ATTACHMENT 2

Pharmacology and Toxicology Review

By

Dr. Satish Tripathi

September 17, 1997

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 2

IND No.:	46,687
Serial No(s).:	017
Date(s) of Submission:	04/09/97
Information to be Conveyed to Sponsor:	Yes (X), No ( )
Reviewer:	Satish C. Tripathi, Ph.D.
Date Review Completed:	09/17/97
Sponsor:	Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877 (Tel. 203-798-5337/5684).
Manufacturer (if different):	Boehringer Ingelheim KG, Germany
Drug Name: Primary:	Ba 679 BR
Other Names:	Tiotropium bromide
Chemical Name:	[7(S)-(1 ,2 ,4 ,5 ,7)]-7-[(hydroxydi-(2-thienyl)acetyl)oxy]-9,9 dimethyl-3-oxa-9-azonia-tricyclo[3.3.1.$0^{2,4}$]nonane bromide hydrate.
Molecular Weight:	490.4 (hydrate); 472.41 (anhydrous)
Molecular Formula:	$C_{19}H_{24}BrNO_5S_2$ (hydrate)
Related INDs/NDAs/DMFs:	None
Class:	Anticholinergic agent as bronchodilator.

Indication: Treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD).

Clinical Formulation: [redacted] hard gelatin capsules [redacted] containing a white powder for inhalation.

Previous Review, Date, and Reviewer: Original, Review No. 1; 09/03/1996; Satish C. Tripathi

Studies Reviewed in this IND:

PHARMACODYNAMICS
Ba 679 BR: Validation of antagonism against pilocarpine-induced salivation (inhalation route), Vol. C11.1 Ba 679 BR: Validation of measurement of inhibition of pilocarpine-induced salivation (inhalation route), Vol. C11.1 Ba 679 BR: Antagonism against pilocarpine-induced salivation (inhalation route), Vol. C11.1 Ba 679 BR: Antagonism against pilocarpine-induced salivation (subcutaneous route), Vol. C11.2
SAFETY PHARMACOLOGY
Ba 679 BR: Effect on G.I. transit in male mice, Vol. C11.2
GENERAL TOXICITY
Ba 679 BR: Acute (i.v.) toxicity study in Wistar and Fischer rats, Vol. C11.2.
REPRODUCTIVE TOXICITY
Ba 679 BR: Reproductive toxicity (Segment III) study in rats, Vol. C11.2.
SPECIAL TOXICITY
Ba 679 BR: Local tolerance in rats via paravenous injection, Vol. C11.1. Ba 679 BR: Local tolerance in rabbit ear via intravenous route, Vol. C11.1. Ba 679 BR: Local tolerance in rabbit ear via intraarterial route, Vol. C11.1. Ba 679 BR: In vitro hemolytic effect, Vol. C11.1.

Studies Not Reviewed in this IND: None

Studies Previously Reviewed: See Original IND Review of September 03, 1996.

Note: Portions of this review were excerpted directly from the sponsor's submission.

PHARMACODYNAMICS

Mice: Validation of inhibition of pilocarpine-induced salivation (inhalation route): Single Dose

This study was aimed to validate the measurement of salivation inhibition in mice following exposure to drug via inhalation.

Mice (40/Replicate; 10/Replicate/group) were exposed to 0.25, 1.0, and 4.0 µg/kg/day of the drug via snout only inhalation. One hour fifty minutes after the end of inhalation exposure, each animal was administered urethane anesthetic (2.0 g/kg). Ten minutes later, each animal was administered pilocarpine nitrate (1.5 mg/kg) solution in saline subcutaneously in the lower dorsal region towards the tail at a dosing volume of 0.1 mL/10 g body weight. ED_{50} values for pilocarpine-induced salivation inhibition were 2.18 µg/kg for replicate 1 and 1.63 µg/kg for replicate 2. Decreased salivation was associated with increased dose in a dose dependent fashion.

Mice: Validation of inhibition of pilocarpine-induced salivation (inhalation route): Multiple Dose

Mice were exposed to the drug for 12 days (using same dosage and procedure as used in single dose study mentioned above) via snout only inhalation. Treatment resulted in ED_{50} (pilocarpine-induced salivation inhibition) of 0.31 µg/kg. Decreased salivation was associated with increased dose in a dose dependent fashion.

Mice: Inhibition of pilocarpine-induced salivation (inhalation route):

Mice were exposed to 0, 0.25, 1.0, 4.0, and 16.0 µg/kg of the drug via snout only inhalation once to the groups with pretreatment times of 2, 6, and 12 hours and once daily over a period of 12 days to the groups with a pretreatment time of 12 days. Pilocarpine nitrate was administered subcutaneously to each animal using same dose and concentration as mentioned above. Decrease in pilocarpine-induced salivation was measured 2, 6, and 14 hours after inhalation exposure for 2, 6, and 12 hour pretreatment groups and 2 hours after exposure on Day 12 for the group with 12 days pretreatment time. ED_{50} values for pilocarpine-induced salivation inhibition were 3.54, 3.26, and 11.6 µg/kg for 2, 6, and 14 hour pretreatment groups and 1.3 µg/kg for 12 day pretreatment group.

Mice: Inhibition of pilocarpine-induced salivation (subcutaneous route):

Mice were administered 0.5, 1.5, and 5 µg/kg of the drug subcutaneously for 1, 2, 5, and 12 days. Treatment resulted in inhibition of pilocarpine-induced salivation. Data showed lower ED_{50} values [2.0, 1.1, 1.0, and 0.7 µg/kg for 2 hr (after first dose), 2 days, 5 days, and 12 days post dosing, respectively] with increased duration of treatment.

SAFETY PHARMACOLOGY

Mice: Effect of BA 679 BR treatment G.I. transit

Male mice were treated with the drug once daily at doses of 100, 1000, and 10000 µg/kg subcutaneously after acute (2 hours) and subacute (2, 5, and 12 days). Treatment resulted in 22 to 43% inhibition of G.I. transit for the dose range used. ED_{30} (s.c.) inhibition dose of Tiotropium bromide was estimated to be 760 µg/kg. The calculated threshold dose for inhibitory effects on G.I. transit was 80 µg/kg (s.c.).

GENERAL TOXICITY

Acute (i.v.) toxicity study in Wistar and Fischer rats
Boehringer-Ingelheim Study U95-0485, Vol. C11.2.

Wistar and Fischer rats (5/Sex/group for both strains) were treated with 16, 20, and 25 mg/kg of the drug via i.v. which was followed by an observation period of 14 days. Clinical signs of toxicity in Wistar rats were as follows: irregular breathing (all doses), breathing noises (LD), tremor (LD, MD males), ataxia (MD and HD males), dyspnea and respiratory arrest (HD), cardiac arrest (MD females, HD males), reduced motility (females: all doses), gag reflex (MD female), and convulsions (HD females). Signs of toxicity in Fischer rats were: irregular breathing (LD, MD), respiratory arrest (MD, HD), reduced motility (LD, MD), ataxia (LD, MD: male only), dyspnea (MD: both sexes; LD and HD: males), tachypnea (LD: males; MD: both sexes), cardiac arrest (MD: females; HD: both sexes), gag reflex (HD females), and convulsion (MD and HD females). Thus, clinical signs of toxicity were similar in two strains.

Treatment resulted in maximum nonlethal dose of 16 mg/kg and minimum lethal dose of 20 mg/kg for both strains of rats. The LD_{50} values for Wistar and Fischer rats were 20.6 and 22.4 mg/kg, respectively.

REPRODUCTIVE TOXICITY

Rat: Segment III Inhalation Study
Boehringer-Ingelheim Study U96-2493, September 2, 1996, Vol. C11.2.

