5.0	15	150	0.0
Achieved daily dose	[µg/kg]	0.0	4.9	15	160	0.0
MMAD	[µm]	n.a.[a]	2.44	2.92	2.57	n.a.[a]
GSD		n.a.[a]	3.94	3.99	3.36	n.a.[a]

Study no. 05B270: BI 1744 CL – 13-wk tox dog ih

MMAD mass median aerodynamic diameter

GSD geometric standard deviation

[a] not analysed because controls were exposed to vehicle only

⁹ Table taken from study U07-1317 final report

¹⁰ Table taken from study U07-1317 final report

Study no. U08-1740, BI 1744 CL: 52-week inhalation toxicity study in Beagle dogs with a 6-week recovery period. Beagle dogs (4/sex/group) received 0 (vehicle control: 0.01% benzalkonium chloride, (b)(4)% disodium EDTA, and (b)(4)% citric acid), 15, 60, or 330 µg/kg/day olodaterol (achieved doses) via inhalation (face mask with a mouth tube, 10 minutes/day) for 364 consecutive days. The pulmonary deposited doses were estimated as 3.9, 14.6, and 82.6 µg/kg/day olodaterol (refer to Pharm/Tox review under IND 76,362 dated October 28, 2011 (page # 32) for the complete details). An additional 2 dogs/sex were included in the vehicle and HD groups for a 6-week recovery period after the dosing period. At the end of the dosing period, main study animals were sacrificed and necropsies were performed. Assessment of toxicity was based on evaluation of mortality, clinical signs, body weights, food consumption, ECG and ophthalmic examinations, and clinical and anatomic pathology observations.

Survival was not affected by olodaterol treatment. Body weights were increased in olodaterol-treated males at all dose levels (7-16%). In females, increased body weights were observed in the MD and HD groups compared to the vehicle control group (14% and 11%, respectively). Food consumption was not changed in olodaterol treated males or females. In the heart, fibrosis/fibroplasia of the left ventricle was observed in MD and HD animals. This correlates with clinical signs of increased heart force observed frequently in olodaterol-treated animals and also with increased heart rate observed in treated males and females. In addition, these findings of heart toxicity were associated with increases in serum levels of creatinine kinase and cardiac-specific troponin 1. Further, ECG examinations found multiple ventricular premature beats in one MD female and ventricular tachycardia in one HD female. All these findings in the heart were considered related to treatment and dose-limiting.

In the kidney, microscopic findings of increased incidence and severity of mononuclear infiltration (unilateral and bilateral) were observed in the MD and HD and correlated with increases in serum creatinine in olodaterol-treated females. In the liver, increased severity of liver glycogen storage and hemorrhage were observed at the MD and HD dose levels. These hepatic findings correlated with increased ALT levels in MD and HD females. Olodaterol treatment also resulted in moderate palate inflammation with ulceration, observed at the LD and HD levels but not at the MD. Liver and palate findings were recovered after the 6-week recovery period. However, findings in the heart and kidneys were not reversible.

Local (respiratory tract) toxicities were also observed in this study and included atrophy of the nasal cavity at 82.6 µg/kg (PDD) and findings of epithelial atrophy, infiltration, and mineralization in the trachea, also at 82.6 µg/kg (PDD) (refer to pages # 46-49 of the October 28, 2011 Pharm/Tox review under IND 76,362 for the histopathology table). The finding in the nasal cavity is not considered relevant to the clinical route of administration (inhalation by face mask in the dog vs. oral inhalation in humans) and thus, is not considered dose-limiting. However, findings in the trachea were considered dose-limiting. Based on the finding in the trachea, the NOAEL for local toxicities in this study was identified as the mid-dose of 14.6 µg/kg (PDD).

The toxicokinetic evaluation shows that systemic exposure increased almost proportional with dose, that there is no significant drug accumulation over time, and that there is no gender effect. Based on the aforementioned findings in the heart, kidneys, and liver, the NOAEL for systemic toxicities was identified at the low-dose level of 15 µg/kg/day. This dose level was associated with an AUC_{0-24 hr} (at Day 358) of 2160 pmol·hr/L in males and 3560 pmol·hr/L in females (2860 pmol·hr/L combined, Table 35 below excerpted from study U08-1740 final report). Refer to Pharm/Tox review under IND 76,362 dated October 28, 2011 for the complete details.

Table 35: Mean toxicokinetic parameters in the dog.

Parameter	Day	Gender	Target doses of BI 1744 BS		
			15 µg/kg	60 µg/kg	300 µg/kg
C(max) [pmol/L]	1	m	197	1960	13100
	72	m	323	1620	7080
	358	m	381	1620	8990
	1	f	359	2550	15100
	72	f	378	1020	13200
	358	f	423	1490	11500
AUC(0-24h) [pmol·h/L]	1	m	1160	7750	50200
	72	m	2290	9350	36900
	358	m	2160	7970	46000
	1	f	1930	8500	59900
	72	f	3490	7950	47000
	358	f	3560	9910	50200

7 Genetic Toxicology

The genotoxic potential of olodaterol was assessed in bacterial (*in vitro* Ames assay) and mammalian (*in vitro* mouse lymphoma and *in vivo* micronucleus assays) systems. During the review of the initial IND submission, (b) (4)

The sponsor conducted and submitted an (b) (4) *in vivo* micronucleus study using the intravenous route of administration (Study U08-1834). A detailed evaluation of all the genetic toxicology studies is included in Pharm/Tox review for IND 76,362 dated May 10th, 2010. Refer to Section 11 “Integrated Summary and Safety Evaluation” for a discussion of genetic toxicology findings.

8 Carcinogenicity

Two-year carcinogenicity studies were conducted in mice and rats. The FDA Executive Carcinogenicity Assessment Committee (ECAC) provided recommendations for the

selection of doses for the mouse and rat carcinogenicity studies (ECAC meeting minutes dated February 15, 2007). On January 27, 2009, the sponsor submitted a request for feedback from the ECAC of an information amendment to the mouse carcinogenicity protocol. The ECAC recommended that the 2-year mouse study continue as per protocol and this was communicated to the sponsor via facsimile on February 23, 2009. The final reports for the two carcinogenicity studies in rats and mice were submitted to IND 76,362 on January 17, 2012 and March 6, 2012, respectively. A detailed evaluation of the two carcinogenicity studies is included in Pharm/Tox review for IND 76,362 dated May 11, 2012.

The results of the two carcinogenicity studies were discussed with the ECAC on July 10, 2012. The ECAC concurred that both the rat and mouse studies were acceptable, noting prior ECAC concurrence with the protocols. The ECAC concluded that the rat study was positive for olodaterol-induced leiomyomas of the mesovarian tissue in females and that the mouse study was positive for olodaterol-induced combined benign uterine leiomyomas and malignant leiomyosarcomas in females (ECAC meeting minutes dated July 11, 2012).

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity studies were conducted in rats and rabbits. A complete battery of studies was submitted and reviewed under IND 76,362. At the Division's request, the sponsor submitted a communication on October 7, 2011 to address the validity of the inhalation rat embryo-fetal developmental toxicity study. The sponsor's justification was found acceptable. However, the Division requested the sponsor to provide AUC values from the reproductive toxicology studies to ensure adequate exposure to support the validity of these studies, and to enable drug labeling margins based on systemic drug exposure. This information was submitted under IND 76,362 (see below under Section 11, "Integrated Summary and Safety Evaluation"). See Pharm/Tox review for IND 76,362 dated October 26, 2011 for a detailed evaluation of all the reproductive and developmental toxicology studies. Refer to Section 11 "Integrated Summary and Safety Evaluation" for the discussion of reproductive toxicology findings.

10 Special Toxicology Studies

A number of additional toxicology studies were conducted to assess local tolerance of olodaterol. Only the eye and dermal local tolerance studies were reviewed in detail and are summarized below. The results of these studies show that olodaterol has a dose-dependent mild-to-moderate irritating effect in the eye, but was well tolerated when applied on the skin.

Study no. U04-2189, BI 1744 CL: Acute eye irritation/corrosion study in rabbits.

New Zealand White rabbits (4 females) received a single dose of approximately 2 mg (3 animals) or 10 mg (1 animal) olodaterol (solid drug) and were observed for 3 (2 mg group) or 4 (10 mg group) days. This study was conducted following the OECD Guidance for acute eye irritation/corrosion. The drug was applied once to the left conjunctival sac and the untreated eye (right side) served as control.

First, one animal was treated with 2 mg olodaterol and the eyes were examined 1 hour later. At this time point, mild irritation was observed (some injected blood vessels in the conjunctivae and some swelling of the lids above normal) and two additional females were treated in the same way. After 24 hours, the eyes of these two animals were observed and mild irritation was noted (injected blood vessels in the conjunctivae and swelling of the lids above normal). All the animals in this group were observed for up to 72 hours and all the findings were recovered within 48 hours post drug application.

A fourth animal was included in the study and received 10 mg olodaterol and was observed after 1, 7, 24, 48, 72, and 96 hours. Moderate irritation was observed in this animal [in addition to the findings observed at 2 mg; strong lacrimation; diffuse, crimson color in conjunctivae (individual vessels not easily discernible); markedly deepened rugae, congestion, swelling, and moderate circumcorneal hyperaemia or injection in the iris; and iris reactive to light] and was recovered within 72 hours post drug application. Because of these findings, the study was not continued with further animals or higher doses of olodaterol.

Study no. U05-1116, BI 1744 CL: Acute dermal irritation/corrosion study in rabbits. New Zealand White rabbits (3 females) received a single dose of approximately 0.5 g olodaterol as a paste mixture in demineralized water. Initially, only one rabbit was treated sequentially with olodaterol in up to three skin sites for 3 minutes, 1 hour, and 4 hours, respectively. Administration to the next skin area was only performed in the absence of serious skin reactions in the previous one. Because this first animal tolerated the drug very well for up to 4 hours, two additional rabbits were treated on the following day. Untreated skin areas were used as control. Treated skin areas were examined after 4 (end of exposure period), 24, 48, and 72 hours for any skin alterations. Skin areas were evaluated according to the OECD Guideline criteria for dermal tolerance.

Results show that olodaterol is well tolerated when applied on the skin of rabbits as no alterations (skin erythema or edema) were observed.

11 Integrated Summary and Safety Evaluation

Boehringer Ingelheim Pharmaceuticals, Inc. submitted a 505(b)(1) NDA on May 14, 2012 for STRIVERDI Respimat (olodaterol hydrochloride, company code BI 1744) inhalation spray, a NME. Olodaterol is a new long-acting β_2 -adrenergic receptor agonist bronchodilator for the treatment of COPD. The role of LABAs as bronchodilators in the treatment of COPD is well characterized. β_2 -agonists act on smooth muscle in airways as functional antagonists to reverse bronchoconstriction (due to smooth muscle relaxation and increased airflow).

STRIVERDI Respimat inhalation spray consists of a sterile aqueous solution of olodaterol hydrochloride in a cartridge for delivery by the Respimat inhaler (an inhalation device already used for other approved products for COPD). The drug product formulation does not contain any novel excipients and the same formulation was used

for the nonclinical and clinical studies. In addition, all the impurities in the drug substance and drug product are below the ICH threshold or are considered qualified at the proposed product specification (refer to CMC consult review dated June 27, 2012). For the complete chemistry details, refer to Dr. Craig Bertha's review dated July 31, 2012.

The full clinical program for olodaterol consisted of twelve Phase 1 clinical trials, three Phase 2 clinical trials in COPD patients, four Phase 2 clinical trials in asthma patients, and ten Phase 3 clinical trials in COPD patients. The safety, efficacy, and pharmacokinetics of olodaterol were assessed in these clinical trials. Two doses of olodaterol were studied in the Phase 3 clinical studies: 5 µg once daily and 10 µg once daily. The proposed clinical dose for marketing is 5 µg/day (2 actuations of 2.5 µg/day at the same time of the day). The clinical systemic exposure (AUC) at 5 µg/day is estimated as 134.5 pM·hr. For complete details on the clinical program, refer to Dr. Robert Lim's clinical review under NDA 203-108.

The complete nonclinical safety program for olodaterol was submitted with this NDA and included pharmacology (including primary and secondary pharmacology, and safety pharmacology), pharmacokinetics (ADME), general toxicology (single-dose and repeat-dose), genetic toxicology, carcinogenicity, and reproductive and developmental toxicology studies. The nonclinical safety program is considered complete and adequate to support the safety of the proposed clinical dose of 5 µg/day. An integrated summary of the full nonclinical program follows:

Pharmacology

Primary Pharmacology

A number of *in vitro* pharmacology studies were conducted in CHO-K1 cells expressing the cDNA of the human β_1 -, β_2 -, and β_3 -adrenoreceptors to determine the affinity and potency of BI 1744 at each of these receptors. The results of these studies show that BI 1744 has higher affinity for the human β_2 -adrenoreceptor ($K_i = 0.72$ nM) compared to β_1 - and β_3 -adrenoreceptors ($K_i = 47$ nM and 5 µM, respectively). This high affinity for the human β_2 -adrenoreceptor correlates with the potency observed at this receptor ($EC_{50} = 0.1$ nM) compared to the other two subtypes ($EC_{50} = 28$ nM for β_1 , and 269 nM for β_3). Also, the intrinsic activity (percentage of isoprenaline-induced maximal response) was investigated *in vitro* and the results show that BI 1744 is a partial agonist with an intrinsic activity of 88% at the β_2 -adrenoreceptor. The results show that BI 1744 also acts at the β_1 - and β_3 -adrenoreceptors with an intrinsic activity of 52% and 81%, respectively. Table 36 below presents a summary of the affinity (K_i), potency (EC_{50}) and intrinsic activity of BI 1744 at the three human β -adrenoreceptors.

Table 36: Summary of BI 1744 affinity, potency, and intrinsic activity at the three human β -adrenoreceptor subtypes.

	K_i (nM)	EC_{50} (nM)	IA
hβ_1-adrenoreceptor	47	28	52%
hβ_2-adrenoreceptor	0.72	0.11	88%

hβ_3-adrenoreceptor	5500	269	81%
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K_i = dissociation constant

EC₅₀ = effective concentration

IA = intrinsic activity (% compared to isoprenaline, a non-selective full agonist)

A number of *in vivo* pharmacology studies were also conducted to assess the activity of BI 1744 in a model of acetylcholine-induced bronchospasm in anesthetized guinea pigs and dogs. The onset of action of BI 1744's bronchoprotection against acetylcholine-induced bronchospasm and the duration of action were investigated. The results of these studies show that BI 1744 has a fast onset and long duration of action. In the guinea pig model, full (100%) bronchoprotection was observed within 10 minutes after a single dose at 3 $\mu\text{g}/\text{kg}$ (the FED in the guinea pig). In the dog model, maximum (62%) bronchoprotection was observed within 10 minutes at 0.6 $\mu\text{g}/\text{kg}$ (the FED). In both species, BI 1744 showed sustained bronchoprotection for 24 hours (*i.e.*, approximately 19% bronchoprotection still present 24 hours after a single dose).

One potential side effect as a result of β_2 -adrenoreceptor activation is increased heart rate (β_2 -adrenoreceptors are present in cardiac tissue and activation of these receptors leads to tachycardia). This is a well known class effect for β_2 -agonists¹¹. Therefore, in the *in vivo* dog studies, the sponsor investigated the effect of BI 1744 on heart rate. As shown in Table 37 below, BI 1744 induced a transient slight increase in heart rate (19%) within the first 20 minutes after drug administration, which correlates with the C_{max} in the dog. The effect was completely gone after the first 30 minutes.

Table 37: Effect of BI 1744 CL on heart rate in anesthetized dogs, 24 hour recording period.

Time (hours)	Vehicle % bpm	BI 1744 CL (0.3 $\mu\text{g}/\text{kg}$ i.h.) % bpm	BI 1744 CL (0.6 $\mu\text{g}/\text{kg}$ i.h.) % bpm
0	100 \pm 0	100 \pm 0	100 \pm 0
0.1	101 \pm 21	107 \pm 6	114 \pm 7
0.2	98 \pm 8	109 \pm 8	119 \pm 8
0.5	102 \pm 9	104 \pm 6	107 \pm 5
6	106 \pm 7	103 \pm 10	102 \pm 9
12	105 \pm 13	111 \pm 10	107 \pm 13
24	101 \pm 5	104 \pm 19	96 \pm 6

bpm = beats per minute

Secondary Pharmacology

An *in vitro* screen and follow-up *in vivo* assays were conducted to identify potential targets of olodaterol. Data from these studies identified β_2 -adrenergic, β_1 -adrenergic, α_1 -adrenergic, and 5-HT_{2A} serotonin receptors as potential targets. Data from these

¹¹ Petruska JM, Beattie JG, Stuart BO, Pai S, Walters KM, Banks CM, Lulham GW, and Mirro EJ. (1997) Cardiovascular effects after inhalation of large doses of albuterol dry powder in rats, monkeys, and dogs: a species comparison. *Fundamental and Applied Toxicology* 40; 52-62.

studies show that olodaterol exerts partial agonist activity at the β_2 -adrenergic, β_1 -adrenergic receptors and antagonist activity at the α_1 -adrenergic, and 5-HT_{2A} serotonin receptors. Although not detected in these studies, olodaterol also exerts partial agonist activity at the human β_3 -adrenergic receptor (refer to “Primary Pharmacology” above).

Safety Pharmacology

The effects of olodaterol on the cardiovascular, pulmonary, neurological, renal, and gastrointestinal systems were investigated.

Cardiovascular Effects

The effects of olodaterol on the cardiovascular system were investigated *in vitro* and *in vivo*. Data from these studies show that olodaterol induces a dose-dependent increase in force of contraction in guinea pig papillary muscle *in vitro*. In addition, olodaterol produces a dose-dependent (and reversible) decrease in systolic, diastolic, and MAP early after drug administration and up to 18 hours post-dose. This decrease in MAP was associated with a dose-dependent increase in heart rate that persisted for up to 24 hours. These data correlate with the *in vivo* pharmacology data above and data obtained in the 52-week toxicity study in dogs (see below), where a decrease in blood pressure and increase in heart rate were observed.

In the safety pharmacology study, olodaterol also induced a transient (immediately after drug administration and up to 4-5 hours) dose-dependent shortening of the QTc interval after a single oral dose. However, no effects on QTc were observed in the 52-week dog study using the inhalation route of administration. It was not possible to compare the PK parameters in both studies because TK samples were not collected in the safety pharmacology study.

These effects of olodaterol on the cardiovascular system (decreased MAP and increased heart rate) are considered class effects for LABAs. These effects lead to histopathological changes in the heart (discussed below).

Respiratory effects

The effects on the respiratory system were investigated in conscious rats following a single inhalation dose of olodaterol and no effects were observed in respiratory parameters.

Neurological effects

The modified Irwin test in rats was used for assessment of neurological effects of olodaterol. The data show that olodaterol has no effect on behavior or on the physiological state of rats. This correlates with the distribution data that show that olodaterol is not distributed into the brain.

Renal effects

The renal function after olodaterol administration was assessed in rats after a single administration. The results of this study show that olodaterol induces reduction of urine

volume and electrolyte excretion in rats. Although the sponsor claims (and some literature supports¹²) that LABAs induce an antidiuretic effect, the data for olodaterol are variable and not sufficient to support this claim.

Gastrointestinal effects

Two separate studies in rats were conducted to assess the effects of olodaterol on gastric function and secretion. The results show that olodaterol decreases gastric emptying and gastrointestinal transit and reduces gastric secretion. These effects are considered class pharmacodynamic effects of β_2 -adrenergic agonists due to activation of β_2 -receptors in the gastrointestinal tract.

ADME

The Absorption, Distribution, Metabolism, and Excretion of olodaterol were investigated in mice, rats, dogs and female rabbits using different routes of administration (intra-tracheal/inhalation, oral, and IV). Data from these studies show that olodaterol is rapidly absorbed after inhalation administration. The C_{max} was achieved within the first 30 minutes post-dose. After oral administration, the C_{max} was reached at approximately 0.5 to 2 hours post-dose. After inhalation administration, the two rodent species showed the highest bioavailability (54.4% in the mouse and 23.1% in the rat). The bioavailability after inhalation administration was much lower in the dog (9.2%).

Olodaterol is widely distributed into the lungs (after inhalation administration), the pancreas, the gastrointestinal tract, and the eye. In addition, olodaterol is distributed from plasma to blood cells. Protein binding data show moderate binding of olodaterol to plasma proteins (50-70%) in all species tested. Olodaterol is not distributed into the central nervous system. Olodaterol crosses the placenta at the embryonic and fetal stages of development. Due to limitations in the methods used, the distribution into the embryos was not investigated. In fetuses, olodaterol is distributed into the liver, lung, blood, and brain.

Metabolism studies identified two main metabolites of olodaterol: CD992 (a glucuronide metabolite) and SOM1522 (metabolite formed after demethylation of BI 1744). In addition, other glucuronide metabolites were identified in the nonclinical species and human. Boehringer Ingelheim conducted a series of studies and found that, except for SOM1522, which is active at the β_2 -adrenergic receptor, all the other metabolites were classified as pharmacologically inactive.

¹² Levi J, Coburn J, and Kleeman CR. (1976) Mechanism of the antidiuretic effect of β -adrenergic stimulation in man. *Archives of Internal Medicine* 136; 25-29.

Shibouta Y, Inada Y, Terachita ZI, Nishikawa K, and Kikuchi S. (1978) Antidiuresis induced by β_1 - and β_2 -adrenergic agonists in ethanol-anesthetized rats. *European Journal of Pharmacology* 47; 149-157.

Berl T, Cadnapaphornchai P, Harbottle JA, and Schrier RW. (1974) Mechanism of stimulation of vasopressin release during beta adrenergic stimulation with isoproterenol. *The Journal of Clinical Investigation* 53; 857-867.

Olodaterol is excreted primarily via feces in nonclinical species. Urine is the secondary route of excretion. Olodaterol shows a biphasic elimination, with a rapid decrease in drug concentration in the first 2 to 3 hours post-dose and a slower elimination thereafter (for up to 24 hours post-dose). This biphasic elimination supports the fast onset and long duration of action of olodaterol identified in the *in vivo* pharmacology studies described above. In addition, excretion studies found that olodaterol is excreted into milk of lactating rats. The concentration of olodaterol in milk was approximately 6-fold higher than the concentration in plasma.

General Toxicology

Repeat-dose toxicology studies were conducted in CD-1 mice, Wistar Han rats, and Beagle dogs up to 13 weeks, 26 weeks, and 52 weeks of dosing, respectively, using the inhalation route of administration. In all the studies, an aqueous solution of olodaterol in 0.01% benzalkonium chloride, (b) (4)% sodium EDTA, and (b) (4)% citric acid (same as the clinical formulation) was used to generate an aerosol. Inhalation exposure in mice and rats was achieved using a nose (snout)-only technique and in dogs using a face mask with a mouth piece or an oro-pharyngeal tube. Additional repeat-dose toxicity studies were conducted using the oral (gavage) and IV routes but were not considered necessary for the safety determination of olodaterol under this application.

In the mouse, a 13-week repeat-dose toxicity study was conducted in which CD-1 mice were treated with 0 (air or vehicle control), 63, 211, 900, or 3,258 µg/kg/day olodaterol (achieved doses). Olodaterol treatment resulted in a number of β₂-adrenergic-related anabolic effects such as increased body weights and increase in skeletal muscle mass at 3,258 µg/kg. The target organs of toxicity in the mouse include the liver (hepatocyte vacuolation, associated with an increase in bilirubin), thymus (lymphoid hyperplasia), salivary glands (inflammatory cell foci), lung (inflammatory cell foci), nasal cavity (hyaline droplets and regeneration), larynx (squamous metaplasia), and the female reproductive tract (numerous corpora lutea in the ovary and cystic glands/myometrial hypertrophy in the uterus). Although the sponsor identified the systemic NOAEL as the 63 µg/kg dose, because tissues of 63, 211, and 900 µg/kg groups were not examined for many of these findings, a NOAEL for systemic toxicities was not defined in the mouse. The findings in the nasal cavity were considered specific to the nose-only inhalation route in the rodent and not relevant for clinical administration (oral inhalation). Based on microscopic findings in the larynx and lungs, the NOAEL for local (respiratory tract) toxicities was identified at 63 µg/kg. The MTD in female mice was identified as the 3,258 µg/kg dose level, but the MTD was not identified in male mice due to lack of significant toxicity findings.

In the rat, 4-week, 13-week, and 26-week studies were conducted. Because similar findings were observed in all studies, only the 26-week study is discussed in detail for the purposes of this summary. In the 26-week study, Wistar Han rats were treated with 0 (vehicle control), 49, 200, or 3,400 µg/kg/day olodaterol (achieved doses). Olodaterol-related findings included increased food consumption and body weights in both sexes at all dose levels. Increased body weights were also observed in mice and dogs and are

considered due to the anabolic effect of LABAs. In addition, olodaterol-related effects were observed in skeletal muscle (hypertrophy and single cell necrosis) and were associated with a decrease in white adipose tissue. These effects are also associated with the anabolic effect of olodaterol. Skeletal muscle necrosis is considered secondary to hypertrophy as a result of low oxygen supply to the muscle. Literature reports note similar skeletal muscle findings in rodents treated with other drugs of the same class¹³.

Olodaterol-related effects were also observed in the heart (palpable heart beat immediately after dosing in both sexes at ≥ 200 $\mu\text{g}/\text{kg}$, increased heart weights, and microscopic findings of increased congestion at ≥ 200 $\mu\text{g}/\text{kg}$ and left ventricular scar formation at 3,400 $\mu\text{g}/\text{kg}$). Heart toxicity was also observed in the dog (see below) and is considered a class effect for LABAs due to activation of β_1 - and β_2 -adrenoreceptors in cardiac tissue. In the pancreas, lobular atrophy was observed in males and females at 3,400 $\mu\text{g}/\text{kg}$. This finding correlates with the high distribution in the pancreas and very slow elimination of the drug in this tissue. Further, olodaterol treatment resulted in increased incidence and severity of ovarian cysts, which correlates with findings observed with other drugs of the same class in the female rodent reproductive tract and correlates with tumor findings observed in rodents (see below). Most findings were recovered by the end of the recovery period, except for findings of reduced adipose tissue and skeletal muscle hypertrophy, which were only partially recovered.

Olodaterol-treated rats also had increased incidence and severity of epithelial atrophy in the cornea at all dose levels tested. Because β_2 -adrenergic receptors are expressed in the eye and because distribution studies in albino rats showed high distribution in the eye, an information request was sent to the applicant to ask for a toxicological assessment of this finding. In their response dated November 13, 2012; the applicant provided justification and supporting data to suggest that this finding is not of concern or relevant to humans. The epithelial atrophy was considered to be an effect of long term mechanical irritation in custom-made glass restriction tubes used for inhalation administration. According to the applicant, these tubes increased the mechanical contact between the ocular surface and the inner tube wall in cases of exophthalmos, which was observed in most olodaterol-treated animals as an effect of skeletal muscle hypertrophy around the eye. This rationale is supported by the lack of corneal findings in other rat inhalation studies using plastic restriction tubes (4- and 13-week rat studies and the 2-year rat carcinogenicity study). In addition, the applicant submitted another study conducted with the same glass restriction tubes in inhalation studies with salmeterol (an approved LABA). In this study, the same finding of epithelial atrophy in the cornea was observed in a dose-dependent manner in most treated animals at all

¹³ Owen K, Beck SL, and Damment SJP. (2010) The preclinical toxicology of salmeterol hydroxynaphthoate. *Human and Experimental Toxicology* 29(5); 393-407.

Yang YT, and McElligott MA. (1989) Multiple actions of β -adrenergic agonists on skeletal muscle and adipose tissue. *Biochemistry Journal* 261; 1-10.

Suzuki J, Gao M, Xie Z, and Koyama T. (1997) Effects of the β_2 -adrenergic agonist clenbuterol on capillary geometry in cardiac and skeletal muscles in young and middle-aged rats. *Acta Physiologica Scandinavica* 161; 317-326.

dose levels. Based on all the aforementioned, the applicant concluded that this finding is not of human relevance.

After review of the applicant's response, concern for long-term exposure of olodaterol and the development of corneal epithelial atrophy was alleviated, as the finding was not olodaterol-specific, was not observed in the 2-year rat carcinogenicity study (using doses that overlapped with those of the 26-week study), and was not associated with more severe corneal findings such as ulcers or inflammation. Therefore, the reviewer determined that this finding is not dose-limiting and is likely not relevant to humans using this drug via oral inhalation.

In the respiratory tract, a number of findings were observed: squamous cell hyperplasia/metaplasia, epithelial atrophy, degeneration, and regeneration in the larynx at all doses; epithelial atrophy, inflammation, luminal cellular debris, and squamous cell metaplasia in the nasal cavity at the LD and HD; and reversible squamous cell metaplasia in the trachea at 3,400 µg/kg. The findings in the larynx are considered an adaptive response in the rodent after inhalation exposure¹⁴ and are not considered related to olodaterol. The findings in the nasal cavity are considered specific to the route of administration in the rat (nose-only) and are not relevant for the clinical route (oral inhalation). However, the squamous cell metaplasia observed in the trachea was considered dose-limiting in the rat.

After consideration of all data, the systemic NOAEL of the 26-week rat study was identified at the dose level of 49 µg/kg, with an associated AUC_{0-24 hr} (at Day 177) of 9,010 pmol·hr/L in males and 7,880 pmol·hr/L in females (8,445 pmol·hr/L combined). This NOAEL is based on findings in the heart and pancreas. The local NOAEL was identified in the rat at 16.4 µg/kg (PDD) based on the finding of squamous cell metaplasia in the trachea. The sponsor identified the systemic NOAEL in the rat at 200 µg/kg and the local NOAEL at 49 µg/kg. The difference in NOAELs defined by this reviewer do not change the safety evaluation and final recommendation as there is adequate exposure margin compared to the proposed clinical dose (Table 38 below).

In the dog, 4-week, 13-week, and 52-week studies were conducted. Because similar (or less severe) findings were observed in shorter-duration studies, only the 52-week study will be discussed for the purposes of this integrated summary. In the 52-week study, Beagle dogs were treated with 0 (vehicle control), 15, 60, or 330 µg/kg/day olodaterol (achieved doses). Olodaterol-related findings included increased body weights (related to the anabolic effect of olodaterol), an effect that was also observed in

¹⁴ Lewis DJ. (1991) Morphological Assessment of Pathological Changes within the Rat Larynx. *Toxicologic Pathology* 19; 352-357.

Osimitz TG, Droege W, and Finch JM. (2007) Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: A critical review. *Toxicology and Applied Pharmacology* 225; 229-237.

Kaufmann W, Bader R, Ernst H, Harada T, Hardisty J, Kittel B, Kolling A, Pino M, Renne R, Rittinghausen S, Schulte A, Wohrmann T, and Rosenbruch M. (2009) 1st International ESTP Expert Workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. *Experimental and Toxicologic Pathology* 61; 591-603.

the mouse and the rat. In the heart, fibrosis and fibroplasia of the left ventricle were observed at $\geq 60 \mu\text{g}/\text{kg}$ and correlate with clinical signs of increased heart force and the increase in heart rate observed in males and females in electrocardiographic examinations. These findings of heart toxicity were associated with increases in serum creatine kinase and cardiac-specific troponin 1. ECG examinations also found multiple ventricular premature beats at $60 \mu\text{g}/\text{kg}$ and ventricular tachycardia at $330 \mu\text{g}/\text{kg}$. Cardiac findings are considered to be related to olodaterol treatment and are considered dose-limiting. These findings appear to be associated with activation of cardiac $\beta_{1,2}$ -adrenergic receptors. The increase in heart rate was more prominent during the first few weeks of the study, suggesting an adaptive response. In fact, several literature reports suggest an adaptive mechanism to increased sympathetic stimulation in the heart¹⁵.

Additional olodaterol-related findings in the dog included increased incidence and severity of mononuclear infiltration in kidneys (unilateral and bilateral) at $\geq 60 \mu\text{g}/\text{kg}$ (associated with an increase in serum creatinine), increased severity of liver glycogen storage and hemorrhage at $\geq 60 \mu\text{g}/\text{kg}$, and moderate palate inflammation with ulceration at $15 \mu\text{g}/\text{kg}$ and $330 \mu\text{g}/\text{kg}$. In addition, electrolyte changes (increased levels) were observed in olodaterol-treated females. Liver and palate microscopic findings were recovered after a 6-week recovery period.

Olodaterol-related effects were also observed locally in the respiratory tract and included atrophy of the nasal cavity at $82.6 \mu\text{g}/\text{kg}$ (PDD) and epithelial atrophy, infiltration, and mineralization in the trachea at $82.6 \mu\text{g}/\text{kg}$ (PDD). The finding in the nasal cavity is considered related to the route of administration in the dog (face mask) and is not relevant for the clinical route of administration (oral inhalation). However, microscopic findings in the trachea were considered dose-limiting.

After consideration of all findings in the dog, the systemic NOAEL was identified at $15 \mu\text{g}/\text{kg}/\text{day}$ based on findings in the heart, liver, and kidneys. This dose level is associated with an $\text{AUC}_{0-24 \text{ hr}}$ (at Day 358) of $2,160 \text{ pmol}\cdot\text{hr}/\text{L}$ in males and $3,560 \text{ pmol}\cdot\text{hr}/\text{L}$ in females ($2,860 \text{ pmol}\cdot\text{hr}/\text{L}$ combined). The local NOAEL was identified at $14.6 \mu\text{g}/\text{kg}$ (PDD). The sponsor defined the systemic NOAEL in the dog at $15 \mu\text{g}/\text{kg}$ and the local NOAEL at $330 \mu\text{g}/\text{kg}$. The difference in local NOAEL identified by this reviewer does not change the safety evaluation and final recommendation.

In summary, repeat-dose toxicity studies were conducted with olodaterol in three species: mouse, rat, and dog. The target organs of toxicity were identified as the heart (chronotropic effect that led to histopathology findings of fibrosis/fibroplasia over the long term), the female reproductive tract in rodents (which was also a target for

¹⁵ Bernstein D, Fajardo G, Zhao M, Urashima T, Powers J, Berry G, and Kobilka BK. (2005) Differential cardioprotective/cardiotoxic effects mediated by β -adrenergic receptor subtypes. *American Journal of Physiology – Heart and Circulatory Physiology* 289; H2441-H2449.

Spadari-Bratfisch RC, and Nunes dos Santos I. (2008) Adrenoreceptors and Adaptive Mechanisms in the Heart during Stress. *Stress, Neurotransmitters, and Hormones – Annals of the New York Academy of Sciences* 1148; 377-383.

neoplastic findings in the 2-year rodent bioassays), the liver, the kidney in dog, and the skeletal muscle in rat. In addition, in the respiratory tract; the nasal cavity, the larynx, and the trachea were affected by olodaterol. All these are common target organs of toxicity of β -adrenergic agonists. There are no apparent differences between the effects observed with olodaterol compared to other approved LABAs. A NOAEL for systemic toxicities was identified in the rat and the dog and provide adequate exposure margins compared to the exposure at the proposed clinical dose (refer to Table 38 below). Therefore, the safety concern for systemic toxicities in human is very low.

Table 38: Exposure margins for the proposed clinical dose of 5 $\mu\text{g}/\text{day}$ olodaterol – general toxicology studies.

Systemic and Local NOAELs	Safety margins
<p>Rat 26-week study Systemic NOAEL[#]: 4 $\mu\text{g}/\text{kg}$ ($\text{AUC}_{0-24, \text{ss}}$: 8,445 $\text{pM}\cdot\text{hr}$) Local NOAEL[§]: 2.73 $\mu\text{g}/\text{g}$ lung wt</p>	<p>AUC*: 63 Local**: 546</p>
<p>Dog 52-week study Systemic NOAEL[#]: 3.9 $\mu\text{g}/\text{kg}$ ($\text{AUC}_{0-24, \text{ss}}$: 2,860 $\text{pM}\cdot\text{hr}$) Local NOAEL[§]: 1.33 $\mu\text{g}/\text{g}$ lung wt</p>	<p>AUC*: 21 Local**: 266</p>

[#] = Systemic NOAEL in rats (4 $\mu\text{g}/\text{kg}$) is based on systemic toxicities observed in the heart and pancreas. Systemic NOAEL in dogs (3.9 $\mu\text{g}/\text{kg}$) is based on systemic toxicities observed in the heart, kidneys, and liver.

[§] = Local NOAEL in rats (16.4 $\mu\text{g}/\text{kg}$) is based on local toxicities observed in the trachea. Local NOAEL in dogs (14.6 $\mu\text{g}/\text{kg}$) is based on local toxicities observed in the trachea. The local NOAELs in $\mu\text{g}/\text{kg}$ are already adjusted to account for the inhaled dose that is deposited in the lungs of rodents (approximately 10%) and dogs (approximately 25%). These pulmonary deposited doses are then used to calculate the safety margins based on $\mu\text{g}/\text{g}$ lung weight using a lung weight of 1.50 g for rats and of 110 g for dogs.

* = $\text{AUC}_{0-24, \text{ss}}$ of 134.5 $\text{pM}\cdot\text{hr}$ in humans at 5 $\mu\text{g}/\text{day}$ (extrapolated from the AUC at 10 $\mu\text{g}/\text{day}$, assuming linear PK).

** = Based on 0.005 $\mu\text{g}/\text{g}$ lung wt olodaterol (using a lung weight of 1000 g for humans).

Genetic Toxicology

Olodaterol was negative in an *in vitro* bacterial mutagenicity test (Ames assay, Study U05-1046) and in an *in vitro* mouse lymphoma assay (Study U04-2188). In an *in vivo* rat micronucleus assay using the intravenous route of administration (Study U08-1834), olodaterol produced increased percentages of polychromatic erythrocytes (PCEs) in male rats at 10 and 40 mg/kg and a dose-dependent, statistically significant increase in the frequencies of micronucleated polychromatic erythrocytes (MNEs) at 24 and 48 hours post-dose. To explain that the observed response was not the result of DNA damage induced by olodaterol, the sponsor conducted two studies: a cardiovascular safety pharmacology study with rats and a non-GLP mechanistic toxicology study with rats using the intravenous route of administration (Study U09-1845). Review of data

from these studies and the published literature¹⁶ concluded that increases in the frequency of MNEs following treatment with olodaterol most likely occurred as a result of compensatory erythropoiesis and not as a result of a genotoxic event. Tachycardia and a decrease in blood pressure (which were also observed in the general toxicology studies discussed above) induced tissue hypoxia and erythropoietin, which in turn increased erythropoiesis. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Boehringer Ingelheim has concluded that olodaterol is not genotoxic and, taking into account all the aforementioned data, this reviewer agrees with this conclusion.

Carcinogenicity

Two 2-year carcinogenicity bioassays were conducted with olodaterol in rats and mice. In a 24-month inhalation carcinogenicity study in rats (Study U11-2661), CrI:WI(Han) rats were treated with 0 [vehicle control (0.01% benzalkonium chloride, (b) (4)% disodium EDTA, and (b) (4)% citric acid) or air control], 25.8, 75.9, and 270 µg/kg/day olodaterol (achieved doses) over 35 minutes. The pulmonary deposited doses were estimated as 2.58, 7.59, and 27 µg/kg/day. No statistically significant tumor findings were observed in males. In females, olodaterol induced leiomyomas of the mesovarian tissue in the 25.8 µg/kg/day and 270 µg/kg/day treatment groups. The trend and pairwise (HD vs. vehicle) assessments for this tumor finding are considered statistically significant ($p = 0.0092$ for the trend and $p=0.0494$ for the pairwise HD assessment; see statistical review under NDA 203-108, dated December 13, 2012). There was an increase in mortality in treated males relative to controls, but the increase was not dose-dependent or statistically significant. Female survival was not affected. Also, despite an increase in food consumption in olodaterol-treated rats, no changes were observed in body weights when compared to vehicle controls.

In a 24-month inhalation carcinogenicity study in mice (Study U12-1065), CrI:CD1(ICR) mice were treated with 0 [vehicle control (0.01% benzalkonium chloride, (b) (4)% disodium EDTA, and (b) (4)% citric acid) or air control], 26.1, 76.9, and 255 µg/kg/day olodaterol (achieved doses) over 45 minutes. The pulmonary deposited doses were estimated as 2.61, 7.69, and 25.5 µg/kg/day. No statistically significant tumor findings were observed in males. In females, olodaterol induced an increase of benign uterine leiomyomas at all dose levels compared to the vehicle control. Statistical significance was achieved at the high-dose level (255 µg/kg/day, $p = 0.0038$). Malignant leiomyosarcomas were also increased in olodaterol-treated groups, although statistical significance was only achieved at the 76.9 µg/kg/day dose level ($p = 0.0092$). The incidences of combined benign uterine leiomyomas and malignant leiomyosarcomas were higher at all dose levels when compared to the vehicle control group. Statistical

¹⁶ Tweats DJ, Blakey D, Heflich RH, Jacobs A, Jacobsen SD, Morita T, Nohmi T, O'Donovan MR, Sasaki YF, Sofuni T, and Tice R. (2007) Report of the IWGT working group on strategies and interpretation of regulatory in vivo tests I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. *Mutation Research* 627; 78-91.

significance was achieved at doses ≥ 76.9 $\mu\text{g}/\text{kg}/\text{day}$ ($p = 0.0003$ and 0.0018 for the MD and HD groups, respectively).

There were no dose-related increases in mortality in either male or female mice. Females dosed at the 76.9 $\mu\text{g}/\text{kg}/\text{day}$ dose level were terminated a week earlier due to excessive mortality. A slight (less than 10%) transient increase in body weight was observed in both sexes at ≥ 76.9 $\mu\text{g}/\text{kg}/\text{day}$ and was associated with an increase in food consumption.

The ECAC concurred that both the rat and mouse studies were acceptable, noting prior ECAC concurrence with the protocols. The ECAC concluded that the rat study was positive for olodaterol-induced leiomyomas of the mesovarian tissue in females. The ECAC concluded that the mouse study was positive for olodaterol-induced combined benign uterine leiomyomas and malignant leiomyosarcomas in females (refer to ECAC meeting minutes dated July 11, 2012).

Ovarian leiomyomas and uterine leiomyomas/leiomyosarcomas are considered rare tumors in female rodents. These tumor findings in the female rodent genital tract have been judged to be a class effect of β_2 -adrenergic receptors and have been observed with approved drugs of the same class. At this moment, there is no clinical evidence to suggest that these tumor findings are relevant to humans. Although Boehringer Ingelheim concludes that these studies did not reveal evidence of human relevant carcinogenicity, this reviewer considers that the relevance of these findings to human use is still unknown.

Table 39: Exposure margins for the proposed clinical dose of 5 $\mu\text{g}/\text{day}$ olodaterol – carcinogenicity studies.

Nonclinical Doses	Safety margins (AUC*)
Rat carcinogenicity study	
Low-Dose: 25.8 $\mu\text{g}/\text{kg}$ (AUC _{0-24, ss} : 2,650 pM·hr)	20
Mid-Dose: 75.9 $\mu\text{g}/\text{kg}$ (AUC _{0-24, ss} : 8,615 pM·hr)	64
High-Dose: 270 $\mu\text{g}/\text{kg}$ (AUC _{0-24, ss} : 28,800 pM·hr)	214
Mouse carcinogenicity study	
Low-Dose: 26.1 $\mu\text{g}/\text{kg}$ (AUC _{0-24, ss} : 5,715 pM·hr)	42
Mid-Dose: 76.9 $\mu\text{g}/\text{kg}$ (AUC _{0-24, ss} : 14,850 pM·hr)	110
High-Dose: 255 $\mu\text{g}/\text{kg}$ (AUC _{0-24, ss} : 52,000 pM·hr)	387

* = AUC_{0-24, ss} of 134.5 pM·hr in humans at 5 $\mu\text{g}/\text{day}$ (extrapolated from the AUC at 10 $\mu\text{g}/\text{day}$, assuming linear PK).

Reproductive and Developmental Toxicology

Studies of reproductive and developmental toxicology were conducted in rats and rabbits using the inhalation route of administration. These studies evaluated the effects of olodaterol on fertility in rats, teratogenicity in rats and rabbits, and prenatal and postnatal development in rats. Results show no olodaterol-related effects in male or female fertility in rats, teratogenicity in rats, or prenatal and post-natal development in

rats. In the rabbit, olodaterol was teratogenic at 2,489 µg/kg/day (achieved dose after inhalation exposure).

Effects on fertility

The effects of olodaterol on fertility and early embryonic development were evaluated using a rat model (Study U08-2297). Sprague-Dawley rats were administered 0 (vehicle control: 0.01% benzalkonium chloride and (b) (4) % disodium EDTA), 58, 193, or 3068 µg/kg/day olodaterol (achieved doses) over 35 minutes via snout-only inhalation exposure. The pulmonary deposited doses were estimated at 10% of the achieved doses (*i.e.*, 5.8, 19.3, and 306.8 µg/kg/day). Males were treated for 4 weeks prior to mating, then throughout the mating period until sacrifice after mating (up to 9 weeks of treatment). At necropsy, males were given a detailed post-mortem examination. The reproductive organs were weighed (testes, epididymis, seminal vesicles, and prostate) and histopathological examination of the testes and epididymis was performed. Females were treated for 2 weeks prior to mating, then throughout mating and until Day 7 of gestation (Day 0 = day of detection of mating). Females were sacrificed on Day 14, 15, or 16 of gestation and the reproductive tracts examined. During the study, the animals were monitored for clinical signs of toxicity, body weights, food consumption, and mating performance.

There were two olodaterol-related mortalities in the high-dose group, indicating that this dose level exceeded the maximum tolerated dose (MTD). In females, there were no olodaterol-related effects on estrous cyclicity, mating, or pregnancy performance. In males, a significant dose-related decrease in absolute epididymis weights was observed in treated animals compared to vehicle controls. Testes weights were also decreased in all treated groups compared to the vehicle control group. There were no microscopic findings associated with these organ weight effects, therefore the biological significance of these changes is unknown. Furthermore, male reproductive performance was not affected.

The NOAEL for paternal and maternal reproductive toxicity and pregnancy was the high-dose at 3,068 µg/kg/day olodaterol, corresponding to a pulmonary deposited dose of 306.8 µg/kg/day and a concentration of olodaterol ($C_{0.583 \text{ hr}}$) of 208,000 pmol/L in males and 167,000 pmol/L in females. This differed from the sponsor's NOAEL for parental toxicity (*i.e.*, the low-dose) but agreed with the sponsor's NOAEL for pregnancy (*i.e.*, the high-dose). A complete toxicokinetic analysis was not conducted for this study. Blood samples were collected from males on Days 26 and 62, and from females on Day 12, at 35 minutes and 24 hours from the start of inhalation exposure. Only data from olodaterol concentration at 35 minutes after inhalation exposure ($C_{0.583 \text{ hr}}$) were provided in the study report. At the Division's request, the applicant submitted AUC information under IND 76,362. The applicant gathered toxicokinetic data ($AUC_{0-24 \text{ hr}}$ and $C_{0.583 \text{ hr}}$) from all toxicology studies conducted in rats using the inhalation route of administration. These data were evaluated in Microsoft Excel using a linear regression model that was used to calculate the AUCs based on measured $C_{0.583 \text{ hr}}$ in study U08-2297. Thus, calculated $AUC_{0-24 \text{ hr}}$ values for study U08-2297 ranged between 5,876 to 7,798 pM·hr for the LD of 50 µg/kg (target dose), between 14,640 to 24,781 pM·hr for the MD of 200

µg/kg (target dose), and between 312,335 to 550,565 for the HD (target dose) of 3,000 µg/kg.

Because the HD level was associated with two olodaterol-related deaths, the NOAEL for effects on reproduction, fertility and pregnancy exceeded the MTD administered by inhalation in the rat. This is also supported by data from the 13-week MTD study in rats, in which the MTD was identified as 239 µg/kg/day olodaterol ($AUC_{0-24} = 37,400$ pmol·hr/L). Therefore, the study is considered valid for regulatory purposes. The results of this study show that olodaterol does not affect fertility in male or female rats. Other approved drugs of the same class had no effects in rat fertility studies.

Teratogenicity

The effects of olodaterol on development (teratogenicity) were evaluated in rats (Study U05-2534) and rabbits (Study U06-1019). Pregnant Sprague-Dawley rats were administered 0 (vehicle control: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA, and (b) (4) % citric acid), 64, 222, or 1,054 µg/kg/day olodaterol (achieved doses) over 1 hour via snout-only inhalation exposure. The pulmonary deposited doses were estimated at 6.4, 22.2, and 105.4 µg/kg/day (*i.e.*, 10% of the achieved). Dosing was performed from day 6 through day 17 of gestation (12 consecutive days) and necropsies were performed on gestation day 20 for reproductive assessment and fetal examination. Adult females were examined macroscopically. All fetuses were examined macroscopically at necropsy and subsequently by detailed internal visceral examination or skeletal examination. During the study, clinical signs of toxicity, body weights, and food consumption were monitored. Also, blood samples were taken from selected animals at the MD for toxicokinetic evaluation on day 12 of exposure (gestation day 17).

There were no olodaterol-related adverse effects on embryo-fetal survival or growth and on the incidence of malformations or variations. A dose-dependent slight increase in fetuses with incomplete ossification of sternebrae was observed at doses ≥ 222 µg/kg/day. However, this is considered a late developmental process and not a teratogenic effect. The developmental and maternal NOAEL in the rat was the 1,054 µg/kg/day dose, associated with an $AUC_{0-24 \text{ hr}}$ of approximately 530,250 pmol·hr/L (estimated from the quantified $AUC_{0-24 \text{ hr}}$ of 101,000 pmol·hr/L at 222 µg/kg/day, assuming linear pharmacokinetics). This is in agreement with the sponsor's NOAEL (*i.e.*, the high-dose). The results of this study show that olodaterol was not teratogenic in the rat at inhalation doses up to 1,054 µg/kg/day. Other approved drugs of the same class had no teratogenic effects in the rat.

Pregnant New Zealand White rabbits were administered 0 (vehicle control: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA, and (b) (4) % citric acid), 289, 974, or 2,489 µg/kg/day olodaterol (achieved doses) over 90 minutes via a snout-only inhalation exposure system. The pulmonary deposited doses were estimated at 10% of the achieved doses (*i.e.*, 28.9, 97.4, and 248.9 µg/kg/day). Dosing was performed from day 6 through day 19 of gestation (14 consecutive days). Necropsies were performed on gestation day 29 for reproductive assessment and fetal examination. Adult females

were examined macroscopically. All fetuses were examined macroscopically and subsequently examined for skeletal development and abnormalities. In addition, detailed internal visceral examination of the head was performed on approximately one third of the fetuses in each group. During the study, clinical signs of toxicity, body weights and food consumption were monitored. Also, blood samples were collected for toxicokinetic evaluation.

There were no olodaterol-related effects on mean corpora lutea counts, implantation counts, live litter size, mean sex ratio, or mean weights of placenta, litters, and fetuses. At 2,489 µg/kg/day, there were a greater number of fetuses and litters with major abnormalities compared to the controls. At this dose level, most of the fetuses clustered in a single litter and had a specific syndrome of abnormalities which had not been previously seen in this strain or source of rabbits. Eight fetuses in litter no. 53 had a combination of some or all of the following: uneven ossification of the cranial bones, ribs, long bones that included thickened ribs or distorted ribcage; short scapula, humerus, radius, ulna, femur, tibia, fibula; partially opened eyelids or eyelids not fused centrally (which correlates with the anabolic effect of olodaterol); cleft palate; forelimb flexure or malrotated hindlimbs. In addition, the following findings were observed in two fetuses in another litter (no. 9): dilated ascending aorta or aortic arch, narrow pulmonary trunk, enlarged left and small right heart ventricles, and the pulmonary arteries arising directly from the aortic arch (in one fetus). Also, one fetus from another litter (no. 69) presented the following abnormalities: acrania, absent eyes, split sternum, gastroschisis, cervicothoracic scoliosis and lordosis, forelimb flexure and hindlimb hyperflexion. In litter no. 82; one fetus had multiple retinal folds and another fetus had dilated ascending aorta or aortic arch, narrow pulmonary trunk, enlarged left ventricle, and small right atrium and right ventricle. Finally, in litter no. 99, one fetus had dorsoventral distortion of the sternum.

Some of these abnormalities (incidence of fetuses from two litters with great vessel abnormalities) were within the historical control range. However, the incidence of two litters containing fetuses with enlarged or small heart atria or ventricles was outside of the historical control range and thus these are considered related to olodaterol treatment. Eye abnormalities and split or distorted sternum are also considered to be treatment-related, as they were observed in more than one litter.

Based on the lack of toxicities in pregnant females, the maternal NOAEL was the high-dose (in agreement with the sponsor's NOAEL). Based on the aforementioned findings in fetuses at the HD, the developmental NOAEL for olodaterol in the rabbit is the mid-dose of 974 µg/kg/day ($AUC_{0-24 \text{ hr}}$ at this dose level is 182,000 pmol·hr/L; in agreement with the sponsor's NOAEL). The results of this study show that olodaterol, similar to other approved drugs of the same class, is teratogenic in the rabbit at high doses.

Effects on pre-natal and post-natal development

The effects of olodaterol on prenatal and postnatal development were evaluated using a rat model (Study no. U08-1971). Mated female Sprague-Dawley rats were administered 0 (vehicle control: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA, and (b) (4) %

citric acid), 59, 297, or 3,665 µg/kg/day olodaterol (achieved doses) over 35 minutes via snout-only inhalation exposure. The pulmonary deposited doses were estimated at 5.9, 29.7, and 366.5 µg/kg/day. Dosing was performed from gestation day 6 to 20, and then from lactation day 2 to 21 (Day 0 = day of birth of litter). The dams were sacrificed on day 21 of lactation. Two males and two females F₁ pups (when available) were randomly selected from each litter for follow-up observations. The F₁ pups not selected for post-weaning assessments were also sacrificed on day 21 of lactation. F₀ animals were monitored for survival, clinical signs of toxicity, body weights and food consumption. F₁ animals were monitored for normal growth and for physical and functional development. In addition, one F₁ set continued to be examined for reproduction function. A toxicokinetic analysis was not included in this study.

A significant increase in body weight gain was observed in olodaterol-treated female rats (F₀) compared to controls. Also, olodaterol-related clinical signs of toxicity were observed at the HD and included changes in behavior (subdued and excessive general behavior, including paddling) and eye(s) partially closed for the first 2-3 days after the start of dosing (which correlates with the anabolic effect of olodaterol observed in rats in the 26-week toxicity study). In addition, salivation and fur staining were observed in all olodaterol-treated groups.

There were slight differences in litter size by lactation day 21 and reduced survival in the LD and HD groups compared to the vehicle control group. Because of lack of a dose-response, these effects were considered unrelated to olodaterol treatment. In the HD group, litter and pup weights were slightly less, but not statistically significant, compared to the control animals by lactation day 21. A dose-related earlier time to eye opening was observed in both sexes in all olodaterol-treated groups, but was not associated with any ophthalmic findings. In F₁ animals, no olodaterol-related effects were observed on mating, fertility or bearing of live implants to day 14, 15, or 16 of gestation.

The NOAEL was identified as the HD (3,665 µg/kg/day) for maternal toxicity; F₁ survival and development; and F₁ mating, fertility, and pregnancy. This correlates with the sponsor's NOAEL (*i.e.*, the high-dose).

Table 40 below presents the exposure margins for the proposed clinical dose of 5 µg/day olodaterol. As shown, the exposure margins for reproductive and developmental toxicities are 1,353-fold to 3,942-fold for the expected clinical exposures. Therefore, the safety concern for possible human teratogenicity with olodaterol is very low.

Table 40: Exposure margins for the proposed clinical dose of 5 µg/day olodaterol – reproductive and developmental studies.

Nonclinical NOAELs	Safety margins
<p style="text-align: center;">Rat Fertility study</p> <p style="text-align: center;">3,068 µg/kg (AUC_{0-24, ss}: 312,335-550,565 pM·hr)</p>	<p style="text-align: center;">AUC*: 2,322</p>

Rat EFD study 1,054 µg/kg (AUC _{0-24, ss} : 530,250 pM·hr)	AUC*: 3,942
Rabbit EFD study 974 µg/kg (AUC _{0-24, ss} : 182,000 pM·hr)	AUC*: 1,353

* = AUC_{0-24, ss} of 134.5 pM·hr in humans at 5 µg/day (extrapolated from the AUC at 10 µg/day, assuming linear PK).

Local Tolerance

In addition to the standard toxicology studies, a number of special studies were conducted to assess the local tolerance of olodaterol. Only the eye and dermal studies were reviewed and are discussed in this review. Data from these two studies show that olodaterol is a mild to moderate irritant of the eye when applied locally, but has no effect on the skin under the conditions of the study.

Recommendations

The nonclinical safety program for olodaterol is considered complete and adequate to support the safety of the proposed clinical dose of 5 µg/day.

From the nonclinical pharmacology/toxicology perspective, the application is recommended for approval.

Labeling Review¹⁷

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Sponsor's Proposed Labeling Text:

Teratogenic Effects: Pregnancy Category C.

There are no adequate and well-controlled studies with (b) (4) RESPIMAT in pregnant women. (b) (4) RESPIMAT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

(b) (4) RESPIMAT was not teratogenic in rats (b) (4) at inhalation doses (b) (4)

Recommended Labeling Text:

Teratogenic Effects: Pregnancy Category C.

There are no adequate and well-controlled studies with (b) (4) STRIVERDI RESPIMAT in pregnant women. (b) (4) STRIVERDI RESPIMAT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

(b) (4) STRIVERDI RESPIMAT was not teratogenic in rats (b) (4) at inhalation doses (b) (4) approximately (b) (4) times the maximum recommended human daily inhalation dose (MRHDID) (b) (4) on an AUC basis (at a rat maternal inhalation dose of 1,054 mcg/kg/day). Placental transfer of STRIVERDI RESPIMAT was observed in pregnant rats.

STRIVERDI RESPIMAT has been shown to be teratogenic in New Zealand rabbits at inhalation doses approximately 7,130 times the MRHDID in adults on an AUC basis (at a rabbit maternal inhalation dose of 2,489 mcg/kg/day). STRIVERDI RESPIMAT exhibited the following fetal toxicities: enlarged or small heart atria or ventricles, eye abnormalities, and split or distorted sternum. No significant effects occurred at an inhalation dose approximately 1,353 times the MRHDID in adults on an AUC basis (at a rabbit maternal inhalation dose of 974 mcg/kg/day).

Rationale for Changes:

(b) (4)

¹⁷ The Package Insert submitted to the NDA on August 16, 2012 (Sequence 0007) was used for this review.

(b) (4)

8.3 Nursing Mothers

Sponsor's Proposed Labeling Text:

(b) (4)
human milk. (b) (4)
caution should be exercised
when (b) (4) RESPIMAT is administered to nursing women.

Recommended Labeling Text:

Olodaterol, the active component of STRIVERDI RESPIMAT, (b) (4)
Excretion of olodaterol into human milk is probable. There are no human
studies that have investigated the effects of STRIVERDI RESPIMAT on nursing infants.
(b) (4)
Caution should be exercised
when (b) (4) STRIVERDI RESPIMAT is administered to nursing women.

Rationale for Changes:

(b) (4)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Sponsor's Proposed Labeling Text:

Olodaterol (b) (4)
1-adrenoceptors and 1622-fold greater agonist
activity compared to beta₃-adrenoceptors. 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 adenylyl 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.

(b) (4)

Beta-adrenoceptors are divided into three subtypes: beta₁-adrenoceptors predominantly
expressed on cardiac smooth 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.

Recommended Labeling Text:

Olodaterol is a long-acting beta₂-adrenergic agonist (LABA).

(b) (4)
(b) (4)
(b) (4)

(b) (4)

Beta-adrenoceptors are divided into three subtypes: beta₁-adrenoceptors predominantly expressed on cardiac smooth 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.

Rationale for Changes:

(b) (4)
(b) (4)
(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's Proposed Labeling Text:

[Redacted] (b) (4)

[Redacted] (b) (4)

[Redacted] (b) (4)

[Redacted] (b) (4)

Recommended Labeling Text:

[Redacted] (b) (4)

Two-year inhalation studies were conducted in rats and mice to assess the carcinogenic potential of olodaterol. Lifetime treatment of female rats induced [Redacted] (b) (4) leiomyomas of the mesovarium at doses of 25.8 and 270 mcg/kg/day (approximately 18-and 198-fold, respectively, the MRHDID in adults on an AUC basis) [Redacted] (b) (4)

[Redacted] No tumor findings were observed in male rats at doses up to 270 mcg/kg/day (approximately 230-fold the MRHDID in adults on an AUC basis). Lifetime treatment of female mice induced [Redacted] (b) (4) leiomyomas, and leiomyosarcomas) of the uterus at doses \geq 76.9 mcg/kg/day (approximately 106-fold the MRHDID in adults on an AUC basis) [Redacted] (b) (4)

[Redacted]

(b) (4)

No tumor findings were observed in male mice at doses up to 255 mcg/kg/day (approximately 455-fold the MRHDID in adults on an AUC basis). Increases in leiomyomas and leiomyosarcomas of the female rodent reproductive tract have been similarly demonstrated with other β_2 -adrenergic agonist drugs. The relevance of these findings to human use is unknown.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Olodaterol was not mutagenic in the *in vitro* (Ames test, or in the *in vitro* mouse lymphoma assay)

(b) (4)

Olodaterol produced increased frequency of micronuclei in rats after intravenous doses. The increased frequency of micronuclei

-was likely related to drug enhanced (compensatory) erythropoiesis. This 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 3,068 mcg/kg/day (approximately 2,322 times the MRHDID in adults on an AUC basis).

Rationale for Changes: (b) (4)

(b) (4)

(b) (4)

(b) (4)

Also, language was inserted to describe the lack of tumor findings in male rats and male mice.

(b) (4)

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12 Appendix/Attachments

Appendix 1: Pharm/Tox Review of IND 76,362 dated February 28, 2007 (Initial Safety Review)

Appendix 2: Pharm/Tox Review of IND 76,362 dated March 2, 2007 (SPA Review)

Appendix 3: Pharm/Tox Review of IND 76,362 dated February 17, 2009 (Request for ECAC Feedback)

Appendix 4: Pharm/Tox Review of IND 76,362 dated May 10, 2010 (Genetic Toxicology Review)

Appendix 5: Pharm/Tox Review of IND 76,362 dated October 26, 2011 (Reproductive Toxicology Review)

Appendix 6: Pharm/Tox Review of IND 76,362 dated October 28, 2011 (Chronic Toxicology Review)

Appendix 7: Pharm/Tox Review of IND 76,362 dated May 11, 2011 (Carcinogenicity Review)

Appendix 1

Pharm/Tox Review of IND 76,362 dated February 28, 2007 (Initial Safety Review)

***DIVISION OF PULMONARY AND ALLERGY PRODUCTS
PRELIMINARY PHARMACOLOGY SAFETY REVIEW***

IND: 76,362

Drug: BI 1744 Cl

Drug Category: Long acting β_2 agonist

Review completion date: February 28, 2007

Boehringer Ingelheim Pharmaceuticals, Inc. (BI) proposed a Phase 2 clinical study in ~400 adult patients (males and females \geq 40 years of age) with chronic obstructive pulmonary disease (COPD) to assess the safety and efficacy of BI 1744 Cl, a long acting β_2 agonist (LABA). Nonpregnant females on an adequate method of birth control will be included in the study. BI 1744 Cl will be administered via inhalation using the Respimat inhaler at once daily doses of 0 (placebo), 2, 5, 10 and 20 μg for 4 weeks. The primary objective of this study is to determine the optimum dose of BI 1744 Cl.

Three clinical trials have been completed outside of the U.S. Study no. 1222.1 was a single dose study in healthy male and female volunteers (0.5 to 70 μg BI 1744 Cl). Study no. 1222.2 was a multiple rising dose study in healthy male and female volunteers (2.5, 10 and 30 μg BI 1744 Cl for 14 days). Study no. 1222.3 was a single dose study in COPD patients (2, 5, 10 and 20 μg of BI 1744 Cl). There were slight increases in heart rate and in QTcF prolongation (7 ms to 13 ms) with no sign of dose dependency in males treated with \geq 30 μg . Adverse events included increased upper airway secretion, throat irritation, dizziness, headache, nausea, restlessness, palpitations, vertigo, tremor, rash and dry mouth or throat. At a single inhalation dose of 40 μg in healthy subjects, an AUC_{0-Inf} of 400 to 500 pmol*h/L was observed. The proposed 20 μg daily dose is expected to produce an AUC of 250 pmol*h/L.

In vitro pharmacology studies showed BI 1744 Cl as a potent agonist of the human β_2 adrenoceptor with an EC₅₀ = 1.0 \pm 0.4 nM and an intrinsic activity of 68% compared to isoprenaline. BI 1744 stimulated the human β_1 -adrenoceptor with an EC₅₀ = 60 \pm 24 nM and had an intrinsic activity of 16% compared to isoprenaline, demonstrating that BI 1744 is selective for the β_2 receptor. Using the guinea pig and anesthetized dog acetylcholine-induced bronchospasm models, BI 1744 Cl was shown to have antagonistic effects against bronchospasm *in vivo*.

Nonclinical safety studies including central nervous system (CNS), cardiovascular, respiratory, renal, liver and gastrointestinal (GI) safety studies were performed to assess the effects of orally inhaled (IH) and subcutaneously (SC) administered BI 1744 Cl. Notable BI 1744 induced safety effects were observed on the CNS, cardiovascular, renal, liver and GI systems as summarized in the following table.

Study/Species	Doses*	Observations
U05-2401 CNS/Modified Irwin in Wistar Rats	0, 1.71, 6.34, 48.3 $\mu\text{g}/\text{kg}$, IH	<ul style="list-style-type: none"> At 6.34 and 48.3 $\mu\text{g}/\text{kg}$, body temperature \downarrow at 150 and 300 minutes post-dose
U04-1815	0.001- 0.01	<ul style="list-style-type: none"> At 0.01 mg/kg, \downarrow motor coordination and motor

Study/Species	Doses*	Observations
CNS/Modified Irwin in mice	mg/kg, SC	activity at 60 min post-dose
U05-2398 Respiratory studies in conscious Wistar rats	0.1, 0.72, 6.43 and 48.5 µg/kg, IH	<ul style="list-style-type: none"> No effect.
U04-1819 Respiratory studies in conscious Wistar rats	27.0 ad 273.0 µg/mL, IH	<ul style="list-style-type: none"> No effect.
U06-1058 Cardiovascular/telemetry in conscious dogs	0, 0.215, 0.74 and 2.13 µg/kg, IH	<ul style="list-style-type: none"> At 0.74 and 2.13 µg/kg, ↑ HR lasting up to 24 h post-dose; ↓ blood pressure, ↓ RR interval; ↓ QT- interval
U04-1052 Cardiovascular in conscious dogs	0.138 to 0.55 µg/kg, IH	<ul style="list-style-type: none"> At doses of 0.55 µg/kg, systolic blood pressure ↓ by 20% and diastolic blood pressure ↓ by 25% up to 14 h post-dose; Heart rate ↑ by 88%, ↑ contraction force by 33% and ↑ QRS-duration by 6% at HD
U04-1819 Cardiovascular in conscious rats	27.0 to 273.0 µg/mL, IH	<ul style="list-style-type: none"> Marked hypotension (-19%) at HD that lasted over 7 h and ↑ heart rate (50%) at HD from 2 to 7 h post-dose
U04-1436 Renal and liver function in conscious rats	27.0 to 273.0 µg/mL, IH	<ul style="list-style-type: none"> Transient antidiuretic effect up to 8 h post-dose at all doses ↓ albumin excretion at all doses; ↑GGT- and ALP-excretion in the HD
U04-1432/U04-1430 GI effects conscious rats	27 to 273.0 µg/mL, IH	<ul style="list-style-type: none"> ↓ gastric emptying and intestinal transit at doses >91 µg/mL; ↓ gastric secretion at doses ≥91 µg/mL

*Inhaled doses as the pulmonary deposited dose.

Review of the relevant repeat dose inhalation toxicology studies in the rat (4-week and 13-week) and dog (4-week) results 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. The 4-week rat study dosed animals with 0 (vehicle control: 0.01% benzalkonium chloride, (b) (4) % 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. There were no clinical signs of toxicity and no premature deaths in this study. Body weights gains were increased compared to control values for both males (22-32%) and females (44-56%) at all doses by Day 28, which coincided with an increase in food consumption by 12-16%. HD males had an increase in monocyte levels by 89% compared to the vehicle control (VC) group. Males and females from all dose groups showed a decrease in serum glucose levels by 12-17% and 12-25%, respectively, compared to the VC groups. An increase in absolute heart weights (+19% HD males and 15% HD females), lung weights (+12% HD males) and a decrease in absolute thymus weights (-25% HD males) was observed. Relative organ weight to brain weight comparisons showed an increase in heart weights in HD males (+21%) and females (+13%) compared to the VC groups. By Week 4 of the study, the respiratory rate had decreased compared to the VC groups in the male and female MD and HD groups by 44 and 41%, respectively. Histopathology data showed minimal heart cardiomyopathy in the HD male group and hemopoiesis of the spleen that increased in incidence and severity with dose in males. Local toxicity was observed in the larynx (squamous metaplasia in

males, necrosis of U-shaped cartilage in males and females, and ventral pouch inflammation in males and females) and the lungs (alveolar macrophage accumulation).

Findings	VC		7.71 µg/kg		26.2 µg/kg		133.5 µg/kg	
	M	F	M	F	M	F	M	F
Heart n=10 Cardiomyopathy min	0	0	-	-	-	-	2	0
Spleen n=10 Hemopoiesis Min	1	2	4	-	6	-	3	2
Slight	1	3	0	-	2	-	6	1
Larynx n=10 Squamous metaplasia Min	0	2	0	0	2	1	3	0
Slight	0	0	0	1	0	1	1	0
Larynx n=10 Ventral pouch inflam	1	0	0	0	0	0	3	1
Larynx n=10 Cartilage necrosis Min	1	1	0	0	0	1	0	1
Slight	0	0	0	0	2	0	2	4
Lung n=10 Focal alveolar macrophage accumulation Min	1	2	2	5	3	5	5	5
Slight	0	0	0	0	0	0	0	1

-: Not examined

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 (submission no. 001) on December 27, 2006 in support of their proposed 2-year 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 clinical signs of increased salivation and increased heart contractile force. An increase in body weight gains versus control values was observed in MD (100% Males and females) and HD (130% males and 80% females) animals. 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. At Day 3 of dosing, HD males and females had an increase in K⁺ (12-14%), phosphate (29-41%) and a decrease in total bilirubin (25-39%). By Day 28, HD males had increased K⁺ (12%), creatinine (15%), phosphate (20%) and CPK (89%). Clinical chemistry parameters in females had returned to normal by Day 28. The histopathology findings are summarized in the following table.

Findings	VC		0.55 µg/kg		3.43 µg/kg		31.8 µg/kg	
	M	F	M	F	M	F	M	F
Esophagus submucosal gland adenitis, focal	0	0	1	0	0	0	2	0
Kidney inflam, subcapsular chronic focal	0	0	0	0	0	0	1	0
Liver								
Glycogen, periportal diffuse								
Min	0	0	0	0	0	0	0	1
Mild	0	0	0	0	0	1	0	1
Glycogen periportal focal								
Min	0	0	0	0	0	0	0	1
Gall bladder	0	0	0	0	0	0	1	0
Epithelial vacuolation								
Larynx laryngitis								
Min	0	0	0	0	0	0	1	0
mild	0	0	0	0	0	0	1	0
Lung Pleural fibrosis, focal	0	0	0	0	0	0	0	1

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 rat and dog repeat dose studies the animal to human safety ratios were determined and are provided in the table below.