Study Dates: January 06, 1995 to May 23, 1995
Testing Lab: _____
Test Article: Drug solution was prepared in 3 strengths (0.002%, 0.02%, and 0.4% w/v) in an appropriate vehicle (100 mL of vehicle contained 10.0 mg benzalkonium chloride, 50.0 mg disodium edetate, 8.4 mg citric acid monohydrate, 0.8 mL of 0.1 N NaOH, and 0.6 mL of 0.1N HCl).
GLP: Signed GLP Statement was included.

Method:

Species/Strain: Rat (Crl: CD^{R}BR VAF/Plus strain).
Animals: F_0: 108 Females, 25/group; F_1: 20/Sex/group for assessing reproductive capacity and 20/Sex/group for behavioral examinations.
Route: Inhalation (snout only exposure).
Dosage: 0 (Vehicle Control), 0.01 mg/kg/day (LD), 0.1 mg/kg/day (MD), and 2 mg/kg/day (HD).
Study Design: Females were treated from Day 17 of gestation to weaning (Day 21 *post partum* for Control, LD, and MD groups and Day 28 *post partum* for HD group) for one hour each day.

Measurements and observations:

F_0 generation: Clinical signs, mortalities, food consumption, body weight change, pregnancy rate, and duration of pregnancy; in case of litters, reflexes (surface righting, startle, air righting, and pupil), pinnae detachment, incisor eruption, and eye opening. Shortly after weaning (Day 21 *post partum* for Control, LD, and MD groups and Day 28 *post partum* for HD group), excess pups and parents were sacrificed and examined externally and internally for abnormalities

F_1 generation: Clinical signs, Body Weights, and onset of vaginal opening in females from 28 days post partum and occurrence of cleavage of the balanopreputial skinfold in males from 35 days post partum; Developmental/behavioral examinations included actimat test (at 5 weeks age) and one trial passive avoidance test (at 7-8 weeks of age); and assessment of reproductive capacity (at 84 days age; mated on one male to one

female basis for 20 days). On Day 20 of pregnancy, the animals were sacrificed, necropsied, and examined for congenital abnormalities and macroscopic pathological

changes in maternal organs. Live youngs were clinically examined. Parent males were necropsied following completion of the Day 20 sacrifice at about 18 weeks of age. All males were examined for external and internal abnormalities.

Results:

I. F_0 Generation:

a) Adult Animals:

Mortality: There was 1 mortality at MD on Day 19 *post partum*. This animal showed pupil dilation following inhalation exposure and congested lungs during *post mortem* examination. Since there was no mortality at HD, the single mortality at MD should be regarded as not drug-related.

Clinical Observations: Marked post-dose pupil dilation seen for MD and HD animals throughout the treatment period. LD animals showed slight pupil dilation after dosing.

Food Consumption: Treatment resulted in statistically significantly decreased food consumption at all doses (LD 10%, MD 35%, HD 55%) during Day 17 to Day 19 of pregnancy and at HD (Days *Post Partum*: 1-6: 19%; 7-13: 25%; 14-20: 21%) during the *post partum* period.

Body Weights: Treatment resulted in statistically significantly decreased bodyweight gains (LD 22%, MD 61%, HD 98%) on Days 17 to 20 of gestation.

Duration of Pregnancy and Gestation Index: No toxicologically significant treatment-related effect.

Terminal Autopsy: No significant finding was reported.

b) Litter data:

Litter Parameters: Treatment resulted in litter loss at MD (3) and HD (10). There was one LD female that had only one implantation. The sponsor explained this incidence as an atypical finding and indicated that one implant is generally considered insufficient to maintain pregnancy. The Reviewer has asked the sponsor to provide justification for this explanation. There was

decrease in pup weights from the day of pregnancy to Day 21 post partum at MD (12%) and HD (33%). HD resulted in 10% decrease in survival index of pups on Day 4 *post partum*.

Pre-Weaning Development: There was statistically significant delay in mean age for pups attaining startle response (MD: 0.6 day, HD: 1.9 days) or air righting reflex (MD: 0.5 day; HD: 1.8 days).

Terminal Autopsy: No toxicologically significant treatment-related effects.

II. F_1 Generation:

a) Parent Animals:

Mortality and Clinical Observations: There were two mortalities (1 LD, 1 MD). The LD female had congested lungs. The MD female was sacrificed with a suspected damaged hindlimb. The MD female had fractured left hindlimb tibia and swollen paw.

Body Weights: Treatment resulted in statistically significantly decreased body weight gains at MD (11%) and HD (10%) males of F_1.

Vaginal Opening and Balanopreputial Cleavage: Offsprings of both sexes showed statistically significant delay in mean ages of sexual maturation in MD (♂: 1.6 days; ♀: 0.9 days) and HD (♂: 3.2 days; ♀: 2.5 days) groups. There was no effect on sexual maturation in the offsprings (either sex) of LD-treated females.

Post Weaning Behavioral Tests:

Actimat Test: No significant effect.

Passive Avoidance Test: No significant effect on pre-shock entry times (Day 1) and post-shock performance (Day 2 and Day 22)

b) Assessment of Reproductive Capacity:

Mating Performance, Pregnancy Rate, Copulation, and Fertility Indices: No significant effect.

Litter Data:

Litter Values and Sex Ratio: No significant effect.

Abnormalities: Among the dams derived from HD treated F_0 females, the litter of one dam did not survive beyond Day 4 *post partum*. There were no significant effects on mean values for litter and pup weights, pup mortality, litter size, % of males, and survival indices of pups.

Terminal Autopsy: No toxicologically significant treatment-related effects.

SPECIAL TOXICITY

Rat: Local (Paravenous) Tolerance Study

Boehringer-Ingelheim Study U95-0136, Vol. C11.1.

Study Dates: February 01, 1995 to February 02, 1995
Testing Lab: Boehringer-Ingelheim, Department of Experimental Pathology and Toxicology, Germany
Test Article: Injectable solution of the drug (0.006%; 0.06 mg/mL) in saline.
GLP: The study was conducted under GLP compliance of OECD and drug regulating agency (Chemikaliengesetz) of Germany.

Rats (2/Sex/group) were administered Test article, vehicle (negative control) or $CaCl_2$-$MgCl_2$ solution (positive control) laterally (dosing volume: 0.2 mL) and medially (dosing volume: 0.2 mL) to the left jugular vein. Treatment resulted in no toxicologically significant effect. Positive control yielded slight to moderate erythema, slight to moderate bluish red discoloration, slight to severe swelling, and slight necrosis.

Rabbit: Local (Intravenous) Tolerance Study

Boehringer-Ingelheim Study U95-0137, Vol. C11.1.

Study Dates: January 13, 1995 to February 02, 1995
Testing Lab: Boehringer-Ingelheim, Department of Experimental Pathology and Toxicology, Germany
Test Article: Injectable solution of the drug (0.006%; 0.06 mg/mL) in saline.
GLP: The study was conducted under GLP compliance of OECD and drug regulating agency (Chemikaliengesetz) of Germany.

Rabbits (2/Sex/group) were administered drug solution (dosing volume: 2.53 mL) or vehicle into the marginal vein of the ear over a period of 16 minutes. Treatment resulted in erythema (slight) in one animal and lasted one day post dosing and bluish red discoloration at the injection site in another animal that lasted 4 days. The administration of the placebo solution induced slight erythema in one animal and lasted for one day post administration. Thus, intravenous administration of drug induced local effect at the injection site.

Rabbit: Local (Intraarterial) Tolerance Study

Boehringer-Ingelheim Study U95-0138, Vol. C11.1.