	Systemic NOAEL (AUC)	Safety Ratio (AUC)	Safety Ratio (Lung Burden)
Human Dose 20 ug/day	250 pmol*h/L	-	-
Rat 4-wk Systemic NOAEL= 133.5 µg/kg Local NOAEL= 7.71 µg/kg	148,500 pmol*h/L	594	46
Dog 4-wk study Systemic NOAEL= 0.55 µg/kg Local NOAEL= 3.43 µg/kg	309 pmol*h/L	1.24	11.14

- Not applicable

A complete standard genetic toxicology battery (microbial mutagenesis assay, *in vitro* mouse lymphoma assay and an *in vivo* rat micronucleus assay) was performed. 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. 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 the indication of COPD, this deficiency is not a clinical hold issue.

Two teratology studies were conducted in rat (0 (VC), 6.4, 22.2 and 105.4 µg/kg of BI 1744 Cl as pulmonary deposited doses) and rabbit ((0 (VC), 28.9, 97.4 and 248.9 µg/kg of BI 1744 Cl as pulmonary deposited doses) to assess the effects of BI 1744 Cl on embryo-fetal development. BI 1744 Cl showed signs of teratological effects in both rats and rabbits that are typical of the LABAs. In rats, umbilical hernia, anomalous confluence of umbilical and/or hepatic vein with inferior vena cava and slight increase of incomplete ossification of sternbrae compared to controls was noted for the MD and HD groups. The sponsor indicated that these findings were within historical controls. In rabbits, fetuses had low body weights and showed evidence of major abnormalities that included thickened ribs, distorted rib cage, short or bent scapula, humerus, radius, ulna, femur, tibia, fibula and/or limb flexure and cleft palate. The sponsor reported in the Investigator's Brochure that these findings in the rabbit are teratological effects that are common to the LABA drug class.

Recommendation: Based on the calculated safety margins using systemic exposures (AUCs) and lung burden ratios, the proposed clinical trial is supported by the 4-week and 13-week repeat dose inhalation studies in rats and the 4-week repeat dose inhalation study in dogs. The sponsor should monitor heart rate. Additionally, the potential for BI 1744 Cl to induce teratogenic and embryo-lethal effects should be included in the Informed Consent. The potential target organs of toxicity include the heart, lungs, larynx, liver, kidneys, lymph and uterus. These issues were discussed with the Medical Officer. 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.

Non-hold Comments to the Sponsor:

- Your *in vivo* rat micronucleus study conducted as part of the 4-week repeat dose inhalation toxicology study is not considered valid since the test did not use an acceptable limit dose or a dose that produced a dose-limiting toxicity for this endpoint. Therefore, provide adequate justification for the selected doses of this assay based on accepted criteria for evaluation of this endpoint or conduct an additional *in vivo* test for chromosomal damage using accepted criteria for dose selection as part of your on-going drug development program.
- Include a discussion in your Informed Consent regarding the potential of BI 1744 Cl to induce teratologic and/or embryo-lethal effects.

Molly E. Shea, Ph.D.

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

/s/

Molly Shea
2/28/2007 11:48:50 AM
PHARMACOLOGIST

Timothy McGovern
2/28/2007 12:45:48 PM
PHARMACOLOGIST
I concur

Appendix 2

Pharm/Tox Review of IND 76,362 dated March 2, 2007 (SPA Review)

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 76,362 (pre-IND)

Review number: 1

Sequence number/date/type of submission: SN001/December 27, 2006
SN002/January 4, 2007
SN003/January 4, 2007

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Boehringer Ingelheim

Manufacturer for drug substance: not provided

Reviewer name: Molly E. Shea, Ph.D.

Division name: Division of Pulmonary and Allergy Products (DPAP)

Review completion date: March 02, 2007

Drug:

Trade name: not applicable

Generic name: NA

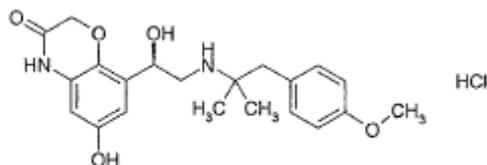
Code name: BI 1744 Cl

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

CAS registry number: NA

Molecular formula/molecular weight: C₂₁H₂₆N₂O₅ X HCl/422.9 g/mol (salt) and 386.5 g/mol (free base)

Structure:



Relevant INDs/NDAs/DMFs: NA

Drug class: Long-acting, human β_2 - adrenoreceptor partial agonist

Intended clinical population: Chronic obstructive pulmonary disease (COPD) and bronchial asthma

Clinical formulation: NA

Route of administration: Oral Inhalation

Proposed clinical protocol: None at this time.

Previous clinical experience: Currently, 3 clinical trials have been completed outside of the U.S.: study no. 1222.1 was a single dose study in healthy male and females volunteers (0.5 to 70 µg BI 1744 CL); study no. 1222.2 was a multiple rising dose study in healthy male and female volunteers (2.5, 10 and 30 µg BI 1744 CL for 14 days), and study no. 1222.3 was a single dose study in COPD patients (2, 5, 10 and 20 µg of BI 1744 Cl). The proposed daily therapeutic dose range is expected to be between 10 and 40 µg per subject. At a single inhalation dose of 40 µg in healthy subjects, an AUC_{0-Inf} of 400 to 500 pM*h was observed. The sponsor based the mouse to human AUC ratios on this value at 40 µg BI 1744 Cl.

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

Studies reviewed within this submission:

Study 666408 (b) (4): BI 1744 Cl: 13-week inhalation MTD study in mice (SN001)

Study 666413 (b) (4): BI 1744 Cl: 13-week inhalation MTD study in rats with a 4-week recovery period (SN001)

Studies not reviewed within this submission: None.

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2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

PK studies of BI 1744 Cl investigated in mice, rats and in humans were not submitted for review but summarized by the sponsor. The plasma protein binding is low in mice, rats and man (50-70%). BI 1744 BS is the dominant compound in plasma, bile and feces after intratracheal and intravenous administration of ¹⁴C BI 1744 Cl to mice and rats. In addition to BI 1744 BS, its glucuronide CD 992, its de-methylated product SOM 1522 and the glucuronide of SOM 1522, a glucuronidated moiety M565(2) and a polar unknown, not extractable metabolite (m6) that is covalently bound to microsomes, were identified as the major metabolites in mice and rats based on in vivo studies. In addition to these metabolites, in vitro rat liver microsome and hepatocyte studies identified two glutathione adducts M694 (1) and M694 (2) as stabilization products of a reactive quinonimine-intermediate. Using human liver microsomes in an in vitro system, the human CYP enzymes (CYP 2C8, 2C9, 3A4) are involved in metabolism of the BI 1744 Cl. From a single-inhalation study in healthy male volunteers, the parent compound, BI 1744 BS) and its glucuronide, CD 922, were found in urine and BI 1744 BS was found in plasma. There was no metabolism by human lung microsomes.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Study title: BI 1744 Cl: 13-week inhalation MTD study in mice

The dose levels selected for this study were based on a single-dose inhalation study in mice up to 54.4 mg/kg. No lethal dose was observed in that study. At this dose there were clinical signs of toxicity with decreased motor activity, abdominal breathing, piloerection and tremor. The HD of 3.2 mg/kg was hypothesized to induce a systemic effect.

Key study findings:

- ↑ group mean BW gains for males and females compared to AC and VC groups, which is a known exaggerated pharmacological effect of the LABAs.
- ↑ total bilirubin levels in males and females compared to AC and VC groups.
- ↑ absolute weights of the lung and spleen for both males and females; and ↑ relative organ weights to BW and to brain weights for ovaries and uteri in females
- Target organs of toxicity were the larynx, nasal cavity, lung, liver, lymphoid, ovary and uterus.
- The MTD in females is considered the HD (3258 µg/kg/day), due to hepatocyte vacuolation and ↑total bilirubin levels. A MTD was not identified in males.
- No NOAEL for systemic effects could be determined due to the absence of histological data in the LD, MD1 and MD2 groups.
- The NOAEL for local toxicity was considered the LD (63 µg/kg/day).

Study no.: 666408 (b) (4) report number 26716)

Volume #, and page #: Vol. 1 and page 2

Conducting laboratory and location: (b) (4)

Date of study initiation: January 17, 2006

GLP compliance: Performed under GLP conditions but draft report

QA report: yes () no (X)

Drug, lot #, and % purity: BI 1744 Cl, batch no. 05_Zo07, and >99.26% pure

Methods

Achieved Doses: 0 (air control), 0 (vehicle control), 63, 211, 900 and 3258 µg/kg/day; pulmonary deposited doses based on 10% lung deposition in rodents are 6.3, 21.1, 90.0 and 325.8 µg/kg/day

Species/strain: Mice/CD-1

Number/sex/group or time point (main study): 15 mice/sex/group

Route, formulation, volume, and infusion rate: Inhalation (snout-only), BI 1744 Cl in 0.01% benzalkonium chloride, (b) (4)% EDTA and citric acid to adjust pH as aerosol and nebulized for 35 minutes

Satellite groups used for toxicokinetics or recovery: 10 mice/sex/group for TK analyses

Age: 4-5 weeks old

Weight: Males: 16-22 g/Females: 14-23 g

Sampling times: See below

Unique study design or methodology: Mice were nose-only exposed using a flow-past system to administer either air as a control, aerosolized vehicle as a control or a BI 1744 Cl inhalation spray for 35 min/day for at least 91 days. A 7th group of mice were used as the sentinel group to monitor environmental and animal contamination with drug. These animals were not dosed or exposed to nebulization. Aerosols were generated using 2 modified Upmist airjet nebulizers. The method of analysis of BI 1744 Cl concentration and particle size was by (b) (4)

Samples were taken for 10 minutes to ensure that there was no overloading of the filter. Particle size distribution of test aerosols was assessed throughout the study using a (b) (4). Measurements were taken at least once weekly. The achieved dose was calculated based on the measured aerosol concentration multiplied by respiratory minute volume (RMV) multiplied by exposure time and divided by actual animal body weight. The McMahon's formula was used to calculate RMV. Mid-week body weights were taken for each animal and used for calculating each dose.

Group	Daily exposure time (min)	Target aerosol concentration (µg/L)	Target dose (µg/kg)
Air-control (AC)	35	0	0
Vehicle control (VC)	35	0	0
Low-dose (LD)	35	1.42	50
Mid-dose1 (MD1)	35	4.75	200
Mid-dose2 (MD2)	35	20.52	800
High-dose (HD)	35	73.51	3200

Observation, Times and Results:

Aerosol mass concentration and estimated inhaled dose:

Parameter	Treatment group					
	AC	VC	LD	MD1	MD2	HD
Analyzed aerosol concentration [$\mu\text{g/L}$]	0	0	1.42	4.75	20.52	73.51
Achieved daily dose of BI 1744 Cl [$\mu\text{g/kg/d}$]	0	0	62.9	211	900	3258
Estimated deposited pulmonary dose of BI 1744 Cl ($\mu\text{g/kg/d}$)*	0	0	6.29	21.1	90.0	325.8
MMAD \pm GSD (μm)**	NA	NA	(b) (4)			
MMAD \pm GSD (μm ***)	NA	NA				

- *Deposited dose based on deposition fraction of 0.1
- ** Analytical results
- *** Gravimetric results

The achieved doses were ~26% above the target dose for the LD group and 13% above the target dose for the MD2 group. The MD1 and HD groups achieved doses were within 10% of the target doses.

Aerosol particle size: The mass median aerodynamic diameter (MMAD) \pm the geometric standard deviation (GSD) was (b) (4) respectively for the low-dose (LD), mid-dose1 (MD1), mid-dose2 (MD2) and high-dose (HD). Particle size distributions were based on both gravimetric and analytical measurements. The particle size showed high variability. However, the MMAD for each dose was within respirable range (b) (4) with some variability exceeding the respirable range.

Measured vs deposited dose: The pulmonary deposited doses were calculated by the reviewer by assuming that 10% of the delivered dose is inhaled by mice. Therefore, the estimated pulmonary deposited doses of the BI 1744 Cl drug product were 6.29, 21.1, 90.0 and 325.8 $\mu\text{g/kg/day}$ for the LD, MD1, MD2 and HD groups, respectively.

Mortality: All animals were checked early morning and as late as possible each day for viability.

There were no deaths prior to scheduled sacrifice.

Clinical signs: Each animal was examined at least once before exposure, continuously during exposure and at approximately 1 h after exposure for clinical signs of toxicity. The onset, intensity and duration of any signs were recorded for each animal.

Males in the vehicle control, LD, MD1, MD2 and HD groups showed excessive salivation in all animals compared to 2 animals in the air control group. All females in the treatment groups showed excessive salivation, which was absent in the vehicle control group and present in 2/15 females in the air control group. Males and females in the AC

showed excessive salivation on Day 49 alone and males VC group also showed excessive salivation on Day 49 alone. The LD, MD1, MD2 and HD groups showed excessive salivation starting Day 30 that continues to Day 40 in males and females. HD males (15/15) and MD2 (1/15) and HD females (7/15) showed increased muscle mass (mild) which was not observed in the control or other treated groups.

Body weights: Body weights were recorded for all treated animals once weekly throughout the study period.

%Δ Body Weight Gain vs Controls

Main study group	LD		MD1		MD 2		HD	
	M	F	M	F	M	F	M	F
Compared to air control	133%	50%	133%	50%	166%	75%	133%	75%
Compared to vehicle control	40%	20%	40%	20%	60%	40%	40%	40%

Both males and females had an increase in body weight gains compared to both air and vehicle controls. This increase is typical for long-acting β_2 agonists (LABA).

Food consumption: The quantity of food consumed was recorded once weekly throughout study. Individual data were reported for males. Female food intake was averaged per cage.

There were transient increases in food intake in the BI 1744 Cl treated groups during Weeks 3, 4, 5, 6 and 7 of the study compared to air and vehicle control treated animals. However, these increases showed significant variability per group and did not reach statistical significance in males. Females in the HD group had a statistically significant increase in food consumption compared to air (ranged from +38 to +44%) and vehicle (ranged from +30 to +35%) controls during Weeks 4 through 10 of the study.

Ophthalmoscopy: Both eyes of each animal were examined once pretrial and during Week 13 of study. Examination used indirect ophthalmoscope after the application of mydriatic agent (Mydriacyl-1%) by a veterinarian.

There were no drug-related effects observed.

EKG: NA

Hematology: Blood samples were taken from the orbital sinus prior to animal sacrifice during Week 14 of study. A complete hematological battery was evaluated with the exception of a blood clotting time sample. However, platelet counts were recorded. Bone marrow smears were also prepared from femur taken at necropsy and were analyzed only if hematological findings were noted. A total of 7 animals/sex/group were sampled.

Statistically significant increases in white blood cell counts (WBC) and neutrophil counts compared to air- or vehicle controls were observed in male mice but not in female mice. A dose related increase was observed from the LD to the MD2 group but not in the HD group for either WBC or neutrophil counts. These changes are most likely not related to treatment since there is no dose-dependent increase observed and the findings are exclusive to males.

%Δ Hematology parameters compared to Air or Vehicle Controls

Parameters		LD		MD1		MD 2		HD	
		M	F	M	F	M	F	M	F
WBC	Compared to AC	+74%	NSC	105%	NSC	140%	NSC	+19%	NSC
	Compared to VC	+30%	NSC	+54%	NSC	+79%	NSC	NSC	NSC
Neutrophils	Compared to AC	+52%	NSC	+115%	NSC	+54%	NSC	NSC	NSC
	Compared to VC	+59%	NSC	125%	NSC	+61%	NSC	NSC	NSC

NSC- No statistically significant change.

Clinical chemistry: Blood samples were taken from the orbital sinus prior to animal sacrifice. A total of 8 animals/sex/group were evaluated. A complete clinical chemistry battery was examined with the exception of blood urea nitrogen. However, urea levels were evaluated.

A slight increase in potassium (K) levels was observed in both males and females treated with BI 1744 CI compared to either air or vehicle control animals. Total bilirubin levels were increased in both males and females compared to air and vehicle control animals. However, there was no dose-related increase for either potassium or bilirubin observed in either males or females. Phosphate levels were significantly increased in HD males compared to either vehicle or air control animals.

%Δ Clinical chemistry parameters compared to Air or Vehicle Controls

Parameters		LD		MD1		MD 2		HD	
		M	F	M	F	M	F	M	F
Phosphate	Compared to AC	NSC	NSC	NSC	NSC	NSC	NSC	+21%	NSC
	Compared to VC	NSC	NSC	NSC	NSC	NSC	NSC	+27%	NSC
Total bilirubin	Compared to AC	+34%	+13%	NSC	-36%	NSC	NSC	+44%	+40%
	Compared to VC	+65%	+79%	+27%	NSC	+31%	+53%	+77%	+121%

NSC- No statistically significant change.

Urinalysis: Not performed.

Gross pathology: All surviving animals were sacrificed on Days 92, 94 or 95 by carbon dioxide asphyxiation and subject to detailed necropsy. At necropsy, the only findings that showed an increase with respect to dose were an increase in muscle mass for (HD only) and thickening or enlarged uterine horns (MD2 and HD).

Findings	AC		VC		63 µg/kg		211 µg/kg		900 µg/kg		3258 µg/kg	
	M N=15	F N=15	M N=15	F N=15								
Skeletal muscle Increased mass	0	0	0	0	0	0	0	0	0	0	1	1
Uterus Thickened, one/both horns	-	0	-	0	-	0	-	0	-	0	-	8
	-	0	-	0	-	0	-	0	-	2	-	0
Enlarged, both horns												

-: Not examined/Not applicable

Organ weights: Organs were weighed from all main study animals. These included: brain, epididymus, heart, kidneys, liver plus gall bladder, lungs, ovaries, prostate, spleen, submaxillary salivary gland, testis, thymus and uterus plus cervix.

%Δ in absolute organ weights relative to controls (AC or VC)

Organ	Group	LD	MD1	MD2	HD
Lung compared to AC	M	+21%	+26%	+42%	+16%
	F	28%	+33%	+28%	NSC
Lung compared to VC	M	+15%	+20%	+35%	+10%
	F	+21%	+26%	+21%	NSC
Spleen compared to AC	M	+17%	+21%	+28%	+19%
	F	+23%	+42%	+35%	NSC
Spleen compared to VC	M	+14%	+18%	+25%	+16%
	F	NSC	NSC	NSC	NSC

AC- air control
 VC- vehicle control
 NSC- No statistically significant change

There were no statistically significant differences in group mean relative organ weights to percent body weight in male mice.

%Δ in relative organ weights to body weights vs. controls (AC or VC)

Organ	Group	LD	MD1	MD2	HD
Ovaries compared to AC	M	-	-	-	-
	F	-7%	+15%	+53%	+82%
Ovaries compared to VC	M	-	-	-	-
	F	-17%	+3%	+37%	+63%
Lung compared to AC	M	+11%	+11%	+27%	11%
	F	+13%	+15%	+10%	11%
Lung compared to VC	M	+15%	+16%	+32%	+15%
	F	+16%	+18%	+13%	+14%
Uterus compared to AC	M	-	-	-	-
	F	+44%	+28%	+32%	+44%
Uterus compared to VC	M	-	-	-	-
	F	+29%	+16%	+19%	+30%

AC- air control
 VC- vehicle control
 - Not applicable

%Δ in relative organ weights to brain weights vs. controls (AC or VC)

Organ	Group	LD	MD1	MD2	HD
Lung compared to AC	M	+17%	+22%	+43%	+19%
	F	+27%	+28%	+28%	+22%
Lung compared to VC	M	+16%	+22%	+42%	+18%
	F	+21%	+23%	+23%	+17%
Spleen compared to AC	M	+16%	+19%	+26%	+20%
	F	+27%	+42%	+40%	+34%
Spleen compared to VC	M	+15%	+18%	+25%	+19%
	F	+16%	+30%	+28%	+22%
Uterus compared to AC	M	-	-	-	-
	F	+64%	+47%	+57%	+61%

Organ	Group	LD	MD1	MD2	HD
Uterus compared to VC	M	-	-	-	-
	F	+29%	+16%	+24%	+26%
Ovaries compared to AC	M	-	-	-	-
	F	+5%	+30%	+77%	+70%
Ovaries compared to VC	M	-	-	-	-
	F	-14%	+6%	+45%	+39%

AC- air control
VC- vehicle control

Both male and females showed an increase in lung and spleen weights when evaluating absolute weights, organ weights relative to body weight and to brain weights. Females treated with BI 1744 CI had a significant increase ovary and uterus weights when evaluating absolute weight and organ weights relative to body and brain weights. These observations are consistent with the LABA class of drugs. These increases in body weight and organ weights and muscle mass are all attributed to the anabolic effects of LABAs.

Histopathology: Adequate Battery: yes (X), no ()
Peer review: yes (X), no ()

All surviving animals were sacrificed on Days 91, 92, 94 or 95 by carbon dioxide asphyxiation and subject to detailed necropsy. Histological examination of all tissues for the main study control and high-dose animals (AC, VC and 3258 µg/kg) was completed. The respiratory tract, heart, liver and thymus were microscopically examined for all groups including the 63, 211 and 900 µg/kg groups.

Findings	AC		VC		63 µg/kg		211 µg/kg		900 µg/kg		3258 µg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
Larynx n=15												
Squamous metaplasia												
Min	0	0	0	0	0	0	3	0	4	3	0	3
Slight	0	0	0	0	0	0	1	0	2	2	9	6
Moderate	0	0	0	0	0	0	0	0	0	0	6	6
Nasal cavity n=15												
Hyaline droplets-resp epith												
Min	1	0	0	1	1	1	2	2	0	4	1	2
Slight	0	0	0	0	0	0	0	0	0	0	3	1
Hyaline droplets-olfactory												
Min	2	0	0	2	3	2	2	6	2	5	0	4
Slight	0	1	1	1	1	2	1	0	4	1	1	1
Regen-nasopalatine												
Min	0	0	0	0	0	0	0	0	3	1	5	2
Lung n=15												
Inflam cell foci												

Findings	AC		VC		63 µg/kg		211 µg/kg		900 µg/kg		3258 µg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
Min	0	1	1	1	0	0	1	0	1	1	0	2
Slight	0	0	0	0	0	0	0	1	0	0	0	0
Bronchiolo-alveolar adenoma	0	0	0	0	0	0	0	0	0	0	0	1
Liver n=15 Hepatocyte vacuolation												
Min	0	1	0	1	0	1	1	0	0	1	1	3
Slight	0	2	0	3	0	0	0	1	0	2	0	2
Moderate	0	0	0	0	0	0	0	0	0	1	0	3
Ovary Numerous corpora lutea	-	3	-	2	-	-	-	-	-	-	-	8
Uterus Cystic glands												
Min	-	2	-	1	-	-	-	-	-	-	-	5
Slight	-	0	-	0	-	-	-	-	-	-	-	1
Moderate	-	0	-	0	-	-	-	-	-	-	-	4
Myometrial hypertrophy												
Min	-	0	-	0	-	-	-	-	-	-	-	4
Slight	-	0	-	0	-	-	-	-	-	-	-	2
Moderate	-	0	-	0	-	-	-	-	-	-	-	9
Salivary gland-submx Infla cell foci												
Min	0	5	1	1	-	-	-	-	-	-	3	4
Slight	0	0	0	0	-	-	-	-	-	-	1	3
Thymus n=15 Lymphoid hyperplasia												
Min	0	0	0	3	0	2	0	3	0	2	1	5
Slight	0	0	0	1	0	1	0	0	0	1	0	0
Moderate	0	1	0	0	0	0	0	0	0	0	0	0

-: Not examined

Toxicokinetics: Approximately 0.6 mL of blood was taken from 2 TK animals/sex/group for each time point from Groups 1 to 6. Blood samples were taken immediately post-dose, 1 h, 3 h, 8 h and 24 h after inhalation after a single dose was administered on Day 1 and one day during Week 13. BI 1744 Cl was analyzed by a validated HPLC-MS/MS method. The TK parameters C_{max} , T_{max} and $AUC_{0-24 h}$ were calculated using Toxkin software.

Dose	Sex	C _{max} (pmol/L)		AUC _{0-24 h} (pmol*h/L)	
		Day 1	Day 87	Day 1	Day 87
LD	M	45300	5110	69900	12100
	F	45800	4320	54300	15400
MD1	M	84600	15500	187000	35000
	F	62200	17600	177000	49700
MD2	M	185000	92200	590000	150000
	F	168000	91200	496000	125000
HD	M	628000	254000	1250000	413000
	F	704000	284000	1410000	699000

The systemic exposure to BI 1744 Cl did increase with dose but was less than dose proportional. However, exposure was extensive at all doses. The sponsor noted that there was a significant difference in AUC on Day 1 to Day 87 with a 3 to 4-fold higher AUC on Day 1 than Day 87. The sponsor did not provide an explanation for this change and stated that technical reasons cannot be ruled out. No gender effects were observed. The T_{max} was 0.538 h for all doses. No metabolites were measured as part of the TK analysis.

Study title: 13-week inhalation MTD study in rats with a 4-week recovery period

The dose levels selected for this study were based on a single-dose inhalation study and a 4-week repeat dose inhalation study in rats. Rats showed decreased motor activity, abdominal breathing, compulsive grooming and chewing at 27.1 mg/kg doses but no lethal dose was obtained. In a 4-week repeat dose study, local effects were observed with epithelial laryngeal squamous metaplasia and necrosis of the U-shaped cartilage at a dose of 1360 µg/kg/day. No systemic effects were observed in this study at these doses. Thus, 2400 µg/kg was selected for the 13-week repeat dose study.

Key study findings:

- A slight ↑group mean BW compared to the AC and VC groups was observed, which is a result of the exaggerated pharmacological effects known from the LABAs.
- ↓ blood glucose levels was observed in males and females compared to AC and VC groups.
- The potential target tissues of toxicity include the lymph nodes, pancreas, uterus and the larynx.
- No NOAEL for systemic toxicity could be determined due to the lack of examination of the LD, MD1 and MD2 groups.
- The NOAEL for local respiratory findings is considered the LD group (61.7 µg/kg/day) due to findings in the larynx.
- Based on laryngeal squamous metaplasia and necrosis, the MTD is the lower mid-dose (239 µg/kg)
- The vehicle control group also showed local irritation of the larynx which was amplified by BI 1744 CL at the lower mid-dose or greater with squamous metaplasia and necrosis of the U-shaped cartilage. All excipients used in the

vehicle are currently used in approved drug products for the same route of administration.

Study no.: 666413 (b) (4) no. 26689)

Volume #, and page #: Vol 3. and page no. 1

Conducting laboratory and location: (b) (4)

Date of study initiation: January 13, 2006

GLP compliance: Performed under GLP conditions but draft report

QA report: yes () no (X)

Drug, lot #, and % purity: BI 1744 Cl, batch no. 05_Zo07, and >99.26% pure

Methods

Achieved Doses: 0 (air control), 0 (vehicle control), 61.7, 239, 971 and 2833 µg/kg/day; pulmonary deposited doses based on 10% lung deposition in rodents are 6.17, 23.9, 97.1 and 283.3 µg/kg/day

Species/strain: Wistar Han Crl:WI rats

Number/sex/group or time point (main study): 10 rats/sex/group

Route, formulation, volume, and infusion rate: Inhalation (snout-only), BI 1744 Cl in 0.01% benzalkonium chloride, (b) (4) % EDTA and citric acid to adjust pH as aerosol and nebulized for 35 minutes

Satellite groups used for toxicokinetics or recovery: 8 rats/sex/group for TK analyses

Age: 4-5 weeks old

Weight: Males: 92-126 g/Females: 69-113 g

Sampling times: See below

Unique study design or methodology (if any): Rats were snout-only exposed using a flow-past system to either air as a control, aerosolized vehicle as a control or BI 1744 Cl inhalation spray for 35 minutes per day for at least 91 days. The air-control, vehicle control and the HD group included recovery animals to assess potential reversal of any drug-induced effects. In addition to these groups, a sentinel group (Group 7) was used to monitor environmental and animal contamination. These animals were not dosed or exposed to nebulization. Aerosols were generated using 2 modified Hospitak airjet nebulizers. The method of analysis of BI 1744 Cl concentration and particle size was by (b) (4)

(b) (4)

Particle size distribution of test aerosols was assessed throughout the study using a (b) (4). Measurements were taken at least once weekly. The achieved dose was calculated based on the measured aerosol concentration multiplied by RMV multiplied by exposure time and divided by actual animal body weight. Initially, the sponsor used the Guyton's formula in place of the McMahon's formula to calculate RMV but later corrected the calculation using the McMahon formula. Mid-week body weights were taken for each animal and dosing was calculated.

Group	Daily exposure time (min)	Target aerosol concentration (µg/L)	Target dose (µg/kg)
Air-control (AC)	35	0	0
Vehicle control (VC)	35	0	0
Low-dose (LD)	35	2.80	50
Mid-dose1 (MD1)	35	10.91	200
Mid-dose2 (MD2)	35	44.9	800
High-dose (HD)	35	129	2400

Observation, Times and Results:

Aerosol mass concentration and estimated inhaled dose:

Parameter	Treatment group					
	AC	VC	LD	MD1	MD2	HD
Analyzed aerosol concentration [µg/L]	0	0	2.80	10.91	44.9	129
Achieved daily dose of BI 1744 Cl [µg/kg/d]	0	0	61.7	239	971	2833
Estimated deposited pulmonary dose of BI 1744 Cl ([µg/kg/d)*	0	0	6.17	23.9	97.1	283.3
MMAD ± GSD (µm)**	NA					(b) (4)
MMAD ± GSD (µm)***	NA	NA	NA	NA	(b) (4)	

- *Deposited dose based on deposition fraction of 0.1
- ** Gravimetric results
- *** Analytical results
- NA- Not available/applicable

The achieved doses were ~18-23% above the target dose for the all dosing groups, based on the McMahon’s formula for RMV. The estimated achieved doses were inadvertently calculated using an incorrect respiratory minute volume during the in-life phase of the study. Therefore, the achieved doses exceeded the target doses.

Aerosol particle size: The mass median aerodynamic diameter (MMAD) ± the geometric standard deviation (GSD) was (b) (4) respectively for the low-dose (LD), mid-dose1 (MD1), mid-dose2 (MD2) and high-dose (HD). Particle size distributions were based on both gravimetric and analytical measurements. The particle size evaluated by the analytical method showed that LD and MD1 groups were not always quantifiable, which was explained by the sponsor as a result of the sensitivity of the analytical method. The MMAD provided by the gravimetric analysis for each dose was within respirable range (b) (4)

Measured vs deposited dose: The pulmonary deposited doses were calculated by the reviewer by assuming that 10% of the delivered dose is deposited in the pulmonary region by rats. Therefore, the estimated pulmonary deposited doses of the BI 1744 C1 drug product were 0 (air control), 6.17, 23.9, 97.1 and 283.3 µg/kg/day for the LD, MD1, MD2 and HD groups, respectively.

Mortality: All animals were checked early morning and as late as possible each day for viability.

There was 1 death prior to scheduled sacrifice (Animal no. 411M) from Group 4. This death was not related to treatment as the animal had an accidental fracture in his leg and was subsequently sacrificed. No other animals died prior to scheduled sacrifice.

Clinical signs: Each animal was examined at least once before exposure, continuously during exposure, immediately after treatment and at approximately 1-2 h post-exposure. Once each week all animals were received a detailed clinical examination that included appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. Observations were made for the main study (10 rats/sex/group) and recovery animals (8 rats/sex/group).

Males showed a drug-related increase of excessive salivation for LD (5/18 rats), MD1 (9/18 rats), MD2 (17/18 rats) and HD (18/18 rats) animals. With increased dose, the onset of excessive salivation was observed earlier in male rats. The LD group was observed Day 65 or Day 72 only, the MD1 group was observed with excessive salivation starting Day 65 or 67 and lasting up to Day 87, the MD2 group started Day 60 and lasted to Days 87 or 91 of dosing and the HD group was noted starting Day 57 and lasted until the end of the dosing period.

Females also showed a drug-related increase of excessive salivation for the LD (0/18 rats), MD1 (6/18 rats), MD2 (16/18 rats), and HD (18/18 rats). The day of onset of excessive salivation was 84 or 87 and lasted until Day 92 for the MD1 group, Day 60 and lasted until days 87 or 92 for the MD2 group and Day 57 and lasted until termination for the HD group.

For both males and females, excessive salivation resolved with arrest of drug administration.

Body weights: Body weights were recorded once weekly throughout the study.

%Δ Body Weight Gain vs Controls (Day 0 to Day 91)

Main study group	LD		MD1		MD 2		HD	
	M	F	M	F	M	F	M	F
Compared to air control	+14%	+10%	+6%	+16%	+11%	+30%	+2%	+24%
Compared to vehicle control	+10%	+3%	+3%	+9%	+8%	+23%	-2%	+17%

Compared to the air- and vehicle control animals, males and females showed a slight increase in body weight gains. Males showed no dose-related increase in body weight gains and the HD male group actually showed a slight decrease in body weight gain compared to the VC group. Females were more sensitive to the weight gain effect of LABA drugs.

Recovery group males treated with the HD showed no significant difference in body weight gain compared to the AC or the VC groups. Recovery groups females in the HD group, showed a decrease in mean body weight gains compared to the AC (-66%) and the VC (-50%) groups. This result is the reversal effects of the administration of drug. The increase in group mean body weight gains observed in females may be a result of this class of drugs anabolic effects.

Food consumption: The quantity of food consumed by each cage of animals was recorded once weekly throughout the study. From these data, the average consumption was calculated.

The sponsor indicated that there was an increase in food consumption in the BI 1744 CI treated animals. After review of these data, the reviewer determined that there were no statistically significant changes in group mean food consumption values in males or females treated with BI 1744 CI compared to air controls and vehicle control groups.

Ophthalmoscopy: Both eyes of each animal were examined once at pretrial and during Week 13 of treatment and Week 4 post-dose for the recovery animals. Examinations used a mydriatic agent and indirect ophthalmoscope by a veterinarian.

There were no ophthalmic findings in this study.

EKG: Not examined.

Hematology: Blood samples were obtained during weeks 7 and 13 from the main study group animals and post-dose week 4 for recovery group animals. During weeks 7 and 13 of dosing, blood samples were collected in the early morning prior to dosing. Approximately 2 mL of whole blood was collected from each animal via the lateral tail vein. Bone marrow smears were prepared from a femur taken at necropsy and were evaluated only with the identification of hematological findings. The sponsor evaluated a complete hematological battery.

Parameters		LD		MD1		MD 2		HD	
		M	F	M	F	M	F	M	F
WK 7 WBC	Compared to AC	+40%	+37%	+26%	+58%	+42%	+49%	+41%	+59%
	Compared to VC	+50%	+49%	+35%	+70%	+52%	+61%	+51%	+71%
Wk 13 WBC	Compared to AC	NSC	NSC	NSC	NSC	NSC	NSC	NSC	NSC
	Compared to VC	NSC	NSC	NSC	NSC	NSC	NSC	NSC	NSC
Wk 7 Neutrophils	Compared to AC	+93%	+74%	+78%	+116%	+89%	+95%	+103%	+88%
	Compared to VC	+99%	+74%	+83%	+116%	+95%	+95%	+109%	+88%
Wk 13 Neutrophils	Compared to AC	+93%	NSC	+84%	NSC	+107	NSC	+116%	NSC
	Compared to VC	+77%	NSC	+69%	NSC	+91%	NSC	+99%	NSC
WK 7	Compared to	+100%	+100%	+83%	+125%	+83%	+125%	+133%	+100%

Parameters		LD		MD1		MD 2		HD	
		M	F	M	F	M	F	M	F
Monocytes	AC								
	Compared to VC	+100%	+167%	+83%	+200%	+83%	+200%	+133%	+167%
Wk 13 Monocytes	Compared to AC	+100%	NSC	+100%	NSC	+100%	NSC	+160%	NSC
	Compared to VC	+67%	NSC	+67%	NSC	+67%	NSC	+117%	NSC
Wk 7 Eosinophils	Compared to AC	+50%	NSC	+42%	NSC	+58%	NSC	+67%	NSC
	Compared to VC	+64%	NSC	+56%	NSC	+72%	NSC	+81%	NSC
Wk 13 Eosinophils	Compared to AC	NSC							
	Compared to VC	NSC							

NSC- No statistically significant change.

There were transient increases in white blood cell counts during the first 7 weeks of the study in males and females compared to the AC and the VC groups. However, by week 13 of study the WBC counts were comparable to both the AC and the VC groups. Males showed a transient increase in eosinophil counts during Week 7 which resolved by Week 13 of study. Neutrophil and monocyte levels in males were increased compared to the AC and VC treated animals at Week 7 and week 13. However, there was no dose-related increase in these values. There were no statistically significant changes for hematological parameters in either males or females in the recovery group compared to the AC or VC groups.

Clinical chemistry: Blood samples were obtained during weeks 7 and 13 from the main study group animals and post-dose week 4 for recovery group animals. During weeks 7 and 13 of dosing, blood samples were collected in the early morning prior to dosing. Approximately 2 mL of whole blood was collected from each animal via the lateral tail vein. The sponsor excluded blood urea nitrogen from their clinical chemistry battery but did measure urea levels. All other clinical chemistry parameters were evaluated to complete the standard battery.

Parameters		LD		MD1		MD 2		HD	
		M	F	M	F	M	F	M	F
Wk 7 Glucose	Compared to AC	NSC							
	Compared to VC	NSC							
Wk 13 Glucose	Compared to AC	-18%	-16%	-16%	-20%	-18%	-21%	-19%	-16%
	Compared to VC	-24%	-25%	-22%	-29%	-24%	-30%	-23%	-25%

NSC- No statistically significant change.

Blood glucose levels were slightly decreased in both males and females treated with BI 1744 CL compared to AC and VC groups at Week 13 of dosing. This decrease did not show a dose dependent pattern. No other clinical chemistry parameter differed in a significant manner or showed a dose-related response.

Urinalysis: Urine samples were obtained from main study animals during Weeks 7 and 13 of dosing. Urine samples were collected over a 4 hour period from metabolic collection cages. A standard urinalysis was completed by the sponsor.

There was a transient increase in urine volume for males in the MD2 (~ +100%) and HD (~ +200%) groups that resulted in transient decreases in sodium (MD2 -40% and HD -55%), potassium (HD -50%) and chloride (HD -67%) content in urine during Week 7 of study. This pattern was not observed in females. Additionally, male urine volume and electrolyte levels returned to normal by Week 13 of dosing. There were no statistically significant differences between the AC or VC groups and the BI 1744 CL treated males or females in the recovery group. These changes are likely not toxicologically significant.

Gross pathology: At the end of 13 weeks or after the recovery period, all surviving animals were asphyxiated followed by exsanguination. All animals were necropsied.

A slight increase in uterine findings was observed at necropsy during this study. No other macroscopic lesions were observed.

Findings	AC		VC		61.7 µg/kg		239 µg/kg		971 µg/kg		2833 µg/kg	
	M N=10	F N=10	M N=10	F N=10	M N=10	F N=10	M N=10	F N=10	M N=10	F N=10	M N=10	F N=10
Main Study: Uterus N=10 Dilated, with fluid, one/both horns	-	2	-	1	-	1	-	0	-	1	-	3
Recovery: Uterus N=8 Dilated, with fluid, one/both horns	-	2	-	-	-	-	-	-	-	-	-	2

-: Not examined

Organ weights: Organs were weighed from all main study and recovery animals. Paired organs were weighed separately. For animal no. 411M (that was sacrificed prior to scheduled sacrifice due to broken leg bone) did not have its organs weighed. The organs weighed at the time of necropsy included: the adrenals, brain, epididymides, heart, kidney, liver, ovaries, pituitary, prostate, spleen, submaxillary salivary gland, testes, thymus, thyroid, uterus plus cervix and lungs.

There were no statistically significant changes and no dose-dependent changes in absolute organ weights, relative organ weights to body weights and relative organ weights to brain weights compared to AC or VC groups for both males and females in this study.

Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (X), no ()

After necropsy, rats in Groups 1, 2 and 6 (HD group) were histologically examined. A complete histological battery was evaluated for these study groups with the exception of the fallopian tubes and the Zymbal gland. All dosing groups were evaluated for local effects histologically. These tissues included the respiratory tract, heart, liver and thymus.

Findings	AC		VC		61.7 µg/kg		239 µg/kg		971 µg/kg		2833 µg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
Main Study Larynx n=10 Squamous metaplasia, ventral												
Min	0	0	3	2	2	2	2	6	3	2	5	1
Mild	0	0	0	1	0	1	0	0	0	5	0	6
Moderate	0	0	0	0	0	0	0	0	0	0	2	2
Recovery Larynx n=8 Squamous metaplasia, ventral												
Min	0	0	2	2	-	-	-	-	-	-	2	2
Mild	0	0	0	0	-	-	-	-	-	-	0	1
Main study Larynx n=10 Necrosis, U shaped cartilage												
Min	0	0	1	2	1	1	3	4	2	2	3	6
Mild	0	0	0	1	0	0	1	0	0	4	0	2
Recovery Larynx n=8 Necrosis, U shaped cartilage												
Min	0	0	2	3	-	-	-	-	-	-	2	5
Mild	0	0	1	0	-	-	-	-	-	-	0	0
Main study Lymph node, cervical n=10 Lymphoid depletion												
	0	0	0	0	0	0	0	0	0	0	2	0
Hemorrhage	0	0	1	0	0	0	0	1	1	0	2	1
Recovery Lymph node, cervical n=8 Lymphoid depletion												
	0	0	0	0	-	-	-	-	-	-	0	0
Hemorrhage	0	1	0	1	-	-	-	-	-	-	0	1
Main study Lymph node, mandibular n=10 Lymphoid depletion												
	0	0	0	0	-	-	-	-	-	-	1	1
Hemorrhage, sinusoidal	0	0	0	0	-	-	-	-	-	-	1	1
Recovery												

Findings	AC		VC		61.7 µg/kg		239 µg/kg		971 µg/kg		2833 µg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
Lymph node, mandibular n=8												
Lymphoid depletion	0	0	0	0	-	-	-	-	-	-	0	0
Hemorrhage, sinusoidal	0	0	0	0	-	-	-	-	-	-	0	0
Uterus Oestrus dilation	-	1	-	1	-	-	-	-	-	-	-	3
Proliferation, cervical, adventitial	-	0	-	0	-	-	-	-	-	-	-	1
Recovery Uterus	-	0	-	0	-	-	-	-	-	-	-	0
Main study Pancreas exocrine Acinar cell atrophy, focal	0	0	0	0	-	-	-	-	-	-	0	1
Recovery Pancreas exocrine Acinar cell atrophy, focal	0	0	0	0	-	-	-	-	-	-	0	0

The HD group (2833 µg/kg/day) showed slight systemic toxicity with an increase in incidence of uterus oestrus dilation and adventitial cervical cell proliferation, lymph node effects and pancreatic acinar cell atrophy. These findings were not high in incidence and the severity was not reported. No systemic NOAEL can be established due to the lack of microscopic examination of the LD, MD1 and MD2 groups.

Local toxicity was restricted to the larynx and included squamous metaplasia and necrosis of the U-shaped cartilage, both of which increased in incidence and severity with dose. Interestingly, the vehicle treated group also showed squamous metaplasia that resulted in necrosis of the U-shaped cartilage.

Toxicokinetics: Blood samples (~0.45 mL) were collected from TK satellite animals in each group at each time point on Days 1 and 91. Four TK animals/sex/group were sampled for 3 of the time points and a further 4 TK animals/sex/group were used to obtain the remaining time points. Animals were sampled: immediately post-dose, 1 h, 2 h, 4 h, 8 h and 24 h after the start of the inhalation dose. BI 1744 BS was analyzed using a validated HPLC-MS/MS method. The TK parameters calculated were T_{max} , C_{max} and $AUC_{0-24 h}$.

Dose	Sex	C_{max} (pmol/L)		$AUC_{0-24 h}$ (pmol*h/L)	
		Day 1	Day 91	Day 1	Day 91
LD	M	2240	1970	7220	7820
	F	1540	3130	6720	9470
MD1	M	23800	16400	58100	34800
	F	18000	19200	33700	40000

MD2	M	90600	87700	201000	191000
	F	85200	98800	161000	188000
HD	M	331000	249000	712000	533000
	F	315000	310000	535000	495000

There was an increase of systemic exposure (AUC) with dose. However, this increase was supra-proportional to dose. There was no significant gender difference in exposure levels at each dose. As well, there was no significant difference in blood levels from Day 1 of study to Day 91 of study, indicating no accumulation of drug after repeat dosing. The t_{max} was 0.583 h for all doses. No metabolites were measured as part of the TK analysis.

2.6.6.4 Genetic toxicology

The sponsor provided a summary of the genetic toxicology studies performed to date without the submission of the actual study reports. Based on the summary, BI 1744 did not induce genetic toxicity in the presence and absence of metabolic activation in the bacterial reverse mutation assay, the mouse lymphoma assay and in an *in vivo* bone marrow micronucleus assay conducted in the rat based on summary data. The bone marrow micronucleus assay dosed rats for 4 weeks up to the nominal dose of 1360 µg/kg by inhalation (136 µg/kg as the pulmonary deposited dose). The following table summarizes the genetic toxicity studies currently performed.

Assay	Test System	Concentration/Dose	Results
Bacterial Reversion (Ames test)	<i>S. typhimurium</i> (TA 1535, 1537, 98, 100 and 102)	100-5000 µg/plate	<ul style="list-style-type: none"> • Strain and technique dependent toxicity > 300 µg/plate • No induction of revertants
Mouse lymphoma Assay	L5178Y tk ⁺ cell line	10-200 µg/mL	<ul style="list-style-type: none"> • Treatment dependent toxicity >100 µg/mL • No increase in mutant frequencies
Bone marrow micronucleus assay	CrI:WI (Han) rats-inhalation exposure (Part of 4-week inhalation study)	78, 260 and 1360 µg/kg	<ul style="list-style-type: none"> • No induction of bone marrow micronuclei • No indication of bone marrow toxicity

The genetic toxicity studies summarized above were submitted with the original IND (SN 000) on January 26, 2007. A detailed review of these studies was completed under the original review of IND 76,362. Under the conditions tested for each the bacterial reversion assay (Ames test), the mouse lymphoma assay and the *in vivo* rat micronucleus assay, BI 1744 Cl was not genotoxic. However, the *in vivo* micronucleus assay does not appear to have included an appropriate high dose that achieves either the MTD for the test or an acceptable limit dose.

2.6.6.9 Discussion and Conclusions

In support of Boehringer’s proposed mouse and rat carcinogenicity protocols, Boehringer submitted two 13-week repeat dose inhalation toxicology studies performed in mice and rats. The 13-week mouse study, exposed mice to nominal doses of BI 1744 Cl at 0 (AC),

0 (VC), 63, 211, 900 and 3258 µg/kg day doses. The rat study exposed animals to nominal doses of 0 (AC), 0 (VC), 61.7, 239, 971 and 2833 µg/kg day of BI 1744 Cl. The target organs of toxicity observed in mice and rats were the larynx, lymph nodes and uterus. Mice showed signs of toxicity in the nasal cavity, lung, liver and ovary, where rats showed toxicity to BI 1744 Cl in the pancreas. Based on hepatocyte vacuolation and an increase in total bilirubin levels observed in female mice, the MTD was considered the 3258 µg/kg/day for female mice. No MTD was identified in male mice. No NOAEL for systemic effects could be determined in the mouse study due to the absence of histological data in the LD, MD1 and MD2 groups. However, the NOAEL for local toxicity was considered the LD (63 µg/kg/day) due to findings in the larynx, nasal cavity and the lung at doses > 63 µg/kg day. In rats, the MTD was considered 239 µg/kg day based on laryngeal squamous metaplasia and necrosis observed at higher doses. The vehicle control group also showed local irritation of the larynx, which was amplified by BI 1744 CL, at the lower mid-dose or greater with squamous metaplasia and necrosis of the U-shaped cartilage. No NOAEL for systemic toxicity could be determined in the rat study due to the lack of examination of the LD, MD1 and MD2 groups. However, the NOAEL for local toxicity was considered 61.7 µg/kg/day due to findings in the larynx.

All excipients used in the vehicle control group in the mouse and rat study are currently used in approved drug products for the same route of administration.

A summary of genetic toxicology studies with BI 1744 Cl indicated negative results under the conditions tested in a full battery. However, the dosing for the in vivo micronucleus assay from a 4-week inhalation toxicity study does not appear to be adequate.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

BI 1744 Cl inhalation spray is a long acting β_2 -agonist (LABA) being developed for the treatment of chronic obstructive pulmonary disease (COPD) and bronchial asthma. The current submissions include the sponsor's special protocol request for carcinogenicity studies in mice and rats. The proposed daily therapeutic dose range is expected to be between 10 and 40 μg per subject. At a single inhalation dose of 40 μg in healthy subjects, an $\text{AUC}_{0-\text{Inf}}$ of 400 to 500 $\text{pM}\cdot\text{h}$ was observed. The sponsor based the mouse to human AUC ratios and the rat: human AUC ratios on this value at 40 μg BI 1744 Cl.

PK studies of BI 1744 Cl investigated in mice, rats and in humans. The plasma protein binding is low in mice (50-70%), rats (51%) and man (~65%). BI 1744 BS is the dominant compound in plasma, bile and feces after intratracheal and intravenous administration of ^{14}C BI 1744 Cl to mice and rats. Using human liver microsomes in an *in vitro* system, the human CYP enzymes (CYP 2C8, 2C9, 3A4) are involved in metabolism of the BI 1744 Cl. From a single-inhalation study in healthy male volunteers, the parent compound, BI 1744 BS and its glucuronide, CD 922, were found in urine and BI 1744 BS was found in plasma. There was no metabolism by human lung microsomes.

Summarized information indicated that BI 1744 did not induce genetic toxicity in the presence and absence of metabolic activation in the bacterial reverse mutation assay, the mouse lymphoma assay and in an *in vivo* bone marrow micronucleus assay conducted in the rat. Of note, subsequent submission and review of the *in vivo* assay suggests that the maximum dosing was not adequate.

The sponsor proposed a 2-year CD-1 mouse carcinogenicity study at nominal doses of 0 (air-control), 0 (vehicle control), 25, 75 and 250 $\mu\text{g}/\text{kg}$ for inhalation. In support of their proposed doses, a 13-week repeat-dose inhalation study was conducted in mice at nominal BI 1744 BS doses of 0 (air control), 0 (vehicle control), 63, 211, 900 and 3258 $\mu\text{g}/\text{kg}$ for the LD, MD1, MD2 and HD groups, respectively. The MTD for this study is the high dose of 3258 $\mu\text{g}/\text{kg}$ based on liver toxicity observed in females. No MTD was established for males. There were no unscheduled deaths in the 13-week repeat dose inhalation study. Excessive salivation in all males in the vehicle control, LD, MD1, MD2 and HD groups and all females in the BI 1744 Cl treatment groups was observed. HD males (15/15) and MD2 males (1/15) and HD females (7/15) showed increased muscle mass (mild) which was not observed in the control or other treated groups. Both males and females had an increase in body weight gains compared to both air and vehicle controls. Females in the HD group had a statistically significant increase in food consumption compared to air (ranged from +38 to +44%) and vehicle (ranged from +30 to +35%) controls during Weeks 4-10 of the study.

Total bilirubin levels were increased in both males (34 to 77%) and females (13-121%) compared to air and vehicle control animals but this increase did not show a dose-related pattern in either males or females except at the highest dose.

At necropsy, the only findings that showed an increase with respect to dose were an increase in muscle mass (1/15 males and 1/15 females) and thickening (8/15) or enlarged uterine horns (2/15) at the HD, and the two highest doses, respectively. Both male and females showed an increase in lung and spleen weights when evaluating absolute weights, organ weights relative to body weight and to brain weights. Females treated with BI 1744 CI had a significant increase in ovary and uterus weights when evaluating absolute weight and organ weights relative to body and brain weights. These observations are consistent with the LABA class of drugs. These increases in body weight, organ weights and muscle mass are all attributed to the anabolic effects of LABAs.

Significant histological findings included local toxicity of the larynx and nasal cavity and systemic toxicity including hepatocyte vacuolation, and uterine and ovarian findings.

(b) (4)
Based on review of the histological data, the reviewer concludes that the local toxicity observed in the larynx did not reach the MTD (no necrosis or ulceration was observed) and therefore, this finding should not restrict dosing at 250 µg/kg for a 2 year period. However, findings in the liver were observed in females at the MD2 (900 µg/kg) and HD (3258 µg/kg) groups that included hepatocyte vacuolation with an increase in total bilirubin at the highest dose, which is considered dose limiting. The MTD appears to be the high dose of 3258 µg/kg for females.

(b) (4)
(b) (4)
(b) (4)
These doses would provide estimated human AUC exposure ratios of 14, 42 and 106-fold based on the total drug exposure.

Group	Nominal Dose (µg/kg)	Expected AUC ₀₋₂₄ (pM*h)	AUC-ratio
LD	25	7,000	14
MD	75	21,000	42
HD	250	53,000	106

In addition to the mouse study, the sponsor proposed a 2-year Wistar rat carcinogenicity study at nominal doses of 0 (air-control), 0 (vehicle control), 25, 75 and 250 µg/kg for inhalation. In support of their proposed doses, a 13-week repeat-dose inhalation study was conducted in rats at nominal aqueous BI 1744 BS doses of 0 (air control), 0 (vehicle control- 0.1 mg/mL BAC, 0.1 mg/mL sodium EDTA and 0.03 mg/mL citric acid), 61.7, 239, 971 and 2833 µg/kg/day as the achieved doses for the LD, MD1, MD2 and HD groups, respectively. The MTD for this study appears to be the lower mid-dose of 239 µg/kg based on laryngeal squamous metaplasia and necrosis. There were no drug-related deaths in this study. Excessive salivation was observed in males and females

treated with BI 1744 Cl. Both males and females had an increase in body weight gain compared to the air- and vehicle control groups. This increase is typical for the LABAs. There was no statistically significant increase in food consumption for either males or females treated with BI 1744 Cl.

Blood glucose levels were slightly decreased in both males (-16 to -24%) and females (-16 to -30%) treated with BI 1744 CL compared to AC and VC groups at Week 13 of dosing. This decrease did not show a dose dependent pattern. At necropsy, a slight increase in the incidence of dilated uterus was observed in HD (3/10 rats) females compared to AC (2/10 rats) and VC (1/10 rats). Based on histological evaluation, the HD group (2833 µg/kg/day) showed slight systemic toxicity with an increase in incidence of uterus oestrus dilation and adventitial cervical cell proliferation, lymph node effects and pancreatic acinar cell atrophy. These findings were not high in incidence. No systemic NOAEL could be established due to the lack of microscopic examination of the LD, MD1 and MD2 groups.

Local toxicity was restricted to the larynx and included squamous metaplasia and necrosis of the U-shaped cartilage. Both findings increased in incidence and severity with dose. The vehicle control group also showed squamous metaplasia that resulted in necrosis of the U-shaped cartilage. Discussion with the Executive Carcinogenicity Assessment Committee regarding use to this vehicle in their proposed study, led to the conclusion that the vehicle may mask potential toxicities induced by the BI 1744 Cl compound but due to its inclusion in the clinical formulation, its use in the study is permitted.

There was an increase of systemic exposure (AUC) with dose. However, this increase was supra-proportional to dose. There was no significant gender difference in exposure levels at each dose. As well, there was no significant difference in blood levels from Day 1 of study to Day 91 of study, indicating no accumulation of drug after repeat dosing.

(b) (4)
[redacted] was based on local toxicity findings in the larynx (squamous metaplasia and necrosis of the U-shaped cartilage) and the > 40- to 90-fold animal to human exposure ratio at this dose. Based on review of the histological data, the reviewer concludes that the MTD is 239 µg/kg. Therefore, th [redacted] (b) (4)

[redacted] These doses would provide estimated human AUC exposure ratios of 3-9, 11-26 and 40-90-fold for the LD, MD and HD, respectively, based on the total drug exposure.

Group	Nominal Dose (g/kg)	Expected AUC ₀₋₂₄ (pM*h)	AUC-ratio
LD	25	1,800- 4,300	3-9
MD	75	5,400-13,000	11-26
HD	250	20,000-47,000	40-90

Internal comments: The Executive CAC meeting was held on February 13, 2007 to discuss the sponsor's proposed mouse and rat carcinogenicity protocols. Based on the

discussion at this meeting the following recommendations were given. These recommendations were captured in the meeting minutes and were faxed to the sponsor.

Mouse:

* The Committee concurred with the sponsor's proposed doses of 0 (air control), 0 (vehicle-control), 25, 75, and 250 µg/kg by nose-only inhalation, based on AUC ratio. The sponsor is cautioned that when doses are selected on the basis of AUC that if the clinical dose changes such that the ratio is no longer at least 25-fold, the study may not be acceptable.

Rat:

* The Committee concurred with the sponsor's proposed doses of 0 (air control), 0 (vehicle-control), 25, 75, and 250 µg/kg by nose-only inhalation, based on MTD (laryngeal squamous metaplasia and necrosis). It is noted that the vehicle could contribute to or mask findings, but that it will be acceptable since it is in the clinical formulation.

* It was noted that there is also an adequate AUC ratios.

General comments: The Committee recommends that the proposed hematology, clinical chemistry and urinalysis assessments be omitted. Additionally, it was noted that the vehicle control group (0.1 mg/mL benzalkonium chloride, 0.1 mg/mL EDTA and 0.03 mg/mL citric acid) also showed squamous metaplasia that resulted in necrosis of the U-shaped cartilage and that the vehicle may mask BI 1744 Cl –related findings in their studies.

External comments (to sponsor): None.

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this page is the manifestation of the electronic signature.**

/s/

Molly Shea
3/2/2007 12:35:54 PM
PHARMACOLOGIST

Timothy McGovern
3/2/2007 12:45:57 PM
PHARMACOLOGIST
I concur.

Appendix 3

Pharm/Tox Review of IND 76,362 dated February 17, 2009 (Request for ECAC Feedback)

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 76,362

Review number: 2

Sequence number/date/type of submission: SDN 54/January 27, 2009/Request for CAC Feedback

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Boehringer Ingelheim

Manufacturer for drug substance:

Boehringer Ingelheim Pharma GmbH & Co. KG

Birkendorfer Strasse 65

D-88397 Biberach an der Riss

Germany

Reviewer name: Molly E. Shea, Ph.D.

Division name: Division of Pulmonary and Allergy Products (DPAP)

Review completion date: February 17, 2009

Drug:

Trade name: NA

Generic name: NA

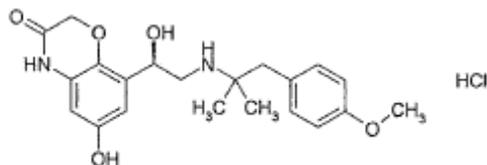
Code name: BI 1744 Cl

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

CAS registry number: NA

Molecular formula/molecular weight: $C_{21}H_{26}N_2O_5 \cdot HCl$ /422.9 g/mol (salt) and 386.5 g/mol (free base)

Structure:



Relevant INDs/NDAs/DMFs: NA

Drug class: Long-acting, human β_2 - adrenoreceptor partial agonist

Intended clinical population: Chronic obstructive pulmonary disease (COPD) and bronchial asthma

Clinical formulation: The composition of BI 1744 Cl Respimat Inhalation Spray at the 1, 2.5, 5 and 10 µg doses and the placebo is summarized below. Two actuations will be administered to achieve the 2, 5, 10 and 20 µg/day doses proposed for the Phase 2 B clinical trial subject to this IND.

Ingredient	Percent formula (g/100 mL)					Function
	2 µg dose	5 µg dose	10 µg dose	20 µg dose	Placebo	
BI 1744 BS	0.0091	0.0226	0.0452	0.0905	-	API as base
BI 1744 Cl	0.0099	0.0248	0.0495	0.0990	-	API as salt
Benzalkonium chloride, NF	(b) (4)					(b) (4)
Edetate sodium, USP						
Citric acid, anhydrous, NF						
						(b) (4)
Total mass	100.0	100.0	100.0	100.0	100.0	(b) (4)

Route of administration: Oral Inhalation (nebulized)

Proposed clinical protocol: NA

Previous clinical experience: NA

Studies reviewed within this submission: None

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OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

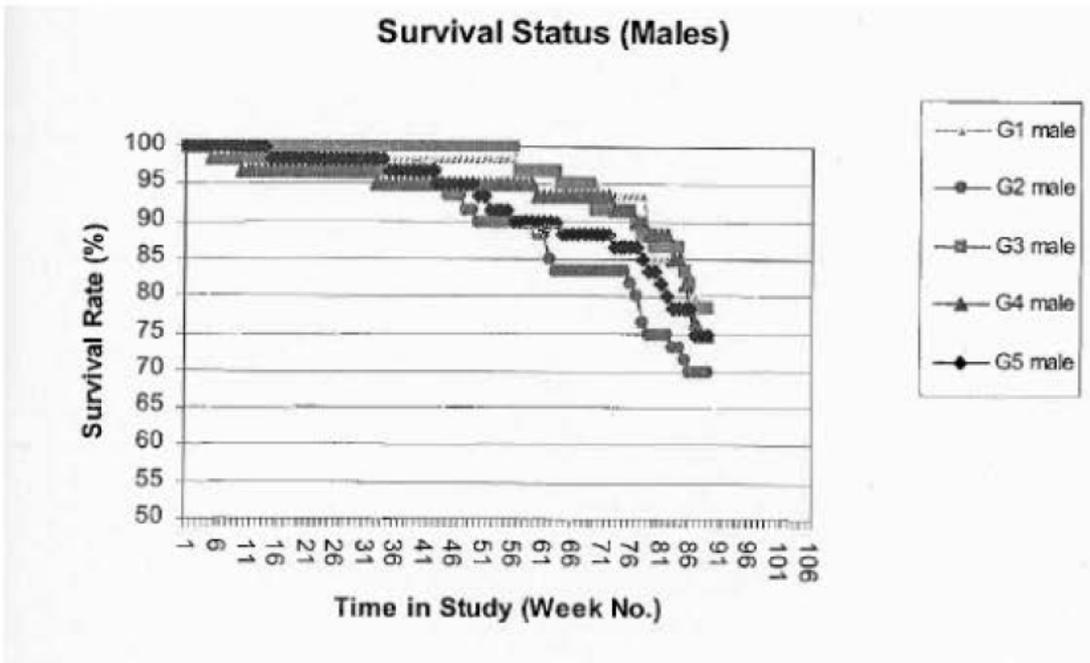
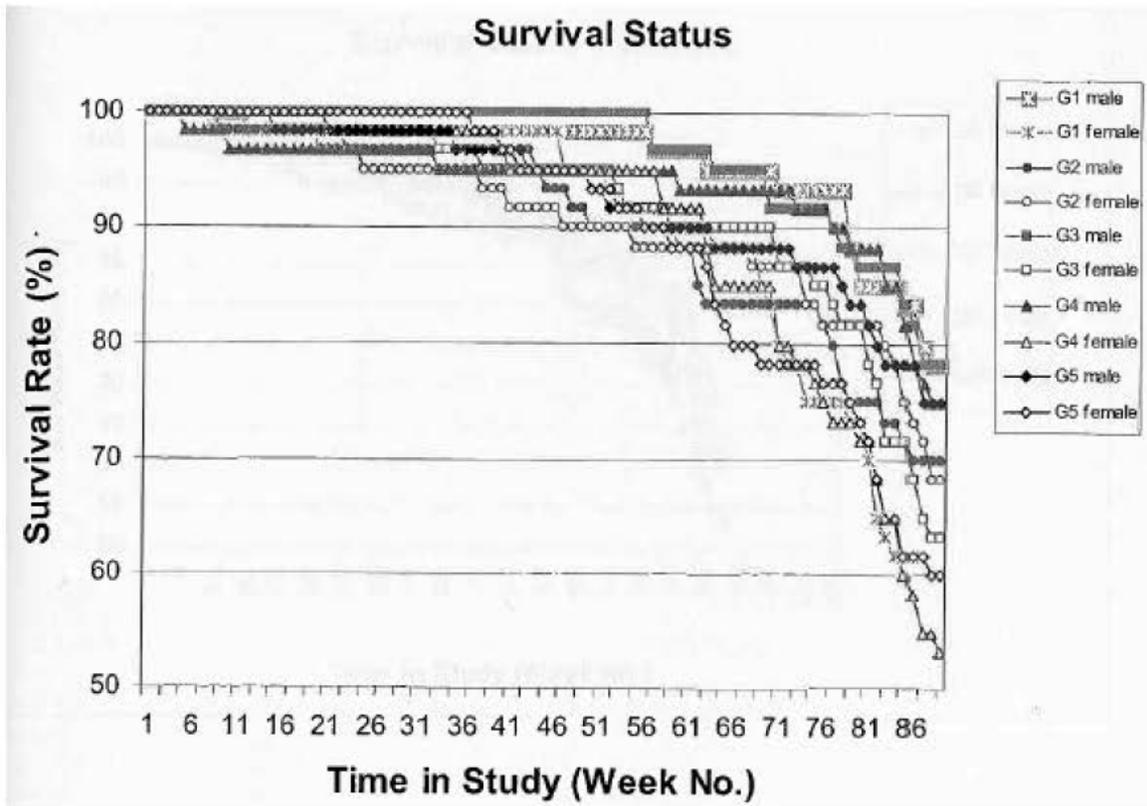
Boehringer Ingelheim (BI) submitted the following survival rate summary for their 2-year mouse study and requested feedback from the Executive Carcinogenicity Assessment Committee (ECAC) regarding dosing termination and animal sacrifice endpoints.

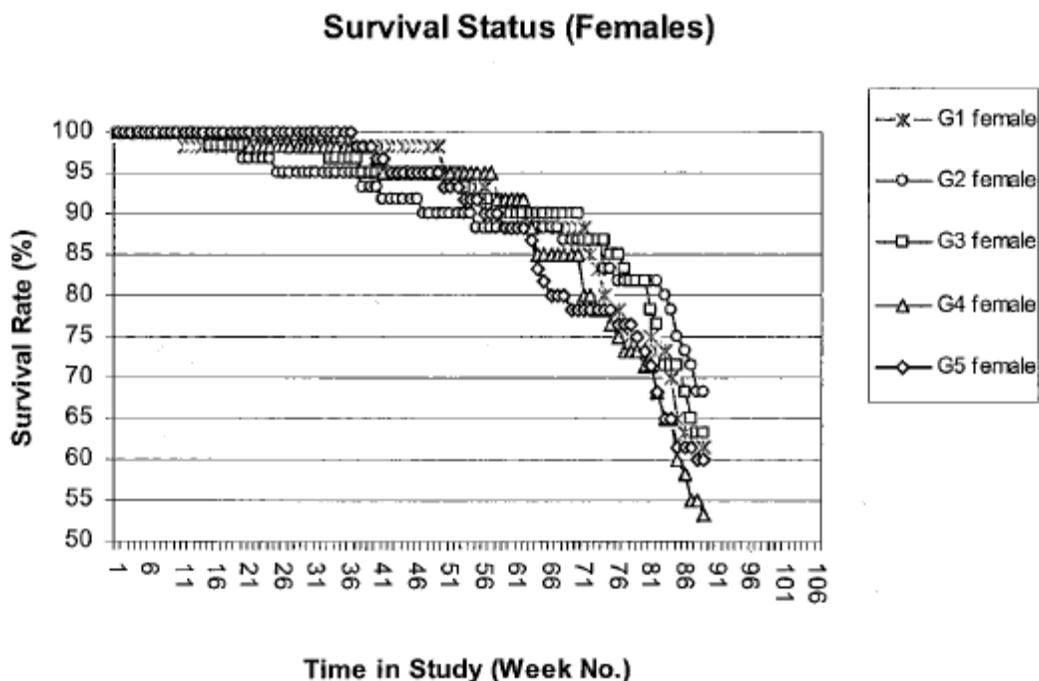
BI indicated that the survival percentages at Study Week 89 were 53% in females for the intermediate-dose group (32 mice surviving) and 60-68% in females for all other groups (36 to 41 mice surviving). In males, the lowest survival rate at Study Week 89 is 70% (vehicle control) and 75-78% for all other groups. Based on these data, BI requested the ECAC's opinion as to how the carcinogenicity study in CD-1 mice may be continued with the following proposal:

When survival of any treatment group (Groups 3, 4 and 5) reaches 22 animals/sex, the study should continue, but dosing of that sex group should cease and the animals be maintained without treatment until survival in the sex/group reaches 11, whereupon the sex/group will be terminated. In addition, when dosing of the last 3 treatment groups (Low, Intermediate and High Dose) has terminated for one particular sex; then dosing of the corresponding control animals (Air and Vehicle Control) should also be terminated. Furthermore, when sacrifice of the last of the 3 treatment groups has occurred, then the corresponding control animals should also be sacrificed.

Would the CAC concur to this proposal or recommend a different proposal?

Review of the submitted survival/mortality data showed that the main study included 60 mice/sex/group for air control, vehicle control, low-dose, mid-dose and high-dose groups. At Week 89 of study survival in males was 78% (47/60), 70% (42/60), 78% (47/60), 75% (45/60) and 75% (45/60) for the AC, VC, LD, MD and HD groups, respectively. At Week 89 for females the survival rates were, 62% (37/60), 68% (41/60), 63% (38/60), 53% (32/60), and 60% (36/60) for the AC, VC, LD, MD and HD groups, respectively. The following Kaplan-Meier curves were provided for both sexes and then individual male and females.





These data were relayed to the ECAC. As the data illustrates, there appears to be no drug-related effect on survival rates for males and females in this study. Therefore, neither study termination nor early animal sacrifice is recommended.

External comments (to sponsor):

- Based on the survival data at Week 89 of study, no drug-related effect is observed. Therefore, the 2-year mouse study should continue per protocol. Should any BI 1744 CL treatment group reach 15 animals/group, it can be terminated. Should a control reach 20 animals/group the study for that sex may be terminated. Please notify the Division when a decision to terminate a study group or study groups is made.

Linked Applications

Sponsor Name

Drug Name / Subject

IND 76362

BOEHRINGER
INGELHEIM
PHARMACEUTICALS
INC

BI 1744 CL RESPIMAT INHALATION
SPRAY

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

/s/

MOLLY E SHEA
02/17/2009

TIMOTHY W ROBISON
02/17/2009
I concur

Appendix 4

Pharm/Tox Review of IND 76,362 dated May 10, 2010 (Genetic Toxicology Review)

**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: 76,362

Supporting document/s: Amendments #005, #032, #034, #039, #044, #059,
and #086

Sponsor's letter date: #005 dated January 26, 2007
#032 dated April 30, 2008
#034 dated May 13, 2008
#039 dated June 16, 2008
#044 dated July 14, 2008
#059 dated March 27, 2009
#086 dated March 16, 2010

CDER stamp date: #005 dated January 29, 2007
#032 dated May 1, 2008
#034 dated May 14, 2008
#039 dated June 17, 2008
#044 dated July 15, 2008
#059 dated March 30, 2009
#086 dated March 17, 2010

Product: BI 1744 CL

Indication: COPD and Asthma

Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877

Review Division: Pulmonary, Allergy, and Rheumatology Products

Reviewer: Timothy W. Robison, Ph.D., D.A.B.T.

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

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

Project Manager: Eunice Chung, Pharm.D.

Template Version: December 7, 2009

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Figure 1 From Mutation Research 627: 78-91, 2007: Increases in micronucleated bone marrow cells in rodents as a result of erythropoiesis that do not indicate genotoxic hazards 64

1 Executive Summary

1.1 Recommendations

Internal Comments: The finding of increased frequencies of micronuclei reported in the study entitled “Intravenous mutagenicity study using micronucleus analysis of rat bone marrow [U08-1834-01]” was more than likely due to a drug enhanced compensatory erythropoiesis and it is unlikely that these findings were due to a genotoxic event. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures. Concurrence was received from Dr. David Jacobson-Kram (see appendices).

Recommendation: None.

External Comments: The following comments should be conveyed to the sponsor.

We have reviewed study reports within your submissions dated January 26, 2007, April 30, 2008, May 13, 2008, June 16, 2008, July 14, 2008, March 27, 2009, and March 16, 2010 to assess the increased frequency of micronuclei reported in the study entitled “Intravenous mutagenicity study using micronucleus analysis of rat bone marrow [U08-1834-01]” and have the following comments.

Question 1: Can the positive findings observed in the in vivo rat micronucleus test [U08-1834-01] be attributed to the exaggerated pharmacological action of BI 1744 CL?

Response: We agree that the finding of increased frequencies of micronuclei was more than likely due to a drug enhanced (compensatory) erythropoiesis and it is unlikely that this finding was due to a genotoxic event based upon the large increase in the percentage PCEs in the high dose group of the in vivo micronucleus study with rats [U08-1834-01] and the non-GLP mechanistic study [U08-1845-01] that provides additional supportive information of induction of erythropoietic activity following treatment with BI 1744 CL.

Question 2: Should the findings of the in vivo micronucleus test with rats be listed in the informed consent?

Response: These findings should be listed in the informed consent; however, the finding may be qualified as follows. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Question 3: Are the assessment of the in vivo micronucleus test with rats adequate to support the NDA filing for Olodaterol?

Response: The studies entitled “Measurement of cardiovascular and respiratory function in conscious rats” [GP2008/0379/PH5] and “Mechanistic investigation of erythropoiesis in male rats after intravenous administration” [U08-1845-01] provide an adequate explanation for the finding of increased frequencies of micronuclei reported in Study #U08-1834-01 and support an NDA filing.

Question 4: Should these findings be placed in the product labeling?

Response: These findings should be listed in the product labeling; however, the finding may be qualified as follows. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

1.2 Brief Discussion of Nonclinical Findings

In the initial IND submission, the sponsor submitted a standard battery of genotoxicity tests with BI 1744 CL that included an in vitro bacterial mutagenicity test, an in vitro mouse lymphoma assay, and an in vivo micronucleus test that was part of a 4-week inhalation toxicology study with rats. The in vitro bacterial mutagenicity test and in vitro mouse lymphoma assay were judged to be negative; however, the in vivo micronucleus test was judged to be inadequate due to low inhalation dosing.

The sponsor subsequently conducted an additional in vivo micronucleus test using the intravenous route. Male rats received BI 1744 CL at doses of 0, 1, 10, and 40 mg/kg. BI 1744 CL produced increased percentages of polychromatic erythrocytes (PCEs) at 10 and 40 mg/kg (i.e., PCE to NCE ratio was increased up to 1.4-fold) although statistical significance was only achieved at 40 mg/kg. Increased PCEs were observed at both 24- and 48-hr postdose for 40 mg/kg. Increased percentages of PCEs suggested induction of erythropoiesis although the range of increase remained within historical control values. At the 24-hr time point, there were dose-dependent, statistically significant increases in the frequencies of micronucleated polychromatic erythrocytes (MNEs) with BI 1744 CL at 10 and 40 mg/kg (up to 2.8-fold of the vehicle-control). At the 48-hr time point, the percentage of MNE remained elevated for rats at 40 mg/kg (up to 2.2-fold of the vehicle-control).

Due to an increased frequency of micronuclei observed in the study, the sponsor conducted a cardiovascular safety pharmacology study with rats (Study number GP2008/0379/PH5) and a non-GLP mechanistic toxicology study with rats using the intravenous route (Study number U08-1845-01) in an effort to explain that the observed response was not the result of DNA damage induced by BI 1744 CL. It is possible that the increased levels of erythropoiesis observed in treatment

groups might have lead to artifactual increases of micronuclei. It is known that conditions that can reduce oxygen tension in the blood (e.g., significant increases of heart rate with ensuing tissue hypoxia) can stimulate the secretion of erythropoietin, which stimulates an increase in cell division of erythroblasts, and hence, increases the numbers of circulating erythrocytes. This improves the overall oxygen carrying capacity of the blood and restores levels of oxygen tension. The increase in cell division will cause more cells to undergo enucleation and this may result in an increase in micronuclei formed 'spontaneously' (Mutation Research 627: 78-91, 2007).

Effects of single intravenous doses of BI 1744 CL at 10 and 40 mg/kg on cardiovascular function (systolic and diastolic arterial pressure and heart rate) were examined in conscious rats. There were dose-dependent and long-lasting (up to 24 hr postdose with 40 mg/kg) decreases of systolic and diastolic blood pressure (up to 50% and 60%, respectively) and increases of heart rate (500 bpm at peak versus 325 bpm at 1 hr prior to the start of treatment) with BI 1744 CL at 10 and 40 mg/kg.

In a non-GLP mechanistic study, induction of erythropoiesis was assessed in male rats that received single intravenous doses of BI 1744 CL at 40 mg/kg. The sponsor has conjectured that the increased frequency of micronuclei in a single intravenous dose micronucleus study with rats might have been due to an induction of erythropoiesis. Percent reticulocytes were significantly elevated (1.32-fold) at 48-hr postdose for rats treated with BI 1744 CL at 40 mg/kg, but not at 24-hr postdose. Erythropoietic activity was evident in bone marrow smears (i.e., increases of the erythroid cell fraction, proerythroblasts, macroblasts, and mitoses of erythroid cells [up to 3-fold increase]). These changes were more pronounced at 48-hr postdose than at 24-hr. Erythropoietin levels were increased at 6- and 24-hr postdose for rats treated with BI 1744 CL at 40 mg/kg (1178 and 234 pg/mL, respectively, versus below the level of quantitation (BLOQ) for the control). Histopathological changes from rats treated with BI 1744 CL at 40 mg/kg were observed in the heart, spleen, liver, and lung. For rats treated with BI 1744 CL at 40 mg/kg and sacrificed at 24 and 48 hr postdose, multifocal degeneration/necrosis of cardiomyocytes with inflammatory reaction was observed. Extramedullary hematopoiesis was observed in the spleen and liver primarily for animals sacrificed at 48-hr postdose and appeared to correlate with observations of increased erythropoietic activity in treated animals. Multifocal acute hemorrhages were observed in the lung for a small number of animals. Circulating levels of erythropoietin significantly increased leading to erythropoietic activity in the bone marrow, spleen, and liver.

In the present situation, BI 1744 CL administered to rats at intravenous doses of 10 and 40 mg/kg produced significant increases of heart rate. It is possible under these conditions that the heart tissue became hypoxic and tissue damage ensued (i.e., multifocal degeneration/necrosis of cardiomyocytes with inflammatory reactions). Circulating levels of erythropoietin significantly

increased leading to erythropoietic activity in the bone marrow, spleen, and liver. The increase in cell division will cause more cells to undergo enucleation and this may result in an increase in micronuclei formed 'spontaneously' (Mutation Research 627: 78-91, 2007). The observed increases in the frequency of micronuclei following treatment with BI 1744 CL most likely occurred as a result of compensatory erythropoiesis. It was unlikely to be the result of a genotoxic event.

2 Drug Information

2.1 Drug

2.1.2 Generic Name

Olodaterol

2.1.3 Code Name

BI 1744 CL

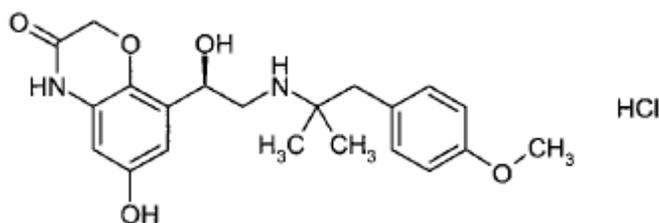
2.1.4 Chemical Name

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

2.1.5 Molecular Formula/Molecular Weight

$C_{21}H_{26}N_2O_5 \times HCl$

2.1.6 Structure



2.1.7 Pharmacologic class

Long-acting beta(2)-agonist (LABA)

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 76,392 (BI 1744 CL + BI 54903 XX)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Table 3.1.4: 1 Composition of BI 1744 CL Respimat® Inhalation Spray (Percentage Formula)

Name of ingredient	Percentage formula [g/100ml] (2 µg dose)	Percentage formula [g/100ml] (5 µg dose)	Percentage formula [g/100ml] (10 µg dose)	Percentage formula [g/100ml] (20 µg dose)	Percentage formula [g/100ml] (placebo)	Function	Specification of ingredient	
BI 1744 BS ¹⁾	0.0091	0.0226	0.0452	0.0905	-	Drug substance	In house standard	
corresponds to BI 1744 CL ²⁾	0.0099	0.0248	0.0495	0.0990	-			
Benzalkonium chloride							(b) (4)	
Edetate disodium								
Citric acid, anhydrous								
Total mass	100.0	100.0	100.0	100.0	100.0	-	-	

¹⁾ Mass per dose and concentration of the active substance depend on the respective dose strength.

²⁾ 1 g of BI 1744 CL corresponds to 0.9138 g of BI 1744 BS.

2.5 Regulatory Background

2.5.2 History of Regulatory Submission

IND 76, 362 was submitted on January 26, 2007. During the review of the initial submission, (b) (4)

The following comment was conveyed to the sponsor on March 21, 2007:

In response, the sponsor conducted an additional in vivo micronucleus report using the intravenous route (Study number U08-1834-01, Draft Report: Submission dated June 16, 2008 and Final Report: Submission dated March 27, 2009). Due to an increased frequency of micronuclei observed in the study, the sponsor conducted a cardiovascular safety pharmacology study with rats (Study number GP2008/0379/PH5) and a non-GLP mechanistic toxicology study with

rats using the intravenous route (Study number U08-1845-01, Draft report provided in Amendment #044 dated July 14, 2008 and Final Report provided in Amendment #086 dated March 16, 2010) in an effort to explain that the observed response was not the result of DNA damage induced by BI 1744 CL.

3 Studies Submitted

3.1 Studies Reviewed

1. Measurement of Cardiovascular and Respiratory Function in Conscious Rats (GP2008/0379/PH5)

(b) (6)

3. BL 1744 CL: Mutagenicity Study using the Salmonella typhimurium /mammalian-microsome assay (Ames test) (04B167)

4. BI 1744 CL: Mutagenicity Study using the Mouse Lymphoma (L5178Y) Assay (04B161)

5. BI 1744 CL: Intravenous Mutagenicity Study Using Micronucleus Analysis of Rat Bone Marrow (U08-1834-01)

6. Mechanistic investigation of erythropoiesis in male rats after intravenous administration (U08-1845-01)

3.2 Studies Not Reviewed

This review was focused on genetic toxicology studies and other supportive studies provided in the referenced submissions. Other nonclinical studies provided in these submissions will be reviewed at a later date.

3.3 Previous Reviews Referenced

Preliminary Safety Review of IND 76,362 dated February 28, 2007

4 Pharmacology

4.3 Safety Pharmacology

Measurement of Cardiovascular and Respiratory Function in Conscious Rats (GP2008/0379/PH5)

Methods: This study examined the effects of single intravenous doses of BI 1744 CL at 10 and 40 mg/kg on cardiovascular function (systolic and diastolic arterial pressure and heart rate), respiration rate, tidal volume, temperature, and motility in conscious rats. Male Han-Wistar rats (approximately 450-600 g obtained from (b) (4)) were chronically instrumented with transmitters for the measurement of blood pressure, heart rate, and temperature. Modification of the receiver circuitry also allowed assessment of animal movement. There were 8 male rats per group. The report stated that rats were monitored over 8 hr, 1 hr prior to drug administration and 7 hr postdose; however, graphs indicate that rats were monitored for 1 hr prior to dosing and up to 24 hr postdose for cardiovascular and body temperature measurements and up to

5 hr postdose for respiratory measurements. In a second study, the effects of an intravenous dose of BI 1744 CL at 10 mg/kg following a 1-hr pretreatment with nadolol at 30 mg/kg were determined. Two rats/group received either BI 1744 CL or nadolol alone and 4 rats/group received the combination. Concurrent control groups were not used in these studies. Animals were placed in separate whole body plethysmograph boxes on the day of the experiment for measurement of respiratory parameters. The parameters, systolic and diastolic arterial blood pressure (mm Hg), heart rate (beats/min), respiration rate (breaths/min), tidal volume (mL/ breath), motility (arbitrary units) and body temperature ($^{\circ}\text{C}$), were summarized by calculating median values of all sequential events over 10 min. Data were expressed as the mean \pm SE for $n=8$.

Results: There were dose-dependent and long-lasting (up to 24 hr postdose with 40 mg/kg) decreases of systolic and diastolic blood pressure (up to 50% and 60%, respectively) and increases of heart rate (500 bpm at peak versus 325 bpm at 1 hr prior to the start of treatment) with BI 1744 CL at 10 and 40 mg/kg. The intensity of this effect was reduced by pre-treatment with nadolol (30 mg/kg) 1 hr prior to the application of BI 1744 CL (i.e., heart rate reached a peak of 475 bpm with BI 1744 CL at 10 mg/kg versus 250 bpm with nadolol; the baseline ranged from 375 to 425 bpm). Respiration rate (baseline of 87 breaths/min to 125-187 breaths/min) and minute volume (baseline of 260 mL/min to 500-750 mL/min) were significantly increased. Tidal volume was unchanged. Body temperature was significantly increased with 40 mg/kg in the first time phase (peak of 38.7°C versus a baseline of 37.5°C).

This was a non-GLP study included as Appendix II in Study U09-1845-01. Descriptions of methods and results by the sponsor were minimal and there were numerous typographical errors. Concurrent control groups were not used in these studies. It is unclear if comparisons to pretreatment values are valid for monitoring changes of physiological parameters over the course of a 24-hr period following dosing.

Table 1 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on systolic blood pressure

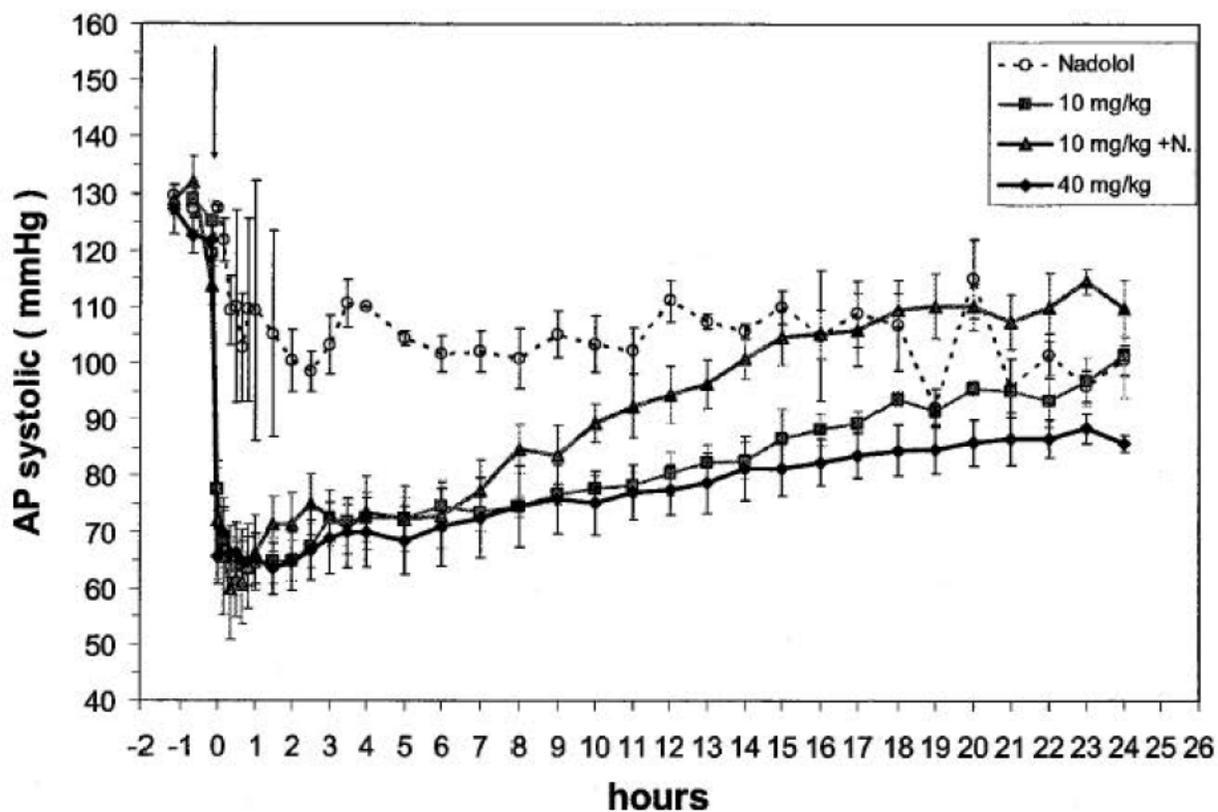


Figure 1: Systolic Arterial Pressure

Table 2 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on diastolic blood pressure

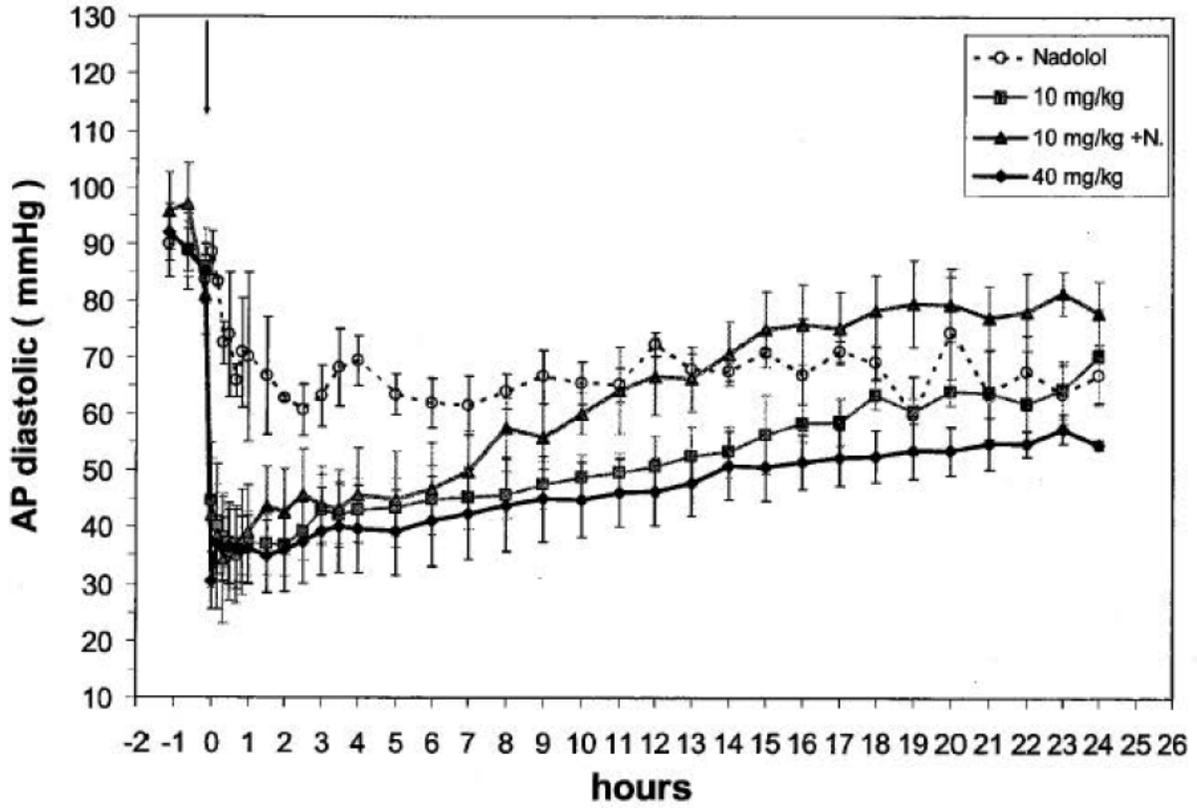


Figure 2: Diastolic Arterial Pressure

Table 3 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on heart rate

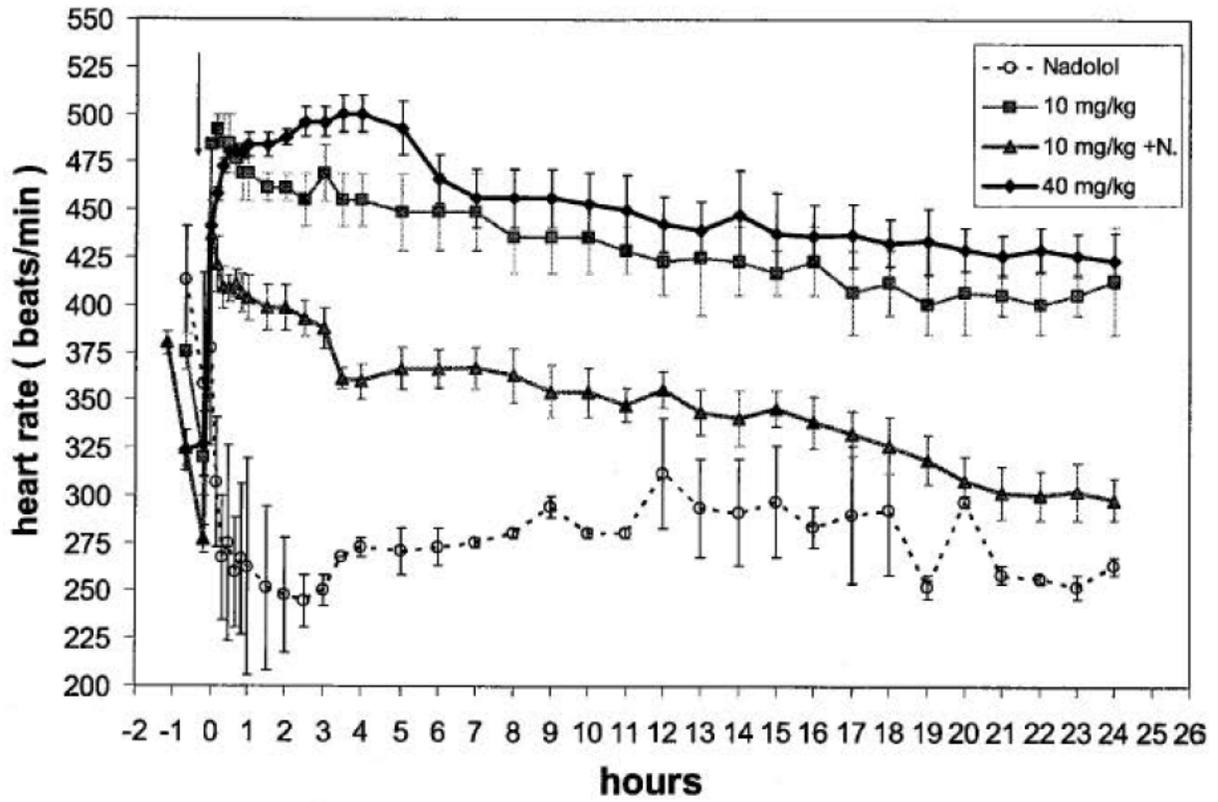


Figure 3: Heart Rate

Table 4 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on respiratory rate

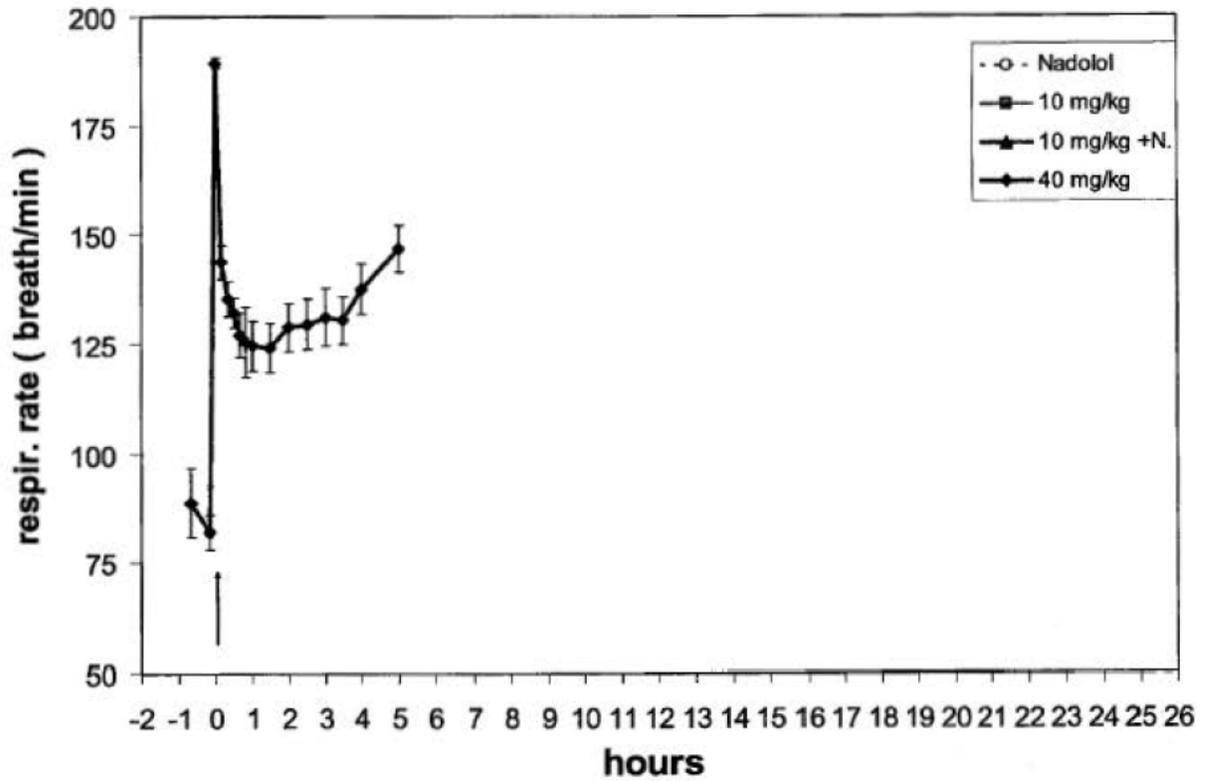


Figure 4: Respiration Rate

Table 5 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on tidal volume

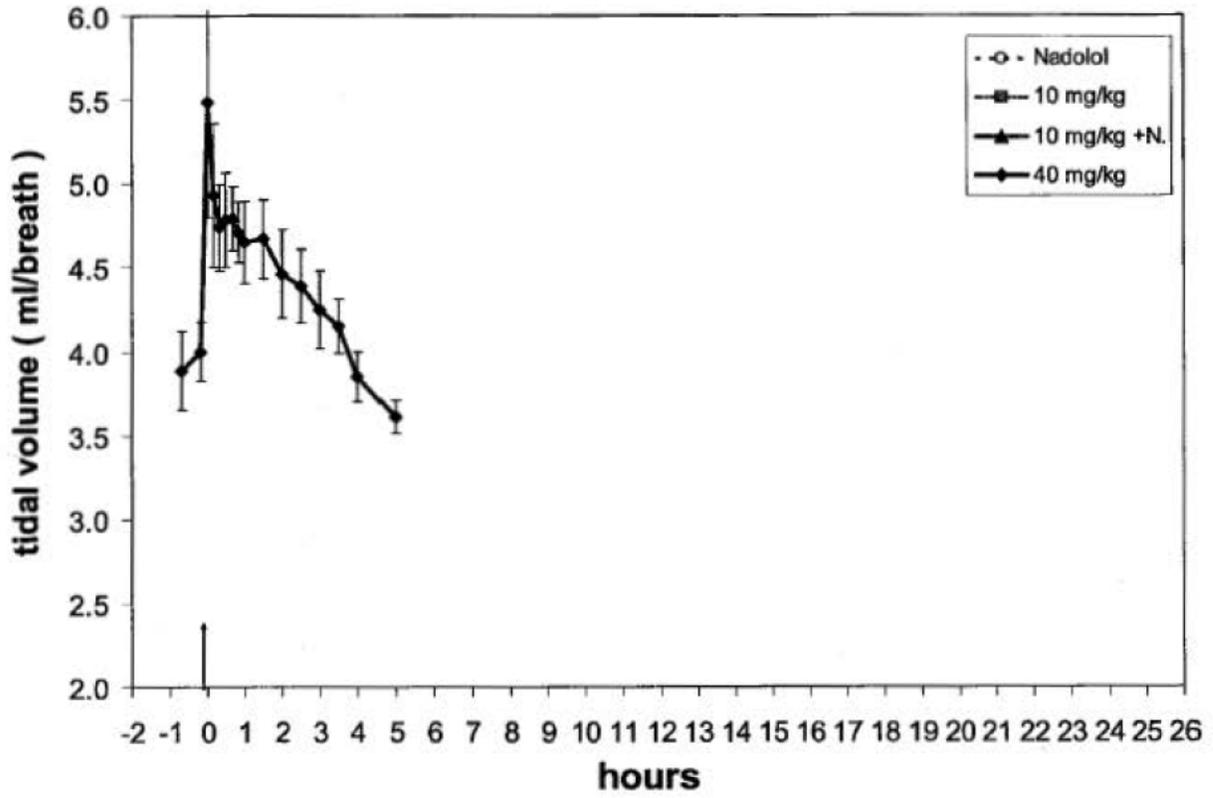


Figure 5: Tidal volume

Table 6 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on minute volume

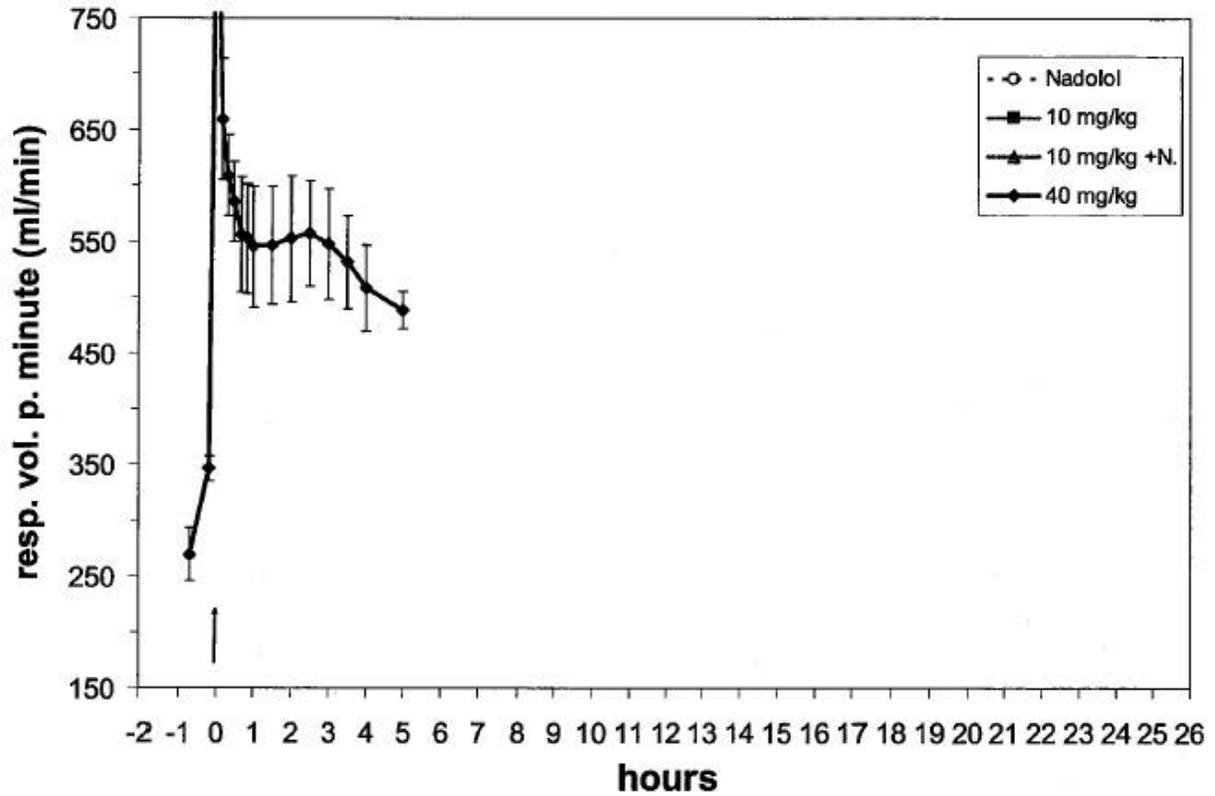


Figure 6: Minute Volume

Table 7 Effects of BI 1744 CL at intravenous doses of 10 and 40 mg/kg administered to male rats on body temperature

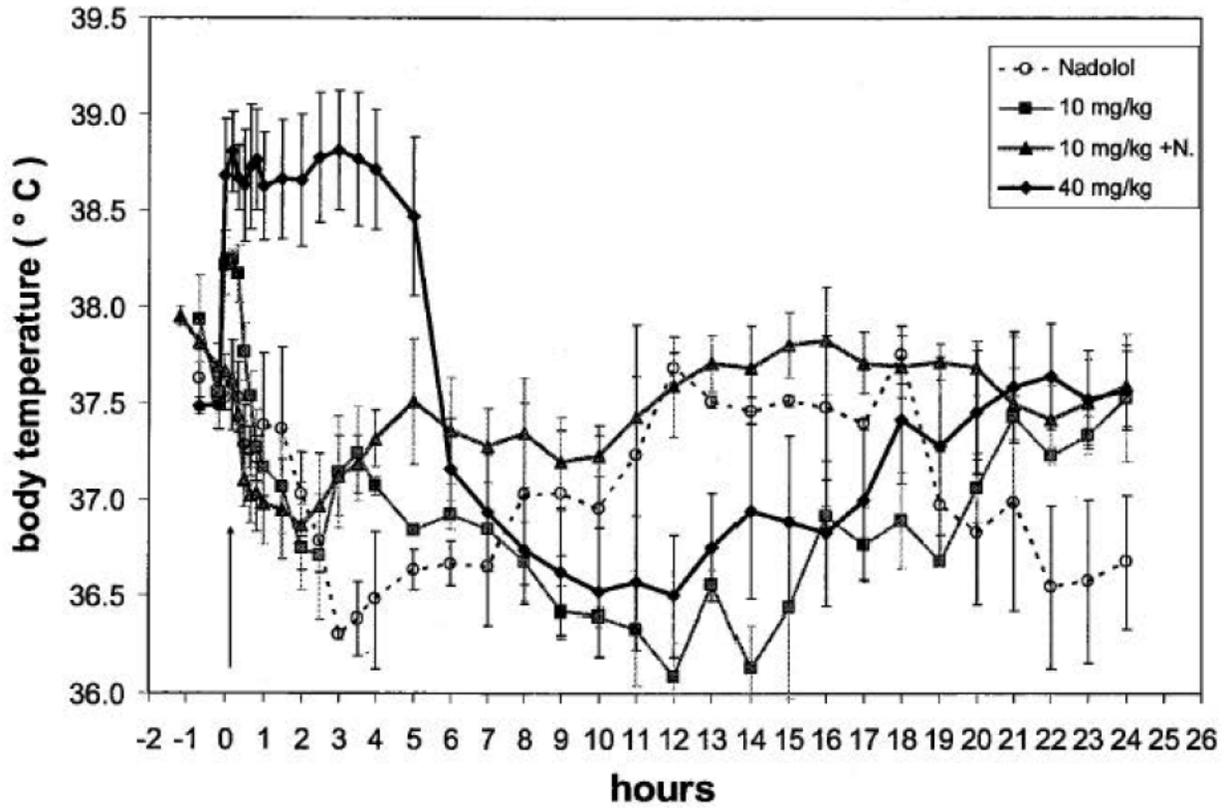


Figure 7: Body Temperature

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7 Genetic Toxicology

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

Study title: **BL 1744 CL: Mutagenicity Study using the Salmonella typhimurium/mammalian-microsome assay (Ames test)**

Study no.:	04B167
Study report location:	Submission dated January 26, 2007
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Department of Non-Clinical Drug Safety Birkendorfer StraBe 65 88397 Biberach an der Riss Germany
Date of study initiation:	July 13, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 1744 CL, Batch number 8460120 (Purity, 97.8%)

Key Study Findings:

- There was no evidence of increased revertant colony counts with BI 1744 CL at doses up to 1000 µg/plate ± S9 using the plate incorporation and pre-incubation methods. Significant toxicity was evident for several tester strains at 5000 µg/plate ± S9 in terms of both reduced bacterial lawn and revertant colony counts; the sponsor might have selected a wider range of lower doses for testing.

Methods

- Strains: TA1537, TA98, TA100, TA1535, and TA102
- Concentrations in definitive study: Using the plate incorporation and pre-incubation methods, doses were 0, 100, 300, 1000, 3000, and 5000 µg/plate ± S9.
- Basis of concentration selection: None
- Negative control: Demineralized water
- Positive control:
- | | |
|-------------------|--------------------------|
| 2-nitrofluorene | 10 µg/plate |
| Sodium azide | 5 µg/plate |
| Mitomycin C | 0.5 µg/plate |
| 9-aminoacridine | 50 µg/plate |
| 2-aminoanthracene | 4 or 10 (TA102) µg/plate |
- Incubation & sampling time: The plate incorporation and pre-incubation methods were used to assess the mutagenicity of BI 1744 CL. S9 was prepared from liver obtained from rats treated with Aroclor 1254 (Batch 1660; (b) (4), MD). Tests were performed in the presence and absence of S9. The concentration of the S9 fraction in the S9 mix was 10%. For the pre-incubation method, vehicle, test article, or positive control, phosphate buffer or S9 mix, and bacteria were pre-incubated and shaken for 20 min at 37°C prior to addition to agar plates. Plates were incubated for 48 hr (TA102: 72 hr). Revertant colonies were counted using an automated plate counter. A reproducible, concentration-dependent increase in the number of revertants of at least one tester strain over the vehicle control value and/or outside the historical control range was considered indicative of genotoxic activity.

Study Validity: The sponsor tested BI 1744 CL up to the limit dose of 5000 µg/plate ± S9 using the plate incorporation and pre-incubation methods. Positive controls produced expected increases of revertant colony counts with and without S9, respectively. Significant toxicity was evident for several tester strains at 5000 µg/plate ± S9 in terms of both reduced bacterial lawn and revertant colony counts; the sponsor might have selected a wider range of lower doses for testing.

Results: Mutagenicity studies were conducted with BI 1744 CL at doses of 0, 100, 300, 1000, 3000, and 5000 µg/plate ± S9 using the plate incorporation and pre-incubation methods. BI 1744 CL precipitated at concentrations ≥3000 µg/plate during the pre-

incubation phase. However, after the 48-72 hr incubation period, precipitation was observed at concentrations ≥ 1000 $\mu\text{g}/\text{plate}$ with S9 and at 5000 $\mu\text{g}/\text{plate}$ without S9. Precipitation was not evident with the plate incorporation method.

Using the plate incorporation method, toxicity was evident at doses ≥ 3000 $\mu\text{g}/\text{plate}$ for strains TA1537, TA98, TA100, and TA102 without S9 and TA1537, TA98, and TA100 with S9 in terms of reduced bacterial lawn and revertant colony counts ($>30\%$). Toxicity was evident for strain TA1535 at 5000 $\mu\text{g}/\text{plate}$ with and without S9 in terms of reduced bacterial lawn. Toxicity was evident for strain TA102 at 5000 $\mu\text{g}/\text{plate}$ with S9 in terms of reduced bacterial lawn and revertant colony counts ($>40\%$).

Using the pre-incubation method, toxicity was evident for strain TA102 at 300 $\mu\text{g}/\text{plate}$ without S9 in terms of reduced bacterial lawn and at ≥ 1000 $\mu\text{g}/\text{plate}$ \pm S9 in terms of reduced bacterial lawn and revertant colony counts ($>20\%$). Toxicity was evident for strain TA1537 at ≥ 1000 $\mu\text{g}/\text{plate}$ without S9 and ≥ 3000 $\mu\text{g}/\text{plate}$ with S9 in terms of reduced bacterial lawns and revertant colony counts (no colonies). Toxicity for TA1537 at 1000 $\mu\text{g}/\text{plate}$ with S9 was limited to reduced bacterial lawn. Toxicity was evident for strains TA98 and TA100 at ≥ 1000 $\mu\text{g}/\text{plate}$ \pm S9 in terms of reduced bacterial lawns and for TA98 at 5000 $\mu\text{g}/\text{plate}$ \pm S9 and TA100 without S9 for reduced revertant colony counts ($>80\%$). Toxicity was evident for strain TA1535 at ≥ 1000 $\mu\text{g}/\text{plate}$ with S9 and ≥ 3000 $\mu\text{g}/\text{plate}$ without S9 in terms of reduced bacterial lawns.

There was no evidence of increased revertant colony counts with BI 1744 CL at doses up to 1000 $\mu\text{g}/\text{plate}$ \pm S9 using the plate incorporation and pre-incubation methods. Significant toxicity was evident for several tester strains at 5000 $\mu\text{g}/\text{plate}$ \pm S9 in terms of both reduced bacterial lawn and revertant colony counts; the sponsor might have selected a wider range of lower doses for testing.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: **BI 1744 CL: Mutagenicity Study using the Mouse Lymphoma (L5178Y) Assay**

Study no.: 04B161

Study report location: Submission dated January 26, 2007

Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co.
KG
Department of Non-Clinical Drug Safety
Birkendorfer StraBe 65
88397 Biberach an der Riss
Germany

Date of study initiation: June 24, 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: BI 1744 CL, Batch number 8460120 (Purity, 97.8%)

Key Study Findings:

- There were no increases of mutant frequencies for the 4- and 20-hr incubations with BI 1744 CL at concentrations up to 200 and 125 µg/mL in the absence of S9, respectively, or the 4-hr incubation with BI 1744 CL at concentrations up to 250 µg/mL in the presence of S9. Under the various conditions, it was judged that BI 1744 CL was tested to sufficiently high concentrations (i.e., reduction of RTG to approximately 10-20% of the control).

Methods

Cell line: L55178Y tk^{+/-} mouse lymphoma cells

Concentrations in definitive study: 4-hr without S9: 25, 50, 100, 125, 150, and 200 µg/mL
20-hr without S9: 10, 25, 50, 75, 100, and 125 µg/mL
4-hr with S9: 25, 50, 100, 150, 200, and 250 µg/mL

Basis of concentration selection: A range finding cytotoxicity study was conducted with doses of 25, 50, 125, 250, and 500 µg/mL. Relative survival following a 4-hr incubation with concentrations of 125 and 250 µg/mL without S9 were 53 and 0%, respectively. Relative survival following a 20-hr incubation with concentrations of 50 and

125 µg/mL without S9 were 103 and 1%, respectively. Relative survival following a 4-hr incubation with concentrations of 125 and 250 µg/mL with S9 were 56 and 0%, respectively.

Negative control: Demineralized water

Positive control: -S9: 0.1 µg/mL 4-nitroquinoline-N-oxide
+S9: 2 µg/mL benzo[a]pyrene

Formulation/Vehicle: Demineralized water

Incubation & sampling time: The microwell method was used in this mutation assay. Cells were treated with BI 1744 CL for 4 hr ± S9 or 20 hr - S9 using duplicate cultures. After treatment, cells were harvested and cell densities were determined. For determination of relative survival, cells were diluted to 8 cells/mL and seeded into 96-well plates and incubated for at least 6 days. For optimal growth and expression of the mutant phenotype, the concentration was adjusted to 2×10^5 cells/mL. Cells were cultured for 2 days to permit expression of the mutant phenotype. Cells were harvested, counted, and diluted to 1×10^4 cells/mL. Trifluorothymidine (TFT) was added at a concentration of 3 µg/mL to allow selection of mutant cells and four 96 well culture plates/culture were filled with 200 µL/well. These cultures were incubated for over a 12-18 day incubation period. Plating efficiency on day 2, as a measure of viability, was determined as described above for relative survival. Plates were examined with a microscope. Plating efficiencies on days 0 and 2, relative survival (RS), relative viability (RV), relative suspension growth (RSG), relative total growth (RTG), and mutant frequency were determined.

A positive response was defined as a concentration-related and/or reproducible increase in the mutant frequency. The average mutant frequency should be at least 2-fold higher than the mean control value.

Study Validity: For the 4- and 20-hr incubations without S9, cells were treated with BI 1744 CL at concentrations up to 200 and 125 µg/mL, respectively. For the 4-hr incubation with S9, cells were treated with BI 1744 CL at concentrations up to 250

µg/mL. For the 4-hr incubation without S9, the RTG value at 200 µg/mL was reduced to 31%. For the 20-hr incubation without S9, the RTG values at 75 and 100 µg/mL were 98 and 14%, respectively. For the 4-hr incubation with S9, the RTG values at 150 and 200 µg/mL were 49 and 18%, respectively. Under the various conditions, it was judged that BI 1744 CL was tested to sufficiently high concentrations (i.e., reduction of RTG to approximately 10-20% of the control). Mutant frequencies for vehicle controls were within the acceptable range ($50-170 \times 10^{-6}$). Positive controls produced expected increases of the mutant frequency in the presence and absence of S9, respectively that met acceptability criteria (mutant frequency of at 300×10^{-6} with at least 40% from small colonies).

Results: There were no increases of mutant frequencies for the 4- and 20-hr incubations with concentrations up to 200 and 125 µg/mL in the absence of S9, respectively, or the 4-hr incubation with concentrations up to 250 µg/mL in the presence of S9.

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

Study title: BI 1744 CL: Intravenous Mutagenicity Study Using Micronucleus Analysis of Rat Bone Marrow

Study no:	U08-1834-01
Study report location:	Draft Report: submission dated June 16, 2008 Final Report: Submission dated March 27, 2009
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Department of Non-Clinical Drug Safety Birkendorfer Str. 65 88397 Biberach an der Riss Germany
Date of study initiation:	March 6, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 1744 CL, batch 4 (Purity, 98.9%)
	BI 1744 BS stock solution (4 mg/mL): batch 5080016
	BI 1744 CL 4.3778 mg (b) (4)
	Citric acid (b) (4)
	Water for injection ad 1.00 mL

Key Study Findings:

- In a single intravenous dose micronucleus test, male rats received BI 1744 CL at doses of 0 (vehicle-control), 1, 10, and 40 mg/kg. A single sampling time (i.e., 24 hr postdose) was used for the control, 1 mg/kg, 10 mg/kg, and positive control groups. Two sampling times (i.e., 24 and 48 hr postdose) were used for the 40 mg/kg group.
- All BI 1744 CL-treated rats showed severe ventral recumbency and deep respiration within 5 min postdose.
- BI 1744 CL produced increased percentages of PCEs (i.e., PCE to NCE ratio was increased up to 1.4-fold) at 10 and 40 mg/kg although statistical significance was only achieved at 40 mg/kg. Increased PCEs were observed at both 24- and 48- hr postdose for 40 mg/kg. Increased percentages of PCEs suggested induction of erythropoiesis although the range of increase remained within historical control values.
- At the 24-hr time point, there were dose-dependent, statistically significant increases in the frequencies of MNEs with BI 1744 CL at 10 and 40 mg/kg (up to 2.8-fold of the vehicle-control). At the 48-hr time point, the percentage of MNE remained elevated for rats at 40 mg/kg (up to 2.2-fold of the vehicle-control).
- It is possible that the increased levels of erythropoiesis observed in treatment groups might have lead to increased frequency of micronuclei. A genotoxic mechanism cannot be excluded based upon available data in the report.
- Due to an increased frequency of micronuclei observed in the study, the sponsor conducted a cardiovascular safety pharmacology study with rats (Study number GP2008/0379/PH5) and a non-GLP mechanistic toxicology study with rats using the intravenous route (Study number U08-1845-01, Draft report provided in Amendment #044 dated July 14, 2008 and Final Report provided in Amendment #086 dated March 16, 2010) in an effort to explain that the observed response was not the result of DNA damage induced by BI 1744 CL. It is possible that the compensatory increases of erythropoiesis observed in treatment groups might have lead to increased micronuclei.

Methods

- Doses in definitive study: 0 (vehicle-control), 1, 10, and 40 mg/kg
- Frequency of dosing: Single dose
- Route of administration: Intravenous (1 mL/min)
- Dose volume: 10 mL/kg
- Formulation/Vehicle: Physiological saline
- Species/Strain: Male Crl: WI(Han) rats from (b) (4)
 [Redacted] Body weight range of 253-283 g on the day of dosing
- Number/Sex/Group: 5 males/group in the 0, 1, and 10 mg/kg groups, 10 males/group in the 40 mg/kg group, and 2 males/group in the positive control group
- Satellite groups: None
- Basis of dose selection: The sponsor conducted a single IV dose toxicity study with male and female rats. Planned doses were 10, 20, 40, and 80 mg/kg. Male and female rats showed dose-dependent reduced motor activity, ventral recumbency, and increased breathing rate at 10, 20, and 40 mg/kg. Female rats that received 80 mg/kg died during or a few hr after administration. Male rats were not treated with 80 mg/kg based upon observed mortality for females. It was expected that doses >40 mg/kg would produce mortality for male rats. There were no differences in toxicity between male and female rats.
- Negative control: A vehicle-control group received physiological saline
- Positive control: Cyclophosphamide, 20 mg/kg

Study Design: Vehicle-control, treatment, and positive control groups are shown in the table below. A single sampling time (i.e., 24 hr postdose) was used for the control, 1 mg/kg, 10 mg/kg, and positive control groups. Two sampling times (i.e., 24- and 48-hr postdose) were used for the 40 mg/kg group. A concurrent vehicle-control group was not included for the 48-hr sampling time. The bone marrow of one femur was flushed and cells suspended in fetal calf serum (FCS). Anucleated erythrocytic cells were separated from other myeloid cells using cellulose column fractionation. One slide per animal was stained with May-Grünwald/Giemsa, coded, and scored. The study protocol stated that 2000 polychromatic erythrocytes (PCE) per animal would be scored for micronucleated cells (MNE); however, due to an increase in the percentage of PCEs, 2000 PCEs would be scored for MNE by each investigator for a total of 4000 PCEs scored. The frequency of polychromatic erythrocytes with micronuclei was determined for each animal and the mean number for the respective dose-group was calculated as percentage (MNE%). As a measure of erythropoiesis or myelotoxicity, the study

protocol stated that the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was determined per animal by counting the first 200 normocytes; however, due to an increase in the percentage of PCEs, 2 x 400 normocytes were evaluated.

The criterion for a positive result is a statistically significant, dose-dependent increase in the frequency of micronucleated polychromatic erythrocytes in treated animals as compared with the negative vehicle control. Additionally, historical control frequencies obtained in similar experiments using this rat strain were taken into consideration.

Table 16 Study design for the single intravenous dose micronucleus test with rats

Test Substance BI 1744 CL	Daily dose (mg/kg)	N	Sampling time (h)	
			24	48
Negative Vehicle Control: Physiological saline	-	5	101-105	
BI 1744 BS				
Low dose	1	5	201-205	
Mid dose	10	5	301-305	
High dose	40	5/5	401-405	406-410
Positive Control: Cyclophosphamide	20	2	501-502	

The stock solution of BI 1744 BS (4 mg/mL) was delivered ready-to-use from the Dept. of Pharmaceutical Research and Development. The analysis of the stock solution was done in the Analytical Sciences Department and resulted in a content of 3.96 mg/mL (99.1%) BI 1744 BS.

Study Validity: Dose selection for BI 1744 CL in the micronucleus study appeared to be appropriate based upon results of a single intravenous dose range finding study. In the dose range finding study, severe clinical signs were observed for both male and female rats that received doses between 10 and 40 mg/kg (i.e., dose-dependent reduced motor activity, ventral recumbency, and increased breathing rate). All female rats that received a dose of 80 mg/kg died. Male rats were not dosed at 80 mg/kg due to observed mortality for females. There were no apparent differences in toxicity between male and female rats following single intravenous doses. Thus, it appeared appropriate to conduct the definitive micronucleus study with male rats only that received BI 1744 CL at intravenous doses up to 40 mg/kg. Comparable clinical signs were observed in male rats that received doses up to 40 mg/kg as described below. Animals treated with the positive control, cyclophosphamide, showed expected increased frequencies of MNE.

Results: There were no deaths with single intravenous doses up to 40 mg/kg in the micronucleus study. All BI 1744 CL-treated rats showed severe ventral recumbency and deep respiration within 5 min postdose. For rats at 1 mg/kg BI 1744 CL, clinical signs lasted less than 5 hr. For rats at 10 and 40 mg/kg, the severe recumbency lasted more than 5 hr.

BI 1744 CL produced increased percentages of PCEs (i.e., PCE to NCE ratio was increased up to 1.4-fold) at 10 and 40 mg/kg although statistical significance was only achieved at 40 mg/kg. Increased PCEs were observed at both 24- and 48- hr postdose for 40 mg/kg. Increased percentages of PCEs suggested induction of erythropoiesis although the range of increase remained within historical control values.

Table 17 Percentages of PCEs (PCE to NCE ratios)

Table 8: 1 Percentages of polychromatic erythrocytes (PCE) in male rats after intravenous treatment with BI 1744 CL - MEAN VALUES

Test substance (mg/kg)	Sampl. time (h)	N	PCE (%) <i>p-value</i> 200 cells	PCE (%) <i>p-value</i> 400 cells 1. Invest.	PCE (%) <i>p-value</i> 400 cells 2. Invest.	PCE (%) <i>p-value</i> 800 cells Summary
Negative vehicle control: Physiological saline	24	5	29.6	27.2	22.9	25.0
BI 1744 free base						
1	24	5	29.6 1.00	28.3 0.74	21.3 0.54	24.8 0.94
10	24	5	30.6 0.72	29.7 0.49	29.2 0.20	29.5 0.28
40	24	5	37.5 0.03*	36.5 0.03*	32.8 0.06	34.6 0.02*
	48	5	36.1 0.10	36.1 0.03*	33.1 0.01*	34.6 0.01*
Positive control: Cyclophosphamide						
20	24	2	15.5 0.05*	14.5 0.05*	13.3 0.05*	13.9 0.05*

* Significantly different from the negative vehicle control ($p \leq 0.05$)

At the 24-hr time point, the IV dose of 1 mg/kg BI 1744 CL produced an increase in the percentage of micronucleated polychromatic erythrocytes (MNE) that was within the historical control range of 0.07-0.20%. However, there were dose-dependent, statistically significant increases (up to 2.8-fold of the vehicle-control) in the frequencies of MNEs with BI 1744 CL at 10 and 40 mg/kg. At the 48-hr time point, the percentage of MNE remained elevated for rats at 40 mg/kg (up to 2.2-fold).

Increased levels of erythropoiesis observed in treatment groups and might have lead to artifactual increases of micronuclei. It is known that conditions that can reduce oxygen tension in the blood can stimulate the secretion of additional erythropoietin, which stimulates an increase in cell division of erythroblasts, and hence, increases the numbers of circulating erythrocytes. This improves the overall oxygen carrying capacity of the blood and restores levels of oxygen tension. The increase in cell division will cause more cells to undergo enucleation and this may result in an increase in micronuclei formed 'spontaneously' (Mutation Research 627: 78-91, 2007). In a cardiovascular safety pharmacology study (GP2008/0379/PH5), BI 1744 CL administered to rats at intravenous doses of 10 and 40 mg/kg produced significant increases of heart rate (500 bpm at peak versus 325 bpm at 1 hr prior to the start of treatment). It is possible under these conditions that the heart tissue became hypoxic. In a non-GLP mechanistic study (U08-1845-01), histopathological examination of hearts

from rats treated with an intravenous dose of 40 mg/kg found multifocal degeneration/necrosis of cardiomyocytes with inflammatory reactions observed primarily in the left ventricle, but also in the septum and right ventricle. Circulating levels of erythropoietin as well as percent reticulocytes were found to be elevated in these animals. Analysis of bone marrow smears from these animals found increases of the erythroid cell fraction, proerythroblasts, macroblasts, and mitoses of erythroid cells (up to 3-fold increase). Further, extramedullary hematopoiesis was also evident in the spleen and liver. The changes in bone marrow cell populations as well as the spleen and liver reflected increased erythropoietic activity and correlated with observed increases of percent reticulocytes and erythropoietin levels.

A genotoxic mechanism for the findings of increased frequencies of micronuclei following intravenous treatment with BI 1744 CL cannot be completely excluded although the findings may have most likely occurred as an artifact of increased erythropoiesis.

Table 18 Frequencies of MNE and percentages of PCE in male rats treated with BI 1744 CL

Table 8: 2 Micronucleated polychromatic erythrocytes (MNE) and mean percentages of polychromatic erythrocytes (PCE) in male rats after intravenous treatment with BI 1744 CL - MEAN VALUES

Test substance (mg/kg)	Sampl. time (h)	N	PCE (%) <i>p-value</i>	MNE (%) <i>p-value</i> 1. Investigator	MNE (%) <i>p-value</i> 2. Investigator	MNE (%) <i>p-value</i> Summary
Negative vehicle control: Physiological saline	24	5	25.0	0.10	0.09	0.10
BI 1744 free base						
1	24	5	24.8 0.94	0.16 0.30	0.16 0.40	0.16 0.28
10	24	5	29.5 0.28	0.26 0.02*	0.29 0.03*	0.28 0.02*
40	24	5	34.6 0.02*	0.27 0.01*	0.27 0.06	0.27 0.02*
	48	5	34.6 0.01*	0.21 0.02*	0.23 0.10	0.22 0.02*
Positive control: Cyclophosphamide 20	24	2	13.9 0.05*	0.75 0.05*	0.60 0.05*	0.68 0.05*

Table 19 Historical control ranges for MNE and PCE to NCE ratios for 48 studies conducted by the testing laboratory with the same rat strain and study protocol

Table 8: 3 Micronucleated polychromatic erythrocytes (MNE) and ratio of poly- to normochromatic erythrocytes in male rats - HISTORICAL CONTROL VALUES

	Male rats, Crl:WI(Han)	
	PCE (%)	MNE (%)
Studies (n)	48	
Mean	32.0	0.14
Range	18.3-43.5	0.07-0.20

The interpretation of bone marrow micronucleus tests can be complicated by compounds that induce toxicity followed by a recovery that results in a rebound in erythropoiesis, or because of their pharmacology, induce cell division in red blood cell precursor cells such as erythroblasts (Mutation Research 627: 78-91, 2007). It has been demonstrated that bleeding or splenectomy can cause increases in micronucleus frequencies in peripheral blood cells of mice. Recombinant EPOs also cause small increases in micronucleated PCEs. When a compound shows little or no genotoxic potential *in silico* or *in vitro* yet induces small increases in micronucleated PCE frequencies at >24 hr or the effect is more pronounced at 48 hr compared to 24 hr, then an effect on erythropoiesis may be suspected (Mutation Research 627: 78-91, 2007).

10 Special Toxicology Studies

Study title: Mechanistic investigation of erythropoiesis in male rats after intravenous administration

Study no.: U08-1845-01
Study report location: Amendment #044 dated July 14, 2008
Amendment #086 dated March 16, 2010
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co.
KG
Department of Non-Clinical Drug Safety
Birkendorfer StraBe 65
88397 Biberach/Riss
Germany
Date of study initiation: May 29, 2008
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BI 1744 CL, Batch number 4 (Purity, 98.9%)
The drug product was supplied as 4 mg/mL
BI 1744 BS in physiological saline,
pH 4.8 buffered with citric acid/NaOH
Batch number 5080050

Key Study Findings:

- In this non-GLP mechanistic study, induction of erythropoiesis was assessed in male rats that received single intravenous doses of BI 1744 CL at 40 mg/kg. The sponsor has conjectured that the increased frequency of micronuclei in a single intravenous dose micronucleus study with rats might have been due to an induction of erythropoiesis.
- Three rats died during intravenous administration of BI 1744 CL at 40 mg/kg.
- Percent reticulocytes were significantly elevated (1.32-fold) at 48 hr postdose for rats treated with BI 1744 CL at 40 mg/kg, but not at 24 hr postdose.
- Analysis of bone marrow smears found increases of the erythroid cell fraction, proerythroblasts, macroblasts, and mitoses of erythroid cells (up to 3-fold increase). These changes were more pronounced at 48 hr postdose than at 24 hr. The changes in bone marrow cell populations reflected increased erythropoietic activity and correlated with observed increases of percent reticulocytes and erythropoietin levels.
- Erythropoietin levels were increased at 6 and 24 hr postdose for rats treated with BI 1744 CL at 40 mg/kg (1178 and 234 pg/mL, respectively, versus BLOQ for the control).

Increases of erythropoietin in treated animals correlate with increases of erythropoietic activity in bone marrow and percent reticulocytes in blood.

- Histopathological examinations were limited to the liver, kidneys, bone marrow (sternum), heart, spleen, and gross lesions. Histopathological changes from rats treated with BI 1744 CL at 40 mg/kg were observed in the heart, spleen, liver, and lung. For rats treated with BI 1744 CL at 40 mg/kg and sacrificed at 24- and 48-hr postdose, multifocal degeneration/necrosis of cardiomyocytes with inflammatory reaction was observed. Extramedullary hematopoiesis was observed in the spleen and liver primarily for animals sacrificed at 48-hr postdose. Extramedullary hematopoiesis appears to correlate with observations of increased erythropoietic activity in treated animals. Multifocal acute hemorrhages were observed in the lung for a small number of animals.
- Analysis of bone marrow cells found a shift from CD71-low (up to a 49% decrease) to CD71-high (up to 28% increase) cells in BI 1744 CL treated rats. The increased expression of CD71 suggests the presence of erythroblastoid precursors.
- Analysis of DNA and RNA content in bone marrow and blood cells appeared to indicate an enhanced maturation of early polychromatic erythroid precursor cells.
- Increases of reticulocytes, erythropoietic activity in bone marrow, erythropoietin levels, and CD71 expression on bone marrow and blood cells, and enhanced maturation of early polychromatic erythroid precursor cells suggest that treatment of male rats with BI 1744 CL at an intravenous dose of 40 mg/kg increased erythropoiesis.

Methods

Doses: 0, 10, or 40 mg/kg
 Frequency of dosing: Single intravenous dose
 Route of administration: Intravenous route
 Dose volume: 10 mL/kg (1 mL/min)
 Formulation/Vehicle: Physiological saline
 Species/Strain: Male Crl:WI(Han) Rats were obtained from (b) (4)

Number/Sex/Group: 10 male rats/group in the main study
 Age: 8-9 weeks old on day 1
 Weight: 253.3 to 295.8 g
 Satellite groups: 2 male rats in the vehicle-control group and 4 male rats/group in the 10 and 40 mg/kg groups
 Unique study design: This non-GLP study was designed to assess parameters of erythropoiesis following a single intravenous dose of BI 1744 BS

Deviation from study protocol: This was a non-GLP study and any deviations were not reported.

Study Design: The purpose of this mechanistic non-GLP study was to assess the underlying working hypothesis for the induction of micronuclei by measuring refined parameters for erythropoiesis following a single intravenous dose of BI 1744 BS. The high dose of 40 mg/kg was identical to the high dose used in the rat micronucleus study (Study number U08-1834-01).

Table 20 Design of single intravenous dose study to assess induction of erythropoiesis

Table 2.5.1: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration. Study design, groups and animal numbers

Group No.	Dose BI 1744 BS [mg/kg]	Administration	Necropsy	Males	
				Main study	Kinetics
1	0	single i.v.	24 h p.a.	101-110	111-112
2	10	single i.v.	-	-	211-214
3	40	single i.v.	24 h p.a.	301-310	311-314
4	0	single i.v.	48 h p.a.	401-410	-
5	40	single i.v.	48 h p.a.	501-510	-

The analysis of the stock solution was done in the Analytical Sciences Department and resulted in a content of 3.998 mg/mL (100%) BI 1744 BS when it was tested according to PV: 1080-B-01I03 (HPLC-UV, 225 nm).

Observations and Results:

Mortality: Animals were observed twice daily for moribundity/mortality.

Three rats treated with BI 1744 CL at 40 mg/kg died during the time of administration (Main study animal #301 and Toxicokinetic animals #311 and 312).

Clinical Signs: Animals were observed twice daily for clinical signs of toxicity.

All rats treated with BI 1744 CL at 40 mg/kg exhibited prone position for at least 8 hr postdose.

Body weight: Body weights were measured during the pretest, on day 1, and prior to necropsy.

Body weight losses of 4.0 and 4.3% were evident at 24 and 48 hr postdose, respectively, for rats treated with BI 1744 CL at 40 mg/kg.

Hematology: Blood samples for assessment of a complete panel of hematology parameters were collected at 24 and 48 hr postdose.

Treatment with BI 1744 CL at 40 mg/kg significantly decreased hemoglobin levels, red blood cell counts, and hematocrit, and increased the percent reticulocytes. Hemoglobin levels, red blood cell counts, and hematocrit were significantly decreased (up to 11%) at 24 and 48-hr postdose. Percent reticulocytes were significantly elevated (1.32-fold) at 48 hr postdose, but not at 24 hr postdose. Neutrophil counts were elevated at 24 hr, but not at 48 hr.

Table 21 Hematology parameters at 24- and 48-hr postdose

Parameter	24-hr postdose		48-hr postdose	
	Control	40 mg/kg	Control	40 mg/kg
Hemoglobin g/dL	14.12	12.82* (91%)	14.30	12.73 (89%)
Red blood cells 10 ⁶ /μL	7.709	7.222* (94%)	7.894	6.995 (89%)
Hematocrit %	41.83	37.74* (90%)	42.79	38.64* (90%)
Reticulocyte %	36.1	31.3	37.1	49.3* (133%)
Neutrophils 10 ³ /μL	1.085	2.428 (224%)	NC	NC
Neutrophils %	16.56	30.90* (187%)	NC	NC
Lymphocytes	5.475	4.981	NC	NC

10 ³ /μL		(91%)		
Lymphocytes %	80.45	66.22* (82%)	NC	NC

* Statistical significance was achieved (p≤0.05)

NC = No change

Clinical Chemistry: Blood samples for assessment of a complete panel of clinical chemistry parameters were collected at 24 and 48 hr postdose.

A number of changes of clinical chemistry parameters were observed at 24- and/or 48-hr postdose following treatment with an intravenous dose of BI 1744 CL at 40 mg/kg. AST activity was increased to 1.9-fold of the control at 24 hr postdose; however, there was no difference evident at 48-hr postdose. The elevation of ALT activity might correlate with histopathological findings of degeneration and necrosis in the heart. Triglyceride levels were decreased to 42 and 24% of the control, respectively. Calcium and magnesium levels were decreased up to 13% at 24-hr postdose, but no changes were evident at 48-hr postdose. Globulins (Total, A-, and B-) at 24- and 48-hr postdose were increased by up to 22%; however, G-Globulins were decreased up to 26%. Albumin levels and the A/G ratios at 24- and 48-hr postdose were decreased up to 22%.

Table 22 Clinical chemistry parameters at 24- and 48-hr postdose

Parameter	24-hr postdose		48-hr postdose	
	Control	40 mg/kg	Control	40 mg/kg
AST U/L	94.53	178.63* (189%)	63.29	73.24 (116%)
Triglyceride mmol/L	1.189	0.494* (42%)	1.600	0.390 (24%)
Calcium mmol/L	2.832	2.701* (95%)	NC	NC
Magnesium mmol/L	0.963	0.835* (87%)	NC	NC
Total Protein g/L	65.87	63.66	66.17	63.02* (95%)
Albumin g/L	37.45	32.73 (87%)	38.95	33.22 (85%)
Albumin %	56.84	51.43* (91%)	58.86	52.73* (95%)
Globulin g/L	28.42	30.93 (109%)	27.22	29.80 (109%)
Globulin %	43.16	48.57 (113%)	41.14	47.27 (115)
A/G	1.323	1.065 (81%)	1.433	1.117 (78%)
A-Globulin %	23.33	25.31* (109%)	21.06	24.71* (117%)
B-Globulin %	17.96	21.88* (122%)	18.22	20.93* (115%)
G-Globulin %	1.87	1.38 (74%)	1.86	1.63* (89%)

* Statistical significance was achieved (p≤0.05)

NC = No change

Bone marrow smears: Bone marrow smears were prepared at necropsy from all main study animals at 24 and 48 hr postdose.

At 24-hr postdose: Proerythroblasts, macroblasts, and mitoses of erythroid cells were increased by 89.1, 55.5, and 61.6%, respectively. Neutrophilic band cells and lymphocytes were decreased by 33 and 37%, respectively. Segmented neutrophils were increased by 28.5%.

At 48-hr postdose: The erythroid cell fraction, proerythroblasts, macroblasts, and mitoses of erythroid cells were increased by 22.0, 208.3, 127.2, and 150.6%, respectively. The granulocyte to erythroid cell ratio was decreased by 30.4%. Neutrophilic myeloblasts, neutrophilic band cells, and lymphocytes were decreased by 28.4, 46.3, and 33.3%, respectively.

The changes in bone marrow cell populations reflect increased erythropoietic activity and correlated with observed increases of percent reticulocytes and erythropoietin levels.

Table 23 Evaluation of bone marrow cell populations at 24 and 48 hr postdose

Table 3.2.3: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration - bone marrow examination (mean group values)

Dose of BI 1744 BS [mg/kg]	24 h p.a.			48 h p.a.		
	0 (Control)	40		0 (Control)	40	
Group	1	3		4	5	
Parameter	mean	mean	Δ%	mean	mean	Δ%
Erythroid fraction [%]	132	148	12.0	148	↑180	22.0
Normoblast fraction (%)	122	131	7.3	136	↑149.9	10.2
Gran/Ery-ratio	1.12	0.97	-13.6	0.93	↓0.65	-30.4
Proerythroblasts [%]	0.73	↑1.37	89.1	0.92	↑2.82	208.3
Macroblasts [%]	2.07	↑3.22	55.5	2.23	↑5.07	127.2
Mitoses erythroid cells [%]	0.57	0.91	61.6	0.74	↑1.84	150.6
Neutrophilic myeloblasts [%]	5.69	5.07	-10.8	5.16	↓3.69	-28.4
Neutrophilic band cells [%]	10.6	↓7.15	-32.7	9.98	↓5.36	-46.3
Neutrophilic segmented cells [%]	13.6	↑17.5	28.5	12.3	14.5	18.4
Lymphocytes [%]	10.3	↓6.45	-37.4	5.90	↓3.93	-33.3
Rest [%]	35.7	↓25.9	-27.5	21.0	↓15.0	-28.6

Δ% percent deviation from Control (calculated from original raw data/rounded)

↑ statistically significant change compared with Control; $p \leq 0.05$, many to one t-test, two sided

↓ statistically significant change compared with Control; $p \leq 0.05$, many to one t-test, two sided

Erythropoietin: Blood samples for determination of plasma erythropoietin were collected at 6, 24, and 48 hr postdose. Erythropoietin levels were determined in plasma using a sandwich enzyme immunoassay (ELISA).

Erythropoietin levels were increased at 6 and 24 hr postdose for rats treated with BI 1744 CL at 40 mg/kg. Erythropoietin levels were still elevated at 48 hr postdose for 2 rats that received BI 1744 CL (data not provided). Erythropoietin levels were undetectable for control rats.

Increases of erythropoietin in treated animals correlate with increases of erythropoietic activity in bone marrow and percent reticulocytes in blood.

Table 24 Erythropoietin levels at 6, 24, and 48 hr postdose

Table 3.3: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration - plasma erythropoietin concentrations (mean animal values)

Parameter [unit]	Hours p.a.	6 h		24 h		48 h	
	Group	1+4	3+5	1	3	4	5
	Dose of BI 1744 BS [mg/kg]	0 (Control)	40	0 (Control)	40	0 (Control)	40
Erythropoietin [pg/mL]		<i>mean</i>	<i>mean</i>	<i>mean</i>	<i>mean</i>	<i>mean</i>	<i>mean</i>
		#	1178.1	#	234.1	#	#

below detection limit (47 pg/mL)

Gross Pathology: Necropsy examinations were performed on all main study animals at 24 or 48 hr postdose. Summary tables and individual animal listings were not provided.