Study Dates: January 31, 1995 to February 10, 1995
Testing Lab: Boehringer-Ingelheim, Department of Experimental Pathology and Toxicology, Germany
Test Article: Injectable solution of the drug (0.006%; 0.06 mg/mL) in saline.
GLP: The study was conducted under GLP compliance of OECD and drug regulating agency (Chemikaliengesetz) of Germany.

Rabbits (2/Sex/group) were administered Test (dosing volume: 2.53 mL) or Control article (saline) intraarterially into the central artery of the ear over a period of 16 minutes. Administration of the drug resulted in slight bluish red discoloration (3/4 animals) at the injection site that lasted for up to five days. Administration of the placebo also resulted in bluish red discoloration (3/4 animals) that lasted for up to four days. Thus, injection solution in saline induced local effect at the injection site.

In vitro Hemolytic Potential

Boehringer-Ingelheim Study U95-0177, Vol. C11.1.

Study Date: February 03, 1995
Testing Lab: Boehringer-Ingelheim, Department of Experimental Pathology and Toxicology, Germany
Test Article: Injectable solution of the drug (0.003% or 0.03 mg/mL and 0.006% or 0.06 mg/mL) in saline.
GLP: The study was conducted under GLP compliance of OECD and drug regulating agency (Chemikaliengesetz) of Germany.

An *in vitro* test to determine hemolytic effect of the injectable solutions (0.03 and 0.06 mg/mL) of the drug was performed with fresh citrated human blood. One mL of citrated blood was mixed with 1 mL of: 0.003% or 0.006% drug solution; 1% saponin solution (positive control); 0.9% NaCl (saline) solution; placebo solution (Na-citrate 0.11 mol/L); and placebo solution diluted 1:2 with saline solution. The hemoglobin content of the supernatant was determined photometrically.

The percentage hemolysis in the above experiment was as follows: 0.006% drug: 69%; 0.003% drug: <1%; saline: <1%; placebo (sodium citrate): 60%; placebo solution diluted with 1:2 saline: <1%; and saponin solution: 100%. Thus, hemolysis effect in the 0.006% drug group was due to placebo.

SUMMARY AND EVALUATION

Pharmacology: Tiotropium bromide (Ba 679 BR) administered via inhalation (ED_{50} = 3.5 µg/kg for single dose and 1.3 µg/kg for 12 day treatment group) or subcutaneous (2.0 µg/kg after first dose and 0.7 µg/kg for 12 day treatment group) route inhibited pilocarpine-induced salivation in mice indicating anticholinergic effect.

Safety Pharmacology: At a dose range of 0.1 to 10 mg/kg/day, a subcutaneous administration of the drug resulted in 22 to 43% inhibition of G.I. transit in mice. The calculated threshold dose of the drug for inhibitory effects on G.I. transit was 80 µg/kg (s.c.).

Toxicology: In Wistar and Fischer rats, the LD_{50} values for intravenous (acute) administration of the drug were 20.6 and 22.4 mg/kg, respectively. Clinical signs of toxicity (irregular breathing, breathing noises, tachypnea, tremor, ataxia, dyspnea, respiratory arrest, cardiac arrest, reduced motility, gag reflex, and convulsions) were comparable to signs seen in a previous single dose (acute) i.v. study in rats. In a previous study (U90-0493; See Original Review of 09/03/1996 by Dr. Satish Tripathi) conducted on rats (Chbb:THOM), the LD_{50} value for i.v. administration was 20.5 mg/kg and clinical signs of toxicity were reduced motility, dyspnea, tachypnea, tremor, and convulsions.

Reproductive Toxicity: In a Segment III study, treatment of pregnant rats with drug [0.01 mg/kg/day (LD); 0.1 mg/kg/day (MD); and 2.0 mg/kg/day (HD) resulted in a statistically significant dose dependent decrease in body weight gains and food consumption. There was 1 mortality (F_0 dam) reported at 0.1 mg/kg/day. Treatment resulted in fetocidal activity at 0.1 and 2.0 mg/kg/day (litter loss: 3/25 and 10/25, respectively). In addition, embryocidal activity was seen at 0.01 mg/kg/day (litter loss: 1/25). However, this affected female had only one implantation. The sponsor explained this incidence as an atypical finding and indicated that one implant is generally considered insufficient to maintain pregnancy. The sponsor (via teleconference on August 12, 1997) has been asked to provide justification for this explanation. If justified, the NOAEL for this study would be 0.01 mg/kg/day which has also been stated by the sponsor.

The following argument also seems to favor 0.01 mg/kg/day to be the NOAEL: Irrespective of all the doses used in Segment I and III studies in rat were the same, in the Segment I study, there was 1 litter loss at MD (0.1 mg/kg/day) and no litter loss at HD (2.0 mg/kg/day) or LD (0.01 mg/kg/day). Both the MD as well as HD doses caused maternal effects as shown by decreased bodyweight gains in dams (Wk 7-8: MD 86%, HD 100%; Wk 7-12: MD 13%, HD 38%). In Segment III study in rat, incidence of litter loss (LD 1/25, MD 3/25, HD 10/25) was higher although the duration for which the treatment was given was shorter (than Segment I). Since 1 litter loss at MD in Segment I (and no litter loss at LD or HD in Segment I), the single litter loss at LD due to only one implantation in Segment III could be a spurious finding.

Special Toxicity: At a concentration of 0.06 mg/mL used in the paravenous administration of the drug in rats, treatment showed no effect. In rabbits, intravenous or intraarterial administration of the drug (0.06 mg/mL) resulted in no drug-related effects (placebo and drug both caused bluish red discoloration). At a concentration of 0.06 mg/mL (volume: 1.0 mL), Ba 679 BR caused hemolysis when mixed with citrated blood (volume: 1 mL). However, no hemolysis was seen at a concentration of 0.03 mg/mL. Hemolysis was observed in citric acid group.

The sponsor has been asked (via teleconference on August 12, 1997) that the finding of embryotoxicity and fetotoxicity in Segment III and Segment I studies in rats and Segment II study in rabbits should be incorporated in all clinical protocols. Furthermore, the data to support their justification for the litter loss at the LD group was atypical finding should be provided as conveyed in the same teleconference.

RECOMMENDATION

Pursuant to our discussion with the sponsor over a teleconference on August 12, 1997, the sponsor should be asked to provide data to support that total litter loss at the low dose is an atypical finding [redacted] There is no additional pharmacology/ toxicology recommendation at this time.

Draft Letter to the Sponsor:

Please refer to our teleconference on August 12, 1997 and provide data to support that total litter loss at the low dose in Segment III study in rats is an atypical finding. [redacted]

File Name: N:\IND\46687\PHARM\97-04-09.REV

/S/

Satish C. Tripathi, Ph.D.
Pharmacology/Toxicology Reviewer

Original IND
C.C. /Division File
/Joseph Sun, Team Leader (Pharmacology/Toxicology)
/Martin Himmel, Deputy Division Director
/Anne Trontell, Medical Reviewer
/Betty Kuzmik, Project Manager
/Satish Tripathi, Pharmacology/Toxicology Reviewer

ATTACHMENT 3

Pharmacology and Toxicology Review

By

Dr. Satish Tripathi

December 10, 1997

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 3

IND No.:	46,687
Serial No(s).:	030
Date(s) of Submission:	10/10/97
Information to be Conveyed to Sponsor:	Yes ( ), No (X)
Reviewer:	Satish C. Tripathi, Ph.D.
Date Review Completed:	12/10/97
Sponsor:	Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877 (Tel. 203-798-5337/5684).
Manufacturer (if different):	Boehringer Ingelheim KG, Germany
Drug Name: Primary:	Ba 679 BR
Other Names:	Tiotropium bromide
Chemical Name:	[7(S)-(1 ,2 ,4 ,5 ,7)]-7-[(hydroxydi-(2-thienyl)acetyl)oxy]-9,9 dimethyl-3-oxa-9-azonia-tricyclo[3.3.1.$0^{2,4}$]nonane bromide hydrate.
Molecular Weight:	490.4 (hydrate); 472.41 (anhydrous)
Molecular Formula:	$C_{19}H_{24}BrNO_5S_2$ (hydrate)
Related INDs/NDAs/DMFs:	None
Class:	Anticholinergic agent as bronchodilator.