Gross pathologic changes at necropsy were noted in lungs of rats given 40 mg/kg.

Histopathology: Histopathological examinations were limited to the liver, kidneys, bone marrow (sternum), heart, and spleen from main study animals in Groups 1, 3, 4, and 5. If deemed necessary, organs and tissues with macroscopic changes were also examined. Representative sections of all tissues collected at necropsy were processed and embedded in Paraplast® (paraffin). Evaluated tissues were sectioned at a thickness of approximately 4 µm and stained with hematoxylin-eosin (H&E). Samples containing bone were decalcified before further processing. Summary tables and individual animal listings were not provided.

Histopathological changes from rats treated with BI 1744 CL at 40 mg/kg were observed in the heart, spleen, liver, and lung.

Heart: For rats treated with BI 1744 CL at 40 mg/kg and sacrificed at 24 and 48 hr postdose, multifocal degeneration/necrosis of cardiomyocytes with inflammatory reaction was observed primarily in the left ventricle, but also in the septum and right ventricle.

Spleen: At 48 hr postdose, spleens from all rats treated with BI 1744 CL at 40 mg/kg were observed with extramedullary hematopoiesis. Extramedullary hematopoiesis appears to correlate with observations of increased erythropoietic activity in treated animals.

Liver: At 48 hr postdose, livers from 5 of 10 rats treated with BI 1744 CL at 40 mg/kg were observed with extramedullary hematopoiesis. Extramedullary hematopoiesis appears to correlate with observations of increased erythropoietic activity in treated animals.

Lung: Based upon gross pathological findings, 1 rat at 24 hr postdose and 3 rats at 48 hr postdose were observed with multifocal acute hemorrhages.

Table 25 Histopathological changes in the spleen, liver, heart, and lung at 24 and 48 hr postdose from rats that received BI 1744 CL at 40 mg/kg

Table 3.5.2: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration of 40 mg/kg BI 1744 CL – pertinent histopathological changes

Organ	hours p.a.	Group	Pertinent histopathological changes
Spleen	24 h	3	minimal extramedullary erythropoiesis (EP) (1/10)
	48 h	5	mild (6/10) to moderate (4/10) extramedullary EP
Liver	24 h	3	minimal extramedullary hematopoiesis (1/10)
	48 h	5	minimal extramedullary hematopoiesis (5/10)
Heart	24 h	3	minimal (1/10) to mild (7/10) to moderate (1/10), multifocal degeneration / necrosis of cardiomyocytes with inflammatory reaction, primarily in the left ventricle, but also in the septum and the right ventricle
	48 h	5	ditto, but mild (2/10) to moderate /severe (8/10)
Lung	24 h	3	moderate multifocal acute hemorrhages (1 additional sample) correlating with gross pathological findings
	48 h	5	ditto (3 of all 3 additional samples) correlating with gross pathological findings

Immunohistochemistry: Bone marrow (sternum) was immunohistochemically stained for PCNA and Ki-67.

No unequivocal differences between control animals and treated rats were observed with respect to the immunohistochemical staining of hematopoietic cells for PCNA and Ki-67.

Flow cytometry: Bone marrow cells (obtained from the right humerus at necropsy) and peripheral whole blood (collected before necropsy at 24 or 48 hr postdose) were evaluated for signs of erythropoiesis. Bone marrow cells and whole blood were stained with a combination of fluorochrome-labeled antibodies with specificity for CD71 (the transferrin receptor, a marker for erythroblastoid precursors as well as for proliferating cells) and CD45LCA (the leukocyte common antigen). The fluorochrome labels were PE-Cy5 for CD45LCA and R-phycoerythrin for CD71. To determine nonspecific binding, isotype control staining was performed under identical conditions using the OptiClone[®] IgG1-FITC/IgG1-R-phycoerythrin/IgG1-PE-Cy5 mixture of fluorochrome-labeled mouse antibodies.

In addition, glutaraldehyde fixed cells from bone marrow and whole blood were stained with acridine orange to measure cellular DNA and RNA content. Distinct populations

were gated according to their amount of DNA and RNA: nucleated cells (high DNA, high RNA), immature polychromatic erythroid precursor cells (low DNA, low RNA), and mature normochromatic erythroid precursor cells (no DNA, no RNA).

Tibias of all animals killed at scheduled sacrifices were flushed and prepared for micronucleus evaluation; however, no evaluation was performed.

Flow cytometric analysis of all samples was performed with a Coulter EP1CS-XL-MCL flow cytometer, equipped with a 488 nm argon laser. Fifty thousand (50,000) cells per sample were analyzed by the EPICS-XL-MCL.

CD71-expressing bone marrow cells and erythrocytes:

Analysis of bone marrow cells found a shift from CD71-low (48% decrease) to CD71-high (27.8% increase) cells in BI 1744 CL treated rats (Group 3 and 5). This shift was characterized flow cytometrically by a significant decrease in the relative number of CD71-low cells with a concomitant significant increase in the relative number of CD71 high cells. This shift was more pronounced at 24 hr postdose than at 48 hr postdose. The increased expression of CD71 suggests the presence of erythroblastoid precursors.

Table 26 CD71 expressing bone marrow cells

Table 3.6.2.1.1: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – relative number of CD71 expressing bone marrow cells (Group mean values and statistical comparison to the respective Controls)

Relative number of bone marrow cells (%)				
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
CD71^{low} cells (mean)	21.56	11.13 ↓	21.76	17.70 ↓
		p<0.0001*		p=0.0115*
CD71^{high} cells (mean)	19.57	25.01 ↑	22.06	27.30 ↑
		p=0.0027*		p=0.0410*
Ratio CD71^{high} to CD71^{low} cells (mean)	0.92	2.50 ↑	1.03	1.62 ↑
		p<0.0004*		p=0.0089*

* Wilcoxon rank sum test
 ↑ Significantly different, higher than Control
 ↓ Significantly different, lower than Control

Analysis of red blood cells found a significant increase (1.65-fold) in mean expression of CD71 in treated rats at 48 hr postdose, but not at 24 hr postdose. The increased expression of CD71 suggests the presence of erythroblastoid precursors.

Table 27 CD71-expressing blood cells

Table 3.6.3.1: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – relative number and mean fluorescence of CD71 expressing blood cells (Group mean values and statistical comparison to the respective Control)

Relative number of blood cells (%)				
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
CD71 ⁺ cells (mean)	2.2	2.2 n.s.*	2.3	3.8 ↑ p<0.0001*
Mean fluorescence				
Mean fluorescence CD71 ⁺ cells (mean)	70.3	82.8 ↑ p=0.0003*	84.2	129.2 ↑ p<0.0001*

* Wilcoxon rank sum test
 ↑ Significantly different, higher than Control
 ↓ Significantly different, lower than Control
 n.s. not significant

Expression of CD45LCA on bone marrow cells: At 48 hr postdose, there was a significant increase (20%) in the number of CD45LCA expressing cells (CD45LCA⁺). The difference appeared to be primarily due to a decline of CD45LCA⁺ cells in the control group at 48 hr as compared to 24 hr.

Table 28 CD45LCA expressing bone marrow cells

TABLE 3.6.2.1.2: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – relative number and mean fluorescence of CD45LCA expressing bone marrow cells (Group mean values and statistical comparison to Control)

Relative number of bone marrow cells				
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
CD45LCA ⁺ cells (Group mean)	66.71%	71.36%	60.86%	73.29%
Standard deviation of the mean	6.36%	10.21%	7.70%	6.09%
Statistical difference to Control*		n.s.		p=0.0008
Mean fluorescence				
Mean fluorescence CD45LCA ⁺ cells (Group mean)	16.92	18.99	15.92	19.04
Standard deviation of the mean	1.48	2.92	1.65	1.94
Statistical difference to Control*		n.s.		p=0.011

unpaired Student's t-test, performed with Graph Pad Prism, version 5.01

n.s. not significant

Concomitant expression of CD45LCA and CD71 on bone marrow cells:

With double immunostaining to assess expression of both CD71 and CD45LCA, a significant increase in the population of CD45LCA⁻CD71⁻ high bone marrow cells (1.8-fold) as well as the CD45LCA⁺CD71⁻ high (1.2-fold) population was observed 24 hr following a single dose of 40 mg/kg BI 1744 CL. At 48 hr postdose, there was a significant increase in the both the CD45LCA⁺CD71⁻ high (1.3-fold) and the CD45LCA⁺CD71⁺ (1.2-fold) bone marrow cell populations due to a decrease of CD45LCA⁻CD71⁻ cells (35% decrease). The increased expression of CD71 suggests the presence of erythroblastoid precursors.

Table 29 Concomitant expression of CD45LCA and CD71 on bone marrow cells

Table 3.6.2.1.3: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – relative number of bone marrow cells expressing CD45LCA and/or CD71 (Group mean values and statistical comparison to the respective Controls)

	Relative number of bone marrow cells (%)			
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
CD45LCA ⁻ CD71 ⁻ cells (mean)	30.6	24.5 n.s.*	36.8	24.2 ↓ p=0.0007*
CD45LCA ⁺ CD71 ⁻ cells (mean)	50.3	51.0 n.s.*	41.8	49.3 ↑ p=0.0021*
CD45LCA ⁻ CD71 ⁺ cells (mean)	1.9	3.5 ↑ p=0.0003*	2.4	2.5 n.s.*
CD45LCA ⁺ CD71 ⁺ cells (mean)	17.3	21.1 ↑ p=0.0101*	19.0	24.0 ↑ p=0.0433*

* Wilcoxon rank sum test
 ↑ Significantly different, higher than Control
 ↓ Significantly different, lower than Control
 n.s. not significant

Nucleic acid staining of bone marrow and blood cells:

Analysis of bone marrow cells found that the relative number of total nucleated cells (high DNA, high RNA) was not altered at 24 hr postdose, but was significantly increased at 48 hr postdose. It is noted that the increase was relatively modest (6.7% increase). A significant decrease in the relative number of immature polychromatic erythroid precursor cells (low DNA, low RNA) was seen at 24 hr postdose (29.3%). The relative numbers of mature normochromatic erythroid precursor cells (no DNA, no RNA) were decreased at 48 hr postdose (21.2%). As a result, the ratio of immature polychromatic erythroid precursor cells to mature normochromatic erythroid precursor cells was decreased (-43%) at 24 hr postdose and there was a significant increase in the ratio of total nucleated cells versus erythroid precursor cells (polychromatic plus normochromatic ones) at 48 hr postdose (+27%).

Table 30 Relative number of bone marrow cell populations based upon nucleic acid staining

Table 3.6.2.2.1: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – relative number of bone marrow cell populations based on nucleic acid staining (Group mean values and statistical comparison to the respective Control)

Relative number of bone marrow cells (%)				
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
Total nucleated cells (mean)	68.8	71.4 n.s.*	68.6	73.2 ↑ p=0.0274*
Immature polychromatic erythroid precursor cells (mean)	18.8	13.3 ↓ p=0.0004*	19.4	17.3 n.s.*
Mature normochromatic erythroid precursor cells (mean)	12.2	15.2 n.s.*	11.8	9.3 ↓ p=0.0454*

* Wilcoxon rank sum test
 † Significantly different, higher than Control
 ↓ Significantly different, lower than Control
 n.s. not significant

Table 31 Ratio of individual bone marrow cell populations based upon nucleic acid staining

Table 3.6.2.2.1: 2 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – ratio of individual bone marrow cell populations based on nucleic acid staining (Group mean values and statistical comparison to the respective Control)

Ratio of relative numbers of individual bone marrow cell populations				
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
Ratio total nucleated cells to erythroid precursor cells (mean)	2.24	2.68 n.s.*	2.23	2.85 ↑ p=0.0274*
Ratio polychromatic to normochromatic erythroid precursor cells (mean)	1.57	0.90 ↓ p<0.0001*	1.74	1.95 n.s.*

* Wilcoxon rank sum test
 ↑ Significantly different, higher than Control
 ↓ Significantly different, lower than Control
 n.s. not significant

Erythrocytes were the predominant cell type in the blood. In comparison, the number of nucleated cells was very small. Thus, no changes in the relative numbers of these two cell populations were seen following administration of BI 1744 CL. No differences were seen in the number of immature polychromatic erythroid precursor cells.

RNA Content:

Analysis of bone marrow cells found for total nucleated cells that there was a significant decrease in mean RNA at 24 hr postdose (-16.5%). At 48 hr postdose, there was a slight increase in mean RNA that was not statistically significant. For immature polychromatic erythroid precursor cells, the mean RNA content was significantly decreased at 24 hr postdose (-20%), but increased at 48 hr postdose (+92%). These results appear to indicate an enhanced maturation of early polychromatic erythroid precursor cells.

Table 32 RNA content of total nucleated bone marrow cells and immature polychromatic erythroid precursor cells in bone marrow

Table 3.6.2.2.2: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – mean RNA content of total nucleated bone marrow cells and of immature polychromatic erythroid precursor cells in the bone marrow (Group mean values and statistical comparison to the respective Control)

	Mean RNA content			
	24 h p.a.		48 h p.a.	
	Group 1 Control	Group 3 40 mg/kg BI 1744 BS	Group 4 Control	Group 5 40 mg/kg BI 1744 BS
Mean RNA content of total nucleated cells (Group mean)	127.7	106.0 ↓ p=0.0028*	111.8	128.7 n.s.*
Mean RNA content of immature polychromatic erythroid precursor cells (Group mean)	21.0	16.8 ↓ p=0.0206*	11.2	21.5 ↑ p<0.0001*

* Wilcoxon rank sum test
 ↑ Significantly different, higher than Control
 ↓ Significantly different, lower than Control
 n.s. not significant

For immature polychromatic erythroid precursor cells, the mean RNA content was significantly increased at 24 and 48 hr postdose (up to a 2-fold increase). Based on absolute fluorescence intensity, this difference was more pronounced at 48-hr postdose than at 24-hr postdose.

Table 33 RNA content of total nucleated cells and immature polychromatic erythroid precursor cells

TABLE 3.6.3.3: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration – mean RNA content of total nucleated cells and of immature polychromatic erythroid precursor cells (Group mean values and statistical comparison to Control)

Mean RNA content				
	24 h p.a.		48 h p.a.	
	0 mg/kg BI 1744 BS	40 mg/kg BI 1744 BS	0 mg/kg BI 1744 BS	40 mg/kg BI 1744 BS
Mean RNA content of immature polychromatic erythroid precursor cells (Group mean)	10.91	13.62	9.51	19.46
Standard deviation of the mean	1.06	1.32	0.51	2.37
Statistical difference to Control		p<0.0001	n.s.	p<0.0001 not significant

unpaired Student's t-test, performed with Graph Pad Prism, version 5.01

Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected at 0.167, 0.5, 1, 3, 8, and 24 hr postdose. The free base (BI 1744 BS) was measured by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS). The lower limit of quantification (LOQ) was 25.0 pmol/L.

C_{max} and AUC values for BI 1744 CL at 10 and 40 mg/kg increased in an approximate dose proportional manner.

Table 34 Toxicokinetic parameters in male rats following single intravenous doses of BI 1744 CL at 10 and 40 mg/kg

Table 3.4: 1 BI 1744 CL: Mechanistic investigation of erythropoiesis in male rats after intravenous administration - major toxicokinetic parameters of BI 1744 BS

Parameter	Unit	Mean Group 2 (10 mg/kg)	Mean Group 3 (40 mg/kg)
C(max)	[pmol/L]	3,490,000	18,700,000
C(24 h)	[pmol/L]	15,700	21,600
AUC(0-24h)	[pmol·h/L]	8,770,000	33,600,000

11 Integrated Summary and Safety Evaluation

BI 1744CL is a long-acting β_2 -adrenergic agonist under development for the treatment of COPD and asthma. In the initial IND submission, the sponsor submitted a standard battery of genotoxicity tests with BI 1744CL that included an in vitro bacterial mutagenicity test, an in vitro mouse lymphoma assay, and an in vivo micronucleus test that was part of a 4-week inhalation toxicology study with rats. The in vitro bacterial mutagenicity test and in vitro mouse lymphoma assay were judged to be negative; however, the in vivo micronucleus test was judged to be inadequate.

The following comment was conveyed to the sponsor on March 21, 2007: “Your *in vivo* rat micronucleus study conducted as part of the 4-week repeat dose inhalation toxicology study is not considered valid since the test did not use an acceptable limit dose or a dose that produced a dose-limiting toxicity for this endpoint. Therefore, provide adequate justification for the selected doses of this assay based on accepted criteria for evaluation of this endpoint or conduct an additional *in vivo* test for chromosomal damage using accepted criteria for dose selection as part of your on-going drug development program.”

In response, the sponsor conducted an additional in vivo micronucleus report using the intravenous route (Study number U08-1834-01, Draft Report: Submission dated June 16, 2008 and Final Report: Submission dated March 27, 2009). Male rats received BI 1744 CL at doses of 0, 1, 10, and 40 mg/kg. A single sampling time (i.e., 24-hr postdose) was used for the control, 1 mg/kg, 10 mg/kg, and positive control groups. Two sampling times (i.e., 24 and 48 hr postdose) were used for the 40 mg/kg group. All BI 1744 CL-treated rats showed severe ventral recumbency and deep respiration within 5 min postdose. BI 1744 CL produced increased percentages of PCEs at 10 and 40 mg/kg (i.e., PCE to NCE ratio was increased up to 1.4-fold) although statistical significance was only achieved at 40 mg/kg. Increased PCEs were observed at both 24- and 48-hr postdose for 40 mg/kg. Increased percentages of PCEs suggested induction of erythropoiesis although the range of increase remained within historical control values. At the 24-hr time point, there were dose-dependent, statistically significant increases in the frequencies of MNEs with BI 1744 CL at 10 and 40 mg/kg (up to 2.8-fold of the vehicle-control). At the 48-hr time point, the percentage of MNE remained elevated for rats at 40 mg/kg (up to 2.2-fold of the vehicle-control).

Due to an increased frequency of micronuclei observed in the study, the sponsor conducted a cardiovascular safety pharmacology study with rats (Study number GP2008/0379/PH5) and a non-GLP mechanistic toxicology study with rats using the intravenous route (Study number U08-1845-01, Draft report provided in Amendment #044 dated July 14, 2008 and Final Report provided in Amendment #086 dated March 16, 2010) in an effort to explain that the observed response was not the result of DNA damage induced by BI 1744CL. It is possible that the compensatory increases of erythropoiesis observed in treatment groups might have lead to increased micronuclei. It is known that conditions that can reduce oxygen tension in the blood (e.g., significant

increases of heart rate with ensuing tissue hypoxia) can stimulate the secretion of additional erythropoietin, which stimulates an increase in cell division of erythroblasts, and hence, increases the numbers of circulating erythrocytes. This improves the overall oxygen carrying capacity of the blood and restores levels of oxygen tension. The increase in cell division will cause more cells to undergo enucleation and this may result in an increase in micronuclei formed 'spontaneously' (Mutation Research 627: 78-91, 2007).

Effects of single intravenous doses of BI 1744 CL at 10 and 40 mg/kg on cardiovascular function (systolic and diastolic arterial pressure and heart rate) were examined in conscious rats. There were dose-dependent and long-lasting (up to 24 hr postdose with 40 mg/kg) decreases of systolic and diastolic blood pressure (up to 50% and 60%, respectively) and increases of heart rate (500 bpm at peak versus 325 bpm at 1 hr prior to the start of treatment) with BI 1744 CL at 10 and 40 mg/kg. The intensity of this effect was reduced by pre-treatment with nadolol (30 mg/kg) 1 hr prior to the application of BI 1744 CL (i.e., heart rate reached a peak of 475 bpm with BI 1744 CL at 10 mg/kg versus 250 bpm with nadolol; the baseline ranged from 375 to 425 bpm).

In a non-GLP mechanistic study, induction of erythropoiesis was assessed in male rats that received single intravenous doses of BI 1744 CL at 40 mg/kg. The sponsor has conjectured that the increased frequency of micronuclei in a single intravenous dose micronucleus study with rats might have been due to an induction of erythropoiesis. Percent reticulocytes were significantly elevated (1.32-fold) at 48 hr postdose for rats treated with BI 1744 CL at 40 mg/kg, but not at 24 hr postdose. Analysis of bone marrow smears found increases of the erythroid cell fraction, proerythroblasts, macroblasts, and mitoses of erythroid cells (up to 3-fold increase). These changes were more pronounced at 48 hr postdose than at 24 hr. The changes in bone marrow cell populations reflected increased erythropoietic activity and correlated with observed increases of percent reticulocytes and erythropoietin levels. Erythropoietin levels were increased at 6 and 24 hr postdose for rats treated with BI 1744 CL at 40 mg/kg (1178 and 234 pg/mL, respectively, versus BLOQ for the control). Increases of erythropoietin in treated animals correlate with increases of erythropoietic activity in bone marrow and percent reticulocytes in blood. Histopathological examinations were limited to the liver, kidneys, bone marrow (sternum), heart, spleen, and gross lesions. Histopathological changes from rats treated with BI 1744 CL at 40 mg/kg were observed in the heart, spleen, liver, and lung. For rats treated with BI 1744 CL at 40 mg/kg and sacrificed at 24 and 48 hr postdose, multifocal degeneration/necrosis of cardiomyocytes with inflammatory reaction was observed. Extramedullary hematopoiesis was observed in the spleen and liver primarily for animals sacrificed at 48 hr postdose. Extramedullary hematopoiesis appears to correlate with observations of increased erythropoietic activity in treated animals. Multifocal acute hemorrhages were observed in the lung for a small number of animals. Analysis of bone marrow cells found a shift from CD71-low (up to a 49% decrease) to CD71-high (up to 28% increase) cells in BI 1744 CL treated rats. The increased expression of CD71 suggests the presence of erythroblastoid precursors. Analysis of DNA and RNA content in bone marrow and blood cells appeared to indicate an enhanced maturation of early polychromatic erythroid precursor cells. Increases of

reticulocytes, erythropoietic activity in bone marrow, erythropoietin levels, and CD71 expression on bone marrow and blood cells, and enhanced maturation of early polychromatic erythroid precursor cells suggest that treatment of male rats with BI 1744 CL at an intravenous dose of 40 mg/kg increased erythropoiesis.

The interpretation of bone marrow micronucleus tests can be complicated by compounds that induce toxicity followed by a recovery that results in a rebound in erythropoiesis (Mutation Research 627: 78-91, 2007). It has been demonstrated that bleeding or splenectomy can cause increases in micronucleus frequencies in peripheral blood cells of mice. Recombinant EPOs also cause small increases in micronucleated PCEs. When a compound shows little or no genotoxic potential *in silico* or *in vitro* yet induces small increases in micronucleated PCE frequencies at >24 hr or the effect is more pronounced at 48 hr compared to 24 hr, then an effect on erythropoiesis may be suspected (Mutation Research 627: 78-91, 2007). A flow chart for dealing with chemicals that induce a positive non-relevant response in the *in vivo* micronucleus test through induction of erythropoiesis.

Figure 1 From Mutation Research 627: 78-91, 2007: Increases in micronucleated bone marrow cells in rodents as a result of erythropoiesis that do not indicate genotoxic hazards

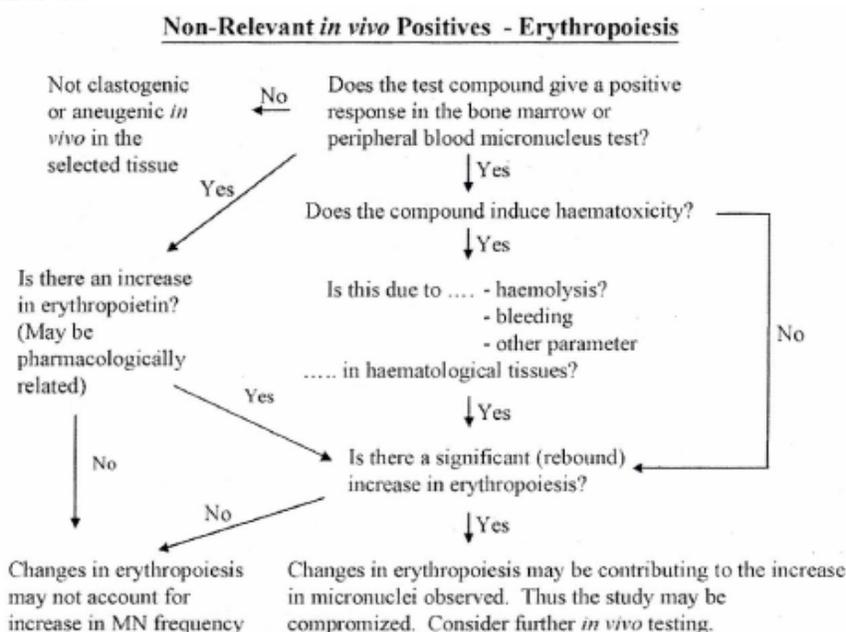


Fig. 3. Flow chart showing the approaches to be taken when an effect on erythropoiesis occurs in rodent bone marrow micronucleus tests.

In the present situation, BI 1744CL administered to rats at intravenous doses of 10 and 40 mg/kg produced significant increases of heart rate. It is possible under these conditions that the heart tissue became hypoxic and tissue damage ensued (i.e., multifocal degeneration/necrosis of cardiomyocytes with inflammatory reactions). Circulating levels of erythropoietin significantly increased leading to erythropoietic activity in the bone marrow, spleen, and liver. The increase in cell division will cause more cells to undergo enucleation and this may result in an increase in micronuclei

formed 'spontaneously' (Mutation Research 627: 78-91, 2007). The observed increases in the frequency of micronuclei following treatment with BI 1744CL most likely occurred as a consequence of increased erythropoiesis. It appears unlikely that a genotoxic mechanism was responsible for the observed induction of micronuclei.

The sponsor submitted the following questions regarding the findings of an increased frequency of micronuclei in rats treated with BI 1744CL by the intravenous route. These questions are listed below followed by Division responses.

Question 1: Can the positive findings observed in the in vivo rat micronucleus test [U08-1834-01] be attributed to the exaggerated pharmacological action of BI 1744 CL?

Response: We agree that the finding of increased frequencies of micronuclei was more than likely due to a drug enhanced (compensatory) erythropoiesis and it is unlikely that this finding was due to a genotoxic event based upon the large increase in the percentage PCEs in the high dose group of the in vivo micronucleus study with rats [U08-1834-01] and the non-GLP mechanistic study [U08-1845-01] that provides additional supportive information of induction of erythropoietic activity following treatment with BI 1744 CL.

Question 2: Should the findings of the in vivo micronucleus test with rats be listed in the informed consent?

Response: These findings should be listed in the informed consent; however, the finding may be qualified as follows. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

Question 3: Are the assessment of the in vivo micronucleus test with rats adequate to support the NDA filing for Olodaterol?

Response: The studies entitled “Measurement of cardiovascular and respiratory function in conscious rats” [GP2008/0379/PH5] and “Mechanistic investigation of erythropoiesis in male rats after intravenous administration” [U08-1845-01] provide an adequate explanation for the finding of increased frequencies of micronuclei reported in Study #U08-1834-01 and support an NDA filing.

Question 4: Should these findings be placed in the product labeling?

Response: These findings should be listed in the product labeling; however, the finding may be qualified as follows. The increased frequency of micronuclei was likely related to drug enhanced (compensatory) erythropoiesis. This mechanism for induction of micronuclei formation is likely not relevant at clinical exposures.

12 Appendix/Attachments

Appendix 1: Email from Dr. David Jacobson-Kram dated June 9, 2008

Appendix 2: Email from Dr. Robert Heflich dated June 9, 2008

Appendix 1: Email from Dr. David Jacobson-Kram dated June 9, 2008

See below.

From: Robison, Timothy W
Sent: Monday, June 09, 2008 10:17 AM
To: Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Levy, Dan; Jacobson-Kram, David; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu, Chingju W; 'Sotomayor, Rene E'; Yao, Jiaqin
Cc: Shea, Molly; McGovern, Timothy J
Subject: Request for Consult to the CDER Genetic Toxicology Subcommittee (IND 76,362; BI 1744 Cl)

I am forwarding a request for consult to the CDER Genetic Toxicology Subcommittee from the Pulmonary Division regarding the results of an *in vivo* rat micronucleus study. See the reviewer's questions below. Do you accept the sponsor's interpretation for the results obtained in the study? Please forward comments and questions to the entire committee and reviewers. Thanks.

Tim Robison

From: Shea, Molly
Sent: Monday, June 09, 2008 10:05 AM
To: Robison, Timothy W
Cc: McGovern, Timothy J; Shea, Molly
Subject: Genotox. Committee Consult Request: IND 76,362 BI 1744 Cl

Hi Tim,

I have an IND that is currently active for the treatment of COPD and asthma from Boehringer Ingelheim (BI). BI 1744 is a long acting B2 agonist inhalation drug that is in Phase 2 clinical trials as monotherapy administered once daily at 0 (placebo), 2, 5, 10 and 20 µg for 4 weeks. (It is also active in another IND 76,397 in combination with tiotropium bromide also up to 20 ug and for 4 weeks treatment to date) ^{(b) (4)}

 We requested the sponsor to conduct a micronucleus assay using adequate doses of BI 1744.

BI repeated the *in vivo* micronucleus study at single IV doses of 1, 10 and 40 mg/kg in male Wistar rats. The following table summarizes the study outcome followed by the historical control table provided by BI.

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<< OLE Object: Picture (Enhanced Metafile) >>

(b) (4)

<< OLE Object: Picture (Enhanced Metafile) >>

My questions for the Committee are:

(b) (4)



I have a question:

Lastly, the sponsor is currently conducting carcinogenicity studies for BI 1744.

Molly E. Shea, Ph.D.

Pharmacologist

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Building 22, Room 3335

Silver Spring, MD 20993

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Appendix 2: Email from Dr. Robert Heflich dated June 9, 2008

(b) (4)

From: Robison, Timothy W
Sent: Monday, June 09, 2008 9:17 AM
To: Robison, Timothy W; Atrakchi, Aisar H; Elespuru, Rosalie K.; Levy, Dan; Jacobson-Kram, David; Heflich, Robert; Bigger, Anita; Ouyang, Yanli; Moore, Martha; Agarwal, Rajiv; Benz, Robert Daniel; De, Mamata; Jagannath, Devaraya R; McGovern, Timothy J; Nostrandt, Amy C; Sheu, Chingju W; 'Sotomayor, Rene E'; Yao, Jiaqin
Cc: Shea, Molly; McGovern, Timothy J
Subject: Request for Consult to the CDER Genetic Toxicology Subcommittee (IND 76,362; BI 1744 CI)

I am forwarding a request for consult to the CDER Genetic Toxicology Subcommittee from the Pulmonary Division regarding the results (b) (4). See the reviewer's questions below. Do you accept the sponsor's interpretation for the results obtained in the study? Please forward comments and questions to the entire committee and reviewers. Thanks.

Tim Robison

From: Shea, Molly
Sent: Monday, June 09, 2008 10:05 AM
To: Robison, Timothy W
Cc: McGovern, Timothy J; Shea, Molly
Subject: Genotox. Committee Consult Request: IND 76,362 BI 1744 CI

Hi Tim,

I have an IND that is currently active for the treatment of COPD and asthma from Boehringer Ingelheim (BI). BI 1744 is a long acting B2 agonist inhalation drug that is in Phase 2 clinical trials as monotherapy administered once daily at 0 (placebo), 2, 5, 10 and 20 µg for 4 weeks. (It is also active in another IND 76,397 in combination with tiotropium bromide also up to 20 ug and for 4 weeks treatment to date) (b) (4)

(b) (4)

<< OLE Object: Picture (Enhanced Metafile) >>

(b) (4)



Lastly, the sponsor is currently conducting carcinogenicity studies for BI 1744.

Molly E. Shea, Ph.D.
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Building 22, Room 3335
Silver Spring, MD 20993
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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-76362	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICA LS INC	BI 1744 CL RESPIMAT INHALATION SPRAY

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

/s/

TIMOTHY W ROBISON
05/10/2010

(b) (4)

MOLLY E SHEA
05/10/2010

Appendix 5

Pharm/Tox Review of IND 76,362 dated October 26, 2011 (Reproductive Toxicology Review)

**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: **76362**

Supporting documents	Sponsor's letter date	CDER stamp date
SD-5	Jan 26, 2007	Jan 29, 2007
SD-59	Mar 27, 2009	Mar 30, 2009
SD-66	Jul 30, 2009	Jul 31, 2009
SD-124	Oct 7, 2011	Oct 11, 2011

Product: **Respimat Inhalation Spray**

Indication: Chronic obstructive pulmonary disease (COPD) and bronchial asthma

Sponsor: **Boehringer Ingelheim Pharmaceuticals Inc**

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

Reviewer: L. Steven Leshin, D.V.M., Ph.D.

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

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

Project Manager: Eunice H. Chung-Davis

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1 Executive Summary

1.1 Recommendations

1.1.1 Clinical Study(ies) Safe to Proceed

This review was not associated with a specific clinical protocol. At this time Inhalation doses of up to 10 µg/day are being studied for safety and efficacy.

1.1.3 Additional Recommendation(s)

Comments to relayed to the sponsor:

We have reviewed your submission dated Oct 7, 2011 that addresses the validity of the inhalation rat embryo-fetal developmental toxicity study and have the following request for information:

Provide AUC values from the reproductive toxicology studies to ensure adequate exposure to support the validity of these studies, particularly the EDF study, and to enable label margins based on systemic drug exposure.

1.2 Brief Discussion of Nonclinical Findings

Studies of reproductive and developmental toxicology were conducted in rats and rabbits using the inhalation route of drug delivery.

Fertility and Early Embryonic Development in Rats: Rats were exposed by inhalation to doses of BI 1744 CL at 58, 193, and 3068 µg BI 1744 BS/kg/day. There were two mortalities in the high dose group related to BI 1744 CL treatment. The stages of estrous cycle and their mean duration were similar in all groups. There were no effects of treatment on mating or pregnancy performance. Testes and epididymides weights (as a % of body weight) were reduced for all BI 1744 doses, but without histopathological correlates, and there were no paternal effects on mating or pregnancy.

Therefore, the NOAEL for paternal and maternal reproductive, fertility, and pregnancy assessments was 3068 µg BI 1744 BS/kg/day, corresponding to PPD of 306.8 µg/kg/day and a BI 1744 concentration for males of 208000 pmol/L (80.3 ng/mL), and for females 167000 pmol/L (64.5 mg/mL). Since this high dose was associated with mortality (one female and one male), the NOAEL for reproductive, fertility and pregnancy exceeded the maximal tolerated dose administered by inhalation in the rat.

Embryo-Fetal Development in Rats: In the rat study of embryo-fetal development, there were no adverse effects on embryo-fetal survival or growth and the incidence of malformations or variations. BI 1744 CL was inhaled for 1 hour/day during gestation days 6 to 17 at achieved doses of 64, 222, or 1054 µg BI 1744 BS/kg/day. In the 222

and 1054 µg BI 1744 BS/kg/day groups there was a slight increase in fetuses with the incomplete ossification of sternbrae in the mid and high dose groups compared with controls (88% and 91% vs 76% respectively). Incomplete ossification is a late pregnancy effect not attributed to organogenesis and not considered teratogenic. Ossification can be arrested or delayed, but since the rats were all sacrificed, it cannot be ascertained if sternbrae ossification was delayed. It is a common finding with maternal toxicity associated with maternal weight loss, but signs of maternal toxicity related to under nutrition or weight loss were not apparent. BI 1744 treated animals had a small non-dose-dependent increase in weight gain during the first week of dosing, as a sign of appropriate drug exposure, but ossification typically occurs later in development. Other findings were considered incidental due to their low incidence and lack of dose dependency, or within recent historical control range of incidence (single fetus on the study in the mid dose, 222 µg BI 1744 BS/kg/day, had complete situs inversus; two incidences of umbilical hernia occurred in the 222 µg BI 1744 BS/kg/day group and a single incidence in the 1054 µg BI 1744 BS/kg/day group; and one fetus in the mid dose and two fetuses in the high dose group had anomalous confluence of umbilical and/or hepatic vein with inferior vena cava). Therefore, the developmental and maternal NOAEL for BI 1744 CL rat was the high dose of 1054 µg BI 1744 BS.

A comparison of doses and toxicological findings in this study suggests that the maximal tolerated dose was not attained and the dosing was inadequate for regulatory acceptance. A comparison of aerosol concentrations and achieved dose level indicated amounts substantially less than the two other studies in rats (fertility and postnatal development studies). Note that although the exposure is 60 minutes in this study, compared with 35 minutes in the other two rat studies, the aerosol concentration is only 29 µg/L compared to 160 µg/L and 173 µg/L for the two other studies, a 5.5- and 5.9-fold less than those studies.

Embryo-Fetal Development in Rabbits: Exposure of pregnant rabbits to BI 1744 CL at the maximum practicable concentration of approximately 120 µg/L resulted in salivation, an initial transient reduction in food consumption, and embryo-fetal resorptions. Overall, this dose was sufficiently tolerated to be suitable for an embryo-feta toxicity study.

Pregnant rabbits were exposed to BI 1744 CL by inhalation for a 90-minute exposure period on days 6 through 19 inclusive after mating, at doses of 0, 289, 974, or 2489 µg/kg/day. There were no treatment-related adverse effects on embryo-fetal survival or growth. In the high dose 2489 µg BI 1744 BS/kg/day group, a greater number of fetuses/litters had major abnormalities compared with the controls or the historical control range. Most of the fetuses clustered in a single litter and had a specific syndrome of abnormalities which had not been previously seen in this strain/source of rabbits (historical control data). These included acrania, retinal folds, split sternum and distortion of the sternum. Also there was uneven ossification of the cranial bones/ribs/long bones (thickened ribs/distorted ribcage; short scapula, humerus, radius, ulna, femur, tibia, fibula; and/or partially open eyelids/eyelids not fused centrally; cleft palate; forelimb flexure/malrotated hindlimbs). There was no consistency in the type of abnormalities seen in the other affected fetuses in this group. Other major

abnormalities included two fetuses in one litter (#9) had dilated ascending aorta/aortic arch; narrow pulmonary trunk; enlarged left and small right heart ventricles; one of these fetuses also had the pulmonary arteries arising directly from the aortic arch; one fetus in a second litter (#69) had multiple abnormalities including acrania, absent eyes, split sternum, gastroschisis, cervicothoracic scoliosis and lordosis, forelimb flexure and hindlimb hyperflexion; one fetus in a third litter (#82) had multiple retinal folds while another fetus in the same litter had dilated ascending aorta/aortic arch, narrow pulmonary trunk, enlarged left heart ventricle/small right atrium and ventricle; one fetus in a fourth litter (#99) had dorsoventral distortion of the sternum.

The maternal NOAEL was the high dose of 2489 µg BI 1744 CL kg/day. The developmental NOAEL for BI 1744 CL in the rabbit was the mid dose of 974 µg BI 1744 BS/kg/day corresponding to an AUC₀₋₂₄ of 182000 pmol-h/L.

Pre and Postnatal Toxicology in Rats: In the pre and postnatal toxicology study, rats were exposed to BI 1744 CL by inhalation for 35 minutes/day from gestation day 6 to 20, and then from lactation day 2 to 21 of dams (day 0 = day of birth of litter). The achieved doses were 0, 59, 297 and 3665 µg/kg/day close to the target doses of 0, 50, 200 and 3000 µg/kg/day.

A significantly increased weight gain and a transient, but significant, decrease in food consumption were observed following the first day of treatment. Observations such as changes in behavior (subdued and excessive general behavior, including paddling) and eye(s) partially closed were generally seen over the first 2-3 days in all high dose animals. Salivation and fur staining occurred in all BI 1744 CL treatment groups. There were slight differences in litter size by day 21 of lactation, and reduced survival in the low and high dose groups. Litter weight and pup weight in the high dose group were slightly less, but not statistically different than the weights of the control group by day 21 of lactation. There was a dose-related decrease in the time to eye opening in both sexes. There were no effects of treatment on the mating, fertility or bearing of live implants to Day 14/15/16 of gestation in the F₁ animals. Based upon these results the NOAEL was 3665 µg/kg/day BI 1744 BS for maternal toxicity, F₁ survival and development, and F₁ mating, fertility and pregnancy.

General Comments with regards to long acting β-adrenergic agonists

The increase in body weight gains noted in males and females in the fertility study, rat and rabbit embryo studies and rat postnatal development study is a well characterized anabolic effect associated with a β₂-agonist treatment. β₂-agonists both enhance lipolysis on adipocytes and enhance skeletal muscle hypertrophy through adrenergic receptors on these tissue.

The reduced testes and epididymides weights noted in the fertility studies, as well as ovarian changes noted in toxicology studies (6 month inhalation rat toxicology study, U08-1691-01-AM1, submitted Nov 2008, submitted Jan 27 2009, SD-54) could be either direct effects, an alteration of neuroendocrine signals in the brain-pituitary-gonadal axis,

or a combination of both, none of which could be readily distinguished in standard general toxicology studies. Drug distribution studies (Reports U10-1602-01 and U10-4128-01, submitted May 20 2011, SD-114) indicated increased pituitary uptake of radiolabeled BI 1744 as well as relatively high levels in testes. There is also fetal accumulation in mid gestation fetuses which supports direct action of BI 1744 on embryofetal development. These findings are consistent with approved LABA drugs in that studies they had no effect in studies of rat fertility.

Whether the teratogenic effects observed here are typical of approved LABAs cannot be determined from the label information for those LABAs. There does seem to be a difference between findings with inhalation studies and oral administration, in that oral administration of LABA does result in teratogenic effects, while inhalation studies do not. The obtained systemic concentrations are most likely the link; at a high enough systemic exposure, whether by inhalation or oral routes of administration, teratogenic effects appear. For the approved LABA products, as for BI 1744 here, the safety margin is quite high (100s-1000s-fold) for the maximal human dose or proposed human dose, that the risk to a pregnant patients would expected to be minimal.

Comment on Study Validity

Pre-NDA meeting responses were sent to the Sponsor on Sept 28 2011 that included a comment that the Sponsor should justify that the rat embryo-fetal development studies were valid since it appeared that dosing may not have included the maximal tolerated or feasible doses. They responded on Oct 11 2011 (SD-124) with a satisfactory explanation the studies were indeed valid. This is based on the lack of observed maternal weight loss for a class of drugs that are known to cause weight gain, and the fact that higher doses used in the later postnatal study did not result in the potential of cannibalism as an explanation for possible reduced effects on structural abnormalities by the "absence or loss" of these animals. The Sponsor also notes the high safety margins in the current studies for the proposed maximal human clinical dose, however this does not contribute to the determination of a valid study, although it would contribute to the overall risk-benefit relationship for pregnant patient use of this product.

Comment of Safety Margins

Safety margins for fertility and embryo-fetal development are necessary to convey in the label and are based on systemic drug concentrations for an inhaled product. Sufficient blood samples were not collected to determine AUC values with the exception of one dose. C_{max} values were obtained at the end of the exposure period. Since a linear correlation exists between dose administered and C_{max} , a safety margin could be extrapolated based on the C_{max} values. It would be more accurate however, due to the large individual variation in blood concentrations from inhalation exposure to determine AUC exposure from multiple timepoint drug analysis.

Therefore, the Sponsor should provide AUC values from the reproductive toxicology studies to ensure adequate exposure, particularly the EDF study, and to enable label margins based on systemic drug exposure.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number not provided

2.1.2 Generic Name Olodaterol

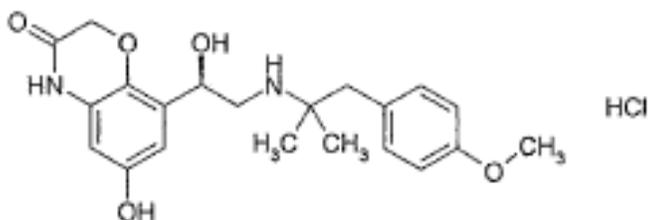
2.1.3 Code Name BI 1744 CL

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

2.1.5 Molecular Formula $C_{21}H_{26}N_2O_5 \cdot HCl$

Molecular Weight 422.9 g/mol (salt) and 386.5 g/mol (free base identified as BI 1744 BS)

2.1.6 Structure



2.1.7 Pharmacologic class Long-acting, human β_2 -adrenoreceptor partial agonist

2.2 Relevant IND/s, NDA/s, and DMF/s

None.

2.3 Clinical Formulation

The composition of BI 1744 CL Respimat Inhalation Spray at the 1, 2.5, 5 and 10 µg doses and the placebo is provided in the Table 1, below. Two actuations will be administered to achieve the 2, 5, 10 and 20 µg/day doses (proposed for the Phase 2B clinical trial).

Table 1: Composition of BI 1744CL Respimat Inhalation Spray

Ingredient	Percent formula (g/100 mL)					Function
	2 µg dose	5 µg dose	10 µg dose	20 µg dose	Placebo	
BI 1744 BS	0.0091	0.0226	0.0452	0.0905	-	API as base
BI 1744 Cl	0.0099	0.0248	0.0495	0.0990	-	API as salt
Benzalkonium chloride, NF	(b) (4)					(b) (4)
Edetate sodium, USP						
Citric acid, anhydrous, NF						
						(b) (4)
Total mass	100.0	100.0	100.0	100.0	100.0	
	(b) (4)					

2.3.1 Drug Formulation

Route of administration: Oral Inhalation (nebulized)

2.3.2 Comments on Novel Excipients

There are no novel excipients.

2.3.3 Comments on Impurities/Degradants of Concern

Refer to previous Pharmacology/Toxicology reviews.

2.4 Proposed Clinical Population and Dosing Regimen

The drug treatment under development is for patients with chronic obstructive pulmonary disease (COPD) and bronchial asthma. Based upon the EOP2 Meeting held on July 17, 2008 (DARRTS Meeting Minutes Aug 11, 2008), Study 1222.6 (report received Dec 22, 2009), and 1222.5 both referred to in the Review of Dr. Karimi-Shah (DARRTS June 17, 2010) and in the July 7, 2010 Teleconference Meeting Minutes (DARRTS Aug 13, 2010), the single daily doses of 10 µg and 20 µg produced similar efficacy results (based on assessment of FEV₁). The internal documents indicate that the Sponsor was notified that their Phase 2 proposed dose and administration frequency clinical studies may have been inadequate to determine appropriate Phase 3

doses and administration frequency. Additional clinical studies or data may be forthcoming.

At this time, to determine human exposure margins based on nonclinical toxicological studies, the 10 µg once daily dose will be used. Based upon information from the review of the tQT study in healthy subjects (reviewed by Drs. Christine Garnett, Justin Earp, Joanne Zhang and Suchitra Balakrishnan; DARRTS Oct 27 2008), a 10 µg dose of inhaled BI 1744 CL resulted in a geometric mean C_{max} of 3.29 µg/mL at a T_{max} mean of 0.367 h, but the AUC was not calculated for this dose due to many samples below the limits of detection.

2.5.1 Previous Clinical Experience

The information readily available (ie., annual reports and clinical submissions not immediately accessible) indicated the following clinical studies had been conducted:

Table 2: Clinical Studies

Study	Patient Population	Frequency / Dose (inhaled µg BI 1744 CL)
1222.1	healthy male and female volunteers	escalating single dose, 0.5 to 70 µg
1222.2	healthy male and female volunteers	once daily dose for 14 days 2.5, 10, or 30 µg
1222.3	COPD patients	single dose 10 µg, 20 µg, 30 µg or 50 µg
1222.4	first US study Asthma patients	single dose 2, 5, 10 or 20 µg
1222.5	COPD patients,	once daily dose for 4 weeks, 0, 2, 5, 10 or 20 µg using the Respimat inhaler
1222.6	COPD patients	once daily dose 0, 5, or 10 µg, or twice daily 2 or 5 µg for 3 weeks using the Respimat inhaler
1222.8	tQT study, healthy adults	single dose 10 µg, 20 µg, 30 µg or 50 µg (10 µg dose x 1, 2, 3, or 5 actuations, respectively)

2.5.2 History of Regulatory Submission

Refer to previous reviews.

3 Studies Submitted

3.1 Studies Reviewed

Table 3: Studies Reviewed

Study Report / Location	Study Title
U08-2297-01 SD-66, Vol. 1-2, July 31 2009, Final (SD-5, Jan 29 2007 Draft)	BI 1744 CL Study for Effects on Fertility and Embryonic Development to Implantation in Rats (Inhalation)
U05-2534 BOI 306/042979 SD-66, vol.22, July 31 2009	BI 1744 CL Study of Effects on Embryo-Fetal Development in CD Rats by Inhalation Administration
U05-2535 BOI308/040116 SD-5, vol. 23, Jan 29, 2007	BI 1744 CL Pilot and Preliminary Embryo-Fetal Study in the Rabbit by Inhalation Administration
U06-1019 BOI 307/043146 SD-5, vols. 23-24, Jan 29, 2007	BI 1744 CL Study of the Effects on Embryo-Fetal Toxicity in the Rabbit by Inhalation Administration
494520 SD-59, Mar 30, 2009, vols. 13-14.	Study for Effects of Pre and Post Natal Development including Maternal Function in Rats (inhalation)

3.2 Studies Not Reviewed

Only reproduction and developmental studies were reviewed here. See other Pharmtox Reviews for additional studies that were reviewed.

3.3 Previous Reviews Referenced

The following PharmTox reviews are filed in DARRTS.

Feb 28, 2007 Preliminary Safety Review (Dr. Molly Shea)

- safety pharmacology
- 4 and 13 week rat tox,
- 4 week dog tox
- genetic tox studies

March 2, 2007 Review, to support carc study dosing proposal (Dr. Molly Shea)

- 13-week mice tox
- 13-week rat tox (draft)

May 10, 2010 Review (Dr. Tim Robison)

- safety pharmacology
- 4-week tox with micronucleus evaluation
- genetic tox studies

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: BI 1744 CL: Study for Effects on Fertility and Early Embryonic Development to Implantation in Rats (Inhalation)

Study no.:	U08-2297-01 (494452/27678)
Study report location:	SD-5, July 31, 2009 (vol 1-2)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	25 September 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 1744 CL, Batch 05 Zo08, Purity 99.26% Batch A06KH08, Purity 99.84% This is from the Certificate of Analysis for the formulated drug product, a certificate of analysis of the drug substance was not provided.

Key Study Findings

Rats were administered by inhalation doses of BI 1744 CL at 58, 193, and 3068 µg BI 1744 BS/kg/day with pulmonary deposited doses (PDD, estimated at 10% of the inhalation dose) of 5.8, 19.3, and 306.8 µg/kg/day. There were two mortalities in the high dose group related to BI 1744 CL treatment.

There were no effects on the stages of estrous cycle and their mean duration, and no effects of treatment on mating or pregnancy performance.

A significant dose related decrease in absolute epididymides weights was seen compared to control. Testes weights in all treated groups were reduced compared to control. There were no histopathological findings related to these organ weight effects or any other histopathological finding and male reproductive performance was not affected.

Although there was an effect on testes and epididymides weights (as a % of body weight) for all BI 1744 doses, there was no effect on paternal toxicity. Therefore, the NOAEL for paternal and maternal reproductive toxicity, and pregnancy, was 3068 µg BI 1744 BS/kg/day, corresponding to PPD of 306.8 µg/kg/day and a BI 1744 concentration

for males of 208000 pmol/L (80.3 ng/mL), and for females 167000 pmol/L (64.5 mg/mL). Since this high dose was associated with mortality (one female and one male), the NOAEL for reproductive, fertility and pregnancy exceeded the maximal tolerated dose administered by inhalation in the rat. Approved LABA drugs also did affect fertility in rat studies.

Methods																																																
Doses:	<table border="1"> <thead> <tr> <th>Dose Group</th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> </tr> </thead> <tbody> <tr> <td>Target dose ($\mu\text{g}/\text{kg}/\text{day}$)</td> <td>0</td> <td>50</td> <td>200</td> <td>3000</td> </tr> <tr> <td>Achieved doses (μg BI 1744 BS/kg/day)</td> <td>0</td> <td>58</td> <td>193</td> <td>3068</td> </tr> <tr> <td>Pulmonary deposited doses ($\mu\text{g}/\text{kg}/\text{day}$)</td> <td>0</td> <td>5.8</td> <td>19.3</td> <td>306.8</td> </tr> </tbody> </table>				Dose Group	1	2	3	4	Target dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	50	200	3000	Achieved doses (μg BI 1744 BS/ kg/day)	0	58	193	3068	Pulmonary deposited doses ($\mu\text{g}/\text{kg}/\text{day}$)	0	5.8	19.3	306.8																								
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Frequency of dosing:	exposure for 35 min each day Males: Day 1 of the study equates to Day 1 of dosing; Females: Day 15 of the study equates to Day 1 of dosing																																															
Route of administration:	Inhalation via snout exposure																																															

Formulation/Vehicle:	Solution consisting of benzalkonium chloride 10 mg/100 mL disodium edetate 10 mg/ 100 mL water
Species/Strain:	Sprague-Dawley rats, Crl: CD®(SD) males were between 6-7 weeks of age and a sample of the males weighed 152-178 g. females were 5-6 weeks of age and a sample weighed 109-137 g.
Number/Sex/Group:	24/sex/dose group
Satellite groups:	Toxicokinetics on Day 26 of the study (Day 26 dosing for males and Day 12 dosing for females) and during Week 9 (Day 62 of dosing) for males only.
Study design:	Males were treated for 4 weeks prior to mating, then throughout the mating period until sacrifice after mating, up to 9 weeks of treatment. Females were treated for 2 weeks prior to mating, then throughout mating and until Day 7 of gestation (Day 0 = day of detection of mating). Females were killed on Day 14/15/16 of gestation and the reproductive tracts examined.
Deviation from study protocol:	The deviations were minor and did not affect the conclusion or interpretation of the study.

Observations and Results

Mortality checked daily

There were 2 deaths one male and one female, both in the high dose group. One male (#86) was found dead on the second day of the study after having received one dose. The animal was not dosed on the second day due to its condition. Clinical observations included weight loss, subdued behavior, irregular respiration and excessive salivation, and necropsy findings consisted of abnormal contents of the stomach, thorax contained red fluid, trachea froth filled, mediastinal lymph node reddened and lung lobes dark. One female (#169) was killed prematurely on welfare grounds on day 24 (day 7 of gestation). Clinical observations included skin cold and pale, weight loss and thin, subdued, piloerection and marked excessive salivation. Necropsy findings included oesophagus distended by contents, spongy and dark lung lobes.

Clinical Signs examined at least once daily; during the treatment period, observations occurred before exposure, continuously during exposure and at approximately 1-3 hours after exposure; also all animals received a detailed physical exam once weekly.

Clinical signs included reddened skin at the extremities and excessive salivation in all groups in males and in mid and high dose groups in females. Subdued behavior was also noted in the high dose animals.

Redness of the skin at the extremities was generally noted from +1 h to +3 h post dose throughout treatment in 23/24 males and all females in the high dose groups starting with the first or second day of treatment. The signs became more persistent after the first few weeks of treatment and were noted prior to dosing as well as up to 3 h post dose. Salivation was also noted for all animals immediately after treatment. Subdued behavior was seen in 11/24 males and 3/24 females but was generally noted up to 1 h post dose only for the first few days of dosing. Isolated instances of respiratory abnormalities, excessive grooming and unkempt coat were also noted, also generally only seen after the first or second day of treatment for up to 1 hour post dose. Most mid-dose animals also tended to show excessive salivation during the first few days of treatment immediately after dosing. Reddened extremities were observed in all males and 2/24 females with a mean onset of day 47 for males and day 19 for females. In the low dose group, males but not females had reddened extremities (mean onset day 54) and salivation (8/24 males).

Table 4: Summary of Clinical Observations

Observation/Finding	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High Dose
<u>Males</u>				
Reddened skin at extremities	0	24	24	23 ✓
Excessive salivation	1	8	22	24 ✓
Subdued	0	0	0	10 ✓
Sneezing respiration	0	0	0	2 ✓
Excessive grooming	0	0	0	4 ✓
Eyelid swollen / eyes partially closed	0	0	0	1 ✓
Staining on dorsal neck / limbs	2	0	0	0
Staining around eyes	0	0	0	1 ✓
Toe encrusted	1	0	1	0
Area(s) of sparse hair / bald area(s)	2	0	2	0
Scab on head	1	0	0	0
Tail damaged	0	2	1	0
Unkempt coat	0	0	0	4 ✓
Animal found dead; signs on day of death were swollen eyelid, partially closed eyes, irregular respiration, subdued, weight loss	0	0	0	1
Total no. of males examined	24	24	24	24

Mean onset of reddened skin for:

Group 2 : Day 54

Group 3 : Day 47

Group 4 : Day 1

Observation/Finding	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High Dose
Females				
Reddened skin at extremities	0	0	2	24
Excessive salivation	0	0	18	24
Subdued	0	0	0	2
Area(s) of sparse hair	1	0	1	0
Staining on head / ventral neck or thorax / dorsal neck / muzzle	2	0	1	1
Animal killed prematurely; signs on day of death were thin, weight loss, pale skin, skin cold to touch, fur staining, tip of tail bent, marked excessive salivation, piloerection, subdued	0	0	0	1
Total no. of females examined	24	24	24	24

Mean onset of reddened skin for:

Group 3 : Day 19

Group 4 : Day 0 (first day of treatment)

Body Weight males and females weighed twice weekly, females were weighed daily until gestation day 8, then on day 11 and 14.

Males

For males, body weight increased during the first 28 days of treatment for all BI 1744 groups (averaging 118% to 122% of control between days 0 prior to the first dose and 28). In the high dose males, between weeks 3 until termination the overall gain was lower than controls. For the low and mid dose male group, body weight gain was similar to the control group until termination. Weight gain is characteristic of LABA administration in rodent studies either by inhalation or oral routes.

Table 5: Summary of Male Body Weights (absolute weight, g, and % of control) (modified from Sponsor's Table 2)

Dose Group	1	2	3	4
Inhaled Dose (ug/kg/day)	0	58	193	3068
Pulmonary Deposited Dose (ug/kg/day)	0	5.8	19.3	306.8
Day -7 (pretreatment)	238	235	239	238
Day 0 (prior to first dose)	297	298	293	295
Day 7	335	355 (106%)	350 (104%)	348 (104%)
Day 14	371	398	395	397

		(107%)	(106%)	(107%)
Day 21	399	428 (107%)	427 (107%)	429 (108%)
Day 28	426	452 (106%)	451 (106%)	447 (105%)
Day 35	423	463	461	458
Day 42	454	483	481	475
Day 49	465	493	493	477
Day 56	476	503	504	489
Day 63	489	517	518	500
Total Gain Days 0-28	129	154 (119%)	158 (122%)	152 (118%)
Total Gain Days 28-63	63	65 (103%)	67 (106%)	53 (83%)
Total Gain Days 0-63	192	219 (114%)	225 (117%)	205 (107%)

Females:

For females, the gain in all treated groups was higher than controls until the cessation of treatment midway through gestation. From Day 8 of gestation, when dosing had been discontinued, the weight gain in groups 2-4 was significantly lower than that of the controls. The overall gain over days 0-14 of gestation was dose related 178%, 227%, and 239% for dose groups 2, 3, and 4 respectively.

Table 6: Summary of Female Body Weights (absolute weight, g, and % of control by week); (modified from Sponsor's Table 3)

Dose Group	1	2	3	4
Inhaled Dose (ug/kg/day)	0	58	193	3068
Pulmonary Deposited Dose (ug/kg/day)	0	5.8	19.3	306.8
Day -7 (pretreatment)	202	205	208	204
Day 0 (prior to first dose)	213	217	213	209
Day 7	223	237	239	237
Day 14	231	249	254	252
Body Weight Gain (Days 0-14)	18	32 (178%)	41 (227%)	43 (239%)

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Table 7: Group Mean Body Weights (g) During Gestation Pregnant Animals Only

Day of Gestation	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High dose
0	234 ± 16	253 ± 16 ***	256 ± 18 ***	255 ± 20 ***
1	243 ± 17	264 ± 17	267 ± 18	267 ± 19
2	249 ± 18	270 ± 17	273 ± 18	271 ± 19
3	253 ± 17	274 ± 17	277 ± 18	274 ± 20
4	257 ± 17	277 ± 18 ***	281 ± 18 ***	280 ± 20 ***
5	261 ± 18	281 ± 18	284 ± 17	282 ± 20
6	264 ± 17	285 ± 18	287 ± 19	285 ± 20
7	262 ± 19	289 ± 19	289 ± 20	288 ± 21
8	263 ± 22	290 ± 22 ***	292 ± 20 ***	291 ± 21 ***
11	287 ± 20	306 ± 19 **	309 ± 27 **	310 ± 22 **
14	306 ± 21	325 ± 21 **	327 ± 24 **	322 ± 22 *
Body weight gain (Day 0-8)	29 ± 20	38 ± 12	36 ± 14	36 ± 10
Body weight gain (Day 8-14)	43 ± 15	35 ± 7 *	35 ± 13 *	31 ± 8 ***

Feed Consumption recorded twice weekly until pairing for mating, commencing one week prior to the start of dosing. For mated females, the amount of food consumed was recorded over Days 1-4, 5-8, 9-11 and 12-14 of gestation.

There was a transient reduction in food consumption during the first few days of treatment in males and females of the high dose group.

**Table 8: Group Mean Food Consumption During Gestation (g/animal/day)
Pregnant Animals Only**

Day of Gestation	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High dose
1-4	22.5	24.9 ***	24.7 ***	24.6 ***
5-8	21.2	23.7 **	23.1 *	23.8 **
9-11	23.6	25.3	24.0	25.0
12-14	25.7	26.8	26.2	26.9

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Toxicokinetics

Blood samples were obtained from the lowest numbered 6 surviving animals per group per sex where possible. Males were sampled on day 26 and 62 at 35 min and 24 hours from the start of inhalation exposure, females were sampled at these times on day 12.

Overall, the increase of C(0.583h, 35 min) was slightly more than proportional to dose. Given that females and males were sampled after differing weeks of exposure, there were no clear differences in the concentration between the genders. However, it does appear that pregnant females would have higher concentrations than males if sampled on the same day since females had greater exposure on day 12, than did males on day 26. Also for males that were sampled on days 26 and 62, there is a slow increase in drug concentration with time.

Table 9: Toxicokinetic Summary

Dose Group		1	2	3	4		
Target dose (ug/kg/day)		0	50	200	3000		
Achieved doses (BS/kg/day)		0	58	193	3068		
Pulmonary deposited doses (ug/kg/day)		0	5.8	19.3	306.8		
Gender		M	F	M	F	M	F
C _(0.583h) (pmol/L)	Day 12		1720		5800		191000
	Day 26	-	1130	-	5630	-	167000
	Day 62	-	1860 (0.0068 ng/mL)	-	8310 (3.2 ng/mL)	-	208000 (80.3 ng/mL)
% increase in			164%		148%		124%

exposure for males								
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* Calculation of ng/mL from pmol/L used MW 386 g/mol: 208000 pmol/L = 80288 pg/mL

Necropsy females were killed on Day 14/15/16 of gestation, males were killed after approximately 9 weeks of treatment.

Reddened extremities were confirmed at necropsy in 19/24 males in the high dose group. There were 4 males with dark focus on lung lobe(s) in the high dose group and one male in the mid and low dose groups also affected. In addition, dark reddened lung lobes or pale focus on lung lobes occurred in males in the low (n=2) and mid dose (n=3) groups. Similar findings did not occur in females.

Table 10: Incidence of Male and Female Necropsy Findings

Observation/Finding	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High Dose
Males				
Reddened extremities	0	0	0	19
Lung lobe(s) dark/ reddened/ pale focus	0	2	3	0
Lung lobe(s) dark focus	0	1	1	4
Mandibular lymph node enlarged / reddened	2	0	1	1
Kidneys discoloured / dark focus	0	0	2	2
Trachea froth filled	0	0	1	0
Abdomen - lesion	0	1	0	0
Prostate - mass	0	1	0	0
Tail damaged	0	1	0	0
Testes and epididymides small	1	0	0	0
Testis flaccid	1	0	0	0
Right testis enlarged with mass; mass on right epididymis	1	0	0	0
Staining on body / head	1	0	0	0
Animal found dead; stomach contents abnormal; thorax contained red fluid; trachea froth filled; mediastinal lymph node reddened and attached to thymus; lung lobes dark (findings excluded from other categories)	0	0	0	1
Total no. of males examined	24	24	24	24
Observation/Finding	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High Dose
Females				
Staining on body / head	0	0	0	4
Hair loss	1	0	0	1
Uterus horns dilated	1	0	0	0
Oesophagus distended; tail bent at tip; lung lobes dark and spongy; brown staining on body/head; reddened ears; animal killed prematurely (findings excluded from other categories)	0	0	0	1
Total no. of females examined	24	24	24	24

Organ Weights and Histopathology the weighed organs are listed below, histology was conducted of the testes and epididymis due to organ weight results

Ovaries
 Uterus (including cervix) and vagina (non-pregnant animals only)
 Testes: weighed individually; both testes fixed in Bouin's fluid
 Epididymides: weighed individually
 Seminal vesicles and coagulating glands: combined weight recorded
 Prostate: weighed
 Pituitary gland

There was a significant dose related decrease in epididymides weights (% of body weight) compared to controls. Testes weights in all treated groups were reduced compared to controls, statistically significance for the mid and high dose groups. There were no histopathological findings associated with these organ weight changes (Table 11).

Table 11: Organ Weights of Males (mean g, % of control)

Dose Group	1	2	3	4
Inhaled Dose (ug/kg/day)	0	58	193	3068
Pulmonary Deposited Dose (ug/kg/day)	0	5.8	19.3	306.8
Epididymides				
g	1.316	1.192	1.175	1.128
% body weight	0.2742	0.2336***	0.2309***	0.2324***
Testes				
g	3.75	3.64	3.61	3.48
% body weight	0.781	0.714***	0.710***	0.716**

Significantly different from control: * p<0.05. **p<0.01, ***p<0.001

Table 12: Summary of Histological Findings

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS			
		Males			
		Grp 1 0 ug/kg /day	Grp 2 50 ug/kg /day	Grp 3 200 ug/kg /day	Grp 4 3000 ug/kg /day
GENITAL SYSTEM					
TESTIS		(24)	(24)	(24)	(24)
No abnormality detected		21	23	24	22
Seminiferous epithelial degeneration moderate		3	0	0	0
Total Incidence		3	0	0	0
Atrophy, seminiferous tubule minimal		0	1	0	2
Total Incidence		0	1	0	2
Periodic acid Schiff stain examined		24	24	24	24
EPIDIDYMISS		(24)	(24)	(24)	(24)
No abnormality detected		16	21	20	13
Sperm granuloma, unilateral		1	0	0	0
Sloughing, spermatogenic cells minimal		1	0	0	1
Total Incidence		1	0	0	1
Oligospermia		1	0	0	0
Inflammatory cell infiltration minimal		4	1	1	0
Total Incidence		4	1	1	0
Epithelial vacuolation minimal		6	2	2	9
mild		0	0	1	1
Total Incidence		6	2	3	10
Inflammatory cell foci		0	0	0	1

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Figures in brackets represent the number of animals from which this tissue was examined microscopically

The absence of a numeral indicates that the lesion specified was not identified

Fertility Parameters

There were no effects on male reproductive performance.

During the pre-mating period, the lengths of the estrus cycles were similar in all groups, and the stages of estrus were also similar. There were no effects of treatment on the mating performance and fertility indicated by similar days to positive mating sign and by the number of pregnant animals.

In all treated groups, the mean number of dead implants, particularly early embryonic deaths, was higher than controls. These early deaths were most noticeable in the low dose dam (#140) primarily due to the 9 deaths.

Table 13: Mating Performance and Fertility Indices

Number of Nights to Positive Mating Sign	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High Dose
	Number of Animals (Number of these not becoming pregnant)			
1	1	4	2	5 (1)
2	5	2	7 (1)	10
3	8	8	8	6
4	8	9	6	3
5	1	0	0	0
7	1 (1)	0	1	0
No indication of mating	0	1	0	0
Median number of nights to positive mating sign	3	3	3	2
Number passing one oestrus	1	0	1	0
Number of males paired	24	24	24	23a
Number of siring males	23	24	23	22
Male Fertility Index (%)	96	100	96	96
Number of females paired	24	24	24	24
Number pregnant	23	24	23	23
Female Fertility Index (%)	96	100	96	96

a = One male paired to 2 females

Table 14: Pregnancy Performance

	Dose Group / Treatment			
	1 Vehicle Control	2 Low Dose	3 Intermediate Dose	4 High Dose
Number of animals mated	24	24	24	24
Percentage Mating	100	100	100	100
Number pregnant	23	24	23	23
Number of premature decedents	0	0	0	1
Number pregnant at necropsy	23	24	23	23
Conception rate %	96	100	96	96
Total corpora lutea graviditatis	350	364	382	338
Total number of implants	349	361	380	326
Pre-implantation loss as %	0	1	1	4
Post – implantation loss as %	3	9	5	7
Total live implants (%)	339 (97)	328 (91)	360 (95)	304 (93)
Total dead implants (%)	10 (3)	33 (9)	20 (5)	22 (7)
Total early embryonic deaths (%)	10 (3)	31 (9)	19 (5)	22 (7)
Total late embryonic deaths (%)	0	2 (1)	1 (0.3)	0
Mean corpora lutea graviditatis †	15.2 ± 1.8	15.2 ± 1.5	16.6 ± 2.1	15.4 ± 1.7
Mean implants †	15.2 ± 1.9	15.0 ± 1.5	16.5 ± 2.2	14.8 ± 2.6
Mean live implants †	14.7 ± 2.0	13.7 ± 2.9	15.7 ± 2.6	13.8 ± 3.0
Mean dead implants †	0.4 ± 0.6	1.4 ± 1.9 *	0.9 ± 1.2	1.0 ± 2.1
Mean early embryonic deaths †	0.4 ± 0.6	1.3 ± 1.9	0.8 ± 1.2	1.0 ± 2.1
Mean late embryonic deaths	0	0.1 ± 0.3	0.04 ± 0.2	0

Means are given ± Standard Deviation

Premature decedent excluded below double line

† = Analysed statistically

Significantly different from Control: *P<0.05, **P<0.01, ***P<0.001

Stability and Homogeneity

The measured concentrations of BI 1744 BS were within 2% to 16% of the targeted concentrations. Stability and homogeneity were not assessed in the study, but it was indicated they had been conducted previously.

9.2 Embryonic Fetal Development

Study title: BI 1744 CL Study of Effects on Embryo-Fetal Development in CD Rats by Inhalation Administration

Study no.: U05-2534
 BOI 306/042979
 Study report location: SD-66 July 31 2009, Vol 22-23?
 Conducting laboratory and location: (b) (4)
 Date of study initiation: July 16, 2004
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: 1744 CL Inhalation Solution (0.5 % w/w)
 Batch 602104, Purity: 98.7%

Key Study Findings

There were no adverse effects of inhalation of BI 1744 CL for 1 hour/day during gestation days 6 to 17 at achieved doses of 64, 222, or 1054 µg BI 1744 BS/kg/day (PDD of 6.4, 22.2, or 105.4 µg/kg/day) on embryo-fetal survival or growth and the incidence of malformations or variations. In the 222 and 1054 µg BI 1744 BS/kg/day groups there was a slight increase in fetuses with the incomplete ossification of sternbrae. While incomplete ossification appears to be drug related it is not considered teratogenic as it is a late developmental process. Ossification can be arrested or delayed, but since the rats were all sacrificed, it cannot be ascertained if sternbrae ossification was either arrested without recovery or delayed. It is a common finding with maternal toxicity associated with maternal weight loss, but signs of maternal toxicity related to undernutrition or weight loss were not apparent in this study and might be difficult to demonstrate by giving increasing doses of a drug that results in weight gain.. The developmental and maternal NOAEL for BI 1744 CL rat was the high dose of 1054 µg BI 1744 BS corresponding to AUC₀₋₂₄ of approximately 530,250 nmol-h/L (linearly extrapolated from the quantified mid-dose AUC of 101,000 nmol-h/L).