Indication: Treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD).

Clinical Formulation: [redacted] hard gelatin capsules [redacted] containing a white powder for inhalation.

Previous Review, Date, and Reviewer:

Date of Submission	Date of Review	Reviewer
11/30/94	08/26/96	Satish C. Tripathi
04/09/97	09/17/97	Satish C. Tripathi

Studies Reviewed in this IND:

REPRODUCTIVE TOXICITY
Ba 679 BR: Preliminary Oral Reproductive toxicity (Segment I) study in rats.
Ba 679 BR: Preliminary Oral Reproductive toxicity (Segment III) study in rats.

Note: Portions of this review were excerpted directly from the sponsor's submission.

REPRODUCTIVE TOXICITY

Rat: Preliminary Segment I Oral Study
Boehringer-Ingelheim Study U90-0539, 11 July, 1990

Study Dates: 29 January, 1990 to 03 April, 1990
Testing Lab: Department of Experimental Pathology and Toxicology, Boehringer-Ingelheim.
Test Article: Batch F. Drug solution was prepared in 3 strengths (1.5%, 5%, and 15% w/v) in 0.5% Tylose (Vehicle).
GLP: Signed GLP Statement was included.

Method:

Species/Strain: Rat (Chbb:THOM strain).
Animals: 40/Sex; 10/Sex/group.
Route: Oral.
Dosage: 0 (Vehicle Control); 75 mg/kg/day (LD); 250 mg/kg/day (MD); 750 mg/kg/day (HD). The dosing volume for all groups was 0.5 mL/100 g.
Study Design: Males were treated 7 days a week for a period of 2 weeks before mating and through copulation. It should be noted that treatment of males for 2 weeks prior to mating is not a standard practice. Females were treated from 2 weeks prior to mating and through gestation and the lactation.

Clinical Observations: F_0 Generation:

1. Adult Animals:

Clinical Signs: Daily.
Mortalities: Daily.
Body Weights: Daily.
Food Consumption: Weekly.
Pregnancy Rate: Vaginal smears examined daily for the state of the estrous cycle from the start of administration until the beginning of insemination. Percentage of pregnant rats determined for all groups.
Mating Performance: Fertility index determined by number of pregnant animals/number of paired animals x100.
Duration of Pregnancy: Gestation period was determined for all groups.

2. Litter data:

Viability Index: Number of young which died by Day 4.
Weaning Index: Number of young which died from Day 4 after adjustment until Day 21.
Body weight of Pups: Days 4, 7, 14, and 21

RESULTS

1. Adult Animals:

Clinical Signs: Treatment resulted in chromodacryorrhea (shedding of tears with blood-like color) of slight to moderate extent (♂: LD 2/10, MD 5/10, HD 10/10; ♀: LD 3/10, MD 7/10, HD 10/10).

Mortalities: Treatment resulted in death of 3 HD males: of these, two males died on Day 15 and had congestion and edema of the lung and one animal had cachexia, bronchopneumonia, and esophagus filled with bedding material. Four females (3 HD and 1 MD) also died during dosing period: 1 HD female had edema and emphysema of the lung, 1 HD female had slightly bloody hydrothorax, and 1 HD female had congestion and edema of the lung; 1 MD female had congestion, edema, and emphysema of the lung and petechial pancreatic hemorrhages.

Body Weights: Treatment resulted in decreased bodyweight gains (♂: LD 31%, MD 52%, HD 38%; ♀: MD 28%, HD 24%) before mating. Pregnant females had decreased bodyweight gains from gestation Day 1 to Day 22 (LD 34%, MD 28%, HD 24%) which was not dose dependent.

Food Consumption: Treatment resulted in decreased food consumption (♂: LD 24%, MD 27%, HD 39%; ♀: LD 20%, MD 30%, HD 30%) during the Week 1 but not during Week 2 prior to mating.

Mating Performance and Pregnancy Rate: There was drug-related decrease in mating index at HD (LD and MD: 10% each; HD:29%). In addition, HD resulted in decreased fertility index/pregnancy rate (Control: 9/10 or 90%; LD: 8/9 or about 90%; MD: 4/8 or 50%; HD 3/5 or 60%; these data are based on number of animals that were inseminated).

Duration of Pregnancy: No toxicologically significant treatment-related effects.

2. Litter Data:

Litter Values: There was total litter loss in one HD dam. No toxicologically significant treatment-related effect was seen on viability index. Lower Weaning Index value was seen in HD group (69.6%) compared to Control group (95.3%).

Fetal Examination: No malformations or variations were seen in pups.

Litter Values to Day 21 Post Partum: There was significantly larger number of loss of pups/litter seen at HD (Control: 0.67, LD: 1.3, MD: 0.75, HD: 3.3) during Days 1-21 of birth.

Pup Weights: Pups from treatment groups had lower (LD 10%, HD 10%) body weights at birth compared to pups from control group. Reduced bodyweight gains were seen in the pups from treatment groups from Day 1 to 4 (LD 37%, HD 44%) as well as from Day 4 to Day 21 (LD 21%, MD 17%, HD 29%) post partum.

Decreased mating performance (HD) and fertility index (MD, HD) were seen at maternal toxic doses (mortality) and, therefore, these findings could be secondary to maternal toxicity. The drug was fetocidal at 750 mg/kg/day. The drug was embryo- and feto- cidal in Segment I inhalation study in rats. No information was provided on the incidence of resorption and/or pre- and post-implantation loss.

Rat: Preliminary Segment III Oral Study
Boehringer-Ingelheim Study U90-0540, 25 July, 1990

Study Dates: February, 1990 to April, 1990
Testing Lab: Department of Experimental Pathology and Toxicology, Boehringer-Ingelheim.
Test Article: Batch F. Drug solution was prepared in 3 strengths (1.5%, 5%, and 15% w/v) in 0.5% Tylose (Vehicle).
GLP: Signed GLP Statement was included.

METHOD

Species/Strain: Rat (Chbb:THOM strain).
Animals: F_0: 40 Females, 10/group; F_1: On Day 4 post partum, the litters were reduced to 8 pups (4 males and 4 females, if possible).
Route: Oral.
Dosage: 0 (Vehicle Control)); 75 mg/kg/day (LD); 250 mg/kg/day (MD); 500 mg/kg/day (HD). The dosing volume for all groups was 0.5 mL/100 g.
Study Design: Pregnant females were treated from Day 16 of gestation to weaning (Day 21 *post partum*).

Measurements and observations:

a) Adult Animals:

Clinical signs, mortalities, food consumption, body weight change, and duration of pregnancy. The litters were assessed for size, stillbirths, number of viable offspring, and gross abnormalities.

b) Litter Data:

Viability Index (number of young which died by Day 4); Weaning Index (number of young which died from Day 4 after adjustment until Day 21); Clinical signs; Body Weights on Days 4, 7, 14, and 21; and growth, function, maturation, and behavior of the young (tests: erection of pinnae: Days 4-6, beginning of fur growth: Days 6-8, eruption of maxillary incisors: Days 11-13, running with raised venter: Days 11-13, and separation of eye lids: days 14-17).

RESULTS

a) Adult Animals:

Clinical Signs: Treatment resulted in chromodacryorrhea (shedding of tears with blood-like color) of mild to moderate extent (LD 5/10, MD 10/10, HD 8/10).

Mortalities: There were 2 mortalities in the HD group: One animal died on Day 5 of lactation and had dilatation of caecum, colon, and rectum and acute hyperemia of liver and kidneys; one animal had to be sacrificed on Day 23 of gestation due to exhaustion and had fetal dystocia (difficult/abnormal labor) which was caused by a transverse presentation of one pup in the birth canal.

Body Weights: Body weight gains were decreased (LD 84%, MD 92%, HD 75%) from Day 16 to Day 22 of gestation.

Food Consumption: Decreased food consumption (LD 39%, MD 48%, HD 35%) was seen during Week 3 of gestation.

Duration of Pregnancy: No toxicologically significant treatment-related effects.

b) Litter data:

Litter Values: Four dams had complete litter loss (1 LD, 1MD, 2 HD). In addition, the sponsor stated that one MD dam ate all its pups before they could be registered; this could not be confirmed since pathology report and individual line listings were not provided. One LD dam showed only 2 implantation sites. It was not indicated whether the dam with 2 implantation sites gave birth to pups or not. There was 1 stillbirth in MD group.

Pup Weights: Treatment resulted in decreased birth weights (LD 14%, MD 14%, HD 12%) and decreased body weight gains from Day 1 to Day 4 (LD 32%, MD 46%, HD 43%) and from Day 4 to Day 21 (LD 15%, MD 18%, HD 18%) of lactation.

Examination of Pups: There were no variations or malformations seen in the pups from treatment groups. Compared to controls, decreased values of viability index in the treatment groups (Control 100%, LD 83%, MD 80%, HD 77%) were present but there was no significant effect on weaning index. No toxicologically significant treatment-related effects were seen on growth, function, maturation, and behavior of the young (erection of pinnae, beginning

of fur growth, eruption of maxillary incisors, running with raised venter, and separation of eye lids).

The drug was fetocidal at 75 mg/kg/day. Fetocidal activity was also seen in Segment III inhalation study in rats. Maternal toxicity (mortality) was seen at HD. Data on effect of drug on reproductive capability of F_1 were not provided.

SUMMARY AND EVALUATION

Reproductive Toxicity: In a Preliminary Segment I oral study in rats, male and female animals were treated with the drug (75, 250, and 750 mg/kg/day) for 2 weeks. It should be noted that treatment of males for 2 weeks prior to mating is not a standard practice. All animals were continued to be dosed during pairing. Females were continued to be dosed to gestation and lactation. Treatment resulted in incidence of chromodacryorrhea (all doses). There were 7 drug-related mortalities (♂: 3 HD; ♀: 1 MD, 3 HD). Bodyweight gains were decreased before mating (both sexes) as well as from gestation Day 1 to Day 22 (females). Food consumption was reduced (all doses) during Week 1 but not during Week 2 prior to mating. There was drug-related decrease in mating index (HD) and fertility index (MD and HD at maternal toxic doses and, therefore, these findings could be secondary to maternal toxicity(mortality). The drug was fetocidal at 750 mg/kg/day. The drug was embryo- and feto- cidal in Segment I inhalation study in rats. There was total litter loss in one HD dam and decreased value of weaning index seen in HD group. MD and HD were maternal toxic doses. There was larger number of pup loss at HD during Days 1-21 of birth. There was drug-related reduction in pup weights at birth (LD, HD) and reduced bodyweight gains in pups from Day 1 to 4 (LD, HD) and Day 4 to Day 21 (all doses) post partum. There was no clear NOAEL in this study. No information was provided on the incidence of resorption and/or pre- and post-implantation loss. Line listings for individual animal data were not provided.

In a Preliminary Segment III oral study, treatment of pregnant rats with drug (75, 250, and 500 mg/kg/day) resulted in chromodacryorrhea (all doses). There were 2 drug-related mortalities (HD). Bodyweight gains and food consumption were significantly decreased (all doses) during Day 16 to Day 22 of gestation. HD was maternal toxic dose. Treatment resulted in litter loss and decreased birth weights and bodyweight gains at all doses. Decreased viability index was reported at all doses. The drug was fetocidal at 75 mg/kg/day. Fetocidal activity was also seen in Segment III inhalation study in rats. Data on effect of drug on reproductive capability of F_1 were not provided. No information was provided on the incidence of resorption and/or pre- and post-implantation loss. Line listings for individual animal data were not provided.

The adjusted (for 1 mg/kg/day body weight) drug plasma levels in the Chbb:THOM rats via inhalation route (3.6 ng/mL) were twice those via p.o. route (1.7 ng/mL). Although bioavailability of the drug via oral route is only 1%, systemic exposures in the Segment I and III oral studies (due to higher doses) were much higher than those in Segment I and III inhalation studies.

Data from above Segment I and Segment III oral rat studies compared to similar studies via inhalation route might suggest that top doses in inhalation studies may not be sufficiently high and that the sponsor might need to conduct additional studies via oral route to fully determine reproductive toxicity potential of the drug. However, there were mortalities of dams seen in all reproductive toxicity studies via inhalation route (Segment I Rat: 2 MD males, 4 HD males, and 2 HD females; Segment III Rat: 1 MD) testifying that Ba 679 BR was adequately tested. Thus, it can be concluded that the reproductive toxicity potential of the drug has been adequately tested and that the sponsor need not conduct complete Segment I and III oral studies in rats.

RECOMMENDATION

There is no specific recommendation at this time.

/S/

Satish C. Tripathi, Ph.D.
Pharmacology/Toxicology Reviewer

Original IND
C.C. /Division File
/Joseph Sun, Team Leader (Pharmacology/Toxicology)
/Martin Himmel, Deputy Division Director
/Anne Trontell, Medical Reviewer
/Betty Kuzmik, Project Manager
/Satish Tripathi, Pharmacology/Toxicology Reviewer

ATTACHMENT 4

Pharmacology and Toxicology Review

By

Dr. Satish Tripathi

January 8, 1998

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 4

IND No.:	46,687
Serial No(s).:	031
Date(s) of Submission:	11/05/97
Information to be Conveyed to Sponsor:	Yes ( ), No (X)
Reviewer:	Satish C. Tripathi, Ph.D.
Date Review Completed:	01/08/98
Sponsor:	Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877 (Tel. 203-798-5337/5684).
Manufacturer (if different):	Boehringer Ingelheim KG, Germany
Drug Name: Primary:	Ba 679 BR
Other Names:	Tiotropium bromide
Chemical Name:	[7(S)-(1 ,2 ,4 ,5 ,7)]-7-[(hydroxydi-(2-thienyl)acetyl)oxy]-9,9 dimethyl-3-oxa-9-azonia-tricyclo[3.3.1.$0^{2,4}$]nonane bromide hydrate.
Molecular Weight:	490.4 (hydrate); 472.41 (anhydrous)
Molecular Formula:	$C_{19}H_{24}BrNO_5S_2$ (hydrate)
Related INDs/NDAs/DMFs:	None
Class:	Anticholinergic agent as bronchodilator.

Indication: *Treatment of bronchospasm associated with chronic obstructive pulmonary* disease (COPD).

Clinical Formulation: [redacted] hard gelatin capsules [redacted], containing a white powder for inhalation.

Previous Review, Date, and Reviewer:

Date of Submission	Date of Review	Reviewer
11/30/94	08/26/96	Satish C. Tripathi
04/09/97	09/17/97	Satish C. Tripathi
10/10/97	12/10/97	Satish C. Tripathi

Studies Reviewed in this IND: Historical data on fate of small litters.