Reviewer Comment: *A comparison of doses and toxicological findings in this study suggests that the maximal tolerated dose was not attained and the dosing was inadequate for regulatory acceptance. The doses were based on a 4 week toxicity study in Han Wistar rats, Study 664861, but none of the toxicities in that study were apparent in this 12 day dosing embryo-fetal study. A comparison of aerosol concentrations and achieved dose levels indicated amounts substantially less than the two other studies in rats (refer to Table in the Methods, below). Note that although the exposure is 60 minutes in this study, compared with 35 minutes in the other two rat studies, the aerosol concentration is only 29 µg/L compared to 160 µg/L and 173 µg/L for the two other studies, a 5.5- and 5.9-fold less than those studies. Although the study should be repeated incorporating a higher maximal dose, the safety margin for the high dose exceeds the human maximal dose by greater than 100-fold, so there is unlikely*

for any additional useful human applicable safety information to be gained by dosing to the maximal tolerated dose.

Methods

Doses:

Dose Group	1	2	3	4
Target dose ug/kg/day	0	75	250	1251
Targeted BS/kg/day	0	69	229	1143
Achieved doses BS/kg/day	0	64	222	1054
PDD µg/kg/day	0	6.4	22.2	105.4

the concentration of BI 1744 CL in the exposure chamber was varied, (doses were based on a 4 week toxicity study in Han Wistar rats, Study 664861).

Group	Target concentration	Achieved concentration	Target inhaled dose	Estimated inhaled dose	Particle size		
	(µg/L)	(µg/L)	(µg/kg/day)	(µg/kg/day)	MMAD (µm)	σg	% <7 µm
2	1.83	1.74	69	64	3.6	3.11	72
3	6.12	6.08	229	222	3.3	3.09	74
4	30.7	28.66	1143	1054	3.1	3.15	76
MMAD	Mass median aerodynamic diameter						
σg	Geometric standard deviation						

Frequency of dosing: once daily for a 60 minute duration, from day 6 through day 17 of gestation (12 consecutive days)

Route of administration: inhalation; in a chamber in which the animals snout projected into a tapered end exposing them to the nebulized test compound

Formulation/Vehicle: Placebo inhalation solution (mg/100 ml) consisting of:
Benzalkonium chloride 10 mg
Disodium edetate (b) (4)
Citric acid (anhydrous) (b) (4)
Purified water 100 ml
pH 4.0

Species/Strain: Crl:CD® (SD) IGS BR strain, pregnant females (approximately 9-10 weeks of age, bodyweight range 192 to 322 g)

Number/Sex/Group: 22/dose group

Satellite groups: Toxicokinetic of the mid dose group only, n= 8

Study design:

Group	Treatment	Target dosage (µg/kg/day)		No. of females	Animal numbers	
		Salt (CL)#	Base (BS)		Main study	Satellite animals
1	Control	0	0	22	1-22	
2	BI 1744 CL	75	69	22	23-44	
3	BI 1744 CL	250	229	30	45-66	89-96
4	BI 1744 CL	1251	1143	22	67-88	

Expressed in terms of test material as supplied. A conversion factor of 0.9138 was used to convert to base.

Deviation from study: There were no deviations from protocol

protocol:

Observations and Results

Mortality checked twice daily

There were no mortalities.

Clinical Signs checked pretreatment, during exposure and post treatment up to 2 hours and late in the day; during exposure observations are restricted due to tube restraint; detailed physical examination was performed once a week

There were no treatment related clinical signs based on observations or weekly physical exams. Signs observed post-exposure included brown staining around the head and wet fur observed in both BI 1744 CL treated and controls. This was thought to be related is restraint of the animals during exposure and is a reasonable explanation.

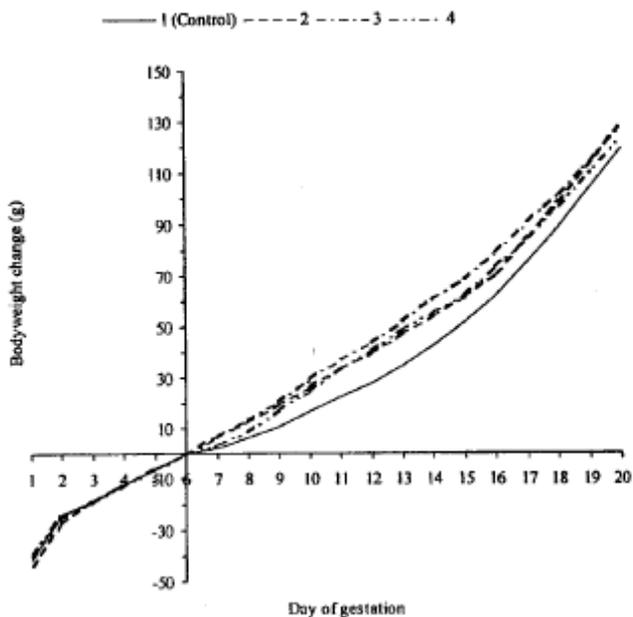
Body Weight monitored daily during exposure days

All BI 1744 CL dose groups had greater mean bodyweight gains during days 6-13 of gestation compared with the control group, but this increase was not dose-dependent (group 2 134%, group 3 148%, and group 4 140% of the control days 6-13 change in weight). After day 13, weight gains were not different than control values.

The lack of a dose-response may indicate that doses were at the maximum of the response curve for weight gain.

Figure 1: Mean Bodyweight Changes

Group	:	1	2	3	4
Compound	:	Control	BI 1744 BS		
Dosage (µg/kg/day)	:	0	64	222	1054



Treatment Day 6 to 17 of gestation inclusive

Food Consumption recorded for 4 day intervals

During days 10-13 of gestation mean food consumption in all BI 1774 CL dose groups was greater than the control group mean.

Table 15: Mean Food Consumption

Group		Day of gestation				
		3-5	6-9	10-13	14-17	18-19
1	Mean	25	25	25	30	31
	SD	2	2	1	2	2
	n	6	6	6	6	6
2	Mean	25	25	28	31	31
	SD	2	2	1	2	2
	n	6	6	6	6	6
3	Mean	25	25	28	31	32
	SD	1	2	2	2	3
	n	6	6	6	6	6
4	Mean	24	23	29	30	31
	SD	2	2	2	2	3
	n	6	6	6	6	6

Group 1: 0 ug/kg/day; Group 2: 64 ug/kg/day; Group 3: 222 ug/kg/day;
Group 4: 1054 ug/kg/day;

Treatment Day 6 to 17 of gestation

Female 16 (Group 1) with total resorption and female 23 (Group 2) not pregnant
- group housed therefore data included in group mean calculations

SD Standard deviation

n Number of cages

Significant when compared with Group 1; ** p<0.01

Toxicokinetics tail vein blood samples obtained on day 17 of gestation corresponding to the last day of dosing exposure. Samples were obtained at 1 hour after the start of exposure (i.e., at the end of exposure) from 5 animals from each dose group, and at 1, 2, 4, 8, and 24 hours after the start of exposure for animals n=8 in the middose group satellite toxicokinetic group.

The plasma concentration levels immediately following the 1 hour exposure increased dose-dependently. For the toxicokinetic group administered the mid dose, the mean maximum plasma concentration of 14400 nmol/L was measured later at 4 hours due to two outliers at this time point.

All five samples from the control animals had relevant BI 1744 CL plasma, with the mean concentrations in controls about two times lower than those in the lowest dose group concentrations but there was an overlap of the ranges (426 to 3450 pmol/L and 2730 to 4120 pmol/L, respectively). Due to these findings, samples were also taken from naive animals on the day of arrival at the testing facility and these were near or below the limit of quantification of 25 pmol/L. The low levels in the control group were

likely due to accidental exposure from the chambers (leak or cross contamination) or contamination during sample analysis. The low levels did not appear to affect the study results, as fetal effects in the control group were similar to historical controls.

Table 16: Toxicokinetic Summary

Dose Group	1	2	3	4
Target dose ug/kg/day	0	75	250	1251
Targeted BS/kg/day	0	69	229	1143
Achieved doses BS/kg/day	0	64	222	1054
PPD µg/kg/day	0	6.4	22.2	105.4
Toxicokinetics				
C_{1h} (pmol/L)	1540 [#]	3390	7200 (2.78* ng/mL) C_{max} at 4 hr =14400	37800
AUC₀₋₂₄ (pmol-h/L)		-	101,000 (38.99 ng-h/mL)	(530,250 ⁺)
[#] likely contaminated samples or chamber leakage or cross contamination, refer to text [*] Calculation of ng/mL from pmol/L used MW 386 g/mol ⁺ linear extrapolation from mid-dose value				

Stability and Homogeneity

This was not determined in this study.

Necropsy animals were killed on day 20 of gestation

There were no effects of BI 1744 CL on macroscopic findings.

Cesarean Section Data

The mean numbers of total resorptions per litter were slightly greater in the high dose group due to a greater incidence of early resorptions and mean percentage incidences of post-implantation loss compared with the control group. There was one litter (#68) with 50% post implantation loss.

The mean number of male fetuses and the mean percentage of males per litter (sex ratio) in the mid (45.1%) and high (46.6%) dose groups were less than in the control group (52.3%). There was no obvious explanation for this effect. The likely explanation would be preferential in utero mortality of males, however in the high dose group the

percentage of males in litters with in utero deaths varied widely ranging from 18.2% to 100% males.

Mean placental weights were similar for all groups. Litter weights were slightly lower in females treated with 222 and 1054 μg BI 1744 BS/kg/day, reflecting the slightly lower number of live young in these groups. Mean weights for male and female fetuses and overall fetal weights were slightly higher in all groups treated with BI 1744 BS compared with controls but there was not a dose response.

Table 17: Litter Data, group means \pm sd

Group	1	2	3	4
Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	64	222	1054
n	21	21	22	22
Copora Lutea	15.1 \pm 1.6	15.0 \pm 2.6	13.9 \pm 2.3	13.9 \pm 2.7
Implantations	13.5 \pm 1.3	13.3 \pm 2.3	12.5 \pm 2.1	13.0 \pm 2.9
Number of Litters Evaluated	21	21	22	22
Number of Fetuses/Litter	13.2	12.9	11.9	12.0
Resorptions				
Early	0.3	0.5	0.6	0.9*
Late	0.0	0.0	0.0	0.1
Total	0.3	0.5	0.6	1.0*
Live Young				
Male	7.0 \pm 2.2	6.9 \pm 2.4	5.5 \pm 2.0	5.4 \pm 2.4
Female	6.2 \pm 2.1	6.0 \pm 1.3	6.4 \pm 1.4	6.6 \pm 2.2
Total	13.2 \pm 1.5	12.9 \pm 2.1	11.9 \pm 2.3	12.0 \pm 3.0
Sex Ratio (% M)	52.3	52.0	45.1*	46.6*
Implantation Loss (%)				
Pre	10.0	10.9	9.8	8.2
Post	2.3	3.4	5.3	8.9**
Treatment Day 6 to 17 of gestation				
Female 16 (Group 1) with total resorption and female 23 (Group 2) not pregnant (these data were excluded from mean calculations)				
Significant when compared with Group 1: * $p < 0.05$, $p < 0.01$				

Table 18: Placental, litter and fetal weights on day 20 of gestation (mean \pm sd)

Group	1	2	3	4
Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	64	222	1054
n	21	21	22	22

Placental weight (g)	0.56 ± 0.08	0.59 ± 0.07	0.60 ± 0.08	0.58 ± 0.10
Litter weight (g)	48.95 ± 6.84	49.43 ± 8.99	44.94 ± 8.36	45.61 ± 10.95
Fetal weight				
males	3.79 ± 0.25	3.94 ± 0.28	3.91 ± 0.25	3.99 ± 0.44
females	3.58 ± 0.23	3.73 ± 0.20	3.70 ± 0.17	3.70 ± 0.19
overall	3.69 ± 0.22	3.84 ± 0.24	3.80 ± 0.20	3.89 ± 0.45
Treatment Day 6 to 17 of gestation				
Female 16 (Group 1) with total resorption and female 23 (Group 2) not pregnant - data excluded from mean				
Significant when compared with Group 1: * p<0.05				

Offspring (Malformations, Variations, etc.)

There were no effects of BI 1744 CL on the incidence of malformations or variations since the treatment related findings were typical of recent historical control values. Although the control group did not exhibit these effects, the historical control data from inhalation studies indicated these were also rare events, occurring in just one or two studies of 6 to 8 studies presented. A single fetus on the study in the mid dose (222 µg BI 1744 BS/kg/day) group had complete situs inversus. Two incidences of umbilical hernia occurred in the 222 µg BI 1744 BS/kg/day group and a single incidence in the 1054 µg BI 1744 BS/kg/day group. One fetus in the mid dose and two fetuses in the high dose group had anomalous confluence of umbilical and/or hepatic vein with inferior vena cava. There was a slight increase in the mean percentage of fetuses with incomplete ossification of sternbrae in the mid and high dose groups compared with controls (88% and 91% vs 76% respectively) which was not related to maternal toxicity since there was no indication of maternal toxicity in this study.

Table 19: Major Fetal Abnormalities (group % fetal and litter incidences)

Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Number examined for skeletal abnormalities	139	135	127	128	21	21	22	21
Number examined for visceral abnormalities	139	135	134	136	21	21	22	22
Number affected #	1	3	7	2	1	3	7	2
Complete situs inversus	-	-	0.7	-	-	-	1	-
Misshapen pituitary; markedly misshapen thymus; thoracogastroschisis; testes overlying kidneys, adrenal overlying kidney; malrotated hindlimb	-	-	0.7	-	-	-	1	-
Dilated ascending aorta/aortic arch; membranous ventricular septal defect[also with the minor abnormality-anomalous confluence of umbilical and or hepatic vein with inferior vena cava]	-	0.7	-	-	-	1	-	-
Medially thickened and kinked ribs, marked; irregularly ossified ribs	0.7	1.5	1.6 ^{a,b}	0.8	1	2	2	1
Bent scapula	-	-	1.6 ^{a,b}	-	-	-	2	-
Umbilical hernia	-	-	1.5	0.7	-	-	2	1
Bent humerus	-	-	0.8 ^b	-	-	-	1	-
Short/thickened humerus	-	-	1.6 ^b	-	-	-	2	-

Individual fetuses/litters may occur in more than one category, superscript denotes same fetus

Group	:	1	2	3	4
Compound	:	Control	----- BI 1744 BS -----		
Dosage (µg/kg/day)	:	0	64	222	1054

]Table 20: Minor Visceral Fetal Abnormalities (group % fetal and litter incidences)

Group	Fetuses				Litters				
	1	2	3	4	1	2	3	4	
Number examined	139	134	130	135	21	21	22	22	
Number affected	25	28	31	20	11	14	17	16	
Visceral abnormalities									
Eye(s)	variation in size	0.7	1.5	3.8	-	1	2	5	-
Oesophagus	displaced	0.7	-	-	-	1	-	-	-
Thymus	partially undescended	0.7	-	0.8	0.7	1	-	1	1
Innominate artery	rudimentary	-	0.7	-	0.7	-	1	-	1
Inferior vena cava*	anomalous confluence of umbilical and/or hepatic vein with inferior vena cava	-	-	0.8	1.5	-	-	1	2
Diaphragm	thin with protruding liver	-	2.2	0.8	0.7	-	3	1	1
Liver	additional lobe	-	1.5	-	-	-	1	-	-
Kidney(s)	rudimentary/absent papilla	0.7	3.0	0.8	-	1	3	1	-
Ureter(s)	dilated	0.7	3.0	0.8	0.7	1	2	1	1
Testis(es)	displaced	2.9	1.5	1.5	2.2	4	2	2	3
Umbilical artery	left sided	0.7	0.7	1.5	-	1	1	1	-
Haemorrhages									
Brain/spinal cord		1.4	1.5	3.1	2.2	2	2	3	3
Intra-abdominal		7.9	6.7	10.0	4.4	9	6	10	4
Hepatic		5.0	1.5	5.4	2.2	5	2	6	3
Subcutaneous		1.4	2.2	1.5	1.5	2	3	2	2

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

Previously reported at this laboratory as duplicated inferior vena cava. Now reclassified as a minor abnormality with amended terminology.

Table 21: Minor Skeletal Fetal Abnormalities (group % fetal and litter incidences)

Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Number examined	138	133	124	127	21	21	22	21
Skeletal abnormalities								
Cranial sutural bone	-	0.8	0.8	-	-	1	1	-
Vertebral element abnormality								
Ribs	medially thickened/kinked							
	2.2	-	0.8	0.8	3	-	1	1
	irregularly ossified							
	0.7	-	-	-	1	-	-	-
Sternebrae	offset alignment							
	1.4	1.5	-	3.9	2	2	-	4
Costal cartilage	offset alignment							
	-	-	-	0.8	-	-	-	1
	misaligned							
	-	-	-	0.8	-	-	-	1
	partially fused							
	-	-	-	0.8	-	-	-	1
	7 th not connected to sternum							
	1.4	2.3	-	0.8	2	3	-	1
	8 th connected to sternum							
	-	-	-	0.8	-	-	-	1
Appendicular	misshapen scapula							
	2.2	1.5	-	-	3	2	-	-
	bent scapula, minimal							
	-	-	-	0.8	-	-	-	1
	irregularly ossified clavicle							
	0.7	-	-	-	1	-	-	-
Total affected by one or more of the above @								
	11	8	2	8	9	6	2	7
Rib and vertebral configuration								
Cervical rib	0.7	2.3	1.6	0.8	1	3	2	1
Small/incompletely ossified 1 st rib	-	-	-	0.8	-	-	-	1
Short 13 th rib	-	2.3	2.4	1.6	-	3	2	2
Number with 13/14 or 14/14 ribs	1.4	6.8	3.2	7.1	2	5	4	7
18 thoracolumbar vertebrae	-	-	0.8	-	-	-	1	-

Incomplete ossification / unossified									
Cranial centres		15.2	12.0	17.7	8.7	12	7	11	8
Hyoid		10.1	9.8	10.5	4.7	9	6	7	6
Clavicle		-	-	-	0.8	-	-	-	1
Vertebrae	cervical	1.4	-	3.2	2.4	2	-	4	3
	thoracic	3.6	3.8	6.5	3.9	5	5	6	5
	lumbar	0.7	-	-	-	1	-	-	-
	sacrocaudal	5.8	9.0	6.5	10.2	5	7	5	7
Sternebrae	5 th and/or 6 th	76.1	69.2	87.9	91.3	21	20	22	21
	Other (1 st to 4 th)	1.4	0.8	2.4	7.1	2	1	2	5
	total	76.1	69.2	87.9	91.3	21	20	22	21
Pelvic bones		1.4	3.8	4.8	5.5	2	3	3	6
Metacarpals		0.7	0.8	0.8	2.4	1	1	1	1
Precocious ossification									
Cervical vertebral centra (>4 ossified)		-	3.0	-	-	-	3	-	-
Additional observations at necropsy									
Left umbilical artery		0.7	-	0.8	0.8	1	-	1	1
Dilated renal pelvis		-	0.8	-	0.8	-	1	-	1
Dilated ureter		-	2.3	-	2.4	-	3	-	2

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded,
 @ one fetus may have more than one observation.

Study title: BI 1744 CL Pilot and Preliminary Embryo-Fetal Study in the Rabbit by Inhalation Administration

Study no.: U05-2535
 BOI308/040116

Study report location: SD-5, vol. 23, Jan 29, 2007

Conducting laboratory and location:  (b) (4)

Date of study initiation: June 30 2004

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: BI 1744 CL, Batch 8460120, Purity 97.8%
 Batch F602104, Purity 98.7%

Key Study Findings

Exposure of pregnant rabbits to BI 1744 CL at the maximum practicable air concentration of approximately 120 µg/L (Group 3: 3921, Group 5: 3706 µg/kg/day; corresponding to PDD 392 and 371 µg/kg/day, respectively) resulted in salivation, an initial transient reduction in food consumption, and embryo-fetal resorptions. Overall, this dose was sufficiently tolerated to be suitable for an embryo-fetal toxicity study.

Methods

Phase 1, Nonpregnant Rabbits: Three groups of 3 non-pregnant female New Zealand White rabbits received BI 1744 CL by snout-only inhalation administration at the maximum practicable concentration (approximately 120 µg/L as a 0.5% w/w concentration in vehicle consisting of benzalkonium chloride, disodium edetate dihydrate, citric acid and purified water) for 30, 60 and 120 minutes a day for 14 consecutive days (estimated dosages of 0.835, 1.80 and 3.92 mg BI 1744 BS/kg/day) respectively. The achieved aerosol concentration, estimated achieved dosages, pulmonary deposited doses and particle size are presented in the Table 22 and 23, below. For phase 1 of the study, animals were sacrificed on the day following the last dosage, and examined macroscopically.

In Phase II, Pregnant Rabbits: Two groups of time-mated rabbits received BI 1744 CL by snout-only inhalation (dosages of 1.74 and 3.71 mg BI 1744 BS/kg/day (PDDs of 0.174 and 0.371 µg/kg/day) for 60 or 120 minutes a day, respectively) from Days 6 to 19 after mating. For phase II, animals were sacrificed on Day 29 after mating and examined macroscopically. The uterus was excised for examination of litter parameters and all fetuses were examined macroscopically at necropsy.

There was no vehicle treatment control group in this study.

Table 22: Study Design

	Phase 1			Phase 2	
Treatment Duration	Gestation Days 6-19				
Dose Group	1	2	3	4	5
Daily Duration of Exposure (min)	30	60	120	60	120
n	3	3	3	3	3
Air Concentration (µg/L)	108	116	124	113	122
Estimation of dose (µg BI 1744 BS/kg/day)	835	1800	3921	1737	3706
Pulmonary deposited doses (µg/kg/day)	83.5	180	392	174	371

The achieved doses (Table 2.4.1: 1) were calculated respectively using the daily actual mean body weight of each group, respiratory minute volumes, exposure time and measured drug concentration in the chamber. The mass median aerodynamic diameter was $4.6 \pm 2.9 \mu\text{m}$ with 65% of the particles $<7 \mu\text{m}$ diameter.

Toxicokinetic blood samples were obtained from all animals on days 1, 5 and 14 of exposure at the end of the exposure period, and at 8 hours after the start of exposure. The conversion factor of 0.9138 was applied to analyzed concentrations to convert from BI 1744 CL to BI 1744 BS (free base).

Results

Phase 1, Nonpregnant Rabbits:

Salivation/wetness around the mouth was noted in some animals at the mid and high doses (longer duration of exposure).

There were no adverse effects of treatment on clinical signs, bodyweight, food consumption, or macropathology. There was a reduction in mean food intake on the first day of exposure (64%, 62%, 74% for dose groups 1, 2, and 3 respectively).

Table 23: Food Intake in Nonpregnant Rabbits (selected data presented)

-1	N	3	3	3
	MEAN	161	121.0	124.3
	S.D.	19.	33.8	13.6
1	N	3	3	3
	MEAN	104	75	92
	S.D.	15.3	2.6	30.4
2	N	3	3	3
	MEAN	146	96	102
	S.D.	2.1	14.7	31.5
3	N	3	3	3
	MEAN	127	108	107
	S.D.	14.9	1.4	9.3
4	N	3	3	3
	MEAN	131	90	124
	S.D.	8.8	13.6	14.5
5	N	3	3	3
	MEAN	124	92	107
	S.D.	13.2	5.5	18.2
6	N	3	3	2
	MEAN	139	115	110
	S.D.	2.3	21.0	11.3
7	N	3	3	3
	MEAN	140	124	119
	S.D.	6.1	13.9	23.7

In Phase II, Pregnant Rabbits:

In pregnant rabbits, there were no overall adverse effects on bodyweight and food consumption. There was a reduction in mean food intake on the first day of exposure as observed in nonpregnant animals (64% and 52% for dose groups 4 and 5, respectively). There was a higher mean number of embryo-fetal resorptions in the high dose (120 minute exposure, 3.71 mg BI 1744 BS/kg/day) group consisting of four resorptions in one litter 16 and a single resorption in the other two litters. There was no adverse effect on embryo-fetal survival or growth. No fetal abnormalities were noted in the 120 minute exposure (3.71 mg BI 1744 BS/kg/day) group, although 3 fetuses had minor fetal abnormalities in the 60 minute exposure dose group.

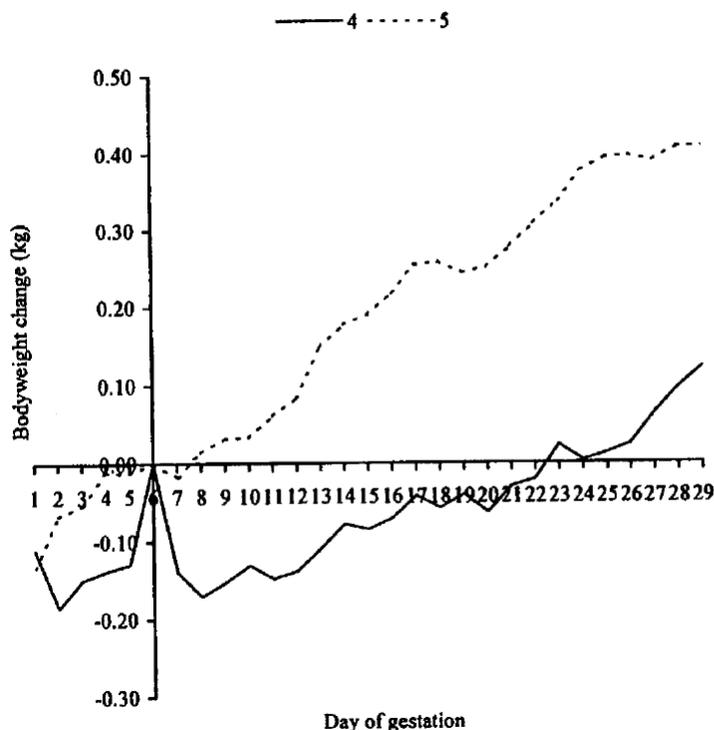
Table 24: Food Intake in Pregnant Rabbits (selected data presented)

Group	Day of gestation															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4																
Mean	66	109	122	113	103	66	65	73	67	61	70	54	49	42	44	72
SD	28	30	29	23	25	10	9	12	10	11	16	20	23	14	11	9
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5																
Mean	127	169	163	151	152	79	123	137	123	116	119	118	105	104	107	122
SD	40	22	24	41	53	65	71	54	69	71	71	83	77	80	74	83
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Treatment period Day 6 to 19 of gestation (inclusive)

Figure 2: Body Weight Change During Gestation**Bodyweight change - group mean values (kg) - Phase II (pregnant animals)**

Group	:	4	5
Compound	:	-----BI 1744 BS-----	
Dosage (mg/kg/day)	:	1.74	3.71
Duration (minutes)	:	60	120



Treatment period Day 6 to 19 after mating, inclusive
 All changes relative to start of treatment

Toxicokinetics

There was a decrease of the BI 1744 BS plasma concentrations between day 1 and day 5. The dosage-normalized BI 1744 BS plasma concentrations in non-mated and pregnant rabbits were similar. The between animal variability in plasma concentrations within dose groups was high.

Table 25: Toxicokinetics (Mean concentration C(8hr) values, pmol/L)

Group	1	2	3	4	5
Achieved doses (µg BI 1744 BS/kg/day)	835	1800	3920	1740	3710
Daily Duration of	30	60	120	60	120

Exposure (min)						
Day	Timepoint	Concentration (pmol/L)				
1	end of inhalation	117000	288000	365000	419000	242000
	8 hr	5500	16000	45000	22800	14600
5	end of inhalation	29200	92100	126000	179000	84800
	8 hr	2380	4430	21400	14000	17300
14	end of inhalation	23400	141000	101000	136000	85500
	8 hr	2330	4520	15000	12200	19100

Study title: BI 1744 CL Study of the Effects on Embryo-Fetal Toxicity in the Rabbit by Inhalation Administration

Study no.: U06-1019
BOI 307/043146

Study report location: SD-66 July 31 2009, Vol 23-24

Conducting laboratory and location: (b) (4)

Date of study initiation: Aug 16 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: BI 1744 CL Inhalation Solution (0.5%),
Batch F602104, Purity 98.7%

Key Study Findings

Pregnant rabbits were exposed to BI 1744 CL by inhalation for a 90-minute exposure period on days 6 through 19 inclusive after mating, at doses of 0, 289, 974, or 2489 µg/kg/day (PDDs of 0, 28.9, 97.4, or 248.9 µg/kg/day, respectively).

There were teratogenic effects at the high dose, but no adverse effects on embryo-fetal survival or growth. In the high dose 2489 µg BI 1744 BS/kg/day group, a greater number of fetuses/litters had major abnormalities compared with the controls or the historical control range. Most of the fetuses clustered in a single litter and had a specific syndrome of abnormalities which had not been previously seen in this strain/source of New Zealand White rabbits. Eight fetuses in one litter (#72) had uneven ossification of the cranial bones/ribs/long bones that included thickened ribs/distorted ribcage; short scapula, humerus, radius, ulna, femur, tibia, fibula; and/or partially open eyelids/eyelids not fused centrally; cleft palate; forelimb flexure/malrotated hindlimb(s). There was no consistency in the type of abnormalities seen in the other affected fetuses in this group.

The maternal NOAEL was the high dose of 2489 µg BI 1744 CL kg/day. The developmental NOAEL for BI 1744 CL in the rabbit was the mid dose of 974 µg BI 1744 BS/kg/day corresponding to an AUC₀₋₂₄ of 182000 pmol-h/L.

Methods

Doses:

Dose Group	1	2	3	4
Target dose ((µg/kg/day)	0	300	1000	3000
Achieved doses (µg/kg/day)	0	289	974	2489
PDDS (µg/kg/day)	0	28.9	97.4	248.9

Frequency of dosing: once daily for 14 consecutive days (gestations days 6 to 19)

Route of administration: inclusive), inhalation duration of 90 minutes/day
inhalation of a nebulized aerosol using a snout only exposure system

Formulation/Vehicle: Placebo inhalation solution (mg/100 ml) consisting of:
Benzalkonium chloride 10 mg
Disodium edetate (b) (4)
Citric acid (anhydrous) (b) (4)
Purified water 100 ml
pH 4.0

Species/Strain: New Zealand White rabbits, pregnant females;
age at day 6 of gestation (start of treatment) 23 to 34 weeks,
bodyweights 3.28 to 4.41 kg

Number/Sex/Group: 22/dose

Satellite groups: none

Study design: test substances BI 1744 CL or placebo were administered to rabbits by inhalation of a nebulized aerosol for 90 minutes a day using a snout only exposure system

Table 26: Summary of the achieved chamber concentrations, estimated achieved doses and particle size data of BI 1744 BS

Group	Batches	Target concentration (µg/L)	Achieved concentration (µg/L)	Target inhaled dose (µg/kg/day)	Estimated inhaled dose (µg/kg/day)	Particle size		
						MMAD	σg	% (b) (4)
2	1-3	11.9	13.6	274	311			
	4-6		11.6					
3	1-3	39.3	45.0	914	1019			
	4-6		40.9					
4	1-3	117	125.4	2741	2866			
	4-6		91.9					
	MMAD	Mass median aerodynamic diameter						
	σg	Geometric standard deviation						

Deviation from study protocol: There were no deviations that affected the study conclusions and interpretation

Observations and Results

Mortality checked daily

There were 3 early deaths that the Sponsor considered unrelated to treatment. A control group female (#4) was sacrificed on gestation day 25 following abortion. A control group female (#17) was sacrificed on gestation day 22 following signs of gastrointestinal disturbance manifested as little/no food intake and low bodyweight gain. Necropsy revealed mottled kidneys in this control female. One mid dose (974 µg BI 1744 BS/kg/day) female (#66) was sacrificed on gestation day 20 following red staining observed on the tray paper. Uterine examination indicated that 2 implantations had died during early gestation.

Clinical Signs checked daily pre exposure, during exposure and within 2 hours after the completion of exposure, and late in the day,

and a detailed physical examination was performed once a week

There were no observed clinical signs or physical exam findings from BI1744 CL administration.

Body Weight recorded daily

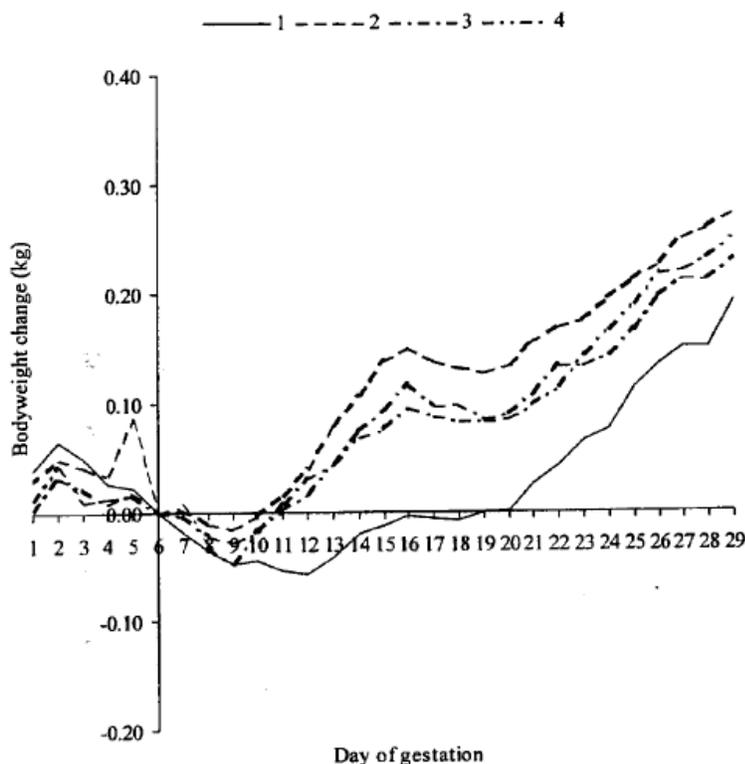
Rabbits in all BI 1744 CL dose groups exhibited a transient mean bodyweight stasis or weight loss during the initial days of treatment (gestation day 6-9), but then exhibited weight gains for the rest of the study. This differed from controls which had a mean weight loss during the first week of inhalation, until day 12, then displayed a weight gain that paralleled the BI 1744 CL treatment group. There was no dose-response relationship for BI 1744 CL dose groups.

The body weight changes BI 1744 CL compared to control vehicle treated rabbits likely reflects the known anabolic effects of β_2 agonist compounds.

Figure 3: Bodyweight Change

Bodyweight change - group mean values for females during gestation (kg)

Group	1	2	3	4
Compound	Vehicle Control		BI 1744 BS	
Dose ($\mu\text{g}/\text{kg}/\text{day}$)	0	289	974	2489



Females with live young Day 29 of gestation
Treatment Day 6 to 19, inclusive
Changes relative to start of treatment

Food consumption measured daily

The high dose group had a small transient reduction in food consumption on the first two days of inhalation exposure (gestation days 6-7) compared to the control group.

Toxicokinetics Blood samples from the central auricular artery were collected from 6 mated animals per group. Blood samples were withdrawn, from batches 1, 4 and 6 on day 5 of exposure (day 10 after mating) at 1.5, 3, 8 and 24 hours after the initiation of dosing.

Maximum mean plasma concentrations (C_{max}) occurred at the end of the 90 minute exposure period (T_{max}).

There was a dose-dependent increase in mean C_{max} and AUC_{0-24} . The variability of BI 1744 BS plasma concentrations was large. The CVs of the $\text{AUC}_{(0-24\text{h})}$ -data were

between 48.2 % and 127.0 % within a given dose. The observed variability is dominated by a significantly higher exposure of the first animal in the first batch of each dose group. In addition, variability in actual achieved doses contributed to the variability in exposure.

Half of the samples (12 of the 24 samples) taken from the control animals and all 4 samples taken from naïve animals contained low levels of BI 1744 BS (< 812 pmol/L) but were much lower than those in the lowest dose group (3810 to 19800 pmol/L). Naïve rabbits were bled prior to any preliminary work with BI 1744 CL and in a different room to the exposure room, thus the weight of evidence suggests that contamination can only have occurred during freezer storage/transport or analysis. Control animals were also bled and samples separated in a different room to that in which animals were exposed.

Table 27: Toxicokinetic Summary

Dose Group	1	2	3	4
Target dose	0	300	1000	3000
Achieved doses (µg/kg/day)	0	289	974	2489
PDDs (µg/kg/day)	0	28.9	97.4	248.9
C_{max} (pmol/L)		8170	30400	161000
AUC₀₋₂₄ (pmol-h/L)		39700	182000	959000

Stability and Homogeneity

These parameters were not measured in this study. On three days during the study, measurements were obtained to calculate particle size and inhalation drug concentrations (refer to Table 27 in the Methods section). There were no significant changes over the duration of the study.

Necropsy necropsy occurred on gestation day 29, except for animals exhibiting pregnancy loss which were killed on the same day that abortion was detected.

There were no effects of BI 1744 CL on macroscopic anatomic pathology.

Cesarean Section Data

There were no BI1744 CL effects on mean corpora lutea counts, implantation counts, live litter size, mean sex ratio, and mean weights of placenta, litters, or fetuses.

Mean fetal weights in the 2489 µg BI 1744 BS/kg/day group were marginally, but not statistically significantly lower than controls. This difference was partly due to 2/21 litters

with atypical fetal weights less than 30 g (Dam #53 and #72); when data for these 2 litters were excluded the group mean overall fetal weight was 42.4 g compared with 44.2 g in the controls.

Table 28: Litter Parameters (mean ± sd)

Group	1	2	3	4
Dose (µg/kg/day)	0	289	974	2489
n (females)	17	22	17	21
Copora Lutea	9.7 ± 2.4	9.0 ± 2.1	10.2 ± 2.4	9.7 ± 1.7
Implantations	8.2 ± 2.5	7.9 ± 2.6	7.7 ± 2.6	8.5 ± 2.8
Resorptions				
Early	0.9	0.5	0.7	0.9
Late	0.3	0.5	0.7	0.5
Total	1.2	1.0	1.4	1.4
Live Young				
Male	3.3 ± 1.6	3.2 ± 1.2	3.1 ± 1.3	3.3 ± 1.5
Female	3.7 ± 1.8	3.6 ± 1.9	3.2 ± 1.6	3.8 ± 1.5
Total	7.0 ± 2.4	6.9 ± 2.4	6.3 ± 2.4	7.1 ± 2.2
Sex Ratio (% M)	46.7	51.9	49.6	45.1
Implantation Loss (%)				
Pre	15.5	14.1	24.3	14.4
Post	14.6	13.1	18.5	14.7

Table 29: Placenta, Litter and Fetal Weights (mean ± sd)

Group	1	2	3	4
Dose (µg/kg/day)	0	289	974	2489
n (females)	17	22	17	21
Placental weight (g)	5.5 ± 0.8	5.8 ± 1.2	5.9 ± 1.1	5.8 ± 0.9
Litter weight (g)	304.0 ± 103.6	290.6 ± 96.5	270.8 ± 92.9	284.2 ± 86.3
Fetal weight				
males	44.2 ± 4.9 ^a	43.9 ± 7.0	43.7 ± 8.1	40.4 ± 6.4
females	43.8 ± 5.4	42.4 ^b ± 7.2	44.3 ± 8.3	41.1 ± 6.9
overall	44.2 ± 4.5	43.6 ± 7.2	43.8 ± 7.7	40.9 ± 6.5
^a no males present in 1 litter				
^b no females present in 2 litters				

Offspring (Malformations, Variations, etc.)

In the high dose (2489 µg BI 1744 BS/kg/day) group there were a greater number of fetuses/litters with major abnormalities compared with the controls. Eight fetuses in one litter (#72) had a combination of some or all of the following: thickened ribs/distorted ribcage; short scapula, humerus, radius, ulna, femur, tibia, fibula; and/or partially open eyelids/eyelids not fused centrally; cleft palate; forelimb flexure/malrotated hindlimb(s). This combination of abnormalities was not present in the historical control data.

An additional 6 fetuses from 4 other litters in the group had other major abnormalities. Two fetuses in one litter (#9) had dilated ascending aorta/aortic arch; narrow pulmonary trunk; enlarged left and small right heart ventricles; one of these fetuses also had the pulmonary arteries arising directly from the aortic arch. One fetus in a second litter (#69) had multiple abnormalities including acrania, absent eyes, split sternum, gastroschisis, cervicothoracic scoliosis and lordosis, forelimb flexure and hindlimb hyperflexion. One fetus in a third litter (#82) had multiple retinal folds while another fetus in the same litter had dilated ascending aorta/aortic arch, narrow pulmonary trunk, enlarged left heart ventricle/small right atrium and ventricle. One fetus in a fourth litter (#99) had dorsoventral distortion of the sternum. The incidence of two litters containing fetuses with great vessel abnormalities was consistent with the historical control range but the incidence of fetuses in two litters with enlarged/small heart atria/ventricles was outside the historical control range. Acrania, retinal folds, split sternum and distortion of the sternum have not been previously recorded in the historical control data. In addition, eight fetuses clustered in a single litter (#53) had the unusual minor abnormality of unossified areas/patchy ossification on the cranial bones/ribs/long bones. As mentioned previously, these litters had atypically low weights.

There was no effect of treatment on the incidence of minor visceral abnormalities.

Table 30: Fetal Abnormalities and Variations (modified from Sponsor's Tables 6, 7, and 8)

Group	1	2	3	4
Dose (µg/kg/day)	0	289	974	2489
PDDS (µg/kg/day)	0	28.9	97.4	248.9
Major Abnormalities				
Number of litters examined	17	22	17	21
Number of fetuses examined	119	152	107	149
Number heads examined at detailed visceral, Fetuses/litters	39/17	53/21	36/17	50/21
Number affected, Fetuses/Litters	2/2	1/1	1/1	14/5
Specific Findings	% fetal incidences / # litter incidences (unfilled cells indicate no abnormality or variation)			
Acrania: absent eyes: cervicothoracic scoliosis and lordosis: multiple cervicothoracic vertebra/Rib abnormalities: split sternum with disorganised sternebrae: forelimb flexure: hindlimb hyperflexion: gastroschisis				0.7/1
Multiple folds retina				2.0/1*
Thickened ribs/distorted rib cage /marked: short/bent scapula, humerus, radius, ulna, femur, tibia, fibula: and/or partially open eyelid/eyelid not fused centrally: cleft palate: forelimb flexure/ hindlimb malrotated				5.4/1
Multiple thoracolumbar vertebral/rib abnormalities/scoliosis: misshapen disorganised and fused sacrocaudal vertebrae, brachyury: additional bone arising from tibia: absent kidney			0.9/1	
Dilated ascending aorta/aortic arch: enlarged left, small right ventricle		0.7/1		
Dilated ascending aorta/aortic arch: narrow pulmonary trunk: pulmonary arteries arising directly from aortic arch: enlarged left, small right ventricle				0.7/1
Dilated ascending aorta/aortic arch: narrow pulmonary trunk: enlarged left, small right ventricle				0.7/1
Dilated ascending aorta/aortic arch, narrow pulmonary trunk, markedly small				0.7/1

right atrium, enlarged left, small right ventricle				
Dorsoventral distortion of sternum				0.7/1
Lumbar scoliosis	0.8/1			
Absent gall bladder	0.8/1			
Minor Visceral Abnormalities				
Number of litters examined	17	22	17	21
Number of fetuses examined	117	151	107	135
Number heads examined at detailed visceral, Fetuses/litters	39/17	53/21	36/17	44/19
Number Affected, Fetuses/Litters	4/4	12/8	7/5	6/6
Specific Findings	% fetal incidences / # litter incidences (Fetuses with major abnormalities excluded; unfilled cells indicate no abnormality or variation)			

* based on % of heads examined at detailed examination

Table 31: Fetal Skeletal Minor Abnormalities/Variants

Number of litters examined	17	22	17	21
Number of fetuses examined	117	151	107	135
Number intact	78/17	98/22	70/17	88/21
Specific Findings	% fetal incidences / # litter incidences (Fetuses with major abnormalities excluded; unfilled cells indicate no abnormality or variation)			
Cranial				
sutural bone	2.6/2	3.1/3		1.1/1
fissures/extra sutures			1.4/1	1.1/1
enlarged anterior fontanelle into frontals	5.1/1		2.9/1	
elongated anterior fontanelle into frontals		1.0/1		
elongated anterior fontanelle				1.1/1
fissured anterior fontanelle		2.0/1	1.4/1	1.1/1
partially fused parietals			1.4/1	
unossified area(s)	1.3/1	2.0/2		2.3/2
bridge of ossification/partially fused maxilla to jugal	3.9/2	4.1/2	7.1/4	5.7/5
bent cornua of hyoid	10.3/3	2.0/1	2.9/1	3.4/3
Vertebral elements abnormality				
additional ossified centre		0.7/1	0.9/1	
thoracic				0.7/1

caudal	0.9/1			1.5/2
Ribs				
medially thickened		0.7/1		1.6/1
branched/partially fused		0.7/1		0.7/1
Sternebrae				
additional centre(s)			0.9/1	0.7/1
bridge of ossification/partially fused/fused	0.9/1	1.3/2	2.8/3	2.2/3
offset alignment	0.9/1	1.3/2	2.8/3	
bipartite ossified	0.9/1		2.8/3	
Costal cartilage				
offset alignment/misaligned		0.7/1	3.8/4	
partially fused			0.9/1	0.7/1
additional/absent			0.9/1	
7th not connected	5.1/5	1.3/2	3.8/3	1.5/1
8th connected				0.7/1
Total affected by one or more of the above	26/13	21/12	18/12	21/14
Rib and vertebral configuration				
Cervical rib	0.9/1	2.6/4	4.7/3	1.5/2
Number with 12/13 or 13/13 ribs	50.4/15	63.6/19	72.6/16	63.7/19
20 thoracolumbar vertebrae	21.4/11	25.8/14	29.2/10	31.1/14
Offset alignment pelvic girdle	2.6/2	1.3/2	4.7/4	5.2/4
Incomplete ossification / unossified				
Enlarged anterior fontanelle		3.3/3	2.8/2	1.5/2
Enlarged posterior fontanelle	3.4/2	3.3/4	2.8/3	1.5/2
Enlarged lachrimal fossa		1.3/1		0.7/1
Enlarged nasal/frontal suture interparietal			0.9/1	0.7/1
Vertebral element cervical thoracic/lumbar	3.4/3	1.3/2 1.3/2	0.9/1	1.5/2 0.7/1
Sternebrae 5th other total	12.0/7 17.9/7 26.5/11	7.3/6 7.3/9 11.9/11	11.3/7 9.4/6 17.0/9	8.1/5 11.1/11 17.0/13
Epiphyses		4.0/3	1.9/1	2.2/2
Phalanges	6.8/4	13.2/9	9.4/5	23.7/11
Metacarpal	0.9/1	7.3/3	5.7/2	7.4/6
Unossified areas/patchy ossification cranial bones/ribs/long bones				5.9/1

9.3 Prenatal and Postnatal Development

Study title: Study for Effects of Pre and Post Natal Development including Maternal Function in Rats (inhalation)

Study no.: 494520
Study report location: SD-59, Mar 27, 2009, Mar 30, 2009
Conducting laboratory and location: (b) (4)
Date of study initiation: June 13 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BI 1744 CL, Batch 5061021, Purity 99.5%

Key Study Findings

BI 1744 CL was administered by inhalation to rats for 35 minutes/day from gestation day 6 to 20, and then from lactation day 2 to 21 of dams (day 0 = day of birth of litter). The achieved doses were 0, 59, 297 and 3665 µg/kg/day (PDDS of 0, 5.9, 29.7 and 366.5 µg/kg/day) close to the target doses of 0, 50, 200 and 3000 µg/kg/day.

A significantly increased weight gain and a transient, but significant, decrease in food consumption were observed following the first day of treatment. Observations such as changes in behavior (subdued and excessive general behaviour, including paddling) and eye(s) partially closed were generally seen over the first 2-3 days in all high dose animals. Salivation and fur staining occurred in all BI 1744 CL treatment groups.

There were slight differences in litter size by day 21 of lactation, and reduced survival in the low and high dose groups. Litter weight and pup weight in the high dose group were slightly less, but not statistically different than the weights of the control group by day 21 of lactation. For all doses of BI 1744 there was a dose-related earlier time to eye opening in both sexes, but this was not associated with deficits in later vision assessments. Earlier eye openings in rats have been reported in the label of one approved LABA (salmeterol) and other short acting β agonists. There were no effects of treatment on the mating, fertility or bearing of live implants to Day 14/15/16 of gestation in the F₁ animals.

Based upon these results the NOAEL was 3665 µg/kg/day BI 1744 BS for maternal toxicity, F₁ survival and development, and F₁ mating, fertility and pregnancy. Toxicokinetics were not conducted for this study and exposure for safety margins could be extrapolated from related studies or based on body surface area comparison.

Methods

Doses:

Dose Group	1	2	3	4
Target dose (µg/kg/day)	0	50	200	3000
Achieved doses (µg/kg/day)	0	59	297	3665
PDDs (µg/kg/day)	0	5.9	29.7	366.5

The test aerosols generated during the study had a bimodal particle size distribution, with one subpopulation of particles with a MMAD of (b) (4) and a second subpopulation of particles with a MMAD of ca (b) (4). The target analytical MMAD range specified on the protocol (MMAD of (b) (4)) was not met, but the test aerosol (analytical) remained within the size range considered to be respirable to rats ((b) (4)).

Frequency of dosing:

once daily for 35 minutes/day from gestation day 6 to 20, and then from lactation day 2 to 21 of dams

Route of administration:

inhalation in a chamber in which the animals snout projected into a tapered end exposing them to the nebulized test compound

Formulation/Vehicle:

Placebo inhalation solution (mg/100 ml) consisting of:

Benzalkonium chloride 10 mg

Disodium edetate (b) (4)

Citric acid (anhydrous) (b) (4)

Purified water 100 ml

pH 4.0

Species/Strain:

mated female Sprague Dawley (CrI:CD®(SD)) rats; approximately 9 weeks of age, 181-267 g body weight

Number/Sex/Group:

F₀: 23-24/dose

F₁: 16-21/sex/dose

Satellite groups:

None

Study design:

At Day 21 of lactation of F₀ females, 2 male and 2 female F₁ pups (where they were available) were randomly selected from each litter. One male and one female were allocated to Set 1 and the other allocated to Set 2.

Deviation from study protocol:

There were a number of deviations, but these did not affect the conclusion or interpretation of the study.

The final report, page 1, indicates "There a number of formal differences between the draft and final report, which are considered unnecessary to be listed in detail, because the overall conclusion of the report has remained the same." These differences are not presented.

Although these differences are not indicated, they pertain to the draft report which, to this Reviewer's knowledge, was not reviewed

nor were any regulatory decisions made based on the draft report. Therefore, is it unnecessary to request those differences for the review of the final study report.

F₀ DAMS OBSERVATIONS AND RESULTS

Survival checked twice daily

There were no unscheduled mortalities.

Clinical signs checked daily and physical exam once weekly

Clinical observations such as changes in behavior (subdued and excessive general behavior including paddling) and eye(s) partially closed were generally seen over the first 2- 3 days in all animals of the high dose group, although some animals exhibited these behaviors (e.g., paddling) throughout the treatment period. Salivation was noted in most animals (92-100% of animals) in all BI 1744 CL groups throughout the exposure period and 25% of the control animals. Staining of the coat occurred in a few animals in all groups and was the only gross necropsy finding.

Table 31: Clinical Observations of F₀

Observation/Finding	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
Subdued behaviour	0	0	0	23
Excessive behaviour	0	0	0	24
Slow respiration	0	0	0	1
Excessive salivation	6	22	24	24
Eye(s) partially closed	0	0	0	24
Scab(s) on abdomen	0	2	0	0
Lesions/ scabs on tail	0	0	1	0
Area(s) of sparse hair	1	3	1	2
Staining (head / neck / eyes)	6	2	5	9
Tail damaged / withered/ missing	0	0	1	0
Unkempt / greasy coat	0	0	0	1
Wet coat / patches	0	0	0	3
Killed prematurely	0	0	0	0
Total number of females examined	24	24	24	24

Body weight recorded daily

Weight gain in all BI 1744 CL groups was significantly greater than that seen in the controls (corresponding to % increase between d 6 and 20 of gestation in groups 2-4 of group 2: 116%, group 3: 121% and group 4: 126% of the control gain).

Similarly during the lactation period, the weight gain was greater than controls, but the extent of the gain was more variable across the groups.

Table 32: F₀ Generation: Group Mean Body Weight (g) During Gestation and Lactation ± Standard Deviation

	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
Day of Gestation ^a				
4	244 ± 18	243 ± 16	249 ± 19	237 ± 21
6	250 ± 17	250 ± 16	254 ± 19	242 ± 21
9	263 ± 17	270 ± 18	275 ± 18	263 ± 21
13	286 ± 19	300 ± 20*	307 ± 18***	296 ± 23
16	306 ± 19	322 ± 23*	331 ± 20***	323 ± 25*
18	329 ± 20	347 ± 25*	355 ± 22***	349 ± 26**
20	349 ± 23	365 ± 29*	374 ± 22**	367 ± 26*
Weight Gain Days 6-20	99	115***	120***	125***
% of Control	-	116	121	126
Day of Lactation ^b				
1	252 ± 20	262 ± 20	275 ± 23	262 ± 24
7	298 ± 23	314 ± 25	322 ± 20	313 ± 24
14	312 ± 20	328 ± 18	332 ± 18	326 ± 22
21	301 ± 21	324 ± 16	329 ± 19	331 ± 21

a = Pregnant animals only

b = Excludes litters where there was a total litter loss

Statistical analysis was performed on gestation body weights only (ANOVA)

Significantly different from the Control: *P<0.05, **P<0.01, ***P<0.001

Feed consumption recorded daily until parturition, then recorded weekly

There was a transient but significant dose related decrease in food consumption in all BI 1744 CL groups on gestation day 7 after dosing was initiated (up to -32% of controls in the high dose group). Thereafter food consumption was essentially similar to that of the controls. During lactation the food consumption was similar or slightly greater than that of controls.

Pregnancy

Treatment with BI 1744 CL resulted in slightly greater gestation length compared to controls (22 or 23 days). The mean values for the duration of gestation were 21.6, 22.0, 21.9 and 21.9 days in Groups 1-4, respectively, statistically different from the low and mid dose groups.

There was no effect of BI 1744 CL on the % gestation index, mean implantation sites per pregnancy, mean total pups born per litter, mean live pups per litter, and number of males and females born per litter.

Table 33: F₀ Generation: Duration of Gestation and Overall Litter Performance

	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
Number Pregnant	21	22	23	23
Duration of Gestation (Days)				
21	9	2	3	4
22	12	19	19	18
23	-	1	1	1
Mean Duration	21.6	22.0*	21.9*	21.9
Number of females producing a live litter	21	22	23	23
Gestation index as %	100	100	100	100
Mean number of implant sites ^a per pregnancy ± standard deviation	13.0 ± 2.4	13.5 ± 2.5	13.0 ± 2.9	13.1 ± 2.0
Mean total number of pups ^a born per litter	12.2 ± 2.1	12.6 ± 2.3	12.1 ± 3.2	12.1 ± 1.6
Mean number of live pups ^a per litter ± standard deviation:				
Day 0 of lactation	12.2 ± 2.1	12.4 ± 2.3	12.0 ± 3.1	11.9 ± 1.7
Day 1 of lactation	12.0 ± 2.2	12.2 ± 2.1	11.7 ± 3.1	11.1 ± 1.8
Day 4 of lactation	11.6 ± 2.7	11.4 ± 2.9	11.6 ± 3.1	10.7 ± 2.4
Day 7 of lactation	11.6 ± 2.7	11.4 ± 2.9	11.6 ± 3.1	10.4 ± 2.3
Day 14 of lactation	11.6 ± 2.6	11.4 ± 2.9	11.6 ± 3.1	10.4 ± 2.3
Day 21 of lactation	11.6 ± 2.6	11.4 ± 2.9	11.6 ± 3.1	10.4 ± 2.3
Total number of males on Day 1 ^a of lactation (%)	122 (48)	114 (49)	142 (52)	106 (53)
Total number of females on Day 1 ^a of lactation (%)	130 (52)	118 (51)	128 (48)	93 (47)

a = Excludes litters where all pups died

Statistical analysis performed on duration of gestation only (Fisher's Exact Test 21 days duration versus 22 or more).

Significantly different from the Control: *P<0.05, **P<0.01, ***P<0.001

Necropsy observation animals were sacrificed on day 21 of lactation; F1 pups not selected for post-weaning assessments were sacrificed at this time also

There was no toxicologically significant necropsy finding attributable to BI 1722 CL administration.

Table 34: Summary of Necropsy Findings

Table 28 F0 Generation: Summary of Necropsy Findings

Dose levels are reported as target dose levels

NECROPSY FINDINGS	GROUP DOSE	GROUP TOTALS			
		Females			
		Grp 1 0 ug/kg /day	Grp 2 50 ug/kg /day	Grp 3 200 ug/kg /day	Grp 4 3000 ug/kg /day
GENERAL COMMENTS					
Number of animals necropsied		24	24	24	24
LUNG					
Dark focus					1
Dark				1	
Pale focus		1	2		
SKIN AND SUBCUTIS					
Scab				1	
Hair loss			1	1	
Staining		2	3	2	8
TAIL					
Abnormal shape				1	

The absence of a numeral indicates that the lesion specified was not identified

NECROPSY FINDINGS	GROUP DOSE	GROUP TOTALS			
		Females			
		Grp 1 0 ug/kg /day	Grp 2 50 ug/kg /day	Grp 3 200 ug/kg /day	Grp 4 3000 ug/kg /day
UTERUS					
Dilated, with fluid, both horns			1	2	
Dark, left horn				1	

The absence of a numeral indicates that the lesion specified was not identified

Toxicokinetics

No blood samples or milk samples were collected and there was no toxicokinetic analysis.

Stability and homogeneity

Stability and homogeneity were not conducted in this study. Previously the Sponsor determined that all formulations were stable for 63 days stored in the dark at ambient temperature (<30°C) when not in use.

The analyses of test formulations prepared and used in the study indicated that the measured concentrations of BI 1744 BS were -0.1 to 0.3% of the target concentrations. (b) (4), a degradation product of BI 1744 CL was not detected. Values for the vehicle components benzalkonium chloride was within -5.8 to -7.0% of target, and disodium edetate was within + 1 to +4% of target.

F₁ GENERATION OBSERVATIONS AND RESULTS

At Day 21 of lactation, 2 male and 2 female pups (where they were available) were randomly selected from each litter. One male and one female were allocated to Set 1 and the other allocated to Set 2.

Survival checked twice daily

Litter size and survival was reduced at the high dose Group 4. At the high dose, 3665 µg/kg/day, the viability through postnatal day 4 was 70% compared to 95% in Controls. Eight litters lost more than 3 pups, and in 5/8 litters all pups were dead by Day 4 of lactation. In Group 2, 5 litters lost more than 3 pups and in 3/5 of those litters all pups were dead by Day 4 of lactation. There were no losses in Group 3. Due to the lack of dose response and the similarity to the background range it was considered most likely that these findings were incidental. In Group 4, litter weight and pup weights were non-significantly slightly lower than controls by day 21 of lactation.

Table 35: F1 Survival Indices

		Dose Group (Treatment)			
		1	2	3	4
		(Control)	(Low)	(Intermediate)	(High)
Birth Index	Mean Litter Index (%)	95	94	93	93
	Number Losing >2 pups	2	1	3	3
	Number of Litters	21	22	23	23
Live Birth Index	Mean Litter Index (%)	100	99	100	98
	Number Losing >1 pup	0	0	0	1
	Number of Litters	21	22	23	23
Viability Index Days 0-4	Mean Litter Index (%)	95	80 (93)	97	70 (89)
	Number Losing >3 pups	1	5 (2)	0	8 (3)
	Number of Litters	21	22 (19)	23	23 (18)
Lactation Index Days 4-21	Mean Litter Index (%)	100	100	100	98
	Number Losing >1 pup	0	0	0	0
	Number of Litters	21	19	23	18
Overall Survival Index Birth-21	Mean Litter Index (%)	95	79 (91)	96	68 (87)
	Number Losing >4 pups	1	5 (2)	0	8 (3)
	Number of Litters	21	22 (19)	23	23 (18)
Post-weaning Survival Index	Pups selected	84	75	91	69
	Pups surviving at necropsy	84	75	91	68
	Index (%)	100	100	100	99

() = value excluding total litter losses

Clinical signs checked daily and physical exam once weekly starting at 1 week

There were no toxicologically significant clinical signs in the F₁ generation that was attributed to BI 1744 CL administration.

Table 36: F1 Males Clinical Observations and Necropsy Findings**(Set 1 and Set 2)**

Observation/Finding	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
Staining on fur, eye(s)	2	0	0	0
Pale discharge from eyes / eyelids encrusted	2	0	0	0
Teeth overgrown	0	0	1	0
Subdued behaviour	0	0	0	1
Enlarged mandibular lymph node	4	1	3	1
Reddened mediastinal lymph node	0	1	0	0
Speckled thymus	0	1	1	0
Dark lung lobes	0	2	0	1
Pale lung lobes	0	0	0	2
Urinary bladder distended by contents	0	0	0	1
Killed prematurely	0	0	0	0
Total number of males examined	42	35	45	34

Table 37: F1 Females Clinical Observations and Necropsy Findings**(Set 1 and Set 2)**

Observation/Finding	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
Area (s) sparse hair	1	1	0	0
Staining (neck / abdomen / head)	6	2	3	5
Body hunched, thin, discoloured skin on head, head tilt, piloerection, rolling gait, subdued, swollen head, animal killed prematurely. Necropsy revealed brain abnormal shape with 2 frontal lobes absent, cranium enlarged and fluid filled	0	0	0	1
Dark lung lobe(s)	0	0	2	0
Pelvic cyst left kidney	0	0	0	1
Cervix exuded viscous brown fluid	0	0	1	0
Uterus dilated by fluid	2	1	2	0
Staining of fur/ hairless/ scabbing	1	0	0	1
Cyst on mandibular lymph node	1	0	0	0
Mandibular lymph node enlarged	1	0	0	0
Reddened lung lobe(s)	0	1	0	0
Cyst on mesenteric lymph node	0	1	0	0
Prominent lobulation of liver	0	1	0	0
Killed prematurely	0	0	0	1
Total number of females examined	42	38	46	35

Body weight weighed weekly starting 1 week after weaning

There were slight, but non-significant, decreases in mean weight gain in the high dose group males for both Set 1 and Set 2 compared to controls overall for weeks 3 to 13 and weeks 3 to 9, respectively. In Set 1 males, the slightly lower mean absolute weight achieved significance in weeks 7 to 10. In females there was a slight, but non-significant increase in mean weight gain in the high dose during weeks 3 to 10 in Set 1 and during weeks 3 to 9 in Set 2 compared to controls.

During the gestation period (up to day 14), the mean weight gain was similar in all dose groups.

Table 38: F1 Mean Litter and Pup Weight (g, mean \pm sd)

Day of Lactation	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
LITTER				
Day 1	76 \pm 9	80 \pm 11	74 \pm 16	70 \pm 12
Day 4	107 \pm 19	109 \pm 26	112 \pm 22	99 \pm 23
Day 7	156 \pm 28	155 \pm 36	162 \pm 29	142 \pm 32
Day 14	302 \pm 47	287 \pm 62	293 \pm 52	257 \pm 52
Day 21*	496 \pm 70	479 \pm 102	482 \pm 80	436 \pm 91
Mean of Litter Mean Pup Weight				
MALES				
Day 1	6.6 \pm 0.9	6.8 \pm 0.7	6.7 \pm 1.1	6.6 \pm 0.8
Day 4	9.6 \pm 1.5	9.8 \pm 1.1	10.3 \pm 2.1	9.7 \pm 1.2
Day 7	13.8 \pm 1.8	13.9 \pm 1.7	14.9 \pm 3.4	14.1 \pm 1.6
Day 14	26.8 \pm 3.2	25.7 \pm 3.2	26.8 \pm 5.4	25.6 \pm 2.5
Day 21*	44.3 \pm 6.7	43.1 \pm 5.7	44.4 \pm 9.5	42.8 \pm 5.4
FEMALES				
Day 1	6.3 \pm 0.8	6.5 \pm 0.7	6.3 \pm 1.0	6.2 \pm 0.6
Day 4	9.3 \pm 1.5	9.4 \pm 1.3	9.8 \pm 1.8	9.0 \pm 1.2
Day 7	13.6 \pm 1.8	13.5 \pm 1.8	14.4 \pm 2.8	13.2 \pm 1.4
Day 14	26.6 \pm 3.1	25.3 \pm 3.2	26.1 \pm 5.4	25.6 \pm 2.5
Day 21*	43.9 \pm 6.4	42.2 \pm 5.9	42.9 \pm 8.6	41.9 \pm 4.4

Means exclude litters where all pups died

* = statistical analysis performed (ANOVA) – significance was not achieved

Table 39: F1 Males set 1, Body Weights

Dose Group (Treatment)		Nominal Age (Weeks)											Body Weight Gain (g) (Week 3 - Week 13)	
		3	4	5	6	7	8	9	10	11	12	13		
1 (Control)	Number	21	21	21	21	21	21	21	21	21	21	21	21	21
	Mean	74	118	177	235	297	339	374	401	424	457	467	393	
	SD	8	12	17	22	28	35	42	47	47	49	53	49	
2 (Low)	Number	18	18	18	18	18	18	18	20	20	20	18	18	
	Mean	69	110	168	224	283	325	359	380	409	442	454	385	
	SD	11	16	21	26	31	34	42	48	48	51	55	46	
3 (Intermediate)	Number	23	23	23	23	23	23	23	23	23	23	23	23	
	Mean	70	112	168	222	280	321	356	380	403	435	447	377	
	SD	12	15	18	21	25	29	33	34	38	44	43	39	
4 (High)	Number	17	17	17	17	17	17	17	20	20	20	17	17	
	Mean	66	106	162	214	270	307	337	359	387	420	430	364	
	SD	11	16	22	26	30	32	36	39	41	45	48	40	
	Prob.					**	**	**	**					

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Data for set 2 animals (used for mating) included during Weeks 10-12

* = statistical analysis performed (ANOVA)

Table 40: F1 Males set 2, Body Weights

Dose Group (Treatment)		Nominal Age (Weeks)							Body Weight Gain (g) (Week 3 - Week 9)
		3	4	5	6	7	8	9	
1 (Control)	Number	21	21	21	21	21	21	21	21
	Mean	72	115	173	233	294	336	373	301
	SD	10	13	17	22	29	36	43	37
2 (Low)	Number	17	17	17	17	17	17	17	17
	Mean	70	112	170	230	289	330	366	295
	SD	7	11	16	21	26	33	39	34
3 (Intermediate)	Number	22	22	22	22	22	22	22	22
	Mean	68	108	161	217	274	312	346	277
	SD	12	16	20	24	27	31	36	27
4 (High)	Number	17	17	17	17	17	17	17	17
	Mean	69	110	164	219	279	318	352	282
	SD	9	13	18	23	29	32	37	31
	Prob.								

* = statistical analysis performed (ANOVA) – significance was not achieved

Table 41: F1 Females set 1, Body Weights

Dose Group (Treatment)		Nominal Age (Weeks)								Body Weight Gain (g) (Week 3 - Week 10)
		3	4	5	6	7	8	9	10	
1 (Control)	Number	21	21	21	21	21	21	21	21	21
	Mean	70	106	143	172	195	211	230	241	171
	SD	8	10	12	13	17	20	23	26	23
2 (Low)	Number	19	19	19	19	19	19	19	20	19
	Mean	66	100	139	170	193	209	226	238	170
	SD	8	10	12	15	17	18	20	22	17
	Prob.									
3 (Intermediate)	Number	23	23	23	23	23	23	23	23	23
	Mean	66	99	137	167	191	208	224	239	172
	SD	10	12	13	14	17	18	19	21	16
	Prob.									
4 (High)	Number	18	18	18	17	17	17	17	20	17
	Mean	66	100	140	171	196	215	232	246	178
	SD	8	12	15	16	17	18	19	19	15
	Prob.									

Includes Set 2 animals (used for mating) during Week 10

* = statistical analysis performed (ANOVA) – significance was not achieved

Table 42: F1 Females set 2, Body Weights

Dose Group (Treatment)		Nominal Age (Weeks)							Body Weight Gain (g) (Week 3 - Week 9)
		3	4	5	6	7	8	9	
1 (Control)	Number	21	21	21	21	21	21	21	21
	Mean	71	105	144	172	197	212	230	159
	SD	8	9	12	15	19	23	25	22
2 (Low)	Number	19	19	19	19	19	19	19	19
	Mean	66	101	139	169	193	209	227	161
	SD	10	14	17	18	20	22	22	16
	Prob.								
3 (Intermediate)	Number	23	23	23	23	23	23	23	23
	Mean	65	98	136	166	190	208	224	159
	SD	10	11	12	14	16	18	21	15
	Prob.								
4 (High)	Number	16	16	16	16	16	16	16	16
	Mean	67	101	142	172	197	213	230	164
	SD	5	6	9	9	11	12	14	14
	Prob.								

* = statistical analysis performed (ANOVA) – significance was not achieved

Feed consumption not monitored

Physical development

Test Day(s) of Lactation

Test Parameter

1 – Criterion
7 – Criterion

Pinna Detachment
Upper Incisor Eruption

In all treated groups there was a small statistically significant dose related decrease in the time to eye opening for both sexes (eyes opened earlier; mean for the high dose animals was on day 12.9 vs the control animals on day 14.8) . There was no later visual impairment, so there does not appear to be an adverse toxicological consequence to this effect. There were no effects on pinna detachment, upper incisor eruption, negative geotaxis, or auditory function.

The following observations and/ or abnormalities were noted:

Group 1 Litter 29: 1 male pup yellow in appearance on Day 0 and 1 of lactation 1 female pup (#11) small lump on abdomen

Group 2 Litter 33: 1 male pup yellow

Group 3 Litter 51: 1 female pup (#3) flap (of skin) attached to left side of mouth Days 7-17 of lactation Litter 55: 1 male pup (#5) has a lump under right forearm on Days 9-10 of lactation 1 female pup has a swollen and longer left forelimb noted from Day 13-18 of lactation. Litter 68 1 male pup (#3) flaps of skin protruding downwards from side of mouth; large flap appears to cover upper part of mouth (roof of mouth) Days 10-17 of lactation Litter 70: 1 male pup (#7) has round growth hanging from right side of mouth (assume means skin flap) Days 9-13 of lactation. Litter 72 1 male pup appears yellow in color on Day 1 of lactation.

There were no abnormalities recorded for the high dose group. The findings in the control, low and mid dose groups were similar with the exception of the skin flap at the side of the mouth. This finding has not previously been observed but was not evident in the high dose group. There low incidence suggests a spontaneously occurring abnormality that does not reflect an effect of treatment. Findings that were considered to reflect maternal care (e.g. possible artifacts such as bruising), pup survival (e.g pups cold to touch) or small pups that were observed across all groups at similar frequencies to those seen in the controls were not reported.