Note: Portions of this review were excerpted directly from the sponsor's submission.

BACKGROUND

Review of Submission (Serial No. 017 of 04/09/97) resulted in recommendation (Date of Review: 09/17/97) that the sponsor should provide data to support that total litter loss at the low dose in Segment III study in rats is an atypical finding. In addition, the sponsor was asked to incorporate the findings of embryo- and feto-toxicity in Segment I studies in rats and Segment II study in rabbits into the Informed Consent Section of all clinical protocols. This request was made via teleconference (See minutes of August 12, 1997). On the basis of a promise by the sponsor that a response would be provided within short period of time, a written letter asking for such information was not issued. The present submission by the sponsor addresses the above issue.

SUMMARY AND EVALUATION

Historical Control data contained in the above Submission obtained from a Contract Research Organization [redacted] showed that single implantations result in either resorption or birth of a pup that is either dead or live and if live then without survival (See Table below). Therefore, it can be concluded that single implantations may be insufficient to maintain pregnancy.

Group	Pregnancy Outcome	Litter Details
F_0 Control	Total Litter Loss = 1 Total Resorption = 1	1 Implant: 1 Live Pup born 1 Implant: No Young born
F_1 Control	Total Litter Loss = 2 Total Resorption = 1	1 Implant: 1 Dead Pup born 1 Implant: 1 Live Pup born 1 Implant: No Young born

These historical control data support the argument that total litter loss at the low dose in Segment III study in rats could be an atypical finding. In addition, these findings establish LD as a NOAEL for the Segment III study. Although MD and HD resulted in total litter loss, these doses were maternotoxic as reflected by severe decrease in bodyweight gains. Since there was sufficient decrease in bodyweight gain at LD in the Segment III study, the MD and HD (fetotoxic doses), in the face of maternotoxic effect, may not be of concern. It is now imperative that the recommendations made in my review of Submission 017 of 04/09/97 (Date of Review: 09/17/97) are no longer needed to be communicated to the sponsor. It implies that the sponsor need not modify Informed Consent of Clinical Protocols as the findings of embryo- and feto-toxicity in animal studies are no longer of concern.

RECOMMENDATION

Informed Consent of Clinical Protocols for this IND need not be modified to account for the findings of embryo- and feto-toxicity in animal studies.

/S/

Satish C. Tripathi, Ph.D.
Pharmacology/Toxicology Reviewer

Original IND
C.C. /Division File
/Joseph Sun, Team Leader (Pharmacology/Toxicology)
/Anne Trontell, Medical Reviewer
/Betty Kuzmik, Project Manager
/Satish Tripathi, Pharmacology/Toxicology Reviewer

Draft: 12/17/97

ATTACHMENT 5

Pharmacology and Toxicology Review

By

Dr. Timothy MoGovern

November 2, 2001

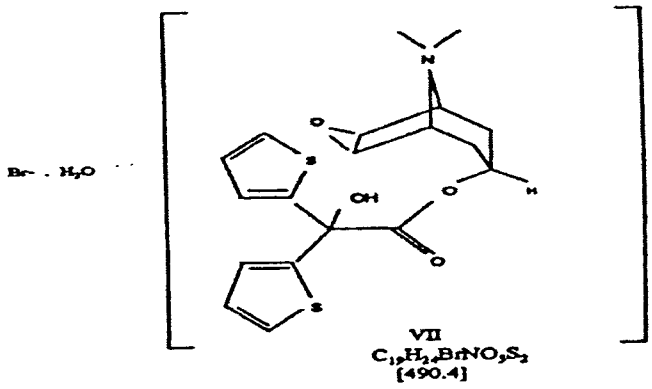
PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 46,687
Review number: 5
Sequence number/date/type of submission: 053/January 7, 2000/IT
Information to sponsor: Yes ( ) No (✔)
Sponsor and/or agent: Boehringer Ingelheim Pharmaceuticals, Inc.; Ridgefield, CT
Manufacturer for drug substance: Boehringer Ingelheim KG, Germany

Reviewer name: Timothy J. McGovern, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: November 2, 2001

Drug:

Trade name: NA
Generic name (list alphabetically): Tiotropium bromide
Code name: BA 679 Br
Chemical name: [7(S)-(1,2,4,5,7)]-7-[(hydroxydi-(2-thienyl)acetyl)oxy]-9,9 dimethyl-3-oxa-9-azonia-tricyclo[3.3.1.$0^{2,4}$]nonane bromide hydrate
CAS registry number: NA
Mole file number: NA
Molecular formula/molecular weight: $C_{19}H_{24}BrNO_5S_2$ (hydrate)/490.4 (hydrate); 472.41 (anhydrous)
Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: Anticholinergic

Indication: COPD/Asthma

Clinical formulation: Hard gelatin capsule containing Ba 679 Br and lactose monohydrate.

Route of administration: Dry powder inhalation

Proposed clinical protocol: None

Previous clinical experience: Phase 1 through Phase 3 clinical trials have been initiated for up to 6 months duration at 18 µg/day.

Previous reviews:

Original IND review	September 6, 1996	S. Tripathi
Review #2	September 17, 1997	S. Tripathi
Addendum to Review #2	September 22, 1997	S. Tripathi
Review #3	December 10, 1997	S. Tripathi
Review #4	January 8, 1998	S. Tripathi

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: Ba 679 BR (tiotropium bromide) is an anticholinergic agent under development for treatment of bronchospasm associated with COPD.

Studies reviewed within this submission:

Study	Res. Report #	Vol.
Safety Pharmacology:		
Inhaled Ba 679 Br and inhaled + i.v. corticosteroids in dogs.	U97-2730	17.1
Investigation of IV administered Tiotropium bromide in mice after i.p. injection of phenyl-p-benzoquinone in the writhing test	U98-2292	17.1
Investigation of IV administered Tiotropium bromide in anesthetized dogs for cardiovascular and respiratory effects	U98-2386	17.1
Investigation of BA 679 Br in isolated rectum from guinea pig for antagonism against the spastic action carbachol, histamine and BaCl2.	U98-2850 U98-2851	17.1 17.1
Effects of BA 679 Br on body temperature in conscious mice after IV administration.	U98-2879	17.1
Phenylenetrazole seizure in mice after IV pretreatment with BA 679 BR.	U99-0166 U99-0167	17.1 17.1
Pharmacokinetics:		
Excretion of ^{14}C-BA 679 BR into milk after IV administration to lactating rats.	U99-0205	17.1
Placental transfer of ^{14}C-BA 679 BR after intravenous administration to pregnant rats.	U99-1322 U99-1336	17.1 17.2
Absorption and distribution of ^{14}C-BA 679 BR in rats after intratracheal administration.	U99-1347	17.2
PK in male and female rats after IV, oral and intratracheal administration.	U99-1349 U99-1357	17.2 17.2
PK in female rabbits after IV and oral administration of 1 mg/kg Ba 679 BR.	U99-1358	17.2
Excretion balance and renal clearance in male and female rats after		

Study	*Res. Report ;*	*Vol.*
IV and IT administration of 10 mg/kg Ba 679 BR.	U99-1400	17.2
Comparative in vitro investigations of Ba 679 BR in human and rat hepatocytes.		
PK in male and female mice after IV and oral administration of 10 mg/kg Ba 679 BR.		
PK in male and female dogs after IV (0.1 mg/kg) and oral (1 mg/kg) administration of Ba 679 BR.		
Dose-dependency of tachycardia induced by IV tiotropium bromide and its antagonism by IV verapamil.		

Studies not reviewed within this submission: None

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. SAFETY PHARMACOLOGY:

Table 1 summarizes the results of six safety pharmacology assays performed with Ba 679 BR.

Neurological effects: Ba 679 Br significantly reduced minimum mouse body temperature (0.5 degrees C) at 100 µg/kg, IV; mice exhibited dry oronasal mucosa and an inability to hold onto a chromium plated decline plate in modified Irwin test. No effects noted at 10 mg/kg. Following administration of 10 µg/kg, IV, no drug-related effects were noted in a mouse writhing test or in pentylenetetrazole-induced seizure.

Cardiovascular effects: Heart rate was increased following administration of 5 µg/kg, IV bolus, in studies assessing Ba 679 Br alone or in interaction with verapamil in conscious dogs. Heart rate remained 30% above control values at 5 hours after dosing. In interaction with verapamil, a rapid and significant drop in heart rate compared to the Ba 679 Br response curve was noted 45-120 minutes after dosing; curves were parallel from that time point on. No effects were noted at doses of 1 or 2 µg/kg. In a separate study, Ba 679 BR produced no significant changes in cardiovascular effects or electrocardiogram following inhalation of 3 µg (0.12 µg/kg).
In anesthetized dogs, dose-related increases in heart rate (4-22%) and diastolic blood pressure (2-15%) were observed with maximal values obtained at 10 µg/kg, IV. Total peripheral resistance increased with maximum values noted at 3 µg/kg. Three of four dogs exhibited reduced QT interval of 10-40 msec at doses of 3-30 µg/kg. No effects were noted on cardiac output, renal and femoral blood flow, systolic arterial and left ventricular pressure, or respiratory parameters.

Pulmonary effects: Inhaled Ba 679 BR (3 µg) significantly inhibited acetylcholine-induced bronchospasm in conscious dogs with no observed significant changes in cardiovascular effects or electrocardiogram. The activity of Ba 679 BR was not significantly affected by pretreatment with corticosteroids. No effects on respiratory parameters were noted in anesthetized dogs following cumulative IV administration of 0.1 to 30 µg/kg.

Gastrointestinal effects: Incubation with Ba 679 Br (0.01-1 µM) induced a rightward shift of histamine and carbachol concentration-response curves with isolated guinea pig rectum and decreased the maximum tissue contraction.

Safety pharmacology summary: Table 1

Model	Species	Dose	Results
Neurological effects	Mouse	10 and 100 µg/kg, IV	Dose of 100 µg/kg reduced the minimum body temperature (~0.5 degrees C). Low dose had no significant effect. Other findings in most drug-treated mice included dry oronasal mucosa and inability to keep hold on the chromium plated decline plate in modified Irwin test.
		10 µg/kg, IV	Ba 679 BR did not influence the number of writhing symptoms associated with IP injections of phenyl-p-benzoquinone.
		10 µg/kg, IV	Pretreatment with BA 679 BR at 5 and 30 minutes prior to pentylenetetrazole (PTZ) infusion did not influence thresholds of PTZ-seizure compared to control animals administered saline 5 and 30 minutes prior to PTZ infusion.
Cardiovascular effects	Conscious dogs	1, 2 and 5 µg/kg, IV (bolus)	↑ heart rate at 5 µg/kg (2.7 to 3.1-fold) in studies assessing Ba 679 alone or in interaction with verapamil (0.3 mg/kg/min for 15 min, administered IV 30 min after Ba 679). Heart rate remained 30% above control values at 5 hrs after dosing. In the interaction study, a rapid and significant drop in heart rate compared to the Ba 679 alone curve from 45-120 minutes after dosing was noted. Curves were parallel from that time point on.
		3 µg (0.12 µg/kg) Ba 679 Br, IH	No effects noted at doses of 1 or 2 µg/kg Ba 679. No biologically relevant changes in cardiovascular effects or in the electrocardiogram were observed.
	Anesthetized dogs	0.1, 0.3, 1, 3, 10 and 30 µg/kg (IV) at 45 minute intervals	Dose-related ↑ in heart rate (4-22%) and diastolic blood pressure (2-15%) was observed with maximal values obtained at 10 µg/kg. Total peripheral resistance ↑ with maximum values noted at 3 µg/kg. No effect on cardiac output, renal and femoral blood flow, systolic arterial and left ventricular pressure. 3 of 4 dogs exhibited ↓QT interval of 10-40 msec at the 3 highest doses.

Respiratory effects	Conscious dogs	3 µg (0.12 µg/kg) Ba 679 Br, IH, alone or following pre-medication with 2 mg/kg methyl-prednisolone IV (1x/d for 2 d or 1 mg/kg beclo-methasone, IH, 1 hr prior to dosing.	Ba 679 BR, alone, inhibited acetylcholine-induced bronchospasm by 52-57%, measured as transpulmonary pressure, pulmonary resistance or dynamic compliance. Activity noted 1 minute following drug inhalation, reaching maximum at ~ 12 minutes; activity half-life of ~ 300 minutes. The activity of Ba 679 BR was not significantly affected by pretreatment with corticosteroids.
	Anesthe-tized dogs	0.1, 0.3, 1, 3, 10, 30 µg/kg, IV, at 45 min intervals	No effect on respiratory volume, transpulmonary pressure, lung compliance, resistance and respiratory minute volume. Blood gas parameters, electrolytes, glucose and lactate were similarly unaffected.
GI effects – isolated rectum	Guinea pig	0.01-1 µM	Agonists (histamine, carbachol, barium chloride) produced a concentration-dependent development of force. Repetition of agonist concentration-response curves (CRC) in the presence of BA 679 BR revealed a dose-related shift to the right of the carbachol and histamine CRCs and decreased the maximum contraction, resembling a "mixed antagonism". Onset of action of BA 679 BR was slow and prevented the achievement of a stable plateau of the agonist effect. The results with barium chloride did not demonstrate an antagonistic potency of BA 679 BR.

Safety pharmacology conclusions: The studies performed to evaluate potential undesirable pharmacodynamic effects were performed at doses exceeding expected clinical doses with the exception of the study on pulmonary effects in conscious dogs. The studies revealed no significant findings except for drug-related increases in heart rate via the IV route. Clinical subjects should be monitored for cardiac effects should the sponsor propose clinical protocols utilizing the IV route, as heart rate effects were observed in dogs at doses approximately 5-fold greater (on mg/kg basis) than those administered clinically. This finding is not expected to be a

concern for the IH route since bioavailability is ~ 6-16% by IH, resulting in a safety factor of ~ 25-fold.

II. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters: Pharmacokinetic parameters were assessed following single dosing in rats, mice, dogs and rabbits. Plasma concentrations were determined by HPLC-MS/MS. The results in male and female rats administered single bolus doses of 10 mg/kg Ba 679*H20 (salt form; 8 mg/kg Ba 679) via IV, IT and oral administration are summarized in Table 2. Following IV administration, plasma levels declined to 1.4% and 0.7% of the maximum attained levels within 1 hour in males and females, respectively. Clearance was rapid and steady state volume indicated extensive tissue distribution. Systemic exposure levels (AUC) were 15% greater in females than in males. Elimination half-life was comparable via IV or IT administration and absolute bioavailability was significantly greater following IT administration (132%) than following oral administration (0.7%).

Table 2. Single dose (10 mg/kg) pharmacokinetics in rats via various routes of administration.