One F₁ generation female pup (#275, Group 4) was killed prematurely shortly after weaning due to its condition. The clinical observations noted included body hunched, thin discolored skin on head, head tilt, piloerection, rolling gait, subdued behavior, irregular respiration and swollen head. Necropsy revealed brain abnormally shaped with 2 frontal lobes absent, cranium enlarged and fluid filled. There were no maternal findings or similar findings in the litter siblings. There were no other findings noted (clinical observations or necropsy findings) that were considered to be atypical for animals of this age and strain.

Neurological assessment

<u>Test Day(s) of Lactation</u>	<u>Test Parameter</u>
11	Negative Geotaxis
11 – Criterion	Eye Opening
16	Auditory Function
18	Visual Function

There were no differences in the auditory (frequency and amplitude), activity (rota-rod, open field and Y-maze tests), or visual assessments between BI 1744 CL and control treatments.

Table 43: F1 Post-Natal Physical Development

	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
MALES				
<u>All Pups Reach Criterion</u>				
Pinna Detachment	3.2 ± 0.9	2.5 ± 0.6	3.0 ± 0.6	3.1 ± 0.7
Upper Incisor Eruption	11.2 ± 1.1	10.9 ± 1.2	10.8 ± 1.4	11.1 ± 1.0
Open Eyes	15.2 ± 0.7	14.0 ± 0.7*	13.3 ± 0.7***	13.5 ± 1.1***
<u>Median Day to Criterion</u>				
Pinna Detachment	2.7 ± 0.7	2.2 ± 0.5	2.5 ± 0.7	2.6 ± 0.7
Upper Incisor Eruption	10.3 ± 1.3	9.8 ± 1.0	9.9 ± 1.1	10.4 ± 0.9
Open Eyes	14.8 ± 0.7	13.5 ± 0.8***	13.0 ± 0.6***	12.9 ± 0.7***
FEMALES				
<u>All Pups Reach Criterion</u>				
Pinna Detachment	3.1 ± 0.7	2.7 ± 0.7	2.9 ± 0.7	3.2 ± 0.7
Upper Incisor Eruption	11.0 ± 1.2	10.8 ± 1.1	10.8 ± 1.2	10.9 ± 1.1
Open Eyes	15.1 ± 0.7	13.9 ± 0.7*	13.3 ± 0.6***	13.2 ± 0.7***
<u>Median Day to Criterion</u>				
Pinna Detachment	2.7 ± 0.7	2.4 ± 0.7	2.3 ± 0.6	2.6 ± 0.7
Upper Incisor Eruption	10.6 ± 1.1	10.0 ± 0.9	9.9 ± 1.2	10.5 ± 0.7
Open Eyes	14.7 ± 0.5	13.6 ± 0.7**	12.9 ± 0.6***	12.9 ± 0.5***
<u>Frequency (%) in pups</u>				
Incisor eruption before Day 11	54.5	70.5	62.9	52.1
Incisor eruption on Day 11	25.0	16.6	25.1	34.0
Incisor eruption after Day 11	20.5	12.9	12.0	13.8
Eye opening before Day 14	0.0	42.4	83.9	79.9
Eye opening on Day 14	33.6	50.7	13.5	16.4
Eye opening after Day 14	65.5	6.9	2.6	3.7

Statistical analysis performed on open eyes only (Fisher's Exact Test less than Day 14 versus more than Day 14)
Significantly different from the Control: *P<0.05, **P<0.01, ***P<0.001

Pups not individually identified at assessment of pinna detachment therefore frequency not calculated

Reproduction

At Day 21 of lactation, 2 male and 2 female pups (where they were available) were randomly selected from each litter. One male and one female were allocated to Set 1 and the other allocated to Set 2. Set 2 animals were necropsied at a convenient time shortly prior to assessment of the reproductive function. Set 1 animals were necropsied following completion of the assessment on the reproductive function. Animals were paired at approximately 10 weeks of age for as long as 14 days.

There were no effects of BI 1744 CL on F₁ mating (time to positive mating sign, number of siring males) male fertility index (%), number of females pregnant and female fertility index.

Post-weaning physical and neurological testingAge of Animals at TestingTest

Starting from 28 days
 Starting from 35 days
 ca 4-5 weeks
 ca 4-5 weeks
 ca 5-6 weeks
 ca 6-8 weeks
 ca 10 weeks

Vaginal Opening (females)
 Balano-preputial Separation (males)
 Detailed Auditory Testing
 Rota-Rod
 Open Field
 Multiple Y Water Maze
 Reproductive Function

The time to vaginal opening was not significantly increased, in the high dose group compared to their respective controls (Set 1 = 36.9 days and Set 2 = 37.1 days). There were no differences between treatment groups of males in the time of preputial separation.

Table 44: F1 Sexual Maturation (Set 1)

Day of Gestation	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
Females				
Age (days) at vaginal opening ^a	34.8 ± 2.27	36.1 ± 2.32	36.4 ± 2.63	36.9 ± 2.80
Weight (g) at vaginal opening ^a	119 ± 16	125 ± 17	125 ± 15	129 ± 21
% incidence less than 36 days	61.9	36.8	34.8	38.9
% incidence 36 days	9.5	5.3	13.0	5.6
% incidence more than 36 days	28.6	57.9	52.2	55.6
Males				
Age (days) at preputial separation ^a	44.1 ± 1.19	43.7 ± 1.01	43.7 ± 0.83	43.8 ± 1.25
Weight (g) at preputial separation	215 ± 18	199 ± 20 *	203 ± 19 *	195 ± 15 **
% incidence less than 44 days	28.6	50.0	39.1	47.1
% incidence 44 days	33.3	27.8	47.8	29.4
% incidence more than 44 days	38.1	22.2	13.0	23.5

All values given ± Standard deviation

a - statistical analysis performed on age (Kruskal-Wallis Test) but significance was not achieved

Weight analysed by ANOVA * - P<0.05, ** - P<0.01

Table 45: F1 Sexual Maturation (Set 2)

Day of Gestation	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
<u>Females</u>				
Age (days) at vaginal opening ^a	35.8 ± 3.21	36.0 ± 2.40	36.7 ± 2.33	37.1 ± 2.19
Weight (g) at vaginal opening ^a	123 ± 18	123 ± 18	126 ± 21	131 ± 15
% incidence less than 36 days	71.4	36.8	34.8	12.5
% incidence 36 days	0.0	15.8	13.0	18.8
% incidence more than 36 days	28.6	47.4	52.2	68.8
<u>Males</u>				
Age (days) at preputial separation ^a	44.3 ± 1.10	43.3 ± 0.99	43.7 ± 1.13	43.8 ± 0.97
Weight (g) at preputial separation	215 ± 25	205 ± 16	199 ± 26 *	195 ± 19 **
% incidence less than 44 days	23.8	58.8	45.5	35.3
% incidence 44 days	33.3	29.4	36.4	41.2
% incidence more than 44 days	42.9	11.8	18.2	23.5

All values given ± Standard deviation

a- statistical analysis performed on age (Kruskal-Wallis Test) but significance was not achieved

Weight analysed by ANOVA * = P<0.05, ** = P<0.01

Table 46: F1 Generation Body Weight During Gestation (mean ± sd)

Day of Gestation ^a	Dose Group (Treatment)			
	1 (Control)	2 (Low)	3 (Intermediate)	4 (High)
0	247 ± 28	242 ± 22	238 ± 20	249 ± 20
7	284 ± 27	278 ± 22	275 ± 23	284 ± 23
14	324 ± 31	317 ± 34	317 ± 26	325 ± 25

a = Pregnant animals only

Excludes premature decedent

11 Integrated Summary and Safety Evaluation

Studies of reproductive and developmental toxicology were conducted in rats and rabbits using the inhalation route of drug delivery.

Fertility and Early Embryonic Development in Rats: Rats were exposed by inhalation to doses of BI 1744 CL at 58, 193, and 3068 µg BI 1744 BS/kg/day. There were two mortalities in the high dose group related to BI 1744 CL treatment. The stages of estrous cycle and their mean duration were similar in all groups. There were no effects of treatment on mating or pregnancy performance. Testes and epididymides weights (as a % of body weight) were reduced for all BI 1744 doses, but without histopathological correlates, and there were no paternal effects on mating or pregnancy.

Therefore, the NOAEL for paternal and maternal reproductive, fertility, and pregnancy assessments was 3068 µg BI 1744 BS/kg/day, corresponding to PPD of 306.8 µg/kg/day and a BI 1744 concentration for males of 208000 pmol/L (80.3 ng/mL), and for females 167000 pmol/L (64.5 mg/mL). Since this high dose was associated with mortality (one female and one male), the NOAEL for reproductive, fertility and pregnancy exceeded the maximal tolerated dose administered by inhalation in the rat.

Embryo-Fetal Development in Rats: In the rat study of embryo-fetal development, there were no adverse effects on embryo-fetal survival or growth and the incidence of malformations or variations. BI 1744 CL was inhaled for 1 hour/day during gestation days 6 to 17 at achieved doses of 64, 222, or 1054 µg BI 1744 BS/kg/day. In the 222 and 1054 µg BI 1744 BS/kg/day groups there was a slight increase in fetuses with the incomplete ossification of sternbrae in the mid and high dose groups compared with controls (88% and 91% vs 76% respectively). Incomplete ossification is a late pregnancy effect not attributed to organogenesis and not considered teratogenic. Ossification can be arrested or delayed, but since the rats were all sacrificed, it cannot be ascertained if sternbrae ossification was delayed. It is a common findings with maternal toxicity associated with maternal weight loss, but signs of maternal toxicity related to under nutrition or weight loss were not apparent. BI 1744 treated animals had a small non-dose-dependent increase in weight gain during the first week of dosing, as a sign of appropriate drug exposure, but ossification typically occurs later in development. Other findings were considered incidental due to their low incidence and lack of dose dependency, or within recent historical control range of incidence (single fetus on the study in the mid dose, 222 µg BI 1744 BS/kg/day, had complete situs inversus; two incidences of umbilical hernia occurred in the 222 µg BI 1744 BS/kg/day group and a single incidence in the 1054 µg BI 1744 BS/kg/day group; and one fetus in the mid dose and two fetuses in the high dose group had anomalous confluence of umbilical and/or hepatic vein with inferior vena cava. Therefore, the developmental and maternal NOAEL for BI 1744 CL rat was the high dose of 1054 µg BI 1744 BS.

A comparison of doses and toxicological findings in this study suggests that the maximal tolerated dose was not attained and the dosing was inadequate for regulatory

acceptance. A comparison of aerosol concentrations and achieved dose level indicated amounts substantially less than the two other studies in rats (fertility and postnatal development studies). Note that although the exposure is 60 minutes in this study, compared with 35 minutes in the other two rat studies, the aerosol concentration is only 29 µg/L compared to 160 µg/L and 173 µg/L for the two other studies, a 5.5- and 5.9-fold less than those studies.

Embryo-Fetal Development in Rabbits: Exposure of pregnant rabbits to BI 1744 CL at the maximum practicable concentration of approximately 120 µg/L resulted in salivation, an initial transient reduction in food consumption, and embryo-fetal resorptions. Overall, this dose was sufficiently tolerated to be suitable for an embryo-feta toxicity study.

Pregnant rabbits were exposed to BI 1744 CL by inhalation for a 90-minute exposure period on days 6 through 19 inclusive after mating, at doses of 0, 289, 974, or 2489 µg/kg/day. There were no treatment-related adverse effects on embryo-fetal survival or growth. In the high dose 2489 µg BI 1744 BS/kg/day group, a greater number of fetuses/litters had major abnormalities compared with the controls or the historical control range. Most of the fetuses clustered in a single litter and had a specific syndrome of abnormalities which had not been previously seen in this strain/source of rabbits (historical control data). These included acrania, retinal folds, split sternum and distortion of the sternum. Also there was uneven ossification of the cranial bones/ribs/long bones (thickened ribs/distorted ribcage; short scapula, humerus, radius, ulna, femur, tibia, fibula; and/or partially open eyelids/eyelids not fused centrally; cleft palate; forelimb flexure/malrotated hindlimbs). There was no consistency in the type of abnormalities seen in the other affected fetuses in this group. Other major abnormalities included two fetuses in one litter (#9) had dilated ascending aorta/aortic arch; narrow pulmonary trunk; enlarged left and small right heart ventricles; one of these fetuses also had the pulmonary arteries arising directly from the aortic arch; one fetus in a second litter (#69) had multiple abnormalities including acrania, absent eyes, split sternum, gastroschisis, cervicothoracic scoliosis and lordosis, forelimb flexure and hindlimb hyperflexion; one fetus in a third litter (#82) had multiple retinal folds while another fetus in the same litter had dilated ascending aorta/aortic arch, narrow pulmonary trunk, enlarged left heart ventricle/small right atrium and ventricle; one fetus in a fourth litter (#99) had dorsoventral distortion of the sternum.

The maternal NOAEL was the high dose of 2489 µg BI 1744 CL kg/day. The developmental NOAEL for BI 1744 CL in the rabbit was the mid dose of 974 µg BI 1744 BS/kg/day corresponding to an AUC₀₋₂₄ of 182000 pmol-h/L.

Pre and Postnatal Toxicology in Rats: In the pre and postnatal toxicology study, rats were exposed to BI 1744 CL by inhalation for 35 minutes/day from gestation day 6 to 20, and then from lactation day 2 to 21 of dams (day 0 = day of birth of litter). The achieved doses were 0, 59, 297 and 3665 µg/kg/day close to the target doses of 0, 50, 200 and 3000 µg/kg/day.

A significantly increased weight gain and a transient, but significant, decrease in food consumption were observed following the first day of treatment. Observations such as changes in behavior (subdued and excessive general behavior, including paddling) and eye(s) partially closed were generally seen over the first 2-3 days in all high dose animals. Salivation and fur staining occurred in all BI 1744 CL treatment groups. There were slight differences in litter size by day 21 of lactation, and reduced survival in the low and high dose groups. Litter weight and pup weight in the high dose group were slightly less, but not statistically different than the weights of the control group by day 21 of lactation. There was a dose-related decrease in the time to eye opening in both sexes. There were no effects of treatment on the mating, fertility or bearing of live implants to Day 14/15/16 of gestation in the F₁ animals. Based upon these results the NOAEL was 3665 µg/kg/day BI 1744 BS for maternal toxicity, F₁ survival and development, and F₁ mating, fertility and pregnancy.

General Comments with regards to long acting β -adrenergic agonists

The increase in body weight gains noted in males and females in the the fertility study, rat and rabbit embryo studies and rat postnatal development study is a well characterized anabolic effect associated with a β_2 -agonist treatment. β_2 -agonist both enhance lipolysis on adipocytes and enhance skeletal muscle hypertrophy through adrenergic receptors on these tissue.

The reduced testes and epididymides weights noted in the fertility studies, as well as ovarian changes noted in toxicology studies (6 month inhalation rat toxicology study, U08-1691-01-AM1, submitted Nov 2008, submitted Jan 27 2009, SD-54) could be either direct effects, an alteration of neuroendocrine signals in the brain-pituitary-gonadal axis, or a combination of both, none of which could be readily distinguished in standard general toxicology studies. Drug distribution studies (Reports U10-1602-01 and U10-4128-01, submitted May 20 2011, SD-114) indicated increased pituitary uptake of radiolabeled BI 1744 as well as relatively high levels in testes. There is also fetal accumulation in mid gestation fetuses which supports direct action of BI 1744 on embryofetal development. These findings are consistent with approved LABA drugs in that studies they had no effect in studies of rat fertility.

Whether the teratogenic effects observed here are typical of approved LABAs cannot be determined from the label information for those LABAs. There does seem to be a difference between findings with inhalation studies and oral administration, in that oral administration of LABA does result in teratogenic effects, while inhalation studies do not. The obtained systemic concentration are most likely the link; at a high enough systemic exposure, whether by inhalation or oral routes of administration, teratogenic effects appear. For the approved LABA products, as for BI 1744 here, the safety margin is quite high (100s-1000s-fold) for the maximal human dose or proposed human dose, that the risk to a pregnant patients would expected to be minimal.

Comment on Study Validity

Pre-NDA meeting responses were sent to the Sponsor on Sept 28 2011 that included a comment that the Sponsor should justify that the rat embryo-fetal development studies were valid since it appeared that dosing may not have included the maximal tolerated or feasible doses. They responded on Oct 11 2011 (SD-124) with a satisfactory explanation the studies were indeed valid. This is based on the lack of observed maternal weight loss for a class of drugs that are known to cause weight gain, and the fact that higher doses used in the later postnatal study did not result in the potential of cannibalism as an explanation for possible reduced effects on structural abnormalities by the "absence or loss" of these animals. The Sponsor also notes the high safety margins in the current studies for the proposed maximal human clinical dose, however this does not contribute to the determination of a valid study, although it would contribute to the overall risk-benefit relationship for pregnant patient use of this product.

Comment of Safety Margins

Safety margins for fertility and embryo-fetal development are necessary to convey in the label and are based on systemic drug concentrations for an inhaled product. Sufficient blood samples were not collected to determine AUC values with the exception of one dose. C_{max} values were obtained at the end of the exposure period. Since a linear correlation exists between dose administered and C_{max} , a safety margin could be extrapolated based on the C_{max} values. It would be more accurate however, due to the large individual variation in blood concentrations from inhalation exposure to determine AUC exposure from multiple timepoint drug analysis.

Therefore, the Sponsor should provide AUC values from the reproductive toxicology studies to ensure adequate exposure, particularly the EDF study, and to enable label margins based on systemic drug exposure.

Table 47: Inter Study Comparison of Drug Exposure and Toxicokinetic Parameters

Study U08-2297-01: Fertility and Early Embryonic Development (Sprague-Dawley rats)									
Target dose (ug/kg/day)	0		50		200		3000		
Aerosol concentration (µg/L), breathe for 35 min	0		2.96		10.1		160		
Achieved doses (µg BS/kg/day)	0		58 paternal, maternal NOAEL		193		3068 early embryonic development NOAEL		
Gender			M	F	M	F	M F		
C_{0.583h} (pmol/L)	Day 12			-	1720	-	5800	-	191000
	Day 26			1130	-	5630	-	167000	-
	Day 62			1860	-	8310	-	208000	-
Study U05-2534: Embryo-Fetal Development in CD Rats (Sprague-Dawley rats)									
Target dose (ug/kg/day)	0		75		250		1251		
Aerosol concentration (µg/L), breathe for 60 min	0		1.74		6.08		28.7		
Achieved doses	0		64		222		1054		

($\mu\text{g BS/kg/day}$)				maternal and developmental NOAEL
C_{1h} (pmol/L)	1540	3390	7200 10900* Cmax at 4 hr =14400	37800
AUC_{0-24} (nmol-h/L)		-	101,000*	-
Study U06-1019: Embryo-Fetal Toxicity in the Rabbit (New Zealand White rabbits)				
Target dose (($\mu\text{g/kg/day}$)	0	300	1000	3000
Achieved doses ($\mu\text{g BS/kg/day}$)	0	289	974 developmental NOAEL	2489 maternal NOAEL
C_{max} (pmol/L)		8170	30400	161000
AUC_{0-24} (pmol-h/L)		39700	182000	959000

Study 494520: Pre and Post Natal Development (Sprague Dawley)				
Target dose (($\mu\text{g}/\text{kg}/\text{day}$)	0	50	200	3000
Aerosol concentration ($\mu\text{g}/\text{L}$), breathe for 35 min	0	2.77	14.1	173
Achieved doses ($\mu\text{g BS}/\text{kg}/\text{day}$)	0	59	297	3665 maternal, F ₁ survival and development and F ₁ reproduction and pregnancy
No toxicokinetics				

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/s/

LAWRENCE S LESHIN
10/26/2011

MOLLY E TOPPER
10/26/2011
I concur.

Appendix 6

Pharm/Tox Review of IND 76,362 dated October 28, 2011 (Chronic Toxicology Review)

**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: 76362, SDN59, Vol. 3-6 and 8-12
Supporting document/s: 1
Sponsor's letter date: March 27, 2009
CDER stamp date: March 30, 2009
Product: Respimat Inhalation Spray
Indication: Chronic Obstructive Pulmonary Disease
Sponsor: Boehringer Ingelheim Pharmaceuticals Inc.
Review Division: Division of Pulmonary, Allergy, and
Rheumatology Products
Reviewer: Hans Rosenfeldt, Ph.D.
Supervisor/Team Leader: Timothy Robison, Ph.D., D.A.B.T.
Division Director: Badrul A. Chowdhury, M.D., Ph.D.
Project Manager: LT Eunice Chung-Davies, PharmD

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1 Executive Summary

1.1 Recommendations

- Based on the calculated safety margins using systemic exposures (AUCs), Phase 3 clinical trials of olodaterol are supported by the submitted 6-month rat and 9-month dog repeat dose oral toxicology studies, Study Nos. 06B234/U08-1691-01 and 06B121/U08-1740-01, respectively. Adequate systemic safety ratios based on AUC levels in rat (41) and dog (5) were achieved.

1.1.1 Clinical Study Safe to Proceed: Yes

1.2 Brief Discussion of Nonclinical Findings

- In the 6-month rat study, daily inhalation of olodaterol produced skeletal and cardiac muscle hypertrophy with a concomitant decrease in white fat, laryngeal degeneration, and increased levels of WBC, neutrophils, and lymphocytes. The skeletal muscle hypertrophy and decreased white fat findings occurred at all dose levels and were not reversible, but were not considered adverse. Effects in the pancreas, ovary, and oviduct occurred at the 288 µg/kg dose level. NOAEL in this study was 4 µg/kg based on observed cardiac effects at doses of 16.4 and 288 µg/kg/day and pancreatic effects at 288 µg/kg/day.
- In the 9-month dog study, daily inhalation of olodaterol resulted in increased weight in males and females, and produced heart, kidney, and liver toxicity. Oral inflammation was also detected in treated dogs. NOAEL in this study was 3.9 µg/kg based on observed heart, kidney, and liver effects at the 42.4 and 87.6 µg/kg dose levels.
- C_{max} and AUC exposures were much greater in the rat, than in the dog. For example, AUC exposure in male rats treated with 4 µg/kg olodaterol was 11-fold higher than doses in male dogs treated at the 3.9 µg/kg dose level. Similarly, AUC exposure in female rats treated with 4 µg/kg olodaterol was 6-fold higher than doses in female dogs treated at the 3.9 µg/kg dose level.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

Not provided

2.1.2 Generic Name

Olodaterol

2.1.3 Code Name

BI 1744 CL

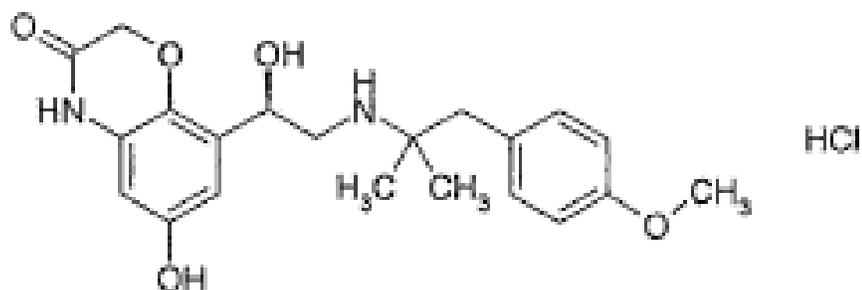
2.1.4 Chemical Name

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

2.1.5 Molecular Formula/Molecular Weight

$C_{21}H_{26}N_2O_5 \times HCl$ / 422.9 g/mol

2.1.6 Structure



2.1.7 Pharmacologic class

Long-acting, β_2 -adrenoreceptor agonist

2.2 Relevant IND/s, NDA/s, and DMF/s

IND [REDACTED] (b) (4)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Component	Conc. in 2 µg dose (g/100 mL)	Conc. in 5 µg dose (g/100 mL)	Conc. in 10 µg dose (g/100 mL)	Conc. in 20 µg dose (g/100 mL)	Function	Specification
BI 1744 CL	(b) (4)				DS	In House
Benzalkonium chloride	(b) (4)				(b) (4)	NF
Edetate disodium	(b) (4)					USP
Citric acid, anhydrous	(b) (4)					NF
Water for injection	(b) (4)					USP
█	(b) (4)					█
Total mass	(b) (4)				--	--

2.3.2 Comments on Novel Excipients

All excipients in the proposed formulation are compendial and are at levels at or below those in currently approved inhalation products.

2.3.3 Comments on Impurities/Degradants of Concern

None. All impurities in the proposed clinical batch are below the ICH Q3A/B thresholds for qualification. Please see the Product Quality Review of this application.

2.4 Proposed Clinical Population and Dosing Regimen

Boehringer Ingelheim Pharmaceuticals, Inc. (BI) is developing daily doses of BI 1744 CL delivered with the Respimat inhaler as a treatment of Chronic Obstructive Pulmonary Disease.

2.5 Regulatory Background

2.5.1 Previous Clinical Experience

The sponsor has completed three clinical trials of BI 1744 CL outside of the U.S. Study no. 1222.1 was a single dose study in healthy male and female volunteers (0.5 to 70 µg BI 1744 CL). Study no. 1222.2 was a multiple rising dose study in healthy male and female volunteers (2.5, 10 and 30 µg BI 1744 CL for 14 days). Study no. 1222.3 was a single dose study in Chronic Obstructive Pulmonary Disease (COPD) patients (2, 5, 10 and 20 µg of BI 1744 CL). There were slight increases in heart rate and in QTcF prolongation (7 ms to 13 ms) with no sign of dose dependency in males treated with ≥ 30 µg. Adverse events included increased upper airway secretion, throat irritation,

dizziness, headache, nausea, restlessness, palpitations, vertigo, tremor, rash and dry mouth or throat. At a single inhalation dose of 40 µg in healthy subjects, an AUC 0-Inf of 400 to 500 pmol*h/L was observed. In total, 169 healthy volunteers and 36 patients with COPD were treated with BI 1744 CL prior to opening of U.S. IND application No. 76362.

The opening clinical trial performed under IND 76362 , protocol 1222.5, was a multi-dose, multi-center, multi-national, randomized, double blind, placebo-controlled, parallel group study with once daily treatment of BI 1744 CL (2, 5, 10, 20 mcg) delivered by the Respimat® inhaler for 4 weeks designed to determine the optimum dose of BI 1744 CL in patients with COPD. In addition the sponsor performed study 1222.6, which is a randomized, double-blind, 4-way cross-over protocol designed to determine the 24-hour FEV1-time profile of orally inhaled BI 1744 CL, delivered with the Respimat inhaler, after 3 weeks of once daily doses of 5 or 10 mcg 20 mcg or twice daily doses of 2 or 5 mcg in 47 COPD patients.

In completed multi-dose clinical trials designed to assess efficacy in COPD patients, the highest dose olodaterol was 20 µg/day.

The sponsor submitted a list of completed clinical trials with olodaterol with their most recent investigator's brochure, submitted on May 31, 2011. However, this list does not include details of several clinical trials with odolaterol, including two completed pivotal parallel group 48-week trials in patients with COPD.

Table 1. List of Completed Clinical Trials (IB 5/31/2011)

Healthy Volunteer Studies

Study No. [Report]	Description [Design]
Inhalation (Respimat®): single dose	
1222.1 [U06-1418-01]	Safety, tolerability, PK of single rising doses of olodaterol (0.5-70 µg) in healthy male and female volunteers [R, DB, PC (within dose)]
1222.8 [U08-1543-01]	Effect of single doses of olodaterol (10, 20, 30, 50 µg) on QT/QTc (open-label positive control) [R, DB, PC, CO]
Inhalation (Respimat®): multiple dose	
1222.2 [U07-2062] [U08-1262-01]	Safety, tolerability, PK of 2.5, 10 and 30 µg olodaterol (14 days once daily) in healthy male and female volunteers [R, DB, PC (within dose)]
1222.21 [U08-3758-01]	Safety, tolerability, PK of 5, 10 and 20 µg olodaterol (14 days once daily) in Japanese healthy male volunteers [R, DB, PC (within dose)]
1222.47 [U10-3390-01]	Effect of ppg inhibitor on the pharmacokinetics of olodaterol [open label, fixed sequence, 10 µg olodaterol without and with 400 mg qd ketoconazole]
1222.48 [U10-3391-01]	Effect of CYP 2C9 inhibitor on the pharmacokinetics of olodaterol [open label, fixed sequence, 10 µg olodaterol without and with 400 mg qd fluconazole]
Intravenous / oral administration; single dose	
1222.7 [U08-1081-01]	Safety, tolerability, PK of olodaterol following single dose (0.5-25 µg) i.v. administration in healthy male volunteers [R, SB, PC (within dose)]
1222.19 [U08-1060-01]	Safety, tolerability, PK of olodaterol following single dose oral administration (15-40 µg) in healthy male volunteers [R, SB, PC (within dose)]
1222.9 [U08-2268-01] [U09-1129-01]	Mass balance, excretion pathways, metabolism following oral (40 µg) and intravenous (20 µg) administration of [¹⁴ C] olodaterol [open-label, PG]

*Excerpted from the sponsor's IB 5/31/2011

Special Populations

Study No. [Report]	Description [Design]
Inhalation (Respimat®): single dose	
1222.20 [U10-2864-01]	Safety, tolerability, PK of 20 µg olodaterol in patients with mild and moderate hepatic impairment compared to healthy volunteers [open label, PG]
1222.35 [U10-2081-01]	Safety, tolerability, PK of 30 µg olodaterol in patients with severe renal impairment compared to healthy volunteers [open label, PG]

*Excerpted from the sponsor's IB 5/31/2011

Patients with COPD

Study No. [Report]	Description [Design]
Inhalation (Respimat®): single dose	
1222.3 [U07-1743] [U08-1263-01]	Efficacy, safety, PK of single doses of olodaterol (2-40 µg) [R, DB, PC, CO]
Inhalation (Respimat®): multiple dose	
1222.5 [U09-3125-01]	Efficacy, safety, PK of 4 weeks once daily treatment with 2-20 µg olodaterol [R, DB, PC, PG]
1237.3 [U09-1422-01]	Safety, PK of 3 weeks once daily treatment of 10 µg olodaterol plus 5 µg tiotropium bromide (fixed dose combination) compared with olodaterol and tiotropium bromide monotherapies [R, DB, CO]
1222.26 [U10-1155-01]	Efficacy (FEV ₁), safety, PK of 3 weeks once daily (5 and 10 µg) vs. twice daily (2 and 5 µg) [R, DB, CO]
1222.22 [U10-2537-01]	Efficacy, safety, PK of 4 weeks once daily treatment with 2, 5 and 10 µg olodaterol in Japanese [R, DB, PC, PG]

*Excerpted from the sponsor's IB 5/31/2011

Patients with Asthma

Study No. [Report]	Description [Design]
Inhalation (RESPIMAT): single dose	
1222.4 [U08-3408-01]	Efficacy, safety, PK of single doses of olodaterol (2-20 µg) [R, DB, PC, CO]
Inhalation (RESPIMAT): multiple dose	
1222.6 [U09-1850-01]	Efficacy, safety, PK of 4 weeks once daily treatment with 2-20 µg olodaterol [R, DB, PC, PG]

*Excerpted from the sponsor's IB 5/31/2011

The Investigator's Brochure submitted on 5/31/2011 also included the following table of pharmacokinetic parameters from clinical trials in healthy volunteers, asthma patients, and COPD patients:

Table 2. Pharmacokinetic Parameters from Selected Clinical Trials (IB 5/31/2011)

Single Dose	Unit	N	COPD 10 µg	gCV [%]	Asthma 10 µg	gCV [%]	HV 10 µg (m/f)	gCV [%]
C_{max}	[pg/mL]	71/44/7/9	5.45	63.5	4.63	59.7	4.12 / 4.28	26.3 / 29.2
t_{max}^*	[h]	71/44/7/9	0.183	0.0500- 1.58	0.234	0.083- 2.83	0.333 / 0.183	0.150-0.333/ 0.150-0.333
AUC_{0-1}	[pg·h/mL]	48/31/6/6	4.93	45.7	3.95	49.4	3.29 / 3.43	23.5 / 25.9
fc_{0-24}		16/24/9/9	1.72 [#]	58.6 [#]	3.68 [§]	44.1 [§]	3.39 / 3.17	47.4 / 36.8
Multiple Dose								
$C_{max,ss}$	[pg/mL]	72/45/8/9	7.13	63.8	5.09	53.0	5.33 / 5.34	57.1 / 34.5
$t_{max,ss}^*$	[h]	72/45/8/9	0.200	0.0500- 1.02	0.333	0.083- 2.93	0.342 / 0.183	0.333-1.00 / 0.167-0.667
$AUC_{0-1,ss}$	[pg·h/mL]	68/43/8/8	5.76	55.8	4.37	50.3	4.50 / 4.85	53.8 / 18.3
$AUC_{0-24,ss}$	[pg·h/mL]	39/20/4/6	104	40.9	83.7	30.3	80.5 / 76.7	26.6 / 19.5
$t_{1/2,ss}$	[h]	31/---/8/8	34.9	60.0	---	---	22.9 / 45.9	205 / 30.5
CL/F_{ss}	[mL/min]	39/20/8/8	1600	40.9	1990	30.3	3290 / 2310	58.2 / 20.5
V_z/F_{ss}	[L]	31/---/8/8	4850	82.4	---	---	6520 / 9200	115 / 34.6
$MRT_{ih,ss}$	[h]	31/---/8/8	49.0	60.5	---	---	31.0 / 65.1	189 / 34.8
$R_{A,Cmax}$		63/35/6/9	1.34	50.1	1.13	50.5	1.45 / 1.25	38.2 / 46.5

1) COPD and asthma patients: once daily for 4 weeks; healthy volunteers: once daily for 2 weeks

* median and range

values from 1222.3; all other data pertaining to COPD patients 10 µg are derived from trial 1222.5;

§ values from 1222.4; all other data pertaining to asthma patients 10 µg are derived from trial 1222.6;

m = male; f = female;

BI Trial No.: 1222.2, 1222.3, 1222.4, 1222.5, 1222.6

The sponsor has submitted the following table detailing the maximum olodaterol exposures achieved in clinical studies:

Maximum dose tested	Single Dose	70 µg [U06-1418-01];
	Multiple Dose	30 µg, once daily for 14 days [U07-2062];
Exposures Achieved at Maximum Tested Dose	Single Dose	C_{max} : 32.7 pg/mL (54.3%), N=5; $AUC_{0-\infty}$: 304 pg·h/mL (105%), N=5; [U06-1418-01];
	Multiple Dose	$C_{max,ss}$: 10.7 pg/mL (45.3%), N=9; $AUC_{0-24,ss}$: 126 pg·h/mL (35.4%), N=9; [U07-2062];
Range of linear PK	Single dose: 2.5 - 70 µg [U06-1418-01]; Multiple dose: 5 - 20 µg [U08-3425-01];	

*Excerpted from the sponsor's EOP2 Briefing Package (June 18, 2008)

2.5.2 History of Regulatory Submission

IND 76, 362 was submitted on January 26, 2007. During the review of the initial submission, deficiencies of the in vivo micronucleus test conducted as part of a 4-week inhalation toxicology study with rats (Study number (b) (4)) were identified.

The following comment was conveyed to the sponsor on March 21, 2007:



In response, the sponsor conducted an additional *in vivo* micronucleus report using the intravenous route (Study number U08-1834-01, Draft Report: Submission dated June 16, 2008 and Final Report: Submission dated March 27, 2009). Due to an increased frequency of micronuclei observed in the study, the sponsor conducted a cardiovascular safety pharmacology study with rats (Study number GP2008/0379/PH5) and a non-GLP mechanistic toxicology study with rats using the intravenous route (Study number U08-1845-01, Draft report provided in Amendment #044 dated July 14, 2008 and Final Report provided in Amendment #086 dated March 16, 2010) in an effort to explain that the observed response was not the result of DNA damage induced by BI 1744 CL.

3 Studies Submitted

3.1 Studies Reviewed

Study #	Title	Submission
06B234/U08-1691-01	26-week inhalation toxicity study in rats with a 4-week recovery period	SDN59
06B121/U08-1740-01	BI 1744 CL: 52-week inhalation toxicity study in Beagle dogs with a 6-week recovery period	SDN59

3.2 Studies Not Reviewed

The following studies will be addressed in future reviews:

Study #	Title	Submission
Pharmacology		
U08-1975-01	BI 1744 CL: Single Dose Inhalation Toxicokinetic Study in Mice	SDN59
U06-2150-02	<i>In vitro</i> pharmacological characterization of CD 992 AC and (b) (4): Determination of agonistic potency and efficacy for the human beta 1 and beta 2 adrenoreceptors using the accumulation of cAMP in CHO-K1 cells expressing the respective receptors as functional readout	SDN59
U08-2038-01	P-gp-dependent limitation of oral absorption and bioavailability of BI 1744 CL in the rat	SDN59

U08-2233-01	Determination of <i>in vitro</i> plasma protein binding of [³ H] BI 1744 BS, in plasma of rat, dog, and human.	SDN59
U08-1317-02	Metabolism of BI 1744 CL in female rabbits – Amendment 2	SDN59
U08-1057-02	Metabolism of [¹⁴ C]BI 1744 CL in dogs – Amendment 2	SDN59
U08-3378-01	in the permeability of BI 1744 CL and its interaction with P-glycoprotein using the Caco-2 cell <i>in vitro</i> absorption model	SDN59
U08-3424-01	<i>In vitro</i> evaluation of the transport and interaction of BI 1744 CL with human P-glycoprotein (P-gp/MDRI)	SDN59
U08-1337-02	Excretion balance, C _C /C _P and pharmacokinetics of radioactivity and parent compound after intravenous, oral, or intraduodenal administration of 0.3 mg/kg of [¹⁴ C] BI 1744 CL to female rabbits	SDN59
Toxicology		
U08-1139-02	BI1744 CL: Escalating dose toxicity study in the beagle dog after oral administration. Letter Report (non-GLP)	SDN59
U08-1442-01	BI1744 CL: Preliminary Inhalation Feasibility Study in Juvenile Beagle Dogs	SDN59
Reproductive and Developmental Toxicity		
U08-1971-01	BI 1744 CL: Study for Effects on Pre and Post Natal Development Including Maternal Function in Rats (Inhalation)	SDN59
Other Toxicology Studies		
U08-1347-01	Development of Methodologies for the Analysis of BI 1744 CL in Oral (Gavage) Dosing Formulations	SDN59
U08-1524-01	Development of Methodologies for the Analysis of BI 1744 CL, Degradation Product (b) (4) Benzalkonium Chloride and Disodium EDTA in Inhalation Dosing Solution	SDN59

3.3 Previous Reviews Referenced

IND	Relevant Studies	Reviewer	Date	Communication PK
76362	Original Submission	Molly Topper, Ph.D.	2/28/2007	1640596
	Carcinogenicity SPA Request	Molly Topper, Ph.D.	3/2/2007	1642809
	Request for ECAC Feedback	Molly Topper, Ph.D.	2/17/2009	1802269
	Safety Pharmacology Secondary Pharmacology Genetic Toxicity	Timothy Robison, Ph.D., D.A.B.T.	5/10/2010	2778471

4 Pharmacology

In vitro pharmacology studies suggest that olodaterol is an agonist of the human β 2 adrenoreceptor with an EC_{50} = 1.0 ± 0.4 nM and that BI 1744 CI has an intrinsic activity of 68% compared to isoprenaline. Olodaterol stimulated the human β 1-adrenoreceptor with an EC_{50} = 60 ± 24 nM and had an intrinsic activity of 16% compared to isoprenaline, demonstrating that BI 1744 is selective for the β 2 receptor. Using the guinea pig and anesthetized dog acetylcholine-induced bronchospasm models, olodaterol was shown to have antagonistic effects against bronchospasm *in vivo*.

5 Pharmacokinetics/ADME/Toxicokinetics

PK studies of olodaterol investigated in mice, rats and in humans are not yet reviewed but summarized in Dr. Molly Topper's review of the original IND submission. Plasma protein binding to olodaterol is low in mice, rats and humans (50-70%). Olodaterol is the dominant compound in plasma, bile and feces after intratracheal and intravenous administration of ^{14}C -olodaterol to mice and rats. In addition to olodaterol, a glucuronide metabolite, CD 992, a product of olodaterol de-methylation, SOM 1522, and the glucuronide metabolite of SOM 1522, M565(2), and a polar unknown and unextractable metabolite covalently bound to microsomes, m6, were identified as the major metabolites in mice and rats based on *in vivo* studies. In addition to these metabolites, *in vitro* rat liver microsome and hepatocyte studies identified two glutathione adducts M694 (1) and M694 (2) as stabilization products of a reactive quinonimine-intermediate. Studies with human liver microsomes in an *in vitro* system, identified that CYP 2C8, 2C9, 3A4 are involved in metabolism of olodaterol. A single-inhalation study of olodaterol in healthy male volunteers showed that, the parent compound, olodaterol and its glucuronide, CD 922, were found in urine; this study found olodaterol. There was no metabolism of olodaterol by human lung microsomes.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: 26-week inhalation toxicity study in rats with a 4-week recovery period Amendment No.: 1

Study no.: 06B234/U08-1691-01-AMI
Study report location: Boehringer Ingelheim Pharma GmbH & Co. KG

Conducting laboratory and location:	88397 Biberach an der Riss Germany
Date of study initiation:	Same as above
GLP compliance:	January 8, 2007
QA statement:	Yes
Drug, lot #, and % purity:	Yes
	5061001, 99%

Key Study Findings

- Daily inhalation of olodaterol produced skeletal muscle hypertrophy and decreased white fat, resulting in increased weight in both males and females in all treatment groups.
- Increased heart weight (in all dosed groups; ≥ 4 $\mu\text{g}/\text{kg}$), increased heart congestion (16.4 and 288 $\mu\text{g}/\text{kg}$) and left ventricular scar formation (288 $\mu\text{g}/\text{kg}$) occurred in males only. Palpable heart beat was detected directly after dosing in both sexes in rats treated at the 16.4 and 288 $\mu\text{g}/\text{kg}$ dose levels.
- Lobular atrophy occurred in the pancreas in 3/10 males and 3/10 females at the 288 $\mu\text{g}/\text{kg}$ dose level.
- Increased incidence and severity of cysts in the ovary and oviduct occurred in females treated at 288 $\mu\text{g}/\text{kg}$ dose level.
- Cardiac and skeletal muscle effects are likely linked to the $\beta 2$ -adrenergic agonist activity of olodaterol.
- olodaterol treatment resulted in non-dose-related decreases in creatine kinase and glucose (males and females), and triglycerides (males only).
- Non-dose related decreases in magnesium also occurred with concomitant increases in potassium, and inorganic phosphate.
- Changes in electrolyte levels together with small increases in blood urea and creatinine (males only) may be related to muscle hypertrophy. Glucose decreases occurring might also be similarly explained by skeletal muscle hypertrophy.
- WBC, neutrophil, and lymphocyte counts were increased in all dosed groups (≥ 4 $\mu\text{g}/\text{kg}$).
- NOAEL was 4 $\mu\text{g}/\text{kg}$ based on observed cardiac effects at doses of 16.4 and 288 $\mu\text{g}/\text{kg}/\text{day}$ and pancreas effects at 288 $\mu\text{g}/\text{kg}/\text{day}$.

Methods

Doses:

Dose Group	MMAD [§] µm	Mean Drug Conc. Aerosol [µg/L]	Mean Tot. Air Breathed Dosing (L)	Depo. Factor	Mean BW (Kg)	Mean Deposited dose [µg/Kg/d]	Mean Deposited dose sponsor calculated [µg/Kg/d]	No. Rats	
								Main/TK/Recovery	
								♂	♀
Control	(b) (4)	0	4.9	0.1	0.27	0	0	20/5/10	20/5/10
LD		2.2	5.1	0.1	0.29	4.0	49	20/5	20/5
MD		9.1	5.2	0.1	0.29	16.4	200	20/5	20/5
HD		160	5.1	0.1	0.28	288	3400	20/5/10	20/5/10

§MMAD: Mass Median Aerodynamic Diameter. MMAD values in the range of (b) (4) are considered to be within the respirable range.

Deposition Factor: 0.1 (DPARP Divisional Practice, rats)

Deposited dose (µg/Kg) = [Mean Drug Conc. Aerosol (µg/L) x Tot. Air breathed (L) x **Deposition Factor**] ÷ Body Weight (Kg)

Mean Deposited dose sponsor calculated (µg/Kg/d): doses calculated with a **Deposition Factor** = 1.0 and an alternative method to calculate Tot. Air breathed (see below)

Mean Deposited dose (µg/Kg/d): average of deposited doses calculated for Days 1 - 182

Tot. Air Breathed Dosing: V_{\min} (L/min) x 35 min

V_{\min} (L/min) = $2.1 \times (\text{Body Weight g})^{0.75} \div 1000$ (to convert to L) (Guyton et al., 1947)¹

Sponsor V_{\min} (L/min) = $4.19 \times (\text{Body Weight g})^{0.66} \div 1000$ (to convert to L) (McMahon et al., 1977)²

V_{\min} separately calculated for Deposited dose determinations for Days 1 - 182

Body Weight: Average body weights (male and female) calculated on Days 1 - 182 Daily x182

Frequency of dosing:

Formulation/ Vehicle: 0.01% benzalkonium chloride, (b) (4)% disodium EDTA x 2 H₂O, (b) (4)% citric acid

Species/ Strain: CrI:WI(Han) rats

Age: ca. 10 weeks

Weight: Males: 236 – 280 g; Females: 162 – 202 g

Deviation from study protocol: No significant deviations

Inhalation Protocol

An aerosol of BI 1744 CL was generated using Heart™ Large Volume nebulizers. Technicians placed approximately 100 mL of BI 1744 CL formulation in each nebulizer and refilled nebulizers with about 30 mL during the inhalation period in rare cases where the formulation in the nebulizers turned out not to be sufficient. The primary aerosol from the nebulizers was diluted with dry air and the resulting test aerosol was passed to the exposure chamber. Prior to animal exposures, the sponsor performed technical trials to assure adequate aerosol quality. Different aerosol concentrations were achieved by dilution of the formulation as described in Table 3.

Table 3. Equipment and Gas Flow Rates; Study No. 06B234



*Excerpted from sponsor submission

The exposure chamber was kept at a negative pressure of (b) (4). Pressurized air (particle, oil, and water free) was obtained from a compressor. Exhaust aerosol was passed through different filters in order to remove the test item before releasing it to the atmosphere. The exposure systems for the Control group and the dose groups were separately located to prevent contamination between the groups.

Drug exposures were performed on rats habituated to the inhalation procedure. The animals were taken from the cages and put into clean glass exposure tubes with an adjustable backstop. The tubes were identified by the individual animal numbers. The tubes with the animals were connected to the exposure chamber when stable aerosol conditions were reached.

The temperature, relative humidity, and air flow rate of the incoming exposure atmosphere were monitored by computer. Aerosol concentration measurements of the test atmosphere were collected on glass fiber filters via the sampling port and aerosol concentration was monitored continuously using aerosol photometers and 10 minute averages were stored for documentation. For retrospective verification of the mass correlated to the photometer signals, the aerosol concentration was determined gravimetrically once a week. The mass median aerodynamic diameter (MMAD) of the aerosol was determined once using a cascade impactor (b) (4). Dose verification was performed daily using an HPLC-UV method.

The sponsor calculated respiratory minute volumes according to McMahon et al² using the last measured body weight:

(b) (4)

Observations and Results

Mortality

- Rats in all groups were assessed at least twice daily, except during the pre-test period, on weekends, and on non-working days when the animals were inspected at least once daily.
- No unscheduled mortality occurred during the course of the study.

Clinical Signs

- The general health of the animals was checked on arrival.
- The overall appearance and behavior of each animal was inspected at least twice daily and any abnormalities were recorded with regard to onset, duration, and intensity. On non-working days, clinical observations were performed in the morning early.
- Detailed examinations of the main study and recovery animals were performed weekly during pretest, administration, and recovery periods.

Cageside Post-Dose Observations

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
Days Observed					
Reduced Motility, directly after dosing	M	--	1-2	1-2	1, 3
	F	--	1-2	1-2	1, 3
Reduced Motility	M	--	--	1-2	1, 3

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
6h after dosing	F	--	--	--	1-3
Abdominal position directly after dosing	M	--	--	--	1
	F	--	--	--	1
More palpable heart beat directly after dosing	M	--	--	1-2	1-3
	F	--	--	1-2	1-3
Wet, sticky and reddish fur around head and chest, directly after dosing	M	--	--	--	1, 4-63
	F	--	--	--	1, 4-63
Reduced grooming 6 h after dosing	M	--	--	--	1-3
	F	--	--	--	1-3
Piloerection, 6 h after dosing	M	--	--	--	1-3
	F	--	--	--	1-3

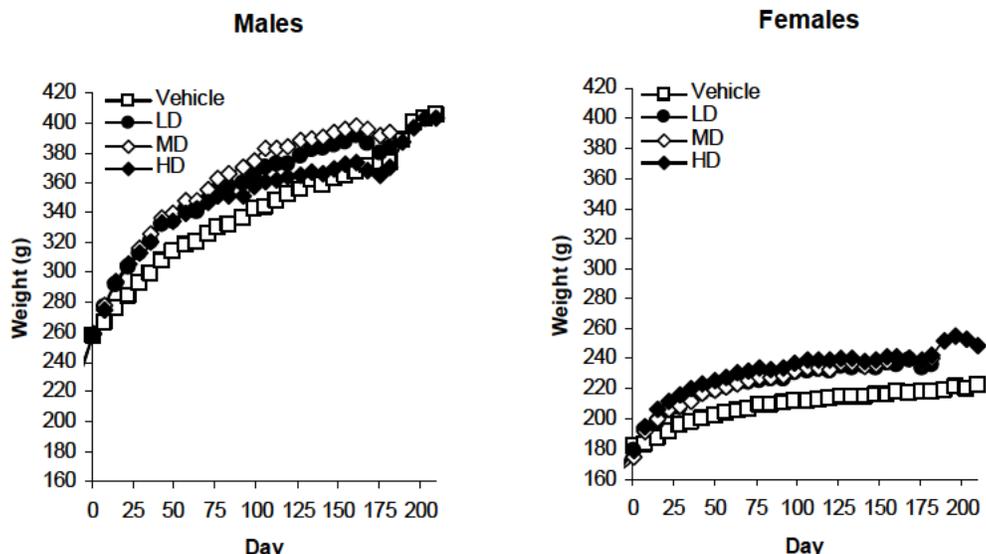
-- No Change

Detailed Observations

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
No. of Animals (Days Observed)					
Left ear swollen (serous liquid)	M	1 (76-94)	--	--	--
	F	--	--	--	2 (77-94)
Rattled Breathing	M	--	--	--	2 (31, 178)
	F	--	--	--	--
Missing/shortened teeth	M	--	--	--	1 (106 – 182)
	F	--	--	--	--

Body Weights

- Individual body weights were recorded at least once a week and just prior to necropsy.
- Statistically significant increases in body weights occurred in all dosed groups and in both sexes during Days 1-57 of the study and in females throughout the study, including the recovery period. However, treated females did not show body weight gain during the recovery period; body weight increases that occurred during the dosing period were not reversed during the recovery period.
- Weight gain differences relative to control recovered in males and females dosed with 288 µg/kg during the recovery period (days 190 – 210).



Increased BW in LD, MD, and HD Groups $p < 0.001$ (2-sided t-test)

Day 57					
Parameter	Sex	Control	4 $\mu\text{g}/\text{kg}$	16.4 $\mu\text{g}/\text{kg}$	288 $\mu\text{g}/\text{kg}$
Av. Body Weight (g)	M	319	342**	348**	339**
	F	204	222**	221**	228**
Day 120					
Av. Body Weight (g)	M	352	371*	384*	364
	F	214	232**	233**	240**
Days 182					
Av. Body Weight (g)	M	373	383	394*	370
	F	218	237**	241**	243**
Day 210 (Recovery Period)					
Av. Body Weight (g)	M	406	N.D.	N.D.	403
	F	222	N.D.	N.D.	249*

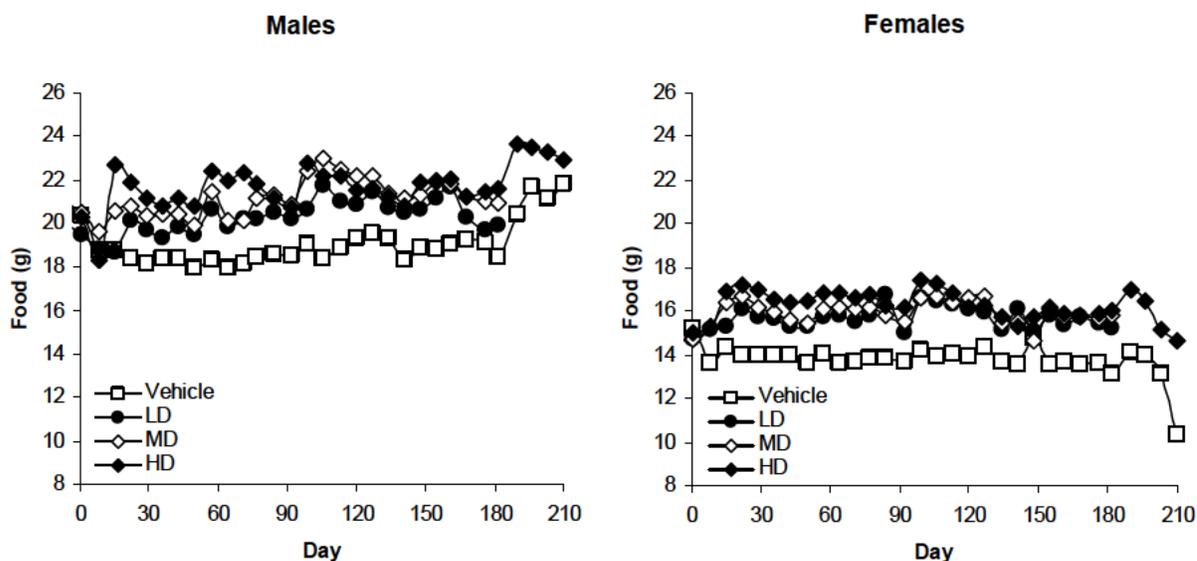
* $p < 0.05\%$; ** $p < 0.001\%$; (2-sided t-test)

Days 1-57					
Parameter	Sex	Control	4 $\mu\text{g}/\text{kg}$	16.4 $\mu\text{g}/\text{kg}$	288 $\mu\text{g}/\text{kg}$
Av. Body Weight Gain (g)	M	61	84**	89**	80**
	F	23	43**	46**	48**
Days 1-120					
Av. Body Weight Gain (g)	M	95	114**	126**	105
	F	33	54**	58**	60**
Days 1-182					
Av. Body Weight Gain (g)	M	116	125	136*	111
	F	36	58**	65**	63**
Days 190-210 (Recovery Period)					
Av. Body Weight Gain (g)	M	17.5	N.D.	N.D.	15.5
	F	3.2	N.D.	N.D.	-3.2

* $p < 0.05\%$; ** $p < 0.001\%$; (2-sided t-test)

Feed Consumption

- Food consumption values were determined during the pre-test, treatment, and recovery periods on a cage basis by weighing of the remaining food on the same days as body weight measurements.
- Water consumption was checked daily by visual assessment along with clinical observations.
- Food Consumption was statistically higher in all dosed groups and in all both sexes.



Increased Food Consumption LD, MD, and HD Groups $p < 0.001$ (2-sided t-test)

Days 1-57					
Parameter	Sex	Control	4 $\mu\text{g}/\text{kg}$	16.4 $\mu\text{g}/\text{kg}$	288 $\mu\text{g}/\text{kg}$
Food Consumption (g)	M	18.8	20.3	21.1	21.5
	F	14.0	15.7	15.9	16.2
Days 1-120					
Food Consumption (g)	M	95	114**	126**	105
	F	33	54**	58**	60**
Days 1-182					
Food Consumption (g)	M	18.8	20.3	21.1	21.5
	F	14.0	15.7	15.9	16.2
Days 190-210 (Recovery Period)					
Food Consumption (g)	M	21.3	N.D.	N.D.	23.5
	F	12.9	N.D.	N.D.	15.8

Ophthalmoscopy

- Ophthalmoscopic examination of conjunctiva, sclera, cornea, anterior chamber of the eye, iris, lens and anterior part of the vitreous body was performed using a slit lamp (Zeiss, Oberkochen/Germany). The fundus was examined using an ophthalmoscope (Zeiss, Oberkochen/ Germany).
- Examinations were performed during the pre-test period on Day -6 (Vehicle) and Day -5 (all other main study and recovery animals), during the treatment period on Day 178 (Vehicle and 288 µg/kg groups) and Day 182 (4 and 16.4 µg/kg groups). Additionally, the recovery animals (Vehicle and 288 µg/kg groups) were examined on Day 207
- Pupillary dilatation was induced approximately 10 min before examination using 1-2 drops of a mydriatic agent (Mydriaticum Stulln® Pharma Stulln GmbH, Stulln/Germany).
- The sponsor did not tabulate male and female findings separately; combined male and female findings are listed below.
- Ophthalmoscopic examinations found increases in monocular and binocular lens opacities.

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
Pre-test Day 178/182 Day 207 (recovery)					
Lens, opacity, subcapsular, posterior diffuse monocular	M+F	-- 10% 15%	-- 28% N.D.	10% 20% N.D.	8% 23% 45%
Lens, opacity, subcapsular, posterior diffuse binocular	M+F	-- 8% 5%	-- 58% N.D.	2.5% 78% N.D.	6.7% 63% 50%

-- No change

ECG

Not conducted

Hematology

- Blood samples were collected during main study on Day 85 and 169 and at the end of the recovery period on Day 205.
- Blood from all animals was obtained from the retrobulbar venous plexus under isoflurane anesthesia.
- Rats were fasted prior to blood sampling (except for blood sampling for CK determination on Day 4).
- olodaterol treatment resulted in increased white blood cell, lymphocyte and neutrophil counts in all treatment groups in both sexes.

- Platelet counts decreased slightly in all treatment groups although there was a concomitant decrease in PT time.

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
Day 85 Day 169 Day 205 (Recovery)					
WBC	M	4.58 x10 ³ /µL	+57%	+58%	+61%
		4.25 x10³/µL	+39%	+49%	+41%
		4.49 x10 ³ /µL	N.D.	N.D.	+27%
	F	3.00 x10 ³ /µL	+44%	+56%	+47%
		2.32 x10³/µL	+47%	+67%	+65%
		2.56 x10 ³ /µL	N.D.	N.D.	+50%
Neutrophils	M	0.729 x10 ³ /µL	+42%	+91%	+108%
		0.837 x10³/µL	+47%	+65%	+77%
		0.790 x10 ³ /µL	N.D.	N.D.	+46%
	F	0.488 x10 ³ /µL	+25%	+52%	+64%
		0.442 x10³/µL	+37%	+64%	+67%
		0.410 x10 ³ /µL	N.D.	N.D.	+75%
Lymphocytes	M	3.64 x10 ³ /µL	+35%	+51%	+50%
		3.20 x10³/µL	+38%	+45%	+32%
		3.50 x10 ³ /µL	N.D.	N.D.	+23%
	F	2.36 x10 ³ /µL	+48%	+57%	+42%
		1.76 x10³/µL	+51%	+68%	+64%
		2.36 x10 ³ /µL	N.D.	N.D.	+45%
Platelets	M	686x10 ³ /µL	--	--	-9%
		766x10³/µL	-15%	-19%	-21%
		681x10 ³ /µL	N.D.	N.D.	--
	F	685x10 ³ /µL	--	--	--
		746x10³/µL	-15%	-16%	-13%
		726x10 ³ /µL	N.D.	N.D.	--
PT	M	18.15 sec	--	-3%	-4%
		17.34 sec	--	--	--
		16.63 sec	--	--	--
	F	17.70 sec	--	-6%	-3%
		16.94 sec	-4%	-6%	-5%
		15.71 sec	N.D.	N.D.	+3%

--No change

Clinical Chemistry

- Blood samples were collected during main study on Day 85 and 169 and at the end of the recovery period on Day 205.
- Blood from all animals was obtained from the retrobulbar venous plexus under isoflurane anesthesia.
- Rats were fasted prior to blood sampling (except for blood sampling for CK determination on Day 4).
- olodaterol treatment resulted in non-dose-related decreases in creatine kinase and glucose (males and females), and triglycerides (males only).
- Non-dose related decreases in magnesium also occurred with concomitant increases in potassium, and inorganic phosphate.

- Increased creatinine and blood urea levels occurred in the 4 and 288 µg/kg dose levels in males.
- Modest increases in bilirubin levels occurred at the 288 µg/kg dose level in males and at the 16.4 and 288 µg/kg dose levels in females. Modest increases in AST were identified in both sexes at the 288 µg/kg dose level.
- Changes in electrolyte levels together with the increases in blood urea and creatinine may be related to muscle hypertrophy, since corresponding histopathology in the kidney was not identified. Glucose decreases might also be similarly explained by skeletal muscle hypertrophy.

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
Day 85 Day 169 Day 205 (Recovery)					
Creatine Kinase	M	274 U/L	-53%	-58%	-58%
	F	229 U/L	-53%	-50%	-48%
Total bilirubin	M	1.1 µmol/L	--	--	+29%
	F	1.0 µmol/L	--	+41%	+81%
Glucose	M	11.7 mmol/L	-29%	-32%	-30%
		11.1 mmol/L	-23%	-25%	-23%
		10.6 mmol/L	N.D.	N.D.	-18%
	F	9.8 mmol/L	-19%	-26%	-28%
		9.1 mmol/L	-12%	-20%	-22%
		9.6 mmol/L	N.D.	N.D.	-12%
Triglycerides	M	1.3 mmol/L	--	-20%	-17%
	F	1.5 mmol/L	-33%	-31%	-33%
		0.9 mmol/L	--	--	--
Creatinine	M	31.4 µmol/L	+11%	--	+16%
	F	36.9 µmol/L	+8%	--	+7%
Urea	M	6.5 mmol/L	+13%	--	+7%
	F	6.4 mmol/L	+12%	--	+12%
		6.5 mmol/L	--	--	--
Magnesium	M	0.8 mmol/L	-5%	-3%	-4%
	F	0.9 mmol/L	--	-5%	-6%
Potassium	M	3.9 mmol/L	+9%	+5%	+6%
		4.0 mmol/L	+3%	+5%	+8%
		3.8 mmol/L	N.D.	N.D.	+12%
	F	3.5 mmol/L	+7%	+7%	+10%
		3.4 mmol/L	+9%	+7%	+13%
		3.3 mmol/L	N.D.	N.D.	+13%
Inorganic phosphate	M	1.2 mmol/L	+17%	+29%	+27%
		1.0 mmol/L	+16%	+28%	+24%
		1.2 mmol/L	N.D.	N.D.	+34%
	F	0.9 mmol/L	+20%	+29%	+31%
		0.8 mmol/L	+24%	+28%	+25%
		0.9 mmol/L	N.D.	N.D.	+28%
AST	M	58.3 U/L	--	--	+106%
	F	68.2 U/L	--	--	+23%

Urinalysis

- Urinalysis was performed on main study animals on Day 78 and 162 to 165 and at the end of the recovery period on Day 197/198.
- The urine was collected during a period of approx. 5 hour using metabolic cages (Urimax).
- Immediately prior to be placed in metabolic cages, the animals received 20 mL/kg drinking water as bolus via gavage.
- The urine was tested with a Clinitek Atlas™ (Bayer AG, Leverkusen, Germany) and the urine sediment was examined microscopically.
- Small statistically significant increases in specific gravity occurred in males treated at 4 and 288 µg/kg on Day 78, males at all doses on Day 162, and in females treated at 4 and 288 µg/kg on Day 162.

Parameter	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
Day 78					
Day 162					
Urine Specific Gravity	M	1.0135 g/mL	1.0177 g/mL	1.0145 g/mL ^s	1.0165 g/mL
	F	1.0133 g/mL	1.0168 g/mL	1.0159 g/mL ^s	1.0172 g/mL

^sNot Statistically Significant

Gross Pathology

- Necropsy was performed on all main study and recovery animals and all gross macroscopic changes were recorded.
- Rats were anesthetized with an injection of ketamine/xylazine and exsanguinated by puncture of the abdominal aorta.
- Common findings included decreases in adipose tissue and skeletal muscle enlargement in all dosed groups.
- Heart enlargement was also detected in males and females at 16.4 and 288 µg/kg.

Macroscopic Signs	Group Size:	Control		4 µg/kg		16.4 µg/kg		288 µg/kg	
		M	F	M	F	M	F	M	F
		20/10	20/10	20	20	20	20	20/10	20/10
	Grade	Terminal/Recovery							
Adipose tissue, white, reduced	Present	--	--	1	--	2	--	15	2
Adrenal Gland, discoloration	Present	--	--	--	--	--	--	--	1
Adrenal Gland, enlargement	Present	--	--	--	--	--	--	--	1
Epididymis, discoloration	Present	--	--	--	--	--	--	1	--
Epididymis, enlargement	Present	--	--	--	--	--	--	1	--

Macroscopic Signs	Group Size:	Control		4 µg/kg		16.4 µg/kg		288 µg/kg	
		M	F	M	F	M	F	M	F
		20/10		20/10		20		20	
	Grade	Terminal/Recovery							
Harderian gland, enlargement	Present	--	--	2	1	4	--	5	1
Heart, dilatation of ventricles	Present	--	--	3	--	3	--	1	--
Heart, enlargement	Present	--	--	3	--	3	--	1	--
Liver, enlargement	Present	--	--	--	--	--	--	--	1
Liver, cyst-like space/pseudocyst	Present	--	--	--	--	--	--	1	--
Ovary, discoloration	Present	--	--	--	--	--	--	--	2
Seminal vesicle, reduced in size	Present	--	--	--	--	--	--	1	--
Skeletal muscle, enlargement	Present	--	--	19	11	20	12	19/9	16
Stomach, discoloration	Present	--	--	--	--	--	--	1	1
testis, reduced in size	Present	--	--	--	--	--	--	1	--
Thymus, discoloration	Present	--	--	1	--	--	--	1	2
Thymus, reduced in size	Present	--	--	1	--	--	--	1	1
Uterus, dilatation	Present	--	1	--	--	--	--	--	4
Axillary region, nodule	Present	--	--	--	--	--	--	--	1
Body as a whole, hyperemia	Present	--	--	--	--	--	--	--	1
Ureter, dilatation	Present	--	--	--	--	--	--	1	--

Organ Weights

- For each necropsy, brain, adrenal glands, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, spleen, testes, and thyroid were weighed.
- Significant weight differences were identified in the adrenal gland and liver in males, and in the heart in both sexes.
- Increased heart weight correlates to gross and microscopic pathology findings

Day 183 Day 211 (Recovery)					
Organ	Sex	Control	4 µg/kg	16.4 µg/kg	288 µg/kg
Heart	M	1.03 g	+16%	+17%	+16%
		1.69 g	N.D.	N.D.	+10%
	F	0.76 g	+12%	+8%	+15%
		0.73 g	N.D.	N.D.	+16%
Liver	M	7.47 g	-6%	--	-9%
	F	4.74 g	--	--	--
Adrenals	M	52.4 mg	+9%	+10%	+12%
	F	65.3 mg	--	--	--

- No change

Histopathology

- The following organs and tissues were examined: adipose tissue (renal pelvis) adrenals, aorta, bone (sternum, femur), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, forestomach, femur joint, glandular stomach, harderian glands, heart, ileum, jejunum, kidneys, knee (joint with femur), submandibular lymph nodes, lacrimal glands, larynx, liver, lungs, female mammary glands, mesenteric and bifurcational lymph nodes, nasal cavity, optic nerves, ovaries, oviducts, pancreas, parathyroids, parotid salivary glands, sciatic nerve, peyer's patches, pharynx, pituitary, prostate, rectum, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, sublingual salivary glands, testes, thymus, thyroid, tongue, trachea, ureters, urinary bladder, uterus, vagina.
- Eyes/optic nerves, testes and epididymides were fixed in modified Davidson's fixative for 24 hours and then in 4% neutral buffered formaldehyde solution. All other organs were fixed in 4% neutral buffered formaldehyde solution. Final storage of all organs/tissues following microscopic examination was in 4% neutral buffered formaldehyde solution.
- Common findings that occurred at all doses included decreases in white adipose tissue, skeletal muscle hypertrophy..

- Heart congestion was also detected in males at 16.4 and 288 µg/kg. One control female and one female treated at the 288 µg/kg dose level also exhibited heart congestion.
- Scar formation in the left ventricle was detected in 2 males treated at the 288 µg/kg dose level.
- Increased incidence and severity of cysts in the ovary and oviduct occurred in females treated at 288 µg/kg dose level.
- Lobular atrophy occurred in the pancreas in 3/10 males and 3/10 females at the 288 µg/kg dose level. β-adrenergic receptors are expressed in the rat pancreas. *In vitro* work suggests that β-adrenergic receptors are positive regulators of islet cell proliferation and insulin secretion³ and thus this finding could correlate with the slight decreases in glucose observed in treated animals.
- Epithelial atrophy, inflammation, and squamous cell hyperplasia occurred in the nasal cavity of males and females treated at the 288 µg/kg dose level. These findings are
- Degeneration of the larynx occurred at all doses. Squamous cell hyperplasia/metaplasia in the larynx was detected in males and females treated at 288 µg/kg. Squamous cell metaplasia and regeneration was detected in males and females treated at 16.4 and 288 µg/kg. These findings are likely to be rat-specific⁴.

Microscopic Signs	Group Size:	Control		4 µg/kg		16.4 µg/kg		288 µg/kg	
		M	F	M	F	M	F	M	F
		20/10	20/10	20	20	20	20	20/10	20/10
	Grade	Terminal/Recovery							
Adipose tissue, white, reduced in size	Minimal	--	--	10	9	4	5	2	2/2
	Slight	--	--	1	2	9	10	5	9/1
	Mod.	--	--	--	--	1	--	15	9
	All	--	--	11	11	14	15	20/2	20/3
Adrenal gland, bilateral cytoplasmic vacuolation	Minimal	--	--	N.D.	N.D.	N.D.	N.D.	1	--
Bone, fibrosis/fibroplasia	Slight	--	--	N.D.	N.D.	N.D.	N.D.	--	1
Brain, glioma, mixed (M)	Present	--	--	--	1	--	--	--	--
Epididymis, spermatocele	Severe	--	--	--	--	--	--	1	--
Heart, acute congestion	Slight	--	1	--	N.D.	1	N.D.	4	1
Heart, left ventricle, scar formation	Minimal	--	--	--	N.D.	--	N.D.	2	--

Microscopic Signs	Group Size:	Control		4 µg/kg		16.4 µg/kg		288 µg/kg	
		M	F	M	F	M	F	M	F
	Grade	Terminal/Recovery							
Kidney, congestion, acute, bilateral	Slight	--	--	--	--	--	--	1	--
Larynx, epithelial atrophy	Slight	--	--	--	--	--	--	--	2
Larynx, degeneration	Minimal	--	--	--	3	3	4	2/4	1/6
	Slight	1	--	--	--	--	4	13/5	16/3
	Mod.	--	--	--	--	--	--	1	--
	All	1	--	--	3	3	8	16/9	17/9
Larynx, squamous cell metaplasia/hyperplasia	Slight	--	--	--	--	--	--	13/1	9
	Mod.	--	--	--	--	--	--	1	--
	All	--	--	--	--	--	--	14/1	9
Larynx, squamous cell metaplasia	Minimal	--	--	--	--	5	11	5/6	1/8
	Slight	--	--	--	--	--	--	7/3	13
	Marked	--	--	--	--	--	--	--	--
	All	--	--	--	--	5	11	12/9	14/8
Larynx, regeneration	Minimal	--	--	--	--	3	3	3/3	4/1
	Slight	--	--	--	--	--	--	3/2	3
	All	--	--	--	--	3	3	6/5	7/1
Liver, focal hypertrophy	Slight	--	--	--	--	--	--	--	1
Liver, scar contraction	Minimal	--	--	--	--	--	--	1	--
Lymph node, mesenteric, foam cell accumulation	Minimal	--	--	N.D.	N.D.	N.D.	N.D.	--	1
Lymph node, mesenteric, pigment storage	Slight	--	--	N.D.	N.D.	N.D.	N.D.	--	1
Nasal cavity, epithelial atrophy	Minimal	2	4/2	2	--	--	--	2/3	10/2
	Slight	--	--	--	--	--	--	16	9
	All	2	4/2	2	--	--	--	18/3	19/2
Nasal cavity, inflammation	Minimal	--	--	--	--	--	--	1	1
	Slight	--	--	--	--	--	--	4	--
	All	--	--	--	--	--	--	5	1
Nasal cavity, luminal cellular debris	Slight	--	--	--	--	--	--	2	--

Microscopic Signs	Group Size:	Control		4 µg/kg		16.4 µg/kg		288 µg/kg	
		M	F	M	F	M	F	M	F
	Grade	Terminal/Recovery							
Nasal cavity, squamous cell metaplasia	Minimal	--	--	--	--	--	--	--	2
	Slight	--	--	--	--	--	--	2	3
	All	--	--	--	--	--	--	2	5
Ovary, cysts	Minimal	--	1	--	4	--	3	--	8/1
	Slight	--	--	--	--	--	--	--	2
	All	--	1	--	4	--	3	--	10/1
Ovary, hemorrhage	Minimal	--	2	--	--	--	--	--	6/1
	Slight	--	--	--	--	--	1	--	1
	All	--	2	--	--	--	1	--	7/1
Oviduct, cyst	Minimal	--	--	--	--	--	--	--	1
	Slight	--	--	--	--	--	--	--	2
	All	--	--	--	--	--	--	--	3
Pancreas, lobular atrophy	Minimal	3	--	--	--	--	--	3	3
Peyer's patch, cystic vacuolation	Slight	--	--	--	--	--	--	1	--
Pituitary, dilatation of Rathke's pouch	Minimal	--	--	N.D.	N.D.	N.D.	N.D.	1	--
	Slight	--	--	N.D.	N.D.	N.D.	N.D.	1	--
Pituitary, eosinophilic exudate	Slight	--	--	N.D.	N.D.	N.D.	N.D.	1	--
Prostate, focal, inflammatory infiltration	Minimal	--	--	N.D.	N.D.	N.D.	N.D.	2	--
	Slight	--	--	N.D.	N.D.	N.D.	N.D.	2	--
	All	--	--	N.D.	N.D.	N.D.	N.D.	4	--
Skeletal Muscle, hypertrophy	Minimal	--	--	7	9	3	5	2/4	4/1
	Slight	--	--	9	2	14	15	13/1	12
	Mod.	--	--	--	--	2	--	4	--
	All	--	--	16/ N.D.	11/ N.D.	19/ N.D.	20/ N.D.	19/5	16/1
Skeletal Muscle, single cell necrosis	Minimal	5	5	13	6	11	10	14	12
Stomach, focal necrosis	Minimal	--	1	--	--	--	--	2	1/1
	Slight	--	--	--	--	--	1	--	--
	All	--	1	--	--	--	1	2	1/1
Testis, seminiferous tubule, atrophy	Minimal	--	--	--	--	--	--	1	--

Microscopic Signs	Group Size:	Control		4 µg/kg		16.4 µg/kg		288 µg/kg	
		M	F	M	F	M	F	M	F
	Grade	Terminal/Recovery							
Testis, Leydig cell Hyperplasia, diffuse, bilateral									
	Slight	--	--	--	--	--	--	1	--
W									
Trachea, squamous cell metaplasia	Minimal	--	--	--	--	--	--	2	1
	Slight	--	--	--	--	--	--	3	--
	All	--	--	--	--	--	--	5	1
Ear, regenerative, chondropathy	Slight	--	--	--	--	--	--	--	1
Body as a whole, congestion	Mod.	--	--	--	--	--	--	--	1
Ureter, luminal distensions	Slight	--	--	--	--	--	--	2	--

Special Evaluation

Not conducted

Toxicokinetics

- Blood samples were taken 35 min, 1, 2, 4, 8, 24 h after the beginning of inhalation on Days 1, 114, and 117.
- Samples were assessed by HPLC-MS/MS using electrospray ionization in the positive ion mode. The lower limit of detection was 25 pmol/L using 100 µl rat plasma.
- The calibration curve of undiluted samples was linear over the range of concentrations from 25.0 to 12500 pmol/L olodaterol

Parameter	Sex	4 µg/kg	16.4 µg/kg	288 µg/kg
Day 1 Day 177				
Cmax (pmol/L)	M	3480 3450	17100 13500	314000 341000
	F	3240 3310	15700 12000	292000 270000
AUC(0-24 h) pmol*h/L	M	13400 9010	54600 43000	881000 878000
	F	11000 7880	44900 32400	785000 668000

Stability and Homogeneity

- Aerosol concentration was determined daily using a validated HPLC-UV method.
- Test atmosphere was collected on glass fiber filters via the sampling port. At least three filters were collected per day for each dosed group and one filter per week for the control groups.

Study title: BI 1744 CL: 52-week inhalation toxicity study in Beagle dogs with a 6-week recovery period

Study no.: 06B121
Study report location: Biberach, Germany
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riss, Germany

Date of study initiation: September 28, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: 5061001, 99%



Key Study Findings

- Daily inhalation of olodaterol resulted in increased weight in males and females, and produced heart, kidney, and liver toxicity. Oral inflammation was also detected in treated dogs.
- Fibrosis/fibroplasia in the left ventricle was detected at the 42.4 and 87.6 µg/kg dose levels. This finding correlated with clinical signs of “increased heart force” which were frequently observed in the olodaterol-treated dogs and with increases in serum levels of creatine kinase and cardiac-specific troponin 1.
- Microscopic kidney findings include increased incidence and severity of infiltration which correlated with increases in serum creatine in females. Electrolyte changes observed predominantly in females may also be related to changes in kidney function.
- Increased severity in liver glycogen stores and hemorrhage in the liver was detected at the 42.4 and 87.6 µg/kg dose levels. These findings correlated with increases in ALT in females treated at these doses.
- Moderate palate inflammation with ulceration detected in the 14.4 and 87.6 µg/kg dose levels, but not in the mid-dose of 42.4 µg/kg.
- NOAEL was 3.9 µg/kg based on observed heart, kidney, and liver effects observed at the 42.4 and 87.6 µg/kg dose levels.

Methods

Doses:

Dose Group	MMAD [§] µm (b) (4)	Mean Drug Conc. Aerosol [µg/L]	Mean Tot. Air Breathed Dosing (L)	Depo. Factor	Mean BW (Kg)	Mean Deposited dose [µg/Kg/d]	Mean Deposited dose <u>sponsor calculated</u> [µg/Kg/d]	No. Dogs	
								Main/Recovery	
								♂	♀
Control		0	42.0	0.25	8.1	0	0	4/2	4/2
LD		2.8	47.0	0.25	8.5	3.9	15	4	4
MD		10	55.2	0.25	9.3	14.6	60	4	4
HD		55	53.3	0.25	9.0	82.6	330	4/2	4/2

[§]MMAD: Mass Median Aerodynamic Diameter. MMAD values in the range of (b) (4) are within an acceptable respirable range.

Mean Deposited dose (µg/Kg/d): average of deposited doses calculated for Days 10 – 346

Deposition Factor: 0.25 (DPARP Divisional Practice, dogs)

Deposited dose (µg/Kg) = [Mean Drug Conc. Aerosol (µg/L) x Tot. Air breathed (L) x **Deposition Factor**] ÷ Body Weight (Kg)

Mean Deposited dose sponsor calculated (µg/Kg/d): doses calculated with a sponsor's Deposition Factor = 1.0

Mean Drug Conc. Aerosol (µg/L): Mean Aerosol conc. (µg/L) x Mean Drug/Vehicle Ratio in Aerosol (HPLC determined)

Tot. Air Breathed Dosing: V_{\min} (L/min) x 15 min

V_{\min} : Experimentally determined (see Figure 1 below).

Frequency of dosing: 10 min inhalation daily x365

Formulation/ Vehicle: 0.01% benzalkonium chloride, (b) (4)% disodium EDTA x 2 H₂O, (b) (4)% citric acid

Species/ Strain: Beagle Dog

Age: Approximately 9 months

Weight: Males: 6.4 – 9.9 Kg; Females: 5.5 – 8.8 Kg

Deviation from study protocol: No significant deviations

Inhalation Protocol

An aerosol of BI 1744 CL was generated using Heart™ Large Volume nebulizers.

The exposure system consisted of mixing and exposure chambers made of glass. Four exposure ports and an additional port identical to the exposure ports for test atmosphere sampling were connected to the exposure chamber. A sensor for monitoring temperature and relative humidity were placed in the central area of the exposure chamber. A continuous flow of aerosol was sent from the exposure chamber to the exposure ports and excess aerosol. Exposures were performed using a face mask with a mouth tube of medium length made of soft silicone. The face mask is connected to the exposure system via a three-way valve allowing for breathing of fresh air (before and after start of inhalation) or for inhalation of the test atmosphere. Exhaled air was filtered and released to the atmosphere via a vacuum pump.

All gas flow rates including aerosol sampling flow rates were controlled by a programmable logic controller (PLC) and mass flow controllers (MFCs).

The apparatus initially used for measurement of the respiratory minute volume consists of a face mask connected to unidirectional restrictor valves, a plastic bag in a plastic box with a plunger, and a gas flow meter. Air exhaled from the dog can be collected for a certain time interval in the plastic bag and the volume can be subsequently determined by the gas flow meter. During the course of the study an improved apparatus using a rubber bellows instead of the plastic bag became available and was employed for V_{\min} measurements. V_{\min} was experimentally determined using the following system:

Figure 1. Apparatus for determining V_{\min} in Dog Study No. 06B121.

Air exhaled from the dog was collected for pre-determined time intervals in a plastic bag, or in later stages of the study, in rubber bellows as depicted above. Air collected in plastic bags or rubber bellows was passed through a gas-flow meter to determine volume.

Technicians placed approximately 120 mL of BI 1744 CL formulation in each nebulizer and refilled nebulizers during the inhalation period in rare cases where the formulation in the nebulizers turned out not to be sufficient. The primary aerosol from the nebulizers was diluted with dry air and the resulting test aerosol was passed to the exposure chamber. Prior to animal exposures, the sponsor performed technical trials to assure adequate aerosol quality. Different aerosol concentrations were achieved by dilution of the formulation as described in Table 4.

Table 4. Equipment and Gas Flow Rates; Study No. 06B121

(b) (4)

*Excerpted from sponsor submission

The exposure chamber was kept at a positive 2 hPa. Pressurized air was obtained from an oil-free compressor. Air was dried and filtered before use. Exhaust aerosol was passed through different filters in order to remove the test item before releasing it to the atmosphere. The exposure systems for the Control group and the dose groups were separately located to prevent contamination between the groups.

Exposure was performed on dogs accustomed to the inhalation procedure over a training period of approximately 3 weeks. The face mask was placed over the animal's snout and secured by an elastic strap. Face masks were cleaned every day after exposure with fresh water.

Observations and Results

Mortality

- Mortality and the general health condition of all animals were inspected at least twice daily.
- During the pre-test period and the recovery period the animals were inspected at least once daily.
- There was no unscheduled mortality during the study.

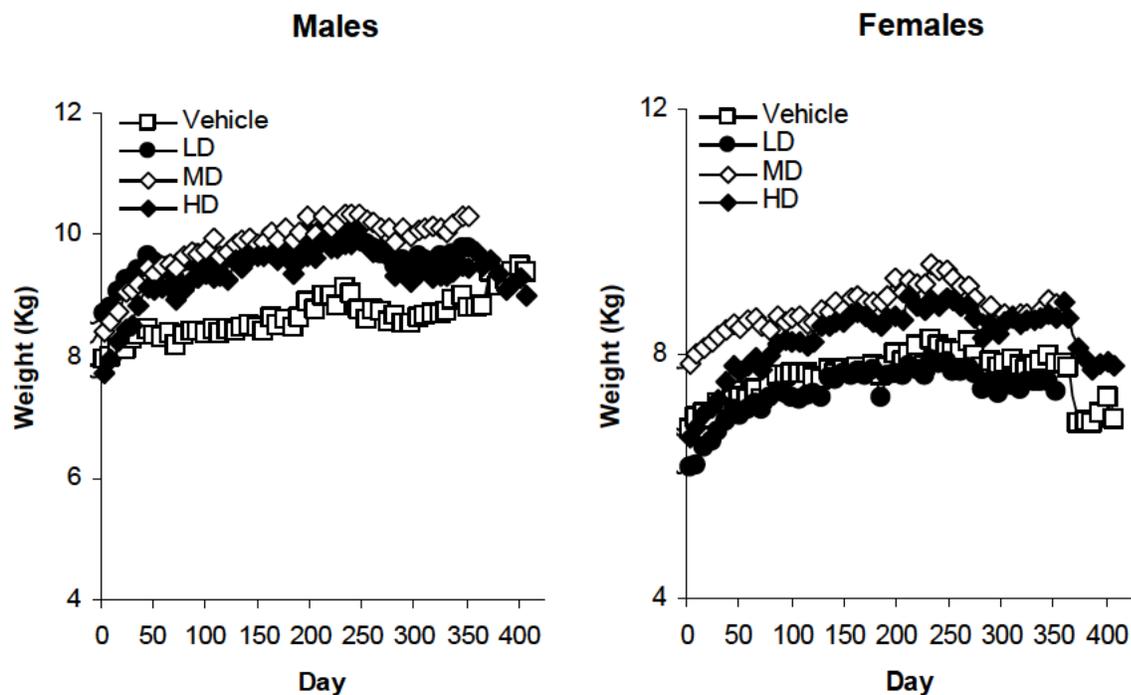
Clinical Signs

- The health status of the animals was checked at supply.
- The overall appearance and behavior of each animal was inspected at least twice daily.
- Any abnormalities were recorded with regard to onset, duration and, if possible, intensity.
- During the pre-test period and the recovery period clinical observations were performed at least once daily.
- The condition of the palate of all animals was visually inspected starting with the pre-test period once a month. Detailed clinical examinations of the main study and recovery animals were performed in the pre-test period, in Weeks 14, 27, 40 and 52 and at the end of the recovery period
- The main observed clinical sign was increased palpable heart beats (reported as "increased heart force."

Parameter	Sex	No. Dogs	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
			n = 6/sex	n = 4/sex	n = 4/sex	n = 6/sex
Number Observations (Days 1-365)						
Increased heart force (No. Dogs)	M	1	11	97	85	79
		2	--	32	62	111
		3	--	11	18	64
		4	--	4	6	32
		5	--	N.D.	N.D.	19
		6	--	N.D.	N.D.	11
	F	1	232	116	114	80
		2	5	30	63	103
		3	--	--	18	79
		4	--	--	6	41
		5	--	N.D.	N.D.	11
		6	--	N.D.	N.D.	3

Body Weights

- Body weights were recorded weekly in the morning, including the pre-test and recovery periods. During the treatment period, the body weight of all animals was recorded prior to dose administration. Body weights were also recorded before necropsy.
- In males, increased body weights occurred in all dosed groups, compared to control. Significant increases in body weight gain also occurred in males. These increases were dose-dependent during the first half of the study (please see table below).
- In females, increased body weights occurred in at the 14.6 and 82.6 $\mu\text{g}/\text{kg}$ dose levels, as compared to control. Significant increases in body weight gain occurred in all dosed groups, but were greater at 3.9 $\mu\text{g}/\text{kg}$ than 14.6 $\mu\text{g}/\text{kg}$. A potential explanation for this discrepancy is that females in the 3.9 $\mu\text{g}/\text{kg}$ group started out at a lower body weight in comparison to all other groups, including the control group.

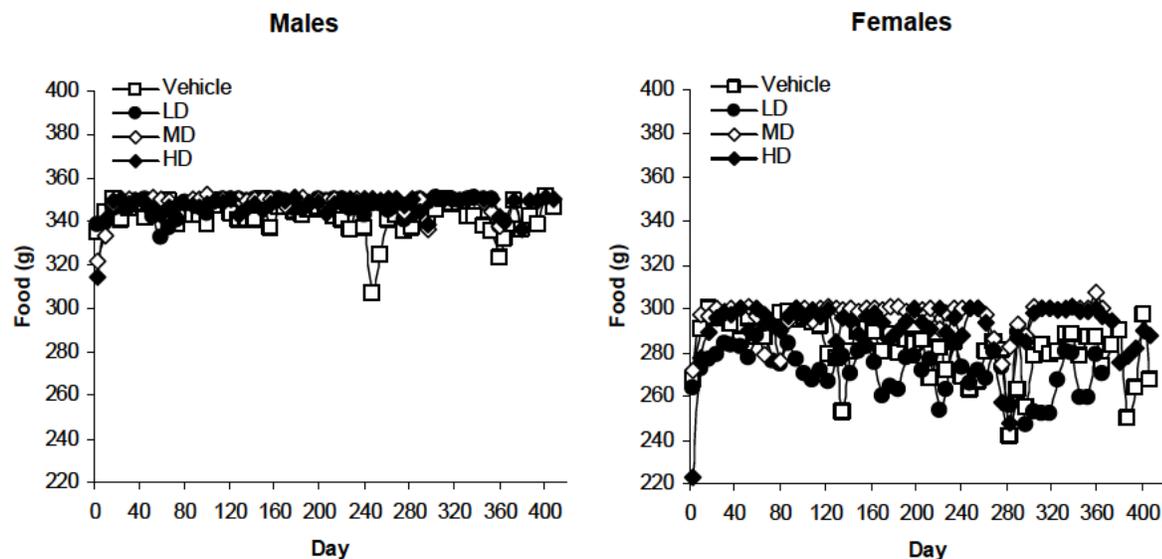


Days 3 – 94					
Parameter	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Av. Body Weight Gain (Kg)	M	0.46	0.97	1.29	1.56
	F	0.87	1.20	0.67	1.58
Days 3 – 185					
Av. Body Weight Gain (Kg)	M	0.51	0.84	1.50	1.65
	F	0.85	1.14	1.00	1.81
Days 3 – 365					
Av. Body Weight Gain (Kg)	M	0.88	0.98	1.88	1.76
	F	0.98	1.39	0.99	1.95
Days 374 – 408 (Recovery Period)					
Av. Body Weight Gain (Kg)	M	0.00	N.D.	N.D.	-0.62
	F	0.08	N.D.	N.D.	-0.30

--No Change; N.D. = No Data

Feed Consumption

- All animals were given a measured quantity of food daily. Remaining food was collected over the time period to the next body weight measurement and the weight was recorded.
- The animals received standard pellet dog food (b) (4)
- The food (350 g per day for male and 300 g per day for female dogs) was offered to the animals in the morning, on administration days approx. 1 hour after completion of exposure to all the animals, and removed again 2 hours later at the earliest.
- From Week 41 on animal No. 451 received a mixture of the standard pellet food and a tinned food (b) (4) from Week 41 on. No food consumption was recorded for this animal.
- There were no detectable changes in food consumption.



Ophthalmoscopy

- Ophthalmoscopical examinations of conjunctiva, sclera, cornea, anterior chamber of the eye, iris, lens and anterior part of the vitreous body were performed using a slit lamp (Zeiss, Oberkochen/Germany). The fundus was examined using an ophthalmoscope (Zeiss, Oberkochen/ Germany).
- Examinations were performed during the pre-test period on Day -15 (all animals), during the treatment period on Day 92, 183 (Control and 82.6 µg/kg group), and 360 (all animals), and during the recovery period on Day 402 (recovery animals of Control and 82.6 µg/kg groups).
- Pupillary dilatation was induced approximately 30-60 minutes before examination using 2-3 drops of a mydriatic agent (Mydriaticum StullnCl, Stulln GmbH, Nabburg/Germany).
- Discoloration in the sclera was observed in one female dosed at 82.6 µg/kg on Day 92.

		Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Parameter	Sex	6	4	4	6
Discoloration, sclera	M	0	0	0	0
	F	0	0	0	1 (Day 92)

ECG

- ECG and arterial pressure recordings were performed during the pre-test period (before and after a training session), and on day 2, in weeks 12, 26 and 51 (before and after inhalation), and at the end of the recovery period.
- From these recordings, heart rate, systolic and diastolic arterial pressure values, and PQ, QRS and QT intervals were evaluated.

- QT duration was corrected for heart rate according to the formulas of Fridericia (QTf) and Sarma (QTs).
- No significant changes in the PQ and QRS intervals were identified.
- Heart rate increased significantly in treated animals after inhalation on Week 1 in males and females.
- Heart rate was higher before inhalation in males and females on Week 1.

Heart Rate

Males	Week 1		Week 12		Week 26		Week 51		Week 58 Recovery
	Before Inhalation	After Inhalation							
Control	109	106	102	95	105	93	91	86	112
3.9 µg/kg	117	131	93	95	97	93	95	96	N.D.
14.6 µg/kg	127	163**	94	107	88	104	87	98	N.D.
82.6 µg/kg	148**	164**	95	108	93	106	93	106**	111

N.D. = No Data; **= p < 0.01; 2-tailed t-test

Females	Week 1		Week 12		Week 26		Week 51		Week 58 Recovery
	Before Inhalation	After Inhalation							
Control	108	99	108	100	113	113	100	103	101
3.9 µg/kg	113	143	93	104	108	113	88	97	N.D.
14.6 µg/kg	133	165**	113	106	116	125	104	111	N.D.
82.6 µg/kg	161**	190**	106	134*	114	133	107	114	96

N.D. = No Data; *p<0.05 2-tailed t-test; **= p < 0.01; 2-tailed t-test

Parameter	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
		6/group	4/group	4/group	6/group
Cardiac Troponin I (No. >0.1 µg/L)	M	0	0	1	4
	F	0	0	2	6

Hematology

- The sponsor conducted a standard hematology battery.
- Blood was obtained in the morning by puncture of the jugular vein of fasted animals without anesthesia.
- Hematology parameters were recorded during the pre-test period (Day -19) and on Day 91, 175, and 357 and on Day 406.
- Samples for the determination of CK activity in serum (in addition to determination of markers for myocardial damage) were taken on Days 87, 169 and 352 and from recovery animals on Day 402.

- Unscheduled analyses of hematology and clinical chemistry parameters were performed on male No. 201 on Day 114, on male No. 106 on Day 248 and female No. 451 on Day 284.
- Bone marrow smears were prepared at necropsy from all main study and recovery animals. The smears were air-dried and stained according to a modified Pappenheim method in a Sakura RSG-61 hematology slide stainer. However, the sponsor did not perform microscopic evaluations of these slides.

%Change relative to control

		Day 91 Day 175 Day 357			
Parameter	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Hemoglobin	M	16.3 g/dL 16.4 g/dL	-- -11%	-17% -16%	-10% -7%
	F	16.5 g/dL	--	--	--
RBC	M	7.0 x10 ⁹ /µL	--	-14%	-7%
	F	6.6 x10 ⁹ /µL	--	--	--
Platelets	M	307 x10 ³ /µL	--	--	+21%
		318 x10³/µL	--	--	+24%
		301 x10 ³ /µL	+19%	--	+28%
	F	300 x10 ³ /µL 293 x10³/µL 344 x10 ³ /µL	-- -- --	-- -- --	-- -- --
PT	M	8.0 s	--	+4%	+3%
	F	8.4 s	--	--	--
APTT	M	12.5 s	--	--	--
	F	12.5 s	--	+8%	+7%

-- No change

Clinical Chemistry

- The sponsor conducted a standard clinical chemistry battery.
- Blood was obtained in the morning by puncture of the jugular vein of fasted animals without anesthesia.
- Clinical Chemistry parameters were recorded during the pre-test period (Day -19) and on Day 91, 175, and 357 and on Day 406.

%Change relative to control

		Day 91 Day 175 Day 357			
Parameter	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
ALT U/L	M	38.0 U/L 34.3 U/L	-- --	-- --	-- --
	F	28.7 U/L 32.3 U/L	--	+92% --	+70% +51%
Glucose	M	5.4 mmol/L	--	--	--
		4.9 mmol/L	--	--	--

Day 91 Day 175 Day 357					
Parameter	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
	F	5.8 mmol/L 5.6 mmol/L	-14% -16%	-- -14%	-13% -8%
Inorg-P	M	1.7 mmol/L 1.5 mmol/L 1.3 mmol/L	-- -- --	-- -- --	-13% -25% -13%
	F	1.8 mmol/L 1.4 mmol/L	-- --	-- --	-- --
Tot. Chol.	M	3.3 mmol/L 3.3 mmol/L	-- -17%	-19% --	-19% -18%
	F	4.0 mmol/L 3.2 mmol/L	-- --	-- --	-- --
Creatinine	M	73.9 µmol/L 64.7 µmol/L	-- --	-- --	-- --
	F	61.8 µmol/L 59.4 µmol/L	+23% +25%	+55% +52%	+37% +37%
		54.8 µmol/L	--	+34%	+28%
Mg	M	0.87 mmol/L 0.83 mmol/L	-- --	-- --	-- --
	F	0.84 mmol/L 0.83 mmol/L	-- --	+10% +11%	+8% +8%
K	M	4.9 mmol/L	--	--	--
	F	4.7 mmol/L	+16%	+10%	-12%
Cl	M	118 mmol/L	-1.4%	-2.3%	-1.7%
	F	117 mmol/L 116 mmol/L	-2.4% -1.7%	-2.7% -2.5%	-1.7% 2.5%

-- No change

Cardiac-specific parameters

- Some assays for cardiac-specific parameters were not performed under GLP standards.
- In non-GLP assays. No significant changes in CK-MB (cardiac specific creatine kinase), CK-MM (skeletal muscle specific creatine kinase), CK-BB, CK-Makro, CK-Mit
- Similar non-GLP assays showed increases in troponin I detected on Day 4:

		Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Parameter	Sex	6/group	4/group	4/group	6/group
Cardiac Troponin I (No. >0.1 µg/L)	M	0	0	1	4
	F	0	0	2	6

- In GLP tests, creatine kinase (CK) analyses showed dose-dependent increases in animals with elevated levels of CK.

Main Study

		Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Parameter	Sex	6/group	4/group	4/group	6/group
Day 87 Day 169 Day 352 Day 357					
CK (No. >300 U/L)	M	1	1	3	3
		0	1	0	4
	F	0	1	1	2
		0	0	0	1
		0	3	2	2
		0	2	2	1
		0	1	1	1
		0	1	1	0

Recovery

		Control	14.4 µg/kg	42.4 µg/kg	87.6 µg/kg
Parameter	Sex	2/group	--	--	2/group
Day 402 Day 406					
CK (No. >300 U/L)	M	1	N.D.	N.D.	0
		0			0
	F	1	N.D.	N.D.	0
		0			0

Urinalysis

- Urine was obtained at necropsy by puncture of the urinary bladder on Days 365 - 368 as well as on Day 409 (recovery animals).
- The urine was tested with a Clinitek Atlas™ (Bayer AG, Leverkusen, Germany) and the urine sediment was examined microscopically.
- No significant changes were detected in dosed groups.

Gross Pathology

- A complete necropsy was performed on all main study and recovery animals, and all gross macroscopic changes were recorded.
- All animals were anesthetised with sodium pentobarbital (intravenous injection) and a supplementary intramuscular injection of ketamine (main study animals) or ketamine/xylazine (recovery animals) for analgesia.
- Animals were exsanguinated by cutting of all major axillar blood vessels.
- Animals were euthanized and necropsied in the following order:

Necropsy Day	Groups	Animal Numbers
365	Control (males), 87.6 µg/kg (males)	101-104; 401-404
366	42.4 µg/kg (males) 14.4 µg/kg (males)	301-304; 201-204
367	Control (females), 87.6 µg/kg (females)	151-154; 451-454
368	42.4 µg/kg (females) 14.4 µg/kg (females)	351-354; 251-254
406	Recovery males and females	105-106 155-156 405-406 455-456

Macroscopic Signs	Group Size:	Control		3.9 µg/kg		14.6 µg/kg		82.6 µg/kg	
		M	F	M	F	M	F	M	F
		4/2	4/2	4	4	4	4	4/2	4/2
Grade		Terminal/Recovery							
Adrenal gland, cyst-like space	Present	--	--	--	--	--	--	--	1
Epididymis, thickening	Present	--	--	--	--	--	--	1	--
Heart, consolidation	Present	--	--	--	--	--	--	1	--
Heart, discoloration	Present	--	--	--	--	--	--	3	2
Lung, discoloration	Present	--	--	--	1	--	--	2	--
Palate, defect	Present	--	--	1	--	--	--	2	1
Rectum, altered surface	Present	--	--	--	--	--	--	--	1
Skin, alopecia	Present	--	--	--	--	--	--	--	1
Skin, discoloration	Present	--	--	--	--	--	--	1	1
Stomach, discoloration	Present	--	--	--	--	--	1	1	--
Lung, discoloration	Present	--	--	--	--	--	--	1	--
				N.D.	N.D.	N.D.	N.D.		

-- No findings

Organ Weights

- The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs (before instillation), ovaries, pituitary gland, prostate (after fixation) spleen, testes, and thyroid gland (including parathyroids).
- Prostate weights were significantly lower at 87.6 µg/kg on an absolute basis.

Absolute weights

Terminal/Recovery					
Organ	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Prostate	M	6.5 g	--	--	-28%

-- No findings

- Prostate and heart weights were significantly lower in males at the 42.4 and 87.6 µg/kg dose levels.
- Heart weights were lower on an absolute basis in BI 1744 CL-treated groups. However, the variance introduced by differences in body weight prevented a valid statistical analysis of absolute heart weights.

Weights Relative to Body Weight

Terminal/Recovery					
Organ	Sex	Control	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Prostate	M	0.076	--	-36%	-35%
Heart	M	0.955	-17%	-18%	-15%
	F	0.845	--	--	--

Histopathology

Adequate Battery

An adequate histopathological battery was performed. All groups were evaluated.

Peer Review

Yes

Histological Findings

- The following organs and tissues were examined: adrenal glands, aorta, bone (sternum), bone marrow, brain, cecum, cervix, colon, duodenum, epididymides, eyes, gall bladder, heart, ileum, jejunum, kidneys, knee joint (with femur), larynx, liver, lungs, lymph nodes, bifurcation, lymph nodes, mesenteric, mammary gland (females only), nasal cavity, esophagus, optic nerves, ovaries, oviducts, palate,

pancreas, parotid salivary glands, peripheral (sciatic) nerve, Peyer's patches, pituitary gland, prostate, rectum, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, sublingual salivary glands, submandibular salivary glands, testes, thymus, thyroid/parathyroid glands, tongue, trachea, ureters, urinary bladder, uterus, vagina.

- The individual histopathology report for control recovery male animal No.105 is missing from the study report.
- Histopathology slides for high-dose recovery male animals Nos. 405 and 406 were mixed.
- The major target organ of toxicity was the heart. Fibrosis/fibroplasia in the left ventricle was detected at the 42.4 and 87.6 µg/kg dose levels.
- Increased severity in liver glycogen stores and hemorrhage in the liver was detected at the 42.4 and 87.6 µg/kg dose levels.
- Moderate palate inflammation with ulceration was detected in the 14.4 and 87.6 µg/kg dose levels, but not in the mid-dose of 42.4 µg/kg.

Microscopic Signs	Group Size:	Control		3.9 µg/kg		14.6 µg/kg		82.6 µg/kg	
		M	F	M	F	M	F	M	F
	Grade	Terminal/Recovery							
Adrenal gland, cyst	Present	--	--	--	--	--	--	--	1
Adrenal gland, mineralisation	Minimal	--	--	--	--	--	--	--	1
Brain, choroid plexus, fibroplasia	Minimal	--	--	--	--	1	--	--	--
Brain, choroid plexus, 4 th ventricle	Minimal	--	--	--	--	--	--	1	--
Colon, hemorrhage	Minimal	--	--	--	--	--	--	--	1
Colon, diffuse infiltration	Slight	--	--	--	--	--	--	1	--
Duodenum, microabscesses of crypts	Minimal	--	--	1	--	--	--	--	2
Epididymis, luminal cellular debris	Minimal	--	--	--	--	--	--	1	--
Esophagus, inflammation	Minimal	--	--	--	--	--	--	--	1
	Slight	--	--	--	--	1	--	--	--
Eye, retina, degeneration	Slight	--	--	--	--	--	--	1	--
Heart, papillary	Minimal	--	--	--	--	--	--	--	1/1

Microscopic Signs	Group Size:	Control		3.9 µg/kg		14.6 µg/kg		82.6 µg/kg	
		M	F	M	F	M	F	M	F
		4/1	4/2	4	4	4	4	4/2*	4/2
	Grade	Terminal/Recovery							
muscle, left ventricle, fibrosis/fibroplasia	Slight	--	--	--	--	1	1	--	1
	Mod.	--	--	--	--	--	--	3	3
	All	--	--	--	--	1	1	3	3/2
Heart, septum, infiltration	Minimal	--	--	--	--	1	--	--	2
Kidney, cyst	Present	--	--	--	--	--	--	--	1
Kidney, fibrosis/fibroplasia	Slight	--	--	--	--	--	--	--	1
Kidney, glomerulopathy	Minimal	--	--	--	--	--	--	--	1/1
Kidney, infiltration	Minimal	--	2	--	1	1	2	1	3
	Slight	--	--	--	1	1	--	1	1
	Mod.	--	--	--	--	--	--	--	1
	Severity not Reported					1			
	All	--	2	--	2	3	2	1/1	4/1
Liver, centrolobular glycogen depletion	Minimal	1	--	1	--	2	1	3	--
	Slight	--	--	--	--	--	1	1	1
	Mod.	--	--	--	--	--	--	--	--
	All	1	--	1	--	2	2	4	1
Liver, peripheral, glycogen storage increased	Minimal	1	--	1	--	2	1	3	--
	Slight	--	--	--	--	--	1	1	1
	All	1	--	1	--	2	2	4	1
Liver, hemorrhage	Minimal	--	--	--	--	--	--	3	--
	Slight	--	--	--	--	--	1	--	--
	All	--	--	--	--	--	1	3	--
Liver, mineralization	Slight	--	--	--	--	--	--	--	1
Liver, kuppfer cell, pigment storage	Slight	--	--	--	--	--	--	--	1
Liver, peripheral lobular region, rarefaction, cytoplasmic	Slight	--	--	--	--	--	--	--	1
Lung, mineralization	Minimal	--	--	1	--	--	--	--	--
	Slight	--	--	--	--	--	--	--	1
	All	--	--	1	--	--	--	--	1

Microscopic Signs	Group Size:	Control		3.9 µg/kg		14.6 µg/kg		82.6 µg/kg	
		M	F	M	F	M	F	M	F
		4/1	4/2	4	4	4	4	4/2*	4/2
	Grade	Terminal/Recovery							
Nasal cavity, atrophy	Minimal	--	--	--	--	--	--	--	1
	Slight	--	--	--	--	--	--	--	1
	All	--	--	--	--	--	--	--	2
Palate, hyperplasia	Minimal	--	--	--	--	--	--	--	--
	Slight	--	--	--	1	--	--	1	--
	Mod.	--	--	1	--	--	--	--	--
	All	--	--	1	1	--	--	1	--
Palate, inflammation, moderate=ulcerating	Slight	--	--	--	--	--	--	1	--
	Mod.	--	--	1	1	--	--	1	1
	All	--	--	1	1	--	--	2	1
Pancreas, apoptosis	Minimal	--	--	--	--	--	--	1	--
	Slight	--	--	1	--	--	--	--	--
	All	--	--	1	--	--	--	1	--
Pancreas, infiltration	Slight	--	--	--	--	--	--	1	1
Pituitary gland, infiltration	Mod.	--	--	--	--	--	--	--	1
Pituitary, inflammation	Slight	--	--	--	--	--	--	--	1
Prostate, glandular atrophy	Minimal	1	--	--	--	2	--	2	--
	Slight	1	--	1	--	1	--	1/2	--
	All	2	--	1	--	3	--	3/2	--
Prostate, dilatation of glands	Slight	--	--	--	--	--	--	1	--
Rectum, luminal distension	Minimal	--	--	--	--	--	--	--	1
Rectum, follicular hyperplasia	Minimal	--	--	--	--	--	--	1	--
Skin, luminal distension	Mod.	--	--	--	--	--	--	--	1
Skin, edema	Mod.	--	--	--	--	--	--	--	1
Skin, squamous cell hyperplasia	Slight	--	--	--	--	--	--	--	1
Spleen, extramedullary hematopoiesis	Minimal	--	--	--	--	--	--	--	1

Microscopic Signs	Group Size:	Control		3.9 µg/kg		14.6 µg/kg		82.6 µg/kg	
		M	F	M	F	M	F	M	F
	Grade	Terminal/Recovery							
Stomach, degeneration	Minimal	--	--	--	--	--	--	--	1
Stomach, microabscesses of crypts	Minimal	--	--	--	--	--	1	--	1
Trachea, epithelial atrophy	Minimal	--	--	--	--	--	--	--	--
	Slight	--	--	--	--	--	--	--	--
	Mod.	--	--	--	--	--	--	--	--
	All	--	--	--	--	--	--	--	1
Trachea, infiltration	Minimal	--	--	--	--	--	--	1	2
Trachea, mineralization	Slight	--	--	--	--	--	--	--	1

-- No findings; *Slides mixed between these two animals.

Special Evaluation

Not conducted.

Toxicokinetics

- AUC exposures in both sexes were higher at low dose on days 72 and 358 compared to Day 1.
- C_{max} exposures at the high-dose were higher in females than in males.

Parameter	Sex	3.9 µg/kg	14.6 µg/kg	82.6 µg/kg
Day 1 Day 72 Day 358				
C _{max} (pmol/L)	M	197	1960	13100
		323	1620	7080
	F	381	1620	8990
		378	1020	13200
Day 1 Day 72 Day 358				
AUC(0-24 h) pmol*h/L	M	1160	7750	50200
		2290	9350	36900
	F	2160	7970	46000
		3490	7950	47000
Day 1 Day 72 Day 358				
Day 1 Day 72 Day 358				
Day 1 Day 72 Day 358				
Day 1 Day 72 Day 358				
Day 1 Day 72 Day 358				
Day 1 Day 72 Day 358				

Stability and Homogeneity

- Samples of each batch of the test item formulation and the vehicle formulation were analyzed for concentration of BI 1744 CL and pH-value before use.
 - Additionally, a sample was taken from each of the dilutions at the time of preparation and at the time of last use.
 - Analyses were performed using a validated HPLC-UV method.
 - Mean test atmosphere concentrations from these analyses are reported in the dosing table in the Methods section above.
-

11 Integrated Summary and Safety Evaluation

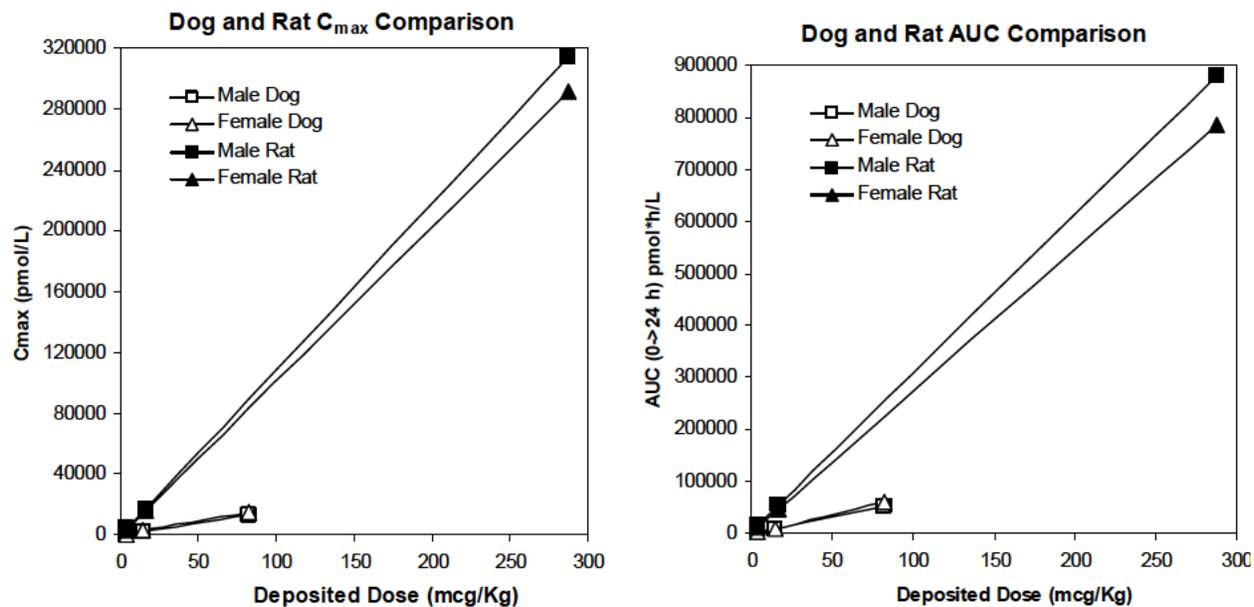
Boehringer Ingelheim Pharmaceuticals, Inc. (BI) is developing BI 1744 CI delivered with the Respimat inhaler as a treatment of Chronic Obstructive Pulmonary Disease. BI 1744 CI is a long-acting agonist of the human β_2 adrenoreceptor with an $EC_{50} = 1.0 \pm 0.4$ nM; BI 1744 CI stimulates the human β_1 -adrenoreceptor with an $EC_{50} = 60 \pm 24$ nM, indicating selectivity for the β_2 adrenoreceptor. The present review covers two long-term inhalational toxicology studies of BI 1744 CL, a 6-month study in rats (06B234/U08-1691-01) and a 9-month study in dogs (06B121/U08-1740-01), submitted as part of SDN 59 (CDER Stamp Date March 30, 2009). SDN 59 also includes more pharmacology, pharmacokinetic, toxicology, and genetic toxicology studies (please see Section 3.2). The genetic toxicology studies have already been reviewed by Dr. Timothy Robison (Communication PK 2778471). The other studies will be evaluated in subsequent reviews.

In the 6-month rat study (Study No. 06B234/U08-1691-01), 20 rats/sex/group were given deposited doses of 0 (vehicle), 4, 16.4, and 288 $\mu\text{g}/\text{kg}$ olodaterol. An additional 10 rats/sex/group were also treated at the control and 288 $\mu\text{g}/\text{kg}$ dose levels to evaluate recovery 4 weeks after the last dose. Daily inhalation of olodaterol produced skeletal and cardiac muscle hypertrophy with a concomitant decrease in white fat, laryngeal degeneration, and increased levels of WBC, neutrophils, and lymphocytes. The skeletal muscle hypertrophy and decreased white fat resulted in increased weight in both males and females in all treatment groups. Heart muscle weight increases also occurred in all dose groups and left ventricular scar formation occurred in animals treated with 288 $\mu\text{g}/\text{kg}$ olodaterol. Lobular atrophy occurred in the pancreas in 3/10 males and 3/10 females at the 288 $\mu\text{g}/\text{kg}$ dose level. Increased incidence and severity of cysts in the ovary and oviduct occurred in females treated at 288 $\mu\text{g}/\text{kg}$ dose level. Clinical signs of palpable heart beat detected directly after dosing at the 16.4 and 288 $\mu\text{g}/\text{kg}$ dose levels

indicated that the cardiac effects are likely linked to the B2-adrenergic agonist activity of olodaterol. In addition, there were a variety of non-dose-related changes in clinical chemistry that might be related to the cardiac and skeletal muscle hypertrophy. These included, decreases in creatine kinase and glucose (males and females), and triglycerides (males only) and decreases in magnesium with concomitant increases in potassium, and inorganic phosphate. In addition, there were small increases in blood urea and creatinine (males only) that may also be related to muscle hypertrophy. WBC, neutrophil, and lymphocyte counts were increased in all dosed groups (≥ 4 $\mu\text{g}/\text{kg}$). Skeletal hypertrophy and white fat decreases did not reverse after the recovery period, but were not considered adverse findings. Taken together, these results indicate that the NOAEL in this study was 4 $\mu\text{g}/\text{kg}$ based on observed cardiac effects at doses of 16.4 and 288 $\mu\text{g}/\text{kg}/\text{day}$ and pancreas effects at 288 $\mu\text{g}/\text{kg}/\text{day}$.

In the 9-month dog study (Study No. 06B121/U08-1740-01), 4 dogs/sex/group were given deposited doses of 0 (vehicle), 14.4, 42.4, and 87.6 $\mu\text{g}/\text{kg}$ olodaterol. An additional 2 dogs/sex/group were also treated at the control and 87.6 $\mu\text{g}/\text{kg}$ dose levels to evaluate recovery 4 weeks after the last dose. Daily inhalation of olodaterol resulted in increased weight in males and females, and produced heart, kidney, and liver toxicity. Oral inflammation was also detected in treated dogs. Fibrosis/fibroplasia in the left ventricle was detected at the 42.4 and 87.6 $\mu\text{g}/\text{kg}$ dose levels. This finding correlated with clinical signs of "increased heart force" which were frequently observed in the olodaterol-treated dogs and with increases in serum levels of creatine kinase and cardiac-specific troponin 1. Microscopic kidney findings included increased incidence and severity of infiltration which correlated with increases in serum creatine and electrolyte changes in females. Increased severity in liver glycogen storage accompanied by hemorrhage in the liver was detected at the 42.4 and 87.6 $\mu\text{g}/\text{kg}$ dose levels. These findings correlated with modest increases (<2-fold) in ALT in females treated at these doses. Moderate palate inflammation with ulceration detected in the 14.4 and 87.6 $\mu\text{g}/\text{kg}$ dose levels, but not in the mid-dose of 42.4 $\mu\text{g}/\text{kg}$. Taken together, these results indicate that the NOAEL was 3.9 $\mu\text{g}/\text{kg}$ based on observed heart, kidney, and liver effects observed at the 42.4 and 87.6 $\mu\text{g}/\text{kg}$ dose levels.

Drug exposures were greater in the rat study, relative to deposited dose. This effect was true both in terms of C_{max} and AUC (Figure 2):

Figure 2. Dog (Study 06B121) and Rat (Study 06B234) C_{max} and AUC exposures**C_{max} values on Day 1 of Study**

Dog Study 06B121 C _{max} (pmol/L)			
Dose $\mu\text{g}/\text{kg}$	3.9	14.6	82.6
M	197	1960	13100
F	359	2550	15100
Rat Study 06B234 C _{max} (pmol/L)			
Dose $\mu\text{g}/\text{kg}$	4	16.4	288
M	3480	17100	314000
F	3240	15700	292000

AUC values on Day 1 of Study

Dog Study 06B121 AUC(0->24) (pmol*h/L)			
Dose $\mu\text{g}/\text{kg}$	3.9	14.6	82.6
M	1160	7750	50200
F	1930	8500	59900
Rat Study 06B234 AUC(0->24) (pmol*h/L)			
Dose $\mu\text{g}/\text{kg}$	4	16.4	288
M	13400	54600	881000
F	11000	44900	785000

AUC exposure in male rats treated with 4 µg/kg olodaterol in rats was 11-fold higher than doses in male dogs treated at the 3.9 µg/kg dose level. Similarly, AUC exposure in female rats treated with 4 µg/kg olodaterol in rats was 6-fold higher than doses in female dogs treated at the 3.9 µg/kg dose level.

Table 5. Safety Margin Calculations from Dog and Rat

		Safety Margin µg/kg	Systemic Exposures AUC 0-24 h (pmol*L)	Safety Margin based on AUC
Highest Human dose 30 µg/day 14-Days No. U07-2062	0.5 µg/kg/day	-	298§	-
Asthma patients: 10 µg/day 4-weeks No. U09-1850-01	0.17 µg/kg/day	-	199§	-
COPD patients: 10 µg/day 4-weeks No. U09-3125	0.17 µg/kg/day	-	246§	-
Dog NOAEL No. 06B121 6 month	3.9 µg/kg/day	Highest: 8 Asthma: 23 COPD: 23	1545*	Highest: 5 Asthma: 8 COPD: 6
Rat NOAEL No. 06B121 9 month	4 µg/kg/day	Highest: 8 Asthma: 23 COPD: 23	12200*	Highest: 41 Asthma: 61 COPD: 50

§ AUC exposure converted from pg/kg*h/mL to pmol*h/L

*Average of male and female values

Recommendation

Based on the calculated safety margins using systemic exposures (AUCs), Phase 3 clinical trials of olodaterol are supported by the submitted 6-month rat and 9-month dog repeat dose oral toxicology studies, Study Nos. 06B234/U08-1691-01 and 06B121/U08-1740-01, respectively. Adequate systemic safety ratios based on AUC levels in rat (41) and dog (5) were achieved at the highest multiple dose given in humans. Similar safety ratios were also achieved in patients with either COPD or asthma.

12 Appendix/Attachments

None

-
- ¹ Guyton, A.C. Measurement of the respiratory volumes of laboratory animals. *Amer. J. Physiol.* 150:7 (1947).
- ² T.A. McMahon, J.D. Brain and S. Lemott, Species differences in aerosol deposition. In: W.H. Walton, Editor, *Inhaled Particles IV*, Pergamon, Oxford (1977), pp. 23–33.
- ³ Das VA, Robinson R, Paulose CS. Enhanced beta-adrenergic receptors in the brain and pancreas during pancreatic regeneration in weanling rats. *Mol Cell Biochem.* 2006 Sep;289(1-2):11-9. Epub 2006 Apr 1.
- ⁴ Lewis, DJ. Morphological Assessment of Pathological Changes within the Rat Larynx. *Toxicologic Pathology.* [Toxicol Pathol.](#) 1991;19(4 Pt 1):352-7.

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/s/

HANS M ROSENFELDT
10/28/2011

TIMOTHY W ROBISON
10/28/2011
I concur

Appendix 7

Pharm/Tox Review of IND 76,362 dated May 11, 2011 (Carcinogenicity Review)

**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: 76362, SDN125, Vol. 1-16; SDN 129 Electronic
Supporting document/s: 1
Sponsor's letter date: SDN 125: January 13, 2012
SDN 129: March 5, 2012
CDER stamp date: SDN 125: January 17, 2012
SDN 129: March 6, 2012
Product: Respimat Inhalation Spray
Indication: Chronic Obstructive Pulmonary Disease
Sponsor: Boehringer Ingelheim Pharmaceuticals Inc.
Review Division: Division of Pulmonary, Allergy, and
Rheumatology Products
Reviewer: Hans Rosenfeldt, Ph.D.
Supervisor/Team Leader: Timothy Robison, Ph.D., D.A.B.T.
Division Director: Badrul A. Chowdhury, M.D., Ph.D.
Project Manager: Christine H. Chung

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1 Executive Summary

1.2 Brief Discussion of Nonclinical Findings

Boehringer Ingelheim Pharmaceuticals Inc. submitted 2-year carcinogenicity studies of olodaterol (BI 1744 CL) in rats and mice (Studies Nos. 667930/U11-2661-01 and 668138/ U12-1065-01, respectively) . These studies were conducted by (b) (4)

Olodaterol was negative for genetic toxicity in bacterial reverse mutagenicity (Ames) studies and in *in vitro* mouse lymphoma assay. However, intravenously administered olodaterol produced increased percentages of polychromatic erythrocytes (PCEs) in male rats at the 10 and 40 mg/kg dose levels and dose-dependent, statistically significant, increases in the frequencies of micronucleated polychromatic erythrocytes (MNEs) 24 and 48 h after treatment (Study No. U08-1834-01). Nonetheless, the overall genotoxicity assessment of olodaterol was judged to be negative because the positive result in the rat micronucleus assay was most likely due to a drug-enhanced compensatory erythropoiesis and not a genotoxic mechanism (please see Dr. Timothy Robison's review of this study; Communication PK 2778471).

Olodaterol was administered by inhalation in both rats and mice. Rats received 25.8, 75.9, and 270 µg/kg olodaterol over 35 minutes exposure, as calculated by the sponsor. Mice received 26.1, 76.9, and 255 µg/kg olodaterol over 40 minutes exposure, as calculated by the sponsor. Both studies included two control groups: a clean air control group and a vehicle group (0.01% benzalkonium chloride, (b) (4) % disodium EDTA x 2 H₂O, (b) (4) % citric acid). The sponsor received concurrence from the CDER Executive Carcinogenicity Advisory Committee (Exec CAC) for the olodaterol doses and study designs used in both studies. There were no treatment-related effects on survival in the either study. Olodaterol induced leiomyomas in the ovaries of female rats at the 25.8 and 270 µg/kg dose levels and combined leiomyomas and leiomyosarcomas in the uteri of female mice at all dose levels. The incidence of benign uterine polyps was increased in female mice at all dose levels, although statistical significance was only achieved at the high dose level of 255 µg/kg. There was also a non-statistically significant increase in the incidence of mesothelioma in the testes of male rats at the 75.9 and 270 µg/kg dose levels.

Olodaterol was tumorigenic in female rats and mice based upon findings of ovarian leiomyomas and uterine leiomyomas/leiomyosarcomas and polyps, respectively. There were no treatment-related tumor findings in male rats or mice.

Increases in leiomyoma/leiomyosarcoma in the female reproductive tract may be related to the olodaterol's mechanism of action as a β_2 - adrenoreceptor agonist. Ovarian leiomyomas are extremely rare spontaneous tumors in rats. Similarly, uterine leiomyomas/leiomyosarcomas occur at low spontaneous incidences in female mice. Proliferation of the mesovarian smooth muscle is considered an adaptive physiologic

response to prolonged stimulation of the β_2 -receptor, which has the effect of causing muscle relaxation. Tumor induction has been shown to be a function of adrenergic stimulation, as concurrent administration of the adrenergic blocker, propranolol, prevents their development. Leiomyomas of ovarian tissue are rare in women, and no increase in incidence has been reported despite the long history of use of β_2 -stimulants in the treatment of bronchial asthma.

Based on toxicokinetic results, achieved AUC exposures associated with the lowest dose given in rats and mice were 9-fold and 19-fold greater than the AUC associated with the maximum human dose (30 $\mu\text{g}/\text{day}$), respectively.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

Not provided

2.1.2 Generic Name

Olodaterol

2.1.3 Code Name

BI 1744 CL

2.1.4 Chemical Name

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

2.1.5 Molecular Formula/Molecular Weight

$\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_5 \times \text{HCl}$ / 422.9 g/mol

2.1.6 Structure

None. All impurities in the proposed clinical batch are below the ICH Q3A/B thresholds for qualification. Please see the Product Quality Review of this application.

2.4 Proposed Clinical Population and Dosing Regimen

Boehringer Ingelheim Pharmaceuticals, Inc. (BI) is developing daily doses of BI 1744 Cl delivered with the Respimat inhaler as a treatment of Chronic Obstructive Pulmonary Disease.

2.5 Regulatory Background

2.5.2 History of Regulatory Submission

IND 76, 362 was submitted on January 26, 2007.

Doses and designs for the 2-year rat and mouse carcinogenicity studies were discussed with the ECAC on February 13, 2007.

3 Studies Submitted

3.1 Studies Reviewed

Study #	Title	Submission
667930/U11-2661-01	BI 1744 CL 24 Month Inhalation Carcinogenicity Study in Rats	SDN125
668138/U12-1065-01	BI 1744 CL:24 Month Inhalation Carcinogenicity Study in Mice	SDN129

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

IND	Relevant Studies	Reviewer	Date	Communication PK
76362	Original Submission	Molly Topper, Ph.D.	2/28/2007	1640596
	Carcinogenicity SPA Request	Molly Topper, Ph.D.	3/2/2007	1642809
	Request for ECAC Feedback	Molly Topper, Ph.D.	2/17/2009	1802269
	Safety Pharmacology Secondary Pharmacology Genetic Toxicity	Timothy Robison, Ph.D., D.A.B.T.	5/10/2010	2778471
	Reproductive and Developmental Toxicology	Lawrence Leshin, Ph.D., D.V.M.	10/26/2011	3034984
	Long-term rat and dog inhalational toxicology studies	Hans Rosenfeldt, Ph.D.	10/28/2011	3036044

8 Carcinogenicity

Study title: BI 1744 CL: 24 Month Inhalation Carcinogenicity Study in Rats

Study no.: 667930/U11-2661-01

Study report location: (b) (4)

Conducting laboratory and location: Same as above

Date of study initiation: May 4, 2007

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 5061001, 99%

CAC concurrence: Yes, Meeting dated February 13, 2007

Key Study Findings

- Daily inhalation of olodaterol induced leiomyomas of the mesovarian tissue in 1/55 females of the 25.8 µg/kg/day dose group, and 4/55 females of the 270 µg/kg/day dose group.
- There was an increase in mortality in males treated with olodaterol, relative to controls, but the increase was not dose-dependent and was not statistically significant. Female mortality was similar across all controls and dose groups.
- The main non-neoplastic findings consisted of an increase in ovarian cysts with and without hyperplasia in the ovary, an increase in mild-cardiomyopathy in females, and vehicle-related squamous metaplasia in the larynx.

Adequacy of Carcinogenicity Study

- The Executive Carcinogenicity Committee concurred with the doses and design of this study in a meeting dated meeting dated February 13, 2007.

Appropriateness of Test Models

- The Wistar rat is an accepted test model for the evaluation of pharmaceutical carcinogenicity.

Evaluation of Tumor Findings

- Daily inhalation of olodaterol induced leiomyomas of the mesovarian tissue in 1/55 females of the 25.8 µg/kg/day dose group, and 4/55 females of the 270 µg/kg/day dose group. Thus, olodaterol was tumorigenic in female rats.
- Although not statistically significant, there was an increase in benign and malignant mesothelioma in the testis. Olodaterol did not appear to be tumorigenic in male rats.

Methods

Inhaled Doses 0, 25.8, 75.9, and 270 µg/kg/day (1.3, 3.9, 13.5 µg/L over 35 minutes)
 Frequency of dosing: Daily x726

Route of administration: Aerosol Inhalation

Formulation/
 Vehicle: 0.01% benzalkonium chloride, 0.01% disodium EDTA x 2 H₂O, 0.003% citric acid

Basis of dose selection: Study 666413 ((b) (4)): BI 1744 Cl: 13-week inhalation MTD study in rats with a 4-week recovery period (SN001); Reviewed by Dr. Molly Topper (1642809)

Species/
 Strain: Crl:WI(Han) rats

Number/sex/
 group: Main Study: 55/sex/group

Age: ca. 10 weeks

Weight: Males: 236 – 280 g; Females: 162 – 202 g

Animal housing: The animals were housed 4 or 5 per cage by sex and dose group (unless reduced by mortality) in solid bottom polycarbonate cages containing wood shavings as bedding material. All cages were supplied with 2 plastic water bottles.

Paradigm for dietary restriction: Not applicable

Dual control employed: No

Interim sacrifice: No

Satellite groups: TK: 10/sex/group; TK Spares: 4/sex/group

Deviation from study protocol: No significant deviations

Inhalation Exposure System

- Each exposure chamber was located in an extract booth to prevent any cross-group contamination and for the protection of the personnel undertaking the animal inhalation exposure procedures.
- Each exposure chamber was operated to sustain a dynamic air flow sufficient to ensure an evenly distributed exposure atmosphere and was operated with an extract attached to the base of the chamber and a vacuum pump system was used to continuously exhaust the generated test atmosphere.
- Each calibrated exhaust flow passed through a HEPA filter system before being passed to the external atmosphere.
- Air flow rates were monitored continuously using calibrated flow meters. Chamber temperature and humidity were monitored and recorded at appropriate intervals during each daily exposure period.
- Test aerosols were generated using Heart airjet nebulizers (Westmed, USA) containing an appropriate prime volume of test formulation.
- The volume of formulation used for each nebulizer was recorded at the end of each generation period. Each nebulizer was operated at a target flow output of 17 L/min.
- Validation of the aerosol generating/exposure system was undertaken prior to commencement of animal exposure to demonstrate satisfactory aerosol performance (aerosol concentration, particle size distribution).
- Respirable particle size distributions were achieved with Mass Median Aerodynamic Diameters ranging from (b) (4).

Figure 1. Diagram Inhalation Exposure System Rat Study #667930/U11-2661-01



Table 1. Technical Parameters Inhalation Rat Study #667930/U11-2661-01

(b) (4)

Mortality

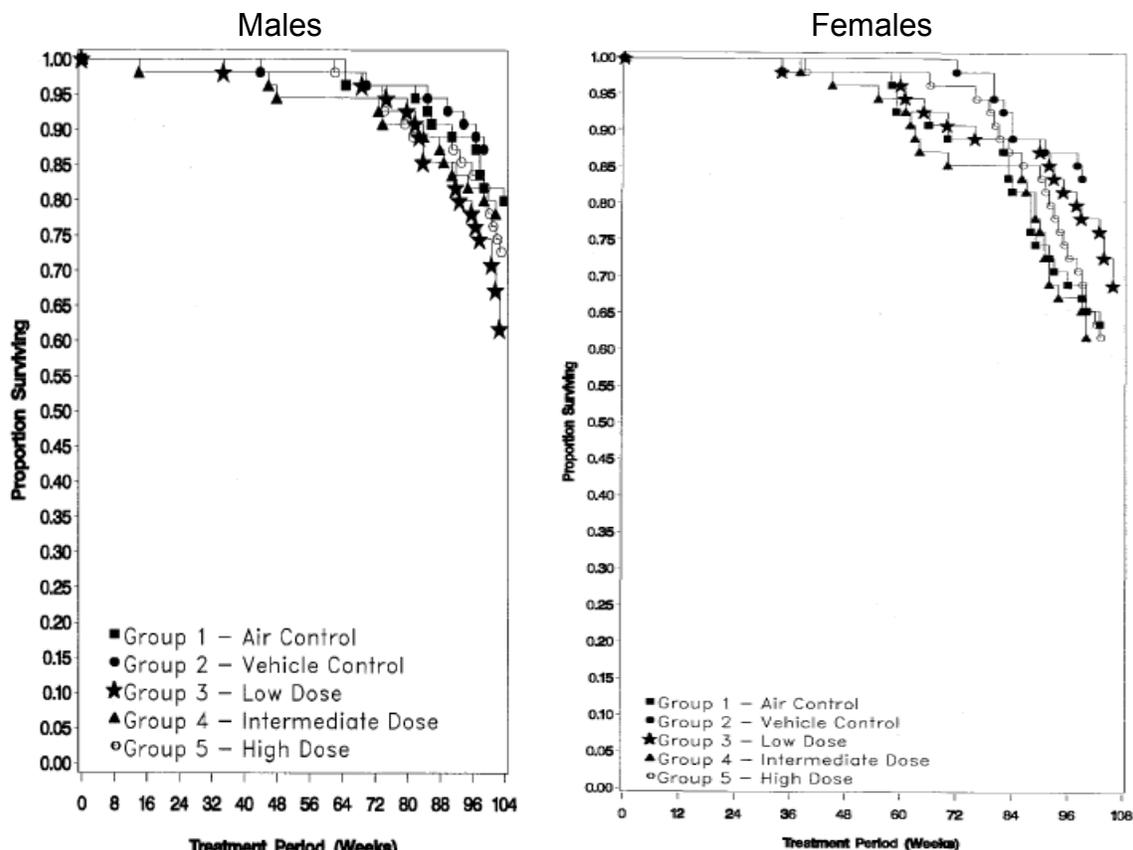
All animals were checked early morning and as late as possible each day for viability. Any animal showing signs of severe debility or intoxication and determined to be moribund or suffering excessively was killed. Any Main Study animal killed prematurely or found dead was subjected to a detailed macroscopic examination as soon as possible after death and tissues retained for histopathological evaluation.

- Mortality was unaffected in olodaterol-treated rats compared to control.

Males (55/group)	A. Control	V. Control	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Accident	0	0	0	1	0
Moribund	9	7	19	11	12
Found Dead	2	0	2	0	3
Total	11	7	21	12	15

Females (55/group)	A. Control	V. Control	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Accident	1	0	1	1	1
Moribund	15	9	13	20	11
Found Dead	4	0	1	0	9
Total	20	9	15	21	21

Figure 2. Kaplan-Meier Survival Curves Rat Study #667930/U11-2661-01



*Excerpted from Sponsor's submission

Clinical Signs

All animals were examined at least once before exposure, continuously during exposure and at approximately 1-2 h after exposure. The results of the observations are retained in study data, but not reported. Once each week all animals received a detailed clinical examination and palpation. The clinical examination included appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. The size, appearance, position and duration of any masses detected were recorded.

- The main clinical finding in this study was increased muscle mass that occurred in all treated groups and in both sexes. This was an expected pharmacological effect of a β_2 -adrenergic agonist.

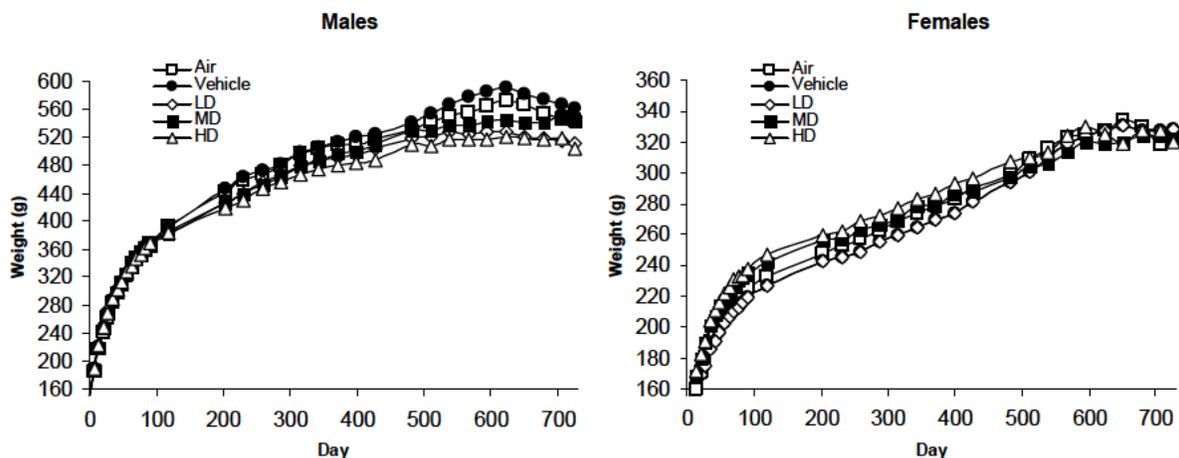
Muscle Mass Increase

	Number of Animals Observed		Number of Animals with Increased Muscle Mass		Mean Time of Onset (Day)	
	Male	Female	Male	Female	Male	Female
A-Control	55	55	0	0	-	-
V-Control	55	55	0	0	-	-
LD	55	55	6	2	474	354
MD	55	55	39	6	236	338
HD	55	55	41	21	126	323

Body Weights

Body weights were recorded once weekly throughout the study pretrial until the end of the first 13 weeks of the study. After this, body weights were recorded once every 4 weeks from Week 14 to Week 102, and once in Week 104. Animals showing weight loss or deterioration in condition were weighed more frequently as necessary.

- Body weights were similar among all treatment groups in both sexes over the course of the study. Absolute body weights for drug-treated male and female rats were decreased by less than 10% of the absolute control body weights.



Body Weights

Males	Day 0	Day 203	Day 371	Day 539	Day 728
A. Control	151.00	443.00	509.00	551.00	544.00
V. Control	154.00	447.00	514.00	566.00	561.00
LD	151.00	426.00	491.00	528.00	512.00
MD	150.00	426.00	496.00	538.00	543.00
HD	150.00	418.00	480.00	516.00	504.00

All weights: grams

Body Weights

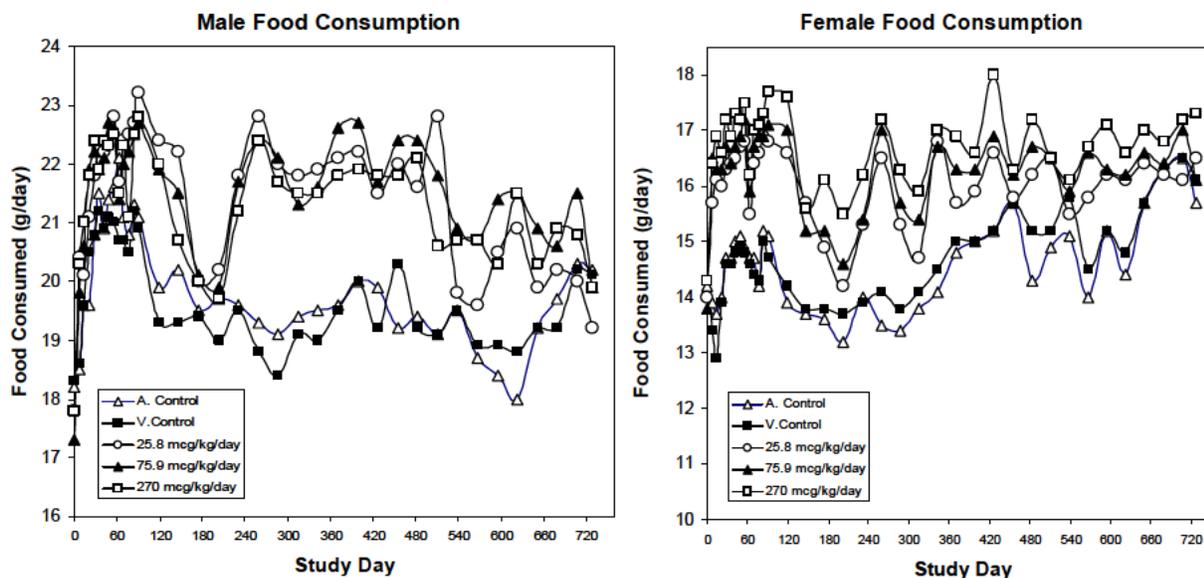
Females	Day 0	Day 203	Day 371	Day 539	Day 728
A. Control	99.00	248.00	279.00	316.00	324.00
V. Control	99.00	243.00	270.00	310.00	328.00
LD	98.00	256.00	279.00	306.00	322.00
MD	99.00	260.00	286.00	313.00	320.00
HD	98.00	267.00	292.00	313.00	317.00

All weights: grams

Food Consumption

The quantity of food consumed by each cage of animals was recorded once weekly, commencing one week pretrial and for the first 13 weeks of the dosing period. After this, the quantity of food consumed was recorded over approximately one week in very 4 weeks from Week 14 to Week 102, and once in Week 104.

- Food consumption was greater among olodaterol-treated animals of both sexes. This is an expected pharmacological effect of a β_2 -adrenergic agonist.



Average Daily Food Consumption (All weights grams)

Males	Day 0	Day 203	Day 371	Day 539	Day 726
A. Control	18.2	19.7	19.6	19.5	20.2
V. Control	18.3	19	19.5	19.5	20.1
LD	17.8	20.2	22.1*	19.8	19.2
MD	17.3	19.9	22.6*	20.9*	19.9
HD	17.8*	19.7	21.8*	20.7*	19.9

Females	Day 0	Day 203	Day 371	Day 539	Day 726
A. Control	14.2	13.2	14.8	14	15.7
V. Control	14.3	13.7	15	14.5	16.1
LD	14	14.2	15.7	15.8	16.5
MD	13.8	14.6*	16.3*	16.6*	16.1
HD	14.3	15.5*	16.9*	16.7*	17.3*

*p<0.05

Ophthalmoscopy

Eyes were examined using an indirect ophthalmoscope after the application of (Mydriacyl- 1% Tropicamide, Alcon Laboratories (UK)). Anterior, lenticular and fundic areas were evaluated. The ophthalmoscopic examination was undertaken on all animals prior to the start of treatment and subsequently performed on Toxicity animals from Groups 1, 2 and 5 in Weeks 50 and 102 of treatment.

- No significant findings were observed.

Hematology

Blood and urine samples were obtained during Week 102-104 from 20 male and 20 female Main Study animals with the lowest numbers in each group. Remaining Main Study animals were evaluated only for RBC, WBC and differential white blood cell counts.

- Neutrophil counts were moderately increased at the mid and high doses.

Parameter	Sex	A. Control	V. Control	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Week 103- 104						
Neutrophils	M	1.35 x10 ⁹ /L	--	--	+40%	+31%
	F	0.92 x10 ⁹ /L	--	--	+157%	+42%

Clinical Chemistry

Blood and urine samples were obtained during Week 102-104 from 20 male and 20 female Main Study animals with the lowest numbers in each group.

- Glucose levels were decreased in males and females at all dose levels.
- Creatinine levels were increased in treated males at all doses.
- Phosphorous levels were increased at the mid and high doses.

Parameter	Sex	A. Control	V. Control	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Week 103- 104						
Glucose	M	7.21 mmol/L	--	-23%	-19%	-17%
	F	5.56 mmol/L	--	-6%	-17%	-13%
Creatinine	M	38 µmol/L	--	+8%	+11%	+13%
	F	39 µmol/L	--	--	--	--
Phosphorous	M	1.23 mmol/L	--	--	+18%	+24%
	F	1.17 mmol/L	--	--	--	--

Gross Pathology

- There was an increased incidence of ovarian cysts in females dosed at 270 µg/kg.
- Increased skeletal muscle mass was observed in males at all dose levels and in females dosed at the 75.9 and 270 µg/kg dose levels.

Parameter	Sex	A. Control	V. Control	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Week 104						
Cranium, contains blood	M	--	--	--	--	1
	F	--	--	--	--	1
Heart, enlarged	M	--	--	--	1	--
	F	--	--	--	--	--
Liver, masses	M	--	--	--	1	1
	F	--	--	--	1	--
Lymph node, mandibular, reddened	M	--	2	2	9	6
	F	3	1	1	6	7
Ovary, masses	F	--	--	1	2	3
Ovary, cyst	F	4	2	7	8	18
Pancreas, masses	M	--	--	--	--	--
	F	--	--	1	1	1
Skeletal Muscle, abnormal shape	M	--	--	--	2	3
	F	--	--	--	--	1
Skeletal Muscle, enlarged	M	--	--	2	18	26
	F	--	--	--	1	6
Prominent mammary	M	--	--	--	--	--
	F	--	--	1	--	1

Organ Weights

Lung weights were the only organ weights recorded at necropsy.

- There was a small non-statistically significant increase in lung weight in both males in females at all doses. The weight increase was similar among all dosed groups.

Parameter	Sex	A. Control	V. Control	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Week 104						
Lung Weight	M	2.04 g	--	+14%	+16%	+10%
	F	1.67 g	--	+10%	+12%	+11%

Histopathology:

The following organs and tissues were examined: abnormal tissue, adrenals, aorta, aortic arch, bone (femur), brain, clitoral gland, duodenum, exorbital lachrymal gland, eyes, forestomach, femur joint, stomach, harderian glands, heart, ileum, jejunum, kidneys, knee (joint with femur), submandibular lymph nodes, lacrimal glands, larynx, liver, lungs, female mammary glands, mesenteric and submandibular lymph nodes, lymph nodes regional to masses, nasal cavity, optic nerves, ovaries, oviducts, pancreas, parathyroids, parotid salivary glands, sciatic nerve, peyer's patches, pharynx, pituitary, prostate, rectum, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, sublingual salivary glands, testes, thymus, thyroid, tongue, trachea, ureters, urinary bladder, uterus, oviduct, and cervix, vagina.

Tissues were fixed in neutral buffered 10% formalin, except for eyes and harderian glands, which were fixed in Davidson's fluid.

Non-neoplastic:

- Squamous metaplasia in the larynx occurred in both males and females and was vehicle-related.
- Mineral deposits in the larynx occurred in males at the 75.9 and 270 µg/kg levels. One female treated at the 75.9 µg/kg level had mineral deposits in the larynx.
- There was a significant dose-dependent increase in ovarian cysts among animals treated with olodaterol, with 22/55 females treated at the 270 µg/kg level. In addition, focal hyperplasia occurred in ovarian smooth muscle at the 75.9 and 270 µg/kg levels.
- Increased levels of mild cardiomyopathy occurred in females at all dose levels. Minimal cardiomyopathy occurred in all groups, including controls. Other low-frequency cardiac effects such as 1/55 pericarditis in the males and 1/55 endocarditis in the females occurred only at the 270 µg/kg level.

Non-neoplastic findings in male rats

Findings	Males					
	Grade	A-Cont.	V-Cont.	25.8 µg/kg	75.9 µg/kg	270 µg/kg
N =		55	55	55	55	55
Larynx, squamous metaplasia	Minimal	--	4	6	1	1
	Mild	--	1	--	2	6
	Total	--	5	6	3	7
Larynx, mineral deposits	Minimal	--	--	--	1	--
	Mild	--	--	--	1	1
	Total	--	--	--	2	1
Larynx, purulent inflammation	Mild	--	--	--	--	1
Liver, inflammatory, cell foci	Minimal	1	--	2	3	4
Lymph node, cervical, erythrophagocytosis	Minimal	--	--	1	--	1
	Mild	1	3	1	8	8
	Total	1	3	2	8	9
	Total	16	25	10	14	4

Non-neoplastic findings in female rats

Findings	Females					
	Grade	A-Cont.	V-Cont.	25.8 µg/kg	75.9 µg/kg	270 µg/kg
N =		55	55	55	55	55
Larynx, squamous metaplasia	Minimal	--	5	5	7	3
	Mild	--	2	9	6	13
	Total	--	7	14	13	16
Larynx, mineral deposits	Minimal	--	--	--	--	1
Lung, alveolar macrophage accumulation	Minimal	5	2	5	2	2
	Mild	19	26	27	30	25
	Moderate	--	--	--	--	3
	Total	24	28	32	32	30

Findings	Females					
	Grade	A-Cont.	V-Cont.	25.8 µg/kg	75.9 µg/kg	270 µg/kg
Ovary, cysts	Present	2	4	13	12	22
Ovary, cystic follicles, multiple		--	--	--	--	1
Ovary, focal hyperplasia, smooth muscle	Minimal	--	--	--	2	3
	Moderate	--	--	--	1	--
	Total	--	--	--	3	3
Heart, progressive cardiomyopathy	Minimal	3	7	6	3	4
	Mild	1	--	3	4	4
	Total	4	7	9	7	8
Heart, endocarditis, acute	Mild	--	--	--	--	1
Vertebrae, increased hemopoiesis, marrow	Mild	1	1	1	2	5

Neoplastic:

- Leiomyomas of the mesovarian tissue were observed in 1/55 females of the 25.8 µg/kg/day dose group, and 4/55 females of the 270 µg/kg/day dose group.
- The incidence of benign mesovarian leiomyomas was significantly higher in the 270 µg/kg/day dose group when compared with the Vehicle Control group (p=0.042). According to the sponsor, the test for a linear trend with dose was also statistically significant (p=0.008) for this rare tumor.
- This tumor type was not observed in animals from any of the other groups.

Neoplastic findings in male rats

Findings	Males						Historical Control Data	
	A-Cont.	V-Cont.	25.8	75.9	270	Trend P-value (Exact method)	(b) (4) *	(b) (4)
Testis N =	55	55	55	55	55		5519	1216
-mesothelioma (B)	0	0	1	0	0		n.a.	n.a.
-mesothelioma (M)	1	1	0	3	3		n.a.	n.a.
-benign + malignant mesothelioma	1	1	1	3	3		n.a.	n.a.

Neoplastic findings in female rats

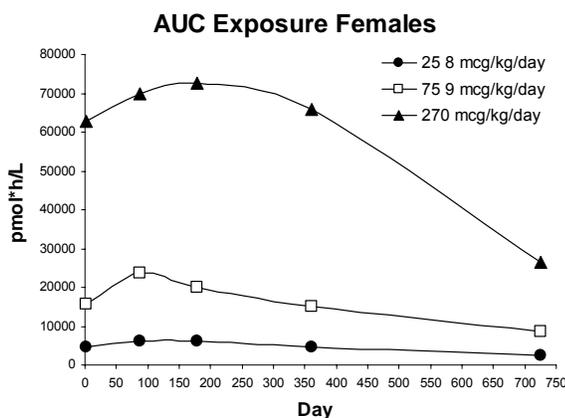
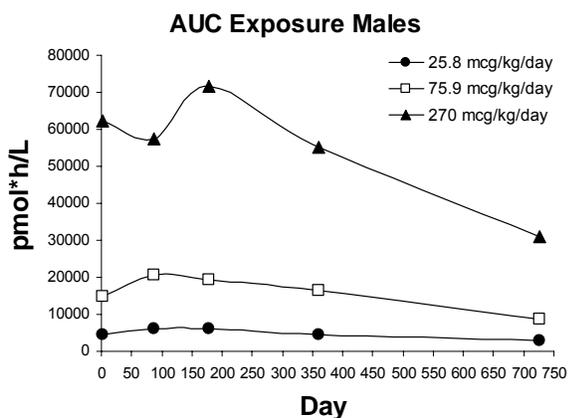
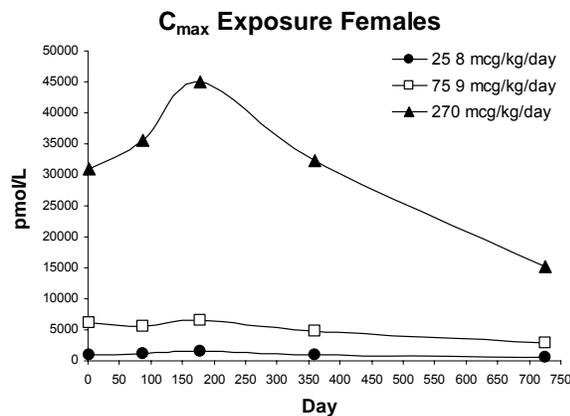
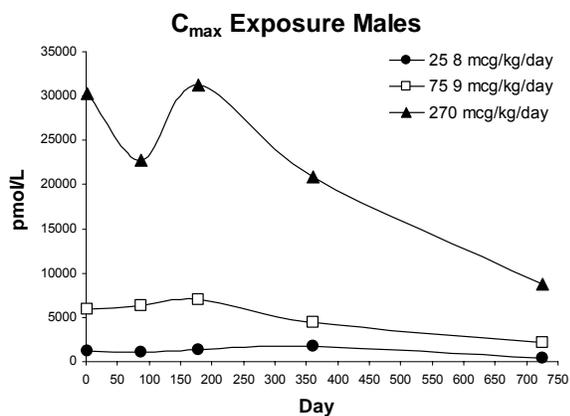
Findings	Females						Historical Control Data	
	A-Cont.	V-Cont.	25.8	75.9	270	Trend P-value (Exact method)	(b) (4) *	(b) (4)
Ovary N =	55	55	55	55	55		5519	1214
leiomyoma	0	0	1 (1.8%)	0	4 (7.3%)	0.0092	<0.1 (0-2)	0

Ovarian leiomyoma + smooth muscle cell hyperplasia

Findings	Females						Historical Control Data	
	A-Cont.	V-Cont.	25.8	75.9	270	Trend P-value (Exact method)	(b) (4) *	(b) (4)
N =	55	55	55	55	55		5519	1214
leiomyoma	0	0	1 (1.8%)	0	4 (7.3%)	0.0092	<0.1 (0.2)	0
hyperplasia, smooth muscle	0	0	0	3 (5.5%)	3 (5.5%)	n.a.	0	n.a.
Total of leiomyoma + hyperplasia, smooth muscle	0	0	1 (1.8%)	3 (5.5%)	7 (12.7%)	0.0324	n.a.	n.a.

Toxicokinetics

- Blood samples were taken immediately post-dose, and 1, 2, 4, 8, 24 h after the start of inhalation.
- Approximately 0.5 mL of blood was obtained from all designated animals at each timepoint.
- Samples from Control Groups 1 and 2 were collected in a separate room by a separate team of technicians from the treated groups.
- C_{max} and AUC exposures peaked on Day 200 for both males and females. While C_{max} was greater in females than in males, AUC exposures were similar between sexes.



C_{max} (pmol/L)

Males

Week	Day	25.8 µg/kg	75.9 µg/kg	270 µg/kg
1	1	1190	5920	30300
13	88	1140	6340	22800
26	178	1380	6970	31200
52	360	1710	4440	20800
104	726	465	2150	8810

Females

Week	Day	25.8 µg/kg	75.9 µg/kg	270 µg/kg
1	1	895	6110	30900
13	88	1060	5650	35600
26	178	1560	6630	45000
52	360	1020	4830	32400
104	726	564	2830	15100

AUC pmol*h/L

Males					
Week	Day	25.8 µg/kg	75.9 µg/kg	270 µg/kg	
1	1	4630	14800	62100	
13	88	6020	20600	57300	
26	178	5970	19200	71700	
52	360	4620	16300	55100	
104	726	2890	8720	31000	
Females					
Week	Day	25.8 µg/kg	75.9 µg/kg	270 µg/kg	
1	1	4760	15800	62700	
13	88	6170	23600	69800	
26	178	6230	20000	72500	
52	360	4550	15000	65700	
104	726	2410	8510	26600	

Study title: BI 1744 CL: 24 Month Inhalation Carcinogenicity Study in Mice

Study no.: 668138/ U12-1065-01

Study report location: (b) (4)

Conducting laboratory and location: Same as above

Date of study initiation: March 30, 2007

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 5061021, 99%

CAC concurrence: Yes, Meeting dated February 13, 2007

Key Study Findings

- There were no treatment-related effects on survival for either males or females.
- Body weights were higher in mid-dose and high-dose groups in both sexes.
- Daily inhalation of olodaterol significantly increased the combined incidences of uterine leiomyomas and leiomyosarcomas at all doses when compared with the vehicle-control group. The incidence of benign uterine leiomyomas were significantly increased at all dose levels when compared with the Vehicle Control group. Malignant leiomyosarcomas were also increased in olodaterol-treated groups although statistical significance was only achieved for the mid dose.
- The incidence of benign uterine polyps was increased in olodaterol-treated groups, compared to vehicle control, although statistical significance was only achieved at the high dose of 255 µg/kg.
- The most prominent histopathological findings in both males and females occurred in the heart. In males and females, major findings included dose-dependent frequency and severity of pericardial fibrosis, myocardial fibrosis, and myocardial vacuolation. Atrial thrombus occurred in males at all dose levels.

- Squamous metaplasia in the larynx occurred in both males and females treated with olodaterol in a dose-dependent manner. There was also an incidence (2/60 in males and 9/60 females) of this finding in the vehicle control.
- Increased alveolar foamy macrophage focal accumulation occurred in males and females at all dose levels, although severity and frequency did not increase greatly with dose.
- There were no ophthalmoscopy findings, but histopathological signs of eye keratitis increased frequency and severity compared to control in males and females occurred at all dose levels.

Adequacy of Carcinogenicity Study

- The Executive Carcinogenicity Committee concurred with the doses and design of this study in a meeting dated February 13, 2007.

Appropriateness of Test Models

- The CD1 mouse is an accepted test model for the evaluation of pharmaceutical carcinogenicity.

Evaluation of Tumor Findings

- There were no significant olodaterol-related neoplastic findings in male mice.
- The incidences of combined uterine leiomyomas and leiomyosarcoma and benign uterine leiomyomas alone were significantly higher at all dose levels when compared with the Vehicle Control group.
- Malignant leiomyosarcomas were also increased in olodaterol-treated groups although statistical significance was only achieved at the mid dose.
- The incidence of benign uterine polyps was increased in olodaterol-treated groups, compared to vehicle control, although statistical significance was only achieved at the high dose of 255 µg/kg.
- Thus, olodaterol was tumorigenic in female mice.

Methods

Inhaled Doses 0, 26.1, 76.9, and 255 µg/kg/day (0.53, 1.56, 5.23 µg/L over 45 minutes)
Frequency of dosing: Daily x726; 76.9 µg/kg/day level Daily x718

Route of administration: Aerosol Inhalation

Formulation/ Vehicle: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA x 2 H₂O, (b) (4) % citric acid

Basis of dose selection: Study 666408 (b) (4) BI 1744 CI: 13-week inhalation MTD study in mice (SN001) ; Reviewed by Dr. Molly Topper (1642809)

Species/ Strain: Crl:CD1(ICR) Mice

Number/sex/ group: Main Study: 60/sex/group

Age: ca. 4 -7 weeks

Weight: Males: 13.4 – 22.3 g; Females: 13.7 – 20.4 g

Animal housing: Male animals were singly housed and female animals were group housed (up to 3 animals per cage unless reduced by mortality) by dose group in solid bottom polypropylene cages containing woodchips as bedding. All cages were supplied with a plastic water bottle.

Paradigm for dietary restriction: Not applicable

Dual control employed: No

Interim sacrifice: No

Satellite groups: TK: 24/sex/clean air and vehicle control groups; TK: 40/sex/26.1, 76.9, and 255 µg/kg/day groups

Deviation from study protocol: No significant deviations

Inhalation Exposure System

- Each exposure chamber was located in an extract booth to prevent any cross-group contamination and for the protection of the personnel undertaking the animal inhalation exposure procedures.
- Each exposure chamber was operated to sustain a dynamic air flow sufficient to ensure an evenly distributed exposure atmosphere and was operated with an extract attached to the base of the chamber and a vacuum pump system was used to continuously exhaust the generated test atmosphere.
- Each calibrated exhaust flow passed through a HEPA filter system before being passed to the external atmosphere.
- Air flow rates were monitored continuously using calibrated flow meters. Chamber temperature and humidity were monitored and recorded at appropriate intervals during each daily exposure period.

- Test aerosols were generated using Heart airjet nebulizers (Westmed, USA) containing an appropriate prime volume of test formulation.
- The volume of formulation used for each nebulizer was recorded at the end of each generation period. Each nebulizer was operated at a target flow output of 11 L/min.
- Validation of the aerosol generating/exposure system was undertaken prior to commencement of animal exposure to demonstrate satisfactory aerosol performance (aerosol concentration, particle size distribution).
- Particle size distributions indicated average Mass Median Aerodynamic Diameters ranging from (b) (4). This size range is smaller than the usual respirable MMAD range of (b) (4). However, toxicokinetic data indicate that adequate drug exposures were achieved in this study.

Figure 3. Inhalation Exposure System Mouse Study #667930/U11-2661-01



Excerpted from sponsor's submission

Table 2. Technical Parameters Inhalation Mouse Study #667930/U11-2661-01

(b) (4)



ca=Approximately

Excerpted from sponsor's submission

Mortality

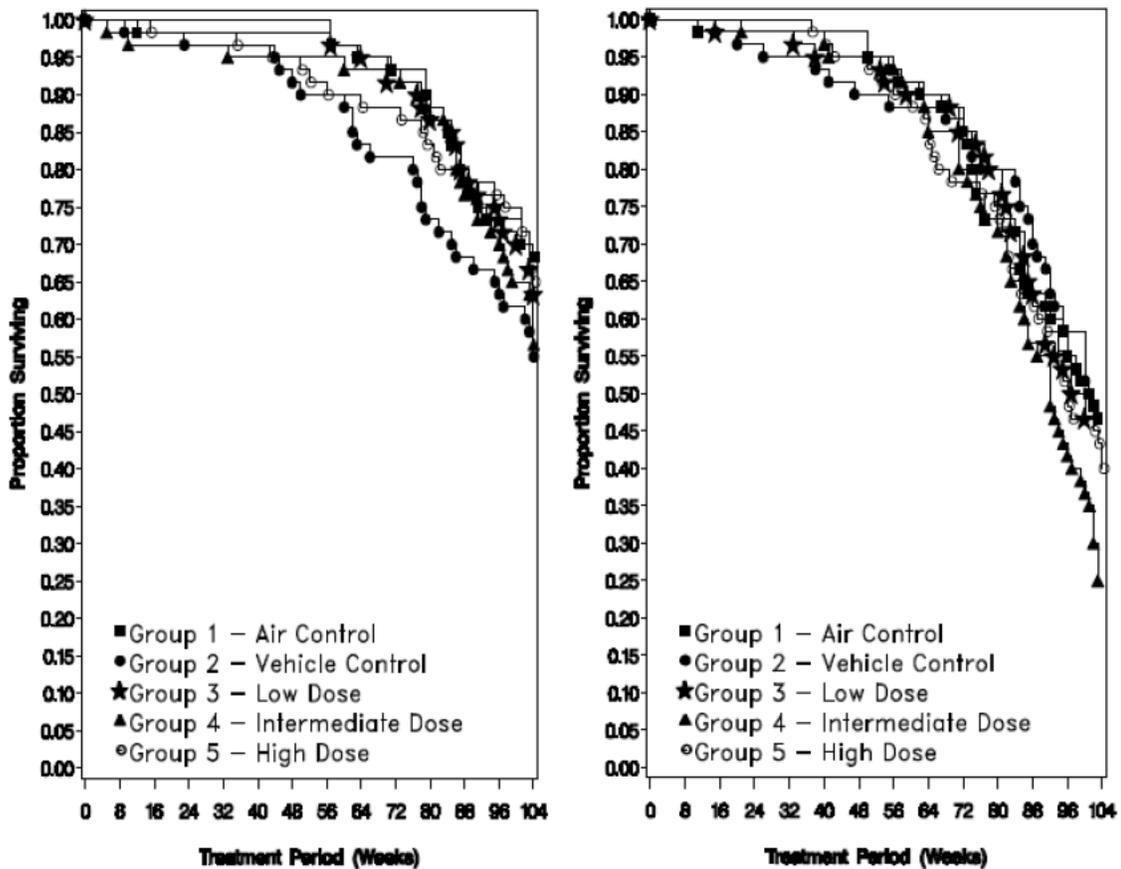
All animals were checked early morning and as late as possible each day for viability. Any animal showing signs of severe debility or intoxication and determined to be moribund or suffering excessively was killed. Any Main Study animal killed prematurely or found dead was subjected to a detailed macroscopic examination as soon as possible after death and tissues retained for histopathological evaluation.

- Females dosed at the 76.9 $\mu\text{g}/\text{kg}$ dose level were terminated on day 718 due to excessive mortality. The sponsor received prior concurrence from the FDA Executive Carcinogenicity Assessment Committee.
- There were no dose-related increases in mortality in either male or female mice.

Males (60/group)	A. Control	V. Control	26.1 $\mu\text{g}/\text{kg}$	76.9 $\mu\text{g}/\text{kg}$	255 $\mu\text{g}/\text{kg}$
Accident	0	1	1	1	1
Moribund	13	19	15	19	18
Found Dead	6	7	7	7	3
Total	19	27	23	27	22

Females (55/group)	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Accident	0	1	0	3	2
Moribund	29	21	27	38	27
Found Dead	3	8	5	4	7
Total	32	30	32	45	36

Figure 4. Kaplan-Meier Survival Curves Mouse Study #667930/U11-2661-01



*Excerpted from Sponsor's submission

Clinical Signs

All animals were examined at least once before exposure, continuously during exposure and at approximately 1-2 h after exposure. Particular attention was given to assessment of increased muscle mass as a known pharmacological effect of olodaterol. Once each week all animals received a detailed clinical examination and palpation. The clinical examination included appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. The size, appearance, position and duration of any masses detected were recorded.

- The main clinical finding in this study was increased muscle mass that occurred in all treated groups and in both sexes. This was an expected pharmacological effect of a β_2 -adrenergic agonist.

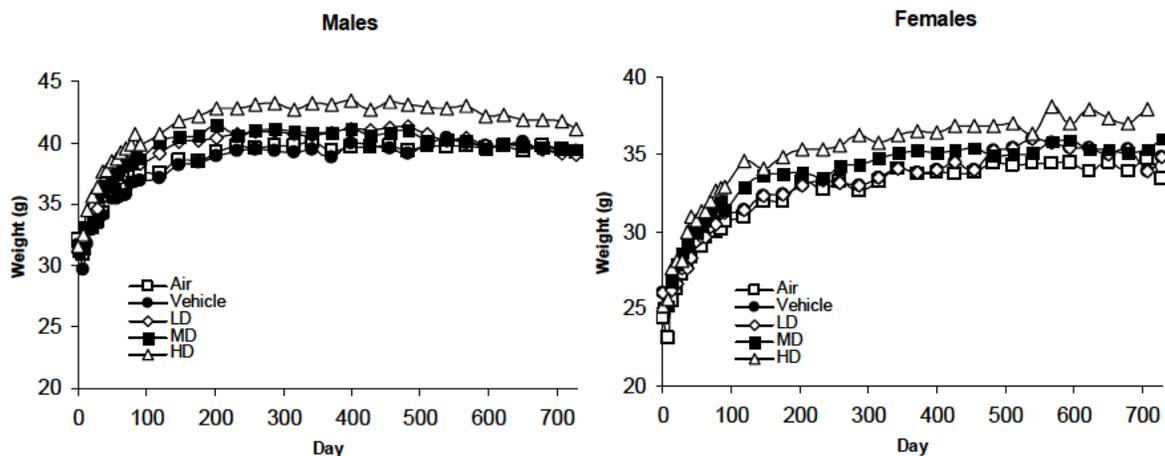
Muscle Mass Increase

	Number of Animals Observed		Number of Animals with Increased Muscle Mass		Mean Time of Onset (Day)	
	Male	Female	Male	Female	Male	Female
A-Control	60	60	0	0	0	0
V-Control	60	60	0	0	0	0
LD	60	60	13	2	268	293
MD	60	60	31	9	238	303
HD	60	60	34	6	237	325

Body Weights

Body weights were recorded once weekly throughout the study pretrial until the end of the first 13 weeks of the study. After this, body weights were recorded once.. Animals showing weight loss or deterioration in condition were weighed more frequently as necessary.

- Body weights were higher in mid-dose and high-dose groups in both sexes. This is an expected pharmacological response of a β_2 -adrenergic agonist.



Body Weights

Males	Day 0	Day 203	Day 371	Day 539	Day 728
A. Control	32.1	39.2	39.3	39.6	39.2
V. Control	31.7	38.9	38.8	40.4	39.3
LD	31.1	40.4*	40.8*	40.2	39
MD	31.1	41.4*	40.8*	40.3	39.4
HD	31.6	42.8*	43.1*	42.8*	41.1

All weights: grams; *p<0.01

Body Weights

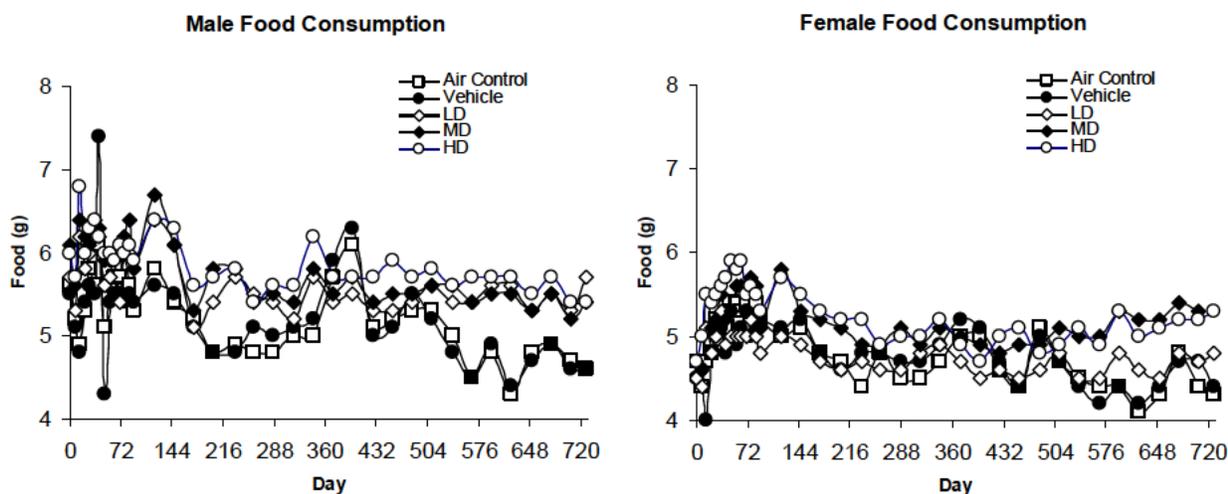
Females	Day 0	Day 203	Day 371	Day 539	Day 728
A. Control	24.4	33.2	33.7	34.4	33.4
V. Control	26	33	33.8	36	34.8
LD	25.1	33.8	35.2	35.1	36
MD	25.2	35.3*	36.5*	36.3	No Data
HD	25.4	34.9*	35.4	35.6	35.8

All weights: grams; *p<0.01

Food Consumption

The quantity of food consumed by each cage of animals was recorded once weekly, commencing one week pretrial and for the first 13 weeks of the dosing period. After this, the quantity of food consumed was recorded over approximately one week in every 4 weeks until the end of the study.

- Food consumption was greater among olodaterol-treated animals of both sexes. This is an expected pharmacological effect of a β_2 -adrenergic agonist.



Average Daily Food Consumption (All weights grams)

Males	Day 0	Day 203	Day 371	Day 539	Day 728
A. Control	5.6	4.8	5.7	5	4.6
V. Control	5.5	4.8	5.9	4.8	4.6
LD	5.7	5.4*	5.4	5.4*	5.7*
MD	6.1	5.8*	5.5	5.6*	5.4*
HD	6	5.7*	5.7	5.6*	5.4*

Females	Day 0	Day 203	Day 371	Day 539	Day 728
A. Control	4.7	4.7	5	4.5	4.3
V. Control	4.5	4.6	5.2	4.4	4.4
LD	4.5	4.6	4.7	4.5	4.8
MD	4.7	5.1*	5	5*	No Data
HD	4.7	5.2*	4.9	5.1*	5.3*

*p<0.001

Ophthalmoscopy

Eyes were examined using an indirect ophthalmoscope after the application of (Mydracil- 1% Tropicamide, Alcon Laboratories (UK)). Anterior, lenticular and fundic areas were evaluated. The ophthalmoscopic examination was undertaken on all animals prior to the start of treatment and subsequently performed on Toxicity animals from Groups 1, 2 and 5 in Weeks 50 and 102 of treatment.

- Due to re-assignment of animals to a new protocol during the pretrial period, up to 21 study animal identification numbers could not be traced in the original Provantis protocol.
- No significant findings are reported in the ophthalmoscopy report. However, there was an increased incidence of histopathological signs of keratitis in the eyes of olodaterol-treated animals (please see histopathology below).

Hematology

Blood and urine samples were obtained during Week 103-104 from 20 male and 20 female Main Study animals with the lowest numbers in each group. The remaining Main Study animals were evaluated only for RBC, WBC and differential white blood cell counts.

- No significant changes were observed.

Clinical Chemistry

Blood and urine samples were obtained during Week 103-104 from 20 male and 20 female Main Study animals with the lowest numbers in each group.

- Blood urea nitrogen was increased in males at all olodaterol doses and females treated with olodaterol at the 26.1 and 255 µg/kg dose levels.

Parameter	Sex	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Week 103- 104						
BUN	M	7.0 mmol/L	--	+11%	+16%	+11%
	F	6.6 mmol/L	--	+18%	--	+35%

Gross Pathology

- There was an increased incidence of pale focus in the hearts of olodaterol-treated males. Concomitantly, there was a low incidence of enlarged heart in 1 male at 26.1 µg/kg, 1 male at 76.9 µg/kg and 2 males at 255 µg/kg. These findings correlated with cardiac histopathology findings, including dose-dependent increases in frequency and severity of pericardial fibrosis, myocardial fibrosis, myocardial vacuolation, and atrial thrombus.
- Increased skeletal muscle mass was observed in 2 males at 76.9 µg/kg and in 2 males at 255 µg/kg. Increased incidence of abnormal skeletal shape was observed in 3 males at 26.1 µg/kg, 2 males at 76.9 µg/kg and 5 males at 255 µg/kg. Increased skeletal muscle mass was an expected pharmacological effect of a β_2 -adrenergic agonist. Findings of abnormal foot shape, which were enhanced primarily in females at the 76.9 and 255 µg/kg dose levels may also be related to olodaterol effects on skeletal muscle.

Parameter	Sex	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Week 104						
Cecum, masses	M	--	--	--	1	1
	F	--	--	--	--	1
Ear, abnormal shape	M	1	--	--	2	3
	F	1	1	--	--	2
Epididymis, small	M	--	--	--	1	1

Parameter	Sex	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Eye, contains fluid	M	--	--	--	--	1
Eye, abnormal shape	M	1	--	--	--	1
	F	--	1	--	2	2
Foot/Leg, abnormal shape	M	--	1	1	2	--
	F	1	1	--	5	4
Harderian Gland, enlarged	M	--	1	1	--	2
	F	1	1	1	2	2
Heart, Pale focus	M	4	2	8	10	12
	F	1	1	1	1	2
Heart, Enlarged	M	--	--	1	1	2
	F	--	--	--	--	--
Intestines, distended	M	--	1	3	1	4
	F	--	1	1	3	3
Lung, spongy	M	1	1	--	3	2
	F	1	--	1	1	4
Ovary, cyst	F	49	43	51	55	55
Pancreas, pale	M	1	--	1	3	3
	F	1	1	4	4	3
Penis, prolapse	M	--	--	--	2	2
Seminal Vesicle, discolored	M	1	--	4	1	2
Skeletal muscle, abnormal shape	M	--	--	3	2	5
	F	--	--	--	1	1
Skeletal muscle, enlarged	M	--	--	--	2	2
	F	--	--	--	--	--
Skin, subcutaneous gelatinous thickening	M	--	--	--	1	--
	F	1	1	1	1	4
Stomach, distended	M	--	--	--	3	--
	F	--	--	2	3	3
Systemic condition, blood watery	M	1	--	--	1	1
	F	2	2	6	3	6
Testis, small	M	--	--	--	2	3

Organ Weights

Lung weights were the only organ weights recorded at necropsy.

- No significant changes were observed.

Histopathology:

The following organs and tissues were examined: abnormal tissue, adrenals, aorta, aortic arch, bone (femur), brain, clitoral gland, duodenum, exorbital lachrymal gland, eyes, forestomach, femur joint, stomach, harderian glands, heart, ileum, jejunum, kidneys, knee (joint with femur), submandibular lymph nodes, lacrimal glands, larynx, liver, lungs, female mammary glands, mesenteric and submandibular lymph nodes, lymph nodes regional to masses, nasal cavity, optic nerves, ovaries, oviducts, pancreas, parathyroids, parotid salivary glands, sciatic nerve, peyer's patches, pharynx, pituitary, prostate, rectum, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, sublingual salivary glands, testes, thymus, thyroid, tongue, trachea, ureters, urinary bladder, uterus, oviduct, and cervix, vagina.

Tissues were fixed in neutral buffered 10% formalin, except for eyes and harderian glands, which were fixed in Davidson's fluid.

Non-neoplastic:

- The most prominent histopathological findings in both males and females occurred in the heart. In males, major findings included dose-dependent frequency and severity of pericardial fibrosis, myocardial fibrosis, and myocardial vacuolation. Atrial thrombus occurred in males at all dose levels. In females, major findings included dose-dependent frequency and severity of pericardial fibrosis, myocardial fibrosis, myocardial vacuolation.
- Squamous metaplasia in the larynx occurred in both males and females treated with olodaterol in a dose-dependent manner. There was also an incidence (2/60 in males and 9/60 females) of this finding in the vehicle control.
- Increased alveolar foamy macrophage focal accumulation occurred in males and females at all dose levels, although severity and frequency did not increase greatly with dose.
- Increased frequency and severity of eye keratitis in males and females at all dose levels occurred, although severity and frequency did not increase greatly with dose.
- Focal hyperplasia in the sex cord/stromal ovary was increased in frequency and severity in a dose-dependent manner.

Non-neoplastic findings in male mice

Findings	Males					
	Grade	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
N =		60	60	60	60	60
Epididymis, sperm granuloma	Minimal	1	--	--	--	1
	Mild	--	--	--	--	2
	Total	1	--	--	--	3
Epididymis, inflammatory cell infiltration	Minimal	--	1	3	1	5
	Mild	--	1	--	--	3
	Total	--	2	3	1	8
Eye, keratitis	Minimal	3	--	3	2	4
	Mild	--	--	2	2	--
	Moderate	--	--	1	--	1
	Total	3	--	6	4	5
Harderian Gland, acinar cell necrosis	Minimal	--	1	--	--	1
	Mild	1	--	3	2	2
	Moderate	1	2	3	2	4
	Marked	1	--	--	--	--
	Total	3	3	6	4	7
Heart, pericardial fibrosis	Minimal	2	1	4	3	6
	Mild	1	--	3	2	6
	Total	3	1	7	5	12
Heart, atrial thrombus	Minimal	--	--	--	2	--
	Mild	--	--	--	2	--
	Moderate	--	--	2	--	2
	Marked	--	--	1	2	1
	Total	--	--	3	6	3
Heart, myocardial fibrosis	Minimal	25	25	33	31	33
	Mild	6	3	10	7	11
	Moderate	--	--	--	--	1
	Total	31	28	43	38	45
Heart, myocardial vacuolation	Minimal	24	13	28	28	31
	Mild	3	3	2	4	5
	Moderate	--	--	3	2	--
	Total	27	16	33	34	36
Heart, myocardial hypertrophy	Present	--	--	--	1	1

Findings	Males					
	Grade	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Heart, ventricular dilation	Mild	--	--	--	1	--
	Moderate	--	--	1	--	1
	Total	--	--	1	1	1
Kidney, cyst	Minimal	1	--	--	2	1
	Mild	--	--	--	--	1
	Total	1	--	--	2	2
Larynx, squamous metaplasia	Minimal	--	2	6	7	14
	Mild	--	--	3	--	11
	Total	--	2	9	7	25
Liver, clear cell focus	Minimal	--	--	--	--	1
	Mild	--	--	--	--	1
	Moderate	--	--	--	1	
	Total	--	--	--	1	2
Liver, pigmented macrophages	Moderate	--	--	1	--	1
Lung, eosinophilic macrophages, alveolar	Minimal	--	2	--	--	--
	Mild	1	--	1	3	2
	Moderate			3	2	--
	Marked			1	2	1
	Severe					
	Total	1	2	5	7	3
Lung, alveolar foamy macrophage accumulation, focal	Minimal	1	--	--	1	1
Nasal cavity, exudate	Minimal	6	2	5	4	10
	Mild	1	--	2	3	1
	Total	7	2	7	7	11
Skeletal muscle, hypertrophy	Mild	--	--	1	2	2
Spinal cord, compression	Present	7	2	6	4	11

Non-neoplastic findings in female mice

Findings	Females					
	Grade	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
N =		60	60	60	60	60
Eye, keratitis	Mild	--	--	--	1	2
	Moderate	--	--	--	--	1
	Total	--	--	--	1	3
Harderian gland, lymphocytic infiltration	Minimal	3	2	--	1	5
	Mild	--	1	--	--	1
	Moderate	--	--	--	--	1
	Total	3	3	1	1	7
Heart, epicardial fibrosis	Minimal	--	--	2	2	2
	Mild	--	1	1	2	1
	Marked	1	--	--	--	--
	Total	1	1	3	4	3
Heart, perifibrosis, pericardial	Mild	--	--	--	2	1
	Marked	--	--	--	--	1
	Total	--	--	--	2	2
Heart, myocardial fibrosis	Minimal	8	6	9	17	20
	Mild	1	--	2	--	6
	Moderate	--	--	--	--	--
	Total	9	6	11	17	26
Heart, myocardial vacuolation	Minimal	5	5	7	15	14
	Mild	--	--	--	--	1
	Total	5	5	7	15	15
Jejunum, focal hyperplasia	Moderate	--	--	--	--	1
Larynx, squamous metaplasia	Minimal	--	9	4	9	13
	Mild	--	--	--	2	7
	Moderate	--	--	--	1	--
	Total	--	9	4	12	20
Liver, centrilobular necrosis	Mild	--	--	--	--	1
	Moderate	--	--	1	--	--
	Total	--	--	1	--	1
Liver, chronic periportal inflammation	Minimal	1	--	--	--	2
	Mild	--	--	1	1	--
	Total	1	--	1	1	2
Liver, pigmented macrophages	Moderate	1	3	1	8	5

Findings	Females					
	Grade	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Lung, eosinophilic macrophages, alveolar	Minimal	--	1	1	1	--
	Mild	1	1	2	3	1
	Moderate	1	--	1	1	--
	Marked	--	1	1	--	1
	Severe	--	--	--	--	1
	Total	2	3	5	5	3
Lung, alveolar foamy macrophage accumulation, focal	Minimal	--	1	11	9	10
	Mild	--	2	1	1	2
	Total	--	3	12	10	12
Ovary, focal hyperplasia, sex cord/stromal	Minimal	--	--	1	--	1
	Mild	--	1	--	3	5
	Moderate	1	--	--	2	1
	Marked	--	--	--	--	2
	Total	1	1	1	5	9
Ovary, focal hyperplasia, luteal	Mild	3	1	3	1	1
	Moderate	1	1	--	2	3
	Marked	--	1	--	--	3
	Total	4	3	3	3	7
Ovary, focal hyperplasia, tubulostromal	Present	--	--	--	--	2
Ovary, hemorrhagic cyst	Present	48	40	51	50	55

Neoplastic:

- There were no significant olodaterol-related neoplastic findings in male mice.
- The incidence of benign uterine leiomyomas was significantly higher at all dose levels when compared with the Vehicle Control group (Trend Test p=0.0138).
- Malignant leiomyosarcomas were also increased in olodaterol-treated groups although statistical significance was only achieved for the mid dose. Combined leiomyosarcoma and leiomyoma incidence was statistically significant at all doses (Trend Test p=0.0141).
- The incidence of benign uterine polyps was increased in olodaterol-treated groups, compared to vehicle control, although statistical significance was only achieved at the high dose of 255 µg/kg (Trend Test 0.0248).

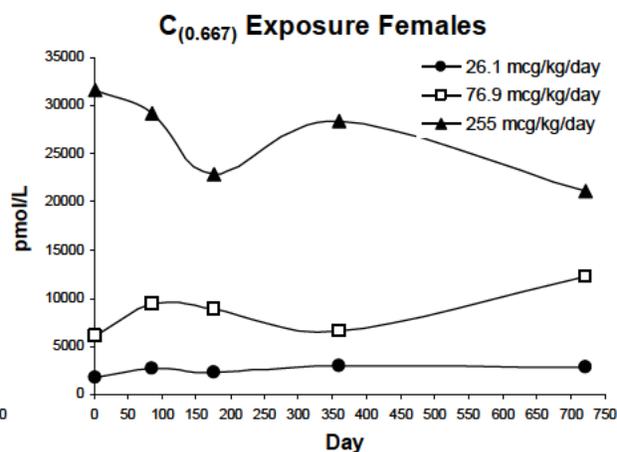
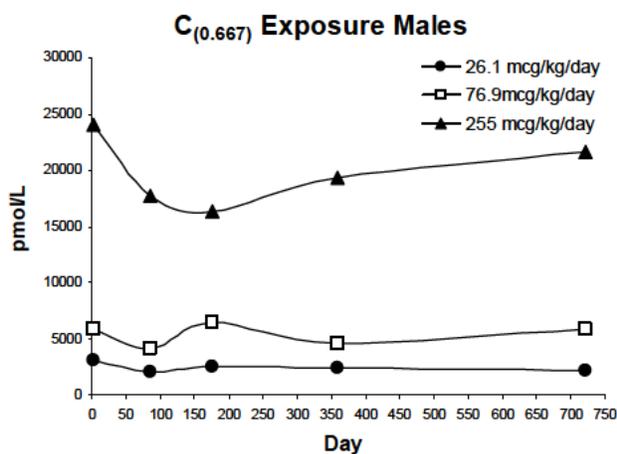
Neoplastic findings in female mice

Findings	Females						Trend P-value (Exact method)	Historical Control Data	
	A. Control	V. Control	26.1 µg/kg	76.9 µg/kg	255 µg/kg	(b) (4) *		(b) (4)	
Uterus N =	60	60	60	60	60				
leiomyosarcoma [M]	1 (1.7%)	2 (3.3%)	5 (8.3%)	10 (16.7%)	4 (6.7%)	0.4108	1.6 (0.0-3.6)	1.4 (0.0-2.7)	
leiomyoma [B]	5 (8.3%)	9 (15%)	18 (30%)	19 (31.7%)	22 (36.7%)	0.0138	2.9 (0.0-8.0)	4.2 (2.0-6.0)	
leiomyoma/leiomyosarcoma	6 (10%)	11 (18%)	23 (38%)	28 (31.7%)	26 (46.7%)	0.0141	n.a.	n.a.	
Polyp [B]	7 (11.6%)	4 (6.7%)	8 (13.2%)	8 (13.2%)	12 (20.1%)	0.0248	n.a.	n.a.	

Toxicokinetics

Blood samples were taken 0.667 h (40 min) after the start of inhalation on Days 1, 85, 176, 359, 722. Blood samples were taken immediately post-dose, and 1, 2, 4, 8, 24 h after the start of inhalation on Day 359. Approximately 0.6 mL of blood was obtained from all designated animals at each timepoint. Samples from Control Groups 1 and 2 were collected in a separate room by a separate team of technicians from the treated groups.

- $C_{0.667}$ values were reported for males and females, verifying exposure on Days 1, 85, 176, 359, and 722. Exposure levels were similar over the course of the study.
- C_{max} and AUC exposures peaked on Day 359 indicate similar dose/exposure relationships in males and females.



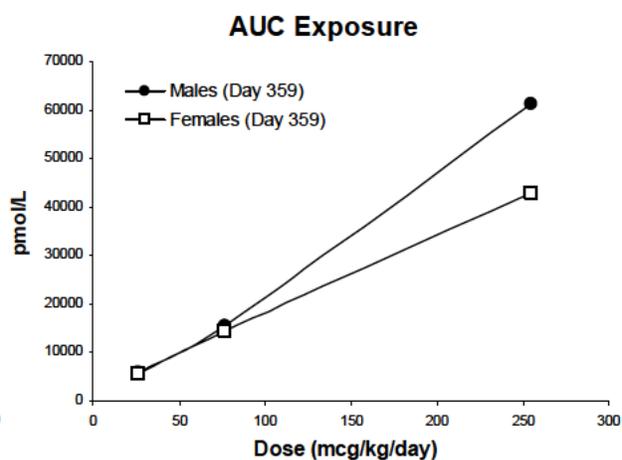
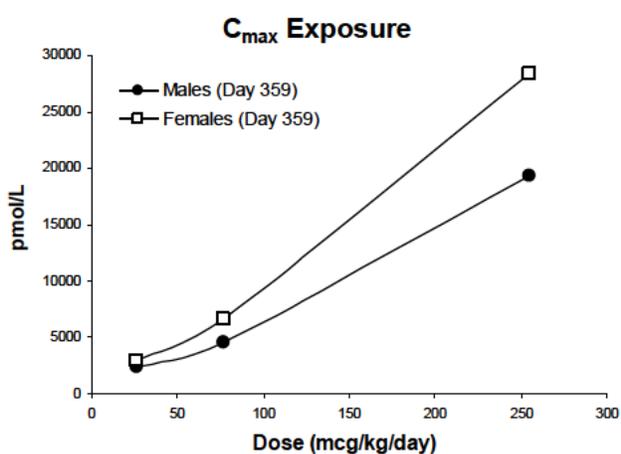
$C_{(0.667)}$ (pmol/L)

Males

Week	Day	26.1 µg/kg	76.9 µg/kg	255 µg/kg
1	1	3060	5840	24000
13	85	2100	4150	17700
26	176	2550	6380	16300
52	359	2410	4610	19300
104	722	2130	5910	21600

Females

Week	Day	26.1 µg/kg	76.9 µg/kg	255 µg/kg
1	1	1710	6010	31700
13	85	2690	9450	29200
26	176	2260	8840	22900
52	359	3020	6650	28400
104	722	2820	12200	21200



	Dose	26.1 µg/kg	76.9 µg/kg	255 µg/kg
Males	C _{max}	2410	4610	19300
	AUC	5750	15400	61200
Females	C _{max}	3020	6650	28400
	AUC	5680	14300	42800

11 Integrated Summary and Safety Evaluation

Boehringer Ingelheim Pharmaceuticals, Inc. (BI) is developing BI 1744 CI delivered with the Respimat inhaler as a treatment of Chronic Obstructive Pulmonary Disease. BI 1744 CI is a long-acting agonist of the human β 2 adrenoreceptor with an EC_{50} = 1.0 ± 0.4 nM; BI 1744 CI stimulates the human β 1-adrenoreceptor with an EC_{50} = 60 ± 24 nM, indicating selectivity for the β 2 adrenoreceptor. The present review covers two 2-year inhalational carcinogenicity studies of BI 1744 CL, Study No. 667930/U11-2661-01 performed in rats submitted as SD125 (CDER Stamp Date January 13, 2012) and Study No. 668138/U12-1065 performed in mice, submitted as SD129 (CDER Stamp date March 6, 2012).

In the rat carcinogenicity study (Study No. 667930/U11-2661-01), 55 rats/sex/group were given doses of 0 (clean air control), 0 (vehicle: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA x 2 H₂O, (b) (4) % citric acid), and 25.8, 75.9, and 270 μ g/kg olodaterol, as calculated by the sponsor. For comparison with doses described in reviews of the general toxicology program, the deposited doses were calculated for this study using a deposition factor of 0.1 and using the method of Guyton et al., 1947¹ to determine minute volume. These deposited doses were 2.3, 6.8, and 23.6 μ g/kg. However, for purposes of this discussion, the sponsor's dose levels will be used. Comparisons of relative drug exposure between this study and clinical doses will be done on an AUC basis, ensuring that any discrepancy in bioavailability, deposition, or other variation in dosing is corrected.

There was an increase in mortality in males treated with olodaterol, relative to controls, but the increase was not dose-dependent, and was not statistically relevant (please see the Statistical Review of this study). Female mortality was similar across all controls and dose groups. Body weights for male and female treatment groups were unaffected.

The main neoplastic finding in rats administered daily inhalation of olodaterol induced leiomyomas of the mesovarian tissue in 1/55 females of the 25.8 μ g/kg/day dose group, and 4/55 females of the 270 μ g/kg/day dose group; the increase was statistically significant at the high dose. The main toxicities recorded in histopathology data consisted of an increase in ovarian cysts with and without hyperplasia in the ovary, an increase in mild-cardiomyopathy in females, and vehicle-related squamous metaplasia in the larynx. AUC exposure at the end of the study was at least 9-fold higher than the AUC associated with the highest clinical dose studied (30 μ g/day; Study No. U07-2062; Table 3).

In the mouse carcinogenicity study (Study No. U11-2661-01/667930), 60 rats/sex/group were given doses of 0 (clean air control), 0 (vehicle: 0.01% benzalkonium chloride, (b) (4) % disodium EDTA x 2 H₂O, (b) (4) % citric acid), and 26.1, 76.9, and 255 μ g/kg olodaterol, as calculated by the sponsor. For comparison with doses described in reviews of the general toxicology program, the deposited doses were calculated for this

study using a deposition factor of 0.1 and using the method of Guyton et al., 1947¹ to determine minute volume. These deposited doses were 2.1, 6.0, and 20.0 µg/kg. However, for purposes of this discussion, the sponsor's dose levels will be used. Comparisons of relative drug exposure between this study and clinical doses will be done on an AUC basis, ensuring that any discrepancy in bioavailability, deposition, or other variation in dosing is corrected.

There were no treatment-related effects on survival for males or females. Body weights were higher in mid-dose and high-dose groups in both sexes.

The main neoplastic finding in mice administered daily inhalation of olodaterol was a statistically significant increased incidence of combined benign uterine leiomyomas and malignant leiomyosarcomas at all dose-levels. Combined, these tumors occurred in 23/60 at 26.1 µg/kg/day, 28/60 at 76.9 µg/kg/day and 26/60 at the 255 µg/kg/day dose levels. By comparison, these tumors occurred in 6/60 air-control mice and 11/60 vehicle control mice. Benign uterine leiomyomas were significantly increased at all doses. Leiomyosarcomas were increased at all doses although statistical significance was only achieved at the mid dose. The incidence of benign uterine polyps was increased in olodaterol-treated groups, compared to vehicle control, although statistical significance was only achieved at the high dose of 255 µg/kg (Trend Test 0.0248).

The most prominent histopathological findings in both males and females occurred in the heart. There were also toxicities identified in the larynx, lung, eye, and ovary. In males, major findings included dose-dependent frequency and severity of pericardial fibrosis, myocardial fibrosis, myocardial vacuolation. Atrial thrombus occurred in males at all dose levels. In females, major findings included dose-dependent frequency and severity of pericardial fibrosis, myocardial fibrosis, myocardial vacuolation. Squamous metaplasia in the larynx occurred in both males and females treated with olodaterol in a dose-dependent manner. There was also an incidence (2/60 in males and 9/60 females) of this finding in the vehicle control. Increased alveolar foamy macrophage focal accumulation occurred in males and females at all dose levels, although severity and frequency did not increase greatly with dose. Increased frequency and severity of eye keratitis in males and females at all dose levels occurred, although severity and frequency did not increase greatly with dose. Focal hyperplasia in the sex cord/stromal ovary was increased in frequency and severity in a dose-dependent manner. There were no significant hematology and clinical chemistry findings. AUC exposure at the end of the study was at least 19-fold higher than the AUC associated with the highest clinical dose studied (30 µg/day; Study No. U07-2062; Table 3).

Olodaterol was negative for genetic toxicity in bacterial reverse mutagenicity (Ames) studies and in *in vitro* mouse lymphoma assay. Although intravenously administered olodaterol produced increased percentages of polychromatic erythrocytes (PCEs) in male rats at the 10 and 40 mg/kg dose levels and dose-dependent, statistically significant, increases in the frequencies of micronucleated polychromatic erythrocytes (MNEs) 24 and 48 h after treatment (Study No. U08-1834-01), the overall genotoxicity assessment of olodaterol was judged to be negative because the positive result in the

rat micronucleus assay was most likely due to a drug-enhanced compensatory erythropoiesis and not a genotoxic mechanism (please see Dr. Timothy Robison's review of this study; Communication PK 2778471).

Olodaterol was tumorigenic in female rats and mice based upon findings of ovarian leiomyomas and uterine leiomyomas/leiomyosarcomas and polyps, respectively. There were no tumor findings in male rats or mice.

However, agonist activity at the β -adrenoreceptor has been previously linked to increased incidence of leiomyomas and leiomyosarcomas in the female rodent genital tract. In the discussion section of the mouse study (Study No. U11-2661-01/667930), the sponsor refers to publicly available documents indicating similar oncologic findings with formoterol fumarate¹ and salmeterol², two long-acting beta-adrenergic receptor agonists that have been approved for use in the treatment of asthma. Further, there is also mechanistic evidence suggesting that beta-adrenergic receptor agonism is tumorigenic in the female genital tract of the rodent. Concurrent administration of the β -adrenergic receptor antagonist propranolol with the adrenergic receptor agonist salbutamol blocked the salbutamol-induced formation of mesovarian tumors in Sprague-Dawley rats^{3,4}. It is important to note that ovarian leiomyomas are extremely rare spontaneous tumors in rats. Similarly, uterine leiomyomas/leiomyosarcomas occur at low spontaneous incidences in female mice. Proliferation of the mesovarian smooth muscle is considered an adaptive physiologic response to prolonged stimulation of the β_2 -receptor and this response may be related to beta-adrenergic receptor agonist-induced tumors in the rodent genital tract. However, leiomyomas of ovarian tissue are rare in women, and no increase in incidence has been reported despite the long history of use of β_2 -stimulants in the treatment of bronchial asthma. Thus, the relevance of these findings to human use is unknown.

A search of Pubmed⁵ did not reveal any link between salmeterol, formoterol, and salbutamol or beta-adrenergic receptor agonists in general and human leiomyomas/leiomyosarcomas or human tumors in general.

Table 3. AUC Exposure Calculations Carcinogenicity Studies vs. Clinical Dose

		Systemic Exposures AUC 0-24 h (pmol*L)	Fold Clinical Exposure based on AUC
Highest Human dose 30 µg/day 14-Days No. U07-2062	0.5 µg/kg/day	298§	-
Asthma patients: 10 µg/day 4-weeks No. U09-1850-01	0.17 µg/kg/day	199§	-
COPD patients: 10 µg/day 4-weeks No. U09-3125	0.17 µg/kg/day	246§	-
Rat Study No. 667930	25.8	2650*	Highest: 9 Asthma: 13 COPD: 11
	75.9	8615*	Highest: 29 Asthma: 43 COPD: 35
	270	28800*	Highest: 97 Asthma: 145 COPD: 117
Mouse Study No. 667930	26.1	5715*	Highest: 19 Asthma: 29 COPD: 23
	76.9	14850*	Highest: 50 Asthma: 75 COPD: 60
	255	52000*	Highest: 175 Asthma: 261 COPD: 211

§ AUC exposure converted from pg/kg*h/mL to pmol*h/L

*Average of male and female values

12 Appendix/Attachments

None

¹ http://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/20831_Foradil_phrmr_P5.pdf

² http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021254s003,020692s031lbl.pdf

³ Gopinath C, Gibson WA. Mesovarian leiomyomas in the rat. [Environ Health Perspect.](#) 1987 Aug;73:107-13.

⁴ Jack D, Poynter D, Spurling NW. Beta-adrenoceptor stimulants and mesovarian leiomyomas in the rat. *Toxicology.* 1983 Jul-Aug;27(3-4):315-20.

⁵ <http://www.ncbi.nlm.nih.gov/pubmed>

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/s/

HANS M ROSENFELDT
05/11/2012

TIMOTHY W ROBISON
05/11/2012
I concur

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/s/

CAROL M RIVERA-LOPEZ
01/17/2013

MARCIE L WOOD
01/17/2013

**PHARMACOLOGY/TOXICOLOGY REVIEW
CHEMISTRY CONSULT REQUEST**

NDA number: 203108
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.
Date of submission: May 14, 2012
Date of consult: June 8, 2012
Reviewer Name: Carol M. Rivera-Lopez, Ph.D.
Supervisor: Molly E. Shea, Ph.D.
Review Division: Division of Pulmonary, Allergy, and Rheumatology Products (DPARP)
Review Completion Date: June 27, 2012

Drug: (b) (4) Respimat Inhalation Spray
Generic Name: Olodaterol
Trade Name: (b) (4)
Route of Administration: Oral inhalation

Subject: Nonclinical qualification of the drug substance specification allowance for up to (b) (4)

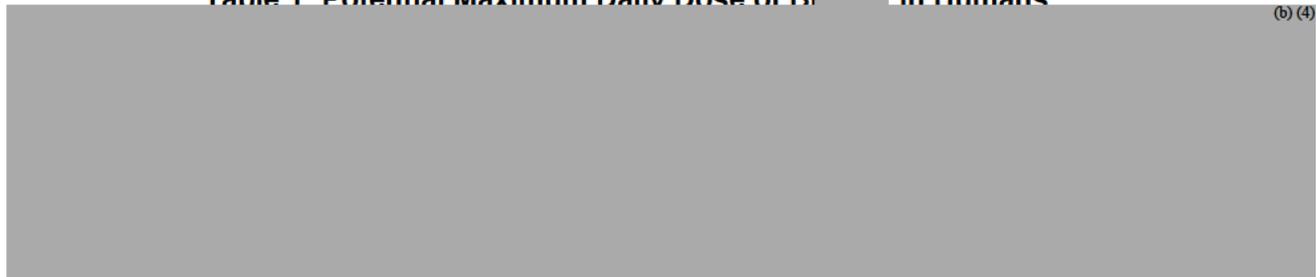
Safety Assessment

This review evaluates the safety of the drug substance specification of up to (b) (4) identified as an impurity in (b) (4) Dr. Craig Bertha, CMC reviewer, submitted a Pharmacology/Toxicology consult on June 8th, 2012 to DPARP requesting a nonclinical evaluation of this specification limit (Attachment 1).

The safety of BI (b) (4) was determined by comparing its potential maximum daily dose in humans to the levels of this impurity in the test article used for the long-term toxicology studies. The estimates are based on a maximum proposed clinical dose of 5 µg/day olodaterol. The potential maximum daily dose of BI (b) (4) using the (b) (4) limit is (b) (4) (b) (4). The potential local maximum dose for human lung is 0.00005 µg/g lung weight (Table 1).

Table 1: Potential Maximum Daily Dose of BI (b) (4) in Humans

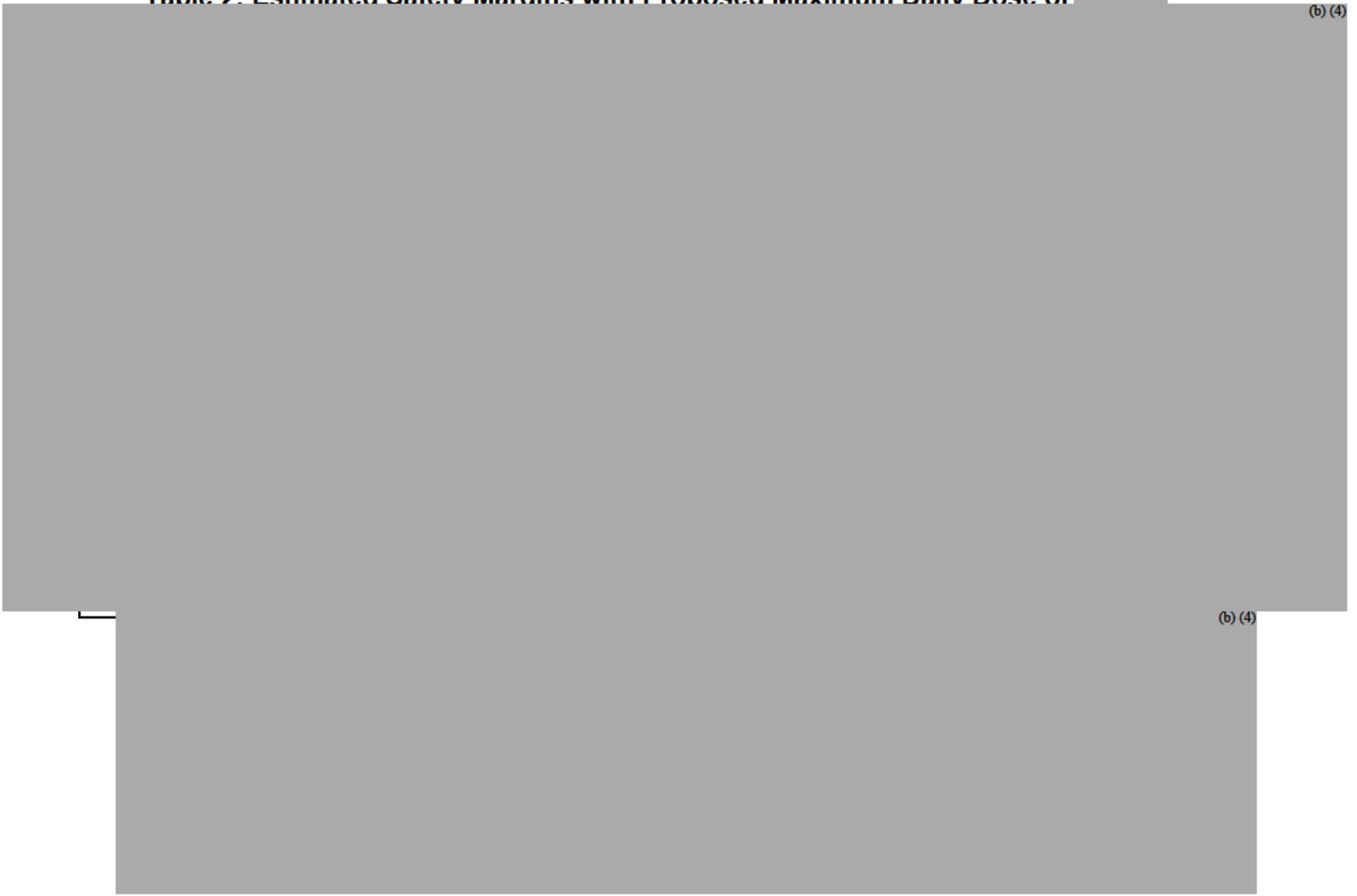
(b) (4)



This dose of (b) (4) was compared to the levels of this impurity present in the chronic toxicology studies [*i.e.*, 0.34% in both the 26-week study in rats (Study U08-1691) and the 52-week study in dogs (Study U08-1740)] and the two carcinogenicity studies [approximately 0.2% in both the rat (Study U11-2661) and mouse (Study U12-1065)]

studies]. Safety margins were calculated based on body weights (BW) and also based on systemic exposure (AUC) comparisons for systemic effects and based on lung weights for local effects (Table 2).

Table 2: Estimated Safety Margins with Proposed Maximum Daily Dose of (b) (4)



The proposed level of (b) (4) is considered qualified based upon adequate safety margins from nonclinical studies and the potential maximum daily dose of (b) (4)

In addition, a consult was sent to the CDER Computational Toxicology Group on 6/12/2012. (b) (4) was evaluated for genetic toxicity (b) (4)



(b) (4) predicted to be negative for (b) (4). These results correlate with the negative results obtained in a (b) (4)

(b) (4) was judged to be non-genotoxic in a full battery of *in vitro* and *in vivo* genetic toxicology studies and NOAELs were identified in the two chronic toxicology studies with

(b) (4) (b) (4) no additional risk is expected with this (b) (4) impurity.

Taking into account all the aforementioned, the proposed specification limit of (b) (4) considered acceptable.

Attachment 1: CMC Consult Request – DARRTS version

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION			REQUEST FOR CONSULTATION	
TO: <i>(Division/Office)</i> Molly Shea, Ph.D., Pharm/Tox Supervisor			FROM: Craig M. Bertha, Ph.D., ONDQA, Div 3	
DATE 05-JUN-2012	IND NO.	NDA NO. N203108	TYPE OF DOCUMENT Original NDA [505(b)(1)]	DATES OF DOCUMENTS 14-MAY-2012
NAME OF DRUG (b)(4) Respimat (olodaterol) Inhalation Spray		PRIORITY CONSIDERATION S	CLASSIFICATION OF DRUG 1	DESIRED COMPLETION DATE 14-OCT-2012 (week before mid-cycle)
NAME OF FIRM: Boehringer Ingelheim, Inc.				
REASON FOR REQUEST				
I. GENERAL				
<input type="checkbox"/> NEW PROTOCOL <input type="checkbox"/> PROGRESS REPORT <input type="checkbox"/> NEW CORRESPONDENCE <input type="checkbox"/> DRUG ADVERTISING <input type="checkbox"/> ADVERSE REACTION REPORT <input type="checkbox"/> MANUFACTURING CHANGE/ADDITION <input type="checkbox"/> MEETING PLANNED BY		<input type="checkbox"/> PRE-NDA MEETING <input type="checkbox"/> END OF PHASE II MEETING <input type="checkbox"/> RESUBMISSION <input type="checkbox"/> SAFETY/EFFICACY <input type="checkbox"/> PAPER NDA <input type="checkbox"/> CONTROL SUPPLEMENT		<input type="checkbox"/> RESPONSE TO DEFICIENCY LETTER <input type="checkbox"/> FINAL PRINTED LABELING <input type="checkbox"/> LABELING REVISION <input type="checkbox"/> ORIGINAL NEW CORRESPONDENCE <input type="checkbox"/> FORMULATIVE REVIEW <input checked="" type="checkbox"/> OTHER <i>(Specify below)</i>
II. BIOMETRICS				
STATISTICAL EVALUATION BRANCH			STATISTICAL APPLICATION BRANCH	
<input type="checkbox"/> TYPE A OR B NDA REVIEW <input type="checkbox"/> END OF PHASE II MEETING <input type="checkbox"/> CONTROLLED STUDIES <input type="checkbox"/> PROTOCOL REVIEW <input type="checkbox"/> OTHER			<input type="checkbox"/> CHEMISTRY <input type="checkbox"/> PHARMACOLOGY <input type="checkbox"/> BIOPHARMACEUTICS <input type="checkbox"/> OTHER	
III. BIOPHARMACEUTICS				
<input type="checkbox"/> DISSOLUTION <input type="checkbox"/> BIOAVAILABILITY STUDIES <input type="checkbox"/> PHASE IV STUDIES			<input type="checkbox"/> DEFICIENCY LETTER RESPONSE <input type="checkbox"/> PROTOCOL-BIOPHARMACEUTICS <input type="checkbox"/> <i>IN-VIVO</i> WAIVER REQUEST	
IV. DRUG EXPERIENCE				
<input type="checkbox"/> PHASE IV SURVEILLANCE/EPIDEMIOLOGY PROTOCOL <input type="checkbox"/> DRUG USE e.g. POPULATION EXPOSURE, ASSOCIATED DIAGNOSES <input type="checkbox"/> CASE REPORTS OF SPECIFIC REACTIONS <i>(List below)</i> <input type="checkbox"/> COMPARATIVE RISK ASSESSMENT ON GENERIC DRUG GROUP			<input type="checkbox"/> REVIEW OF MARKETING EXPERIENCE, DRUG USE AND SAFETY <input type="checkbox"/> SUMMARY OF ADVERSE EXPERIENCE <input type="checkbox"/> POISON RISK ANALYSIS	
V. SCIENTIFIC INVESTIGATIONS				
<input type="checkbox"/> CLINICAL			<input type="checkbox"/> PRECLINICAL	
COMMENTS/SPECIAL INSTRUCTIONS: Please evaluate the acceptability of the drug substance specification allowance for up to (b)(4) of the (b)(4) which is above the ICH Q3A qualification threshold of 0.15%.				
cc: Orig N203108 ONDQA/DIV 3/CBertha(06/05/2012) ONDQA/DIV 3/PPeri OND/DPARP/CChung OND/DPARP/TRobison OND/DPARP/MShea OND/DPARP/CRivera-Lopez ONDQA/DIV 3/ASchroeder				
SIGNATURE OF REQUESTER			METHOD OF DELIVERY <i>(Check one)</i> MAIL HAND	
SIGNATURE OF RECEIVER			SIGNATURE OF DELIVERER	

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/s/

CRAIG M BERTHA
06/08/2012

PRASAD PERI
06/08/2012

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/s/

CAROL M RIVERA-LOPEZ
06/27/2012

MOLLY E SHEA
06/27/2012
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203108

Applicant: Boehringer Ingelheim Pharmaceuticals, Inc. Stamp Date: 5/14/2012

Drug Name: (b)(4)
**(Olodaterol) Respimat
Inhalation Spray**

NDA Type: Standard

On **initial** overview of the NDA application for filing:

76

	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		
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).	X		Formulation used in toxicology studies same as clinical 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 <u>submitted</u> a rationale to justify the alternative route?	X		The inhalation route of administration was used for nonclinical studies.
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		Pivotal studies were conducted in accordance with GLP regulations.

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

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
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 PLR format. Text will be reviewed and edited after review of data.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Chemist identified 1 impurity above ICH threshold (b) (4). Review of data found that it is qualified for the maximum proposed clinical dose of 5 µg/day.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

NDA 203108 is fileable from the Pharmacology/Toxicology perspective.

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

No comments to the applicant.

Carol M. Rivera-Lopez, Ph.D. (see electronic signature)

 Reviewing Pharmacologist Date

Molly E. Shea, Ph.D. (see electronic signature)

 Team Leader/Supervisor Date

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

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/s/

CAROL M RIVERA-LOPEZ
06/26/2012

MOLLY E SHEA
06/26/2012
I concur.