Parameter	IV administration		IT administration		Oral administration	
	Males	Females	Males	Females	Males	Females
Cmax Ba679 (ng/ml)	6370	8452	4232	4784	2.512	1.73
Cmax, Ba679*H_20 (ng/ml)	7963	10570	5290	5980	3.14	2.163
tmax (hr)	0.083	0.083	0.083	0.083	0.5	0.5
AUC0-24h, BA679 (ng.hr/ml)	1357	1564	1591	2263	10.52	9.743
AUC0-24h, Ba679*H_20 (ng.hr/ml)	1696	1955	1988	2829	13.15	12.18
AUC0-inf, Ba679 (ng.hr/ml)	1374	1569	1621	2297	10.83	10.16
AUC0-inf, Ba679*H_20 (ng.hr/ml)	1717	1962	2027	2872	13.53	12.7
MRT0-inf (hr)	1.3	0.6	2.5	2.2	NA	NA
t ½ (hr)	8.2	6.4	7.8	5.8	NA	NA
Cl (ml/min/kg)	98	86.7	NA	NA	NA	NA
Vss (l/kg)	8	3.4	NA	NA	NA	NA

MRT: mean residence time
NA: not applicable.

In mice, females demonstrated a 77% increase in Cmax and a 53% increase in systemic exposure (AUC) compared to males following IV dosing (8 mg/kg, Table 3). Plasma levels declined rapidly in both sexes to ~ 0.6-1% of maximum within 40 minutes. A rapid elimination in terms of clearance and extensive distribution into tissues was noted with values greater in males. No gender difference was noted with elimination half-life or mean residence time. Plasma levels following oral administration reached only 0.2 ng/ml and rapidly declined below the limit of quantitation ([redacted]), absolute bioavailability was estimated to be < 0.02%.

In dogs plasma levels declined rapidly to ~ 5% of maximum within the first hour (Table 3) following an IV dose of 0.08 mg/kg. A rapid elimination in terms of clearance and extensive distribution into tissues was noted. Plasma levels following oral administration (0.8 mg/kg) reached maximum levels at 2 hours after dosing and declined to ~ 3% of maximum within 24 hours; absolute bioavailability was approximately 6.3%. No gender difference was noted in dogs.

In female rabbits administered an intravenous dose of 0.8 mg/kg Ba 679 in saline solution, plasma levels declined rapidly to ~ 2% of peak levels within one hour. Rabbits were similar to dogs in terms of clearance, elimination half-life and distribution. Plasma levels after oral administration (0.8 mg/kg, dissolved in Aqua iniectibila) of the same dose were all below the limit of detection. Absolute bioavailability and absorption were estimated to be less than 0.5% and 11% (urine data).

Table 3. Single IV dose pharmacokinetics in mice.

Parameter	Mouse		Dog		Rabbit
	IV administration 8 mg/kg		IV admin. 0.08 mg/kg	Oral admin. 0.8 mg/kg	IV admin. 0.8 mg/kg
	Male	Female	M+F	M+F	Females
Cmax Ba679 (ng/ml)	3474	6152	122.3	3.573	1048
Cmax, Ba679*H_20 (ng/ml)	4343	7690	152.9	4.467	1310
tmax (hr)	0.083	0.083	0.083	2	0.083
AUC_{0-24h}, Ba679 (ng.hr/ml)	634.5	971.4	$39.23_{(0-8hr)}$	23.93	$262_{(0-8hr)}$
AUC_{0-24h}, Ba679*H_20 (ng.hr/ml)	793.1	1214	$49.04_{(0-8hr)}$	29.91	$327.5_{(0-8hr)}$
AUC_{0-inf}, Ba679 (ng.hr/ml)	641.2	976.8	39.95	25.16	263.4
AUC_{0-inf}, Ba679*H_20 (ng.hr/ml)	801.5	1221	57.22	31.44	329.1
MRT_{0-inf} (hr)	0.8	0.6	1	NA	0.4
t ½ (hr)	10.4	9.5	2.3	NA	2.5
Cl (ml/min/kg)	208	136	33.83	NA	54.53
Vss (l/kg)	9.85	4.8	2	NA	1.3

MRT: mean residence time NA: not applicable.

Distribution: Following intratracheal administration (8 mg/kg) to male rats, radioactivity concentrations in plasma showed a mean value of Cmax (10.905 µg eq/ml) at 5 minutes after administration and the concentration decreased rapidly to 1.563 µg eq/ml after 1 hour. The elimination half-life was 19.4 hours; AUC was 9.111 µg eq*hr/ml and MRT was 10.3 hours. Maximum tissue concentrations were observed at 15 minutes (except for the digestive organ) and the mean distribution ratios are summarized in Table 4. In addition to the trachea and lung, the liver, kidney, pancreas and digestive organ showed high concentrations.

Table 4: Distribution ratios in rats following intratracheal administration.

Tissue	% of dose (15 minutes after administration)
Brain	0.05
Heart	0.18
Thyroid gland	0.01
Trachea	0.45
Lung	33.03
Thymus	0.25
Salivary gland	0.06

Liver	8.12
Kidney	3.78
Spleen	0.04
Pancreas	0.36
Adrenal gland	0.00
Testis	0.07
Stomach	15.89
Small intestine	6.57
Large intestine	0.97
Carcass	21.83

In whole body autoradioluminography, the highest distribution was similar to non-ligated rats. No radioactivity was observed in the stomach when both the esophagus and pyloric regions of the rat were ligated.

Concentrations of radioactivity following a single intravenous administration of ^{14}C-Ba 679 BR (10 mg/kg) to pregnant Sprague-Dawley rats on the 12th and 18th days of gestation are summarized in Table 5. The data demonstrate that Ba 679 BR and/or its metabolites are transferred to the fetus though, generally, at lower levels than observed in maternal plasma. Maximum concentrations on day 12 were observed at 0.25 hours after administration and concentrations of radioactivity in fetal whole body, placenta, and amniotic fluid were lower than that in plasma. A similar pattern was observed at time points up to 24 hours with the exception that placenta and plasma levels were similar from 4 hours onward. In pregnant rats on the 18th day of gestation, maximum concentrations were again noted at 0.25 hours. The concentrations of radioactivity in the fetal whole body, all fetal tissues examined and amniotic fluid were lower than that in plasma at all time points and the concentration of radioactivity in the placenta was higher than that in plasma at all time points.

Table 5: Radioactivity concentrations in rat dams and fetuses following IV administration.

Tissue	Day 12 Concentration at 0.25 hours (ng eq/g or ml)	Day 12 t ½ (hr)	Day 18 Concentration at 0.25 hours (ng eq/g or ml)	Day 18 t ½ (hr)
Maternal				
Blood	1592	10	2107	6.9
Plasma	2275	12.4	2879	6.7
Liver	19472	11.4	30097	9.8
Kidney	27991	10.6	52636	9.7
Lung	2171	9.5	3455	9.6
Heart	1199	26	1819	21.1
Placenta	1326	10.7	4916	8.8
Amniotic fluid	63.3	14.3	31.42	14
Fetal				
Liver	-	-	310	7.7
Kidney	-	-	204	-
Lung	-	-	84	-
Heart	-	-	126	-
Whole body	109.01	-	134	9.8

-: not calculated

Metabolism: The *in vitro* metabolism of Ba 679 was assessed using rat and human hepatocytes at drug concentrations of 1 and 10 µM (~ 0.5 and 5 µg/ml; expected human plasma levels are ~ 10-20 pg/ml at a dose of 18 µg/day). The presence of Ba 679 was significantly reduced (60-83%), within 4 hours of incubation time with rat hepatocytes (Table 6). Dithienylglycolic acid and a variety of other unidentified metabolites were formed. Metabolism with human hepatocytes was quantitatively similar to rats (67-70%) although the rate of metabolism was slower in humans. It should be noted that spontaneous conversion of BA 679 to dithienylglycolic acid occurred in control samples (Ba 679 incubated with collagen matrix without cells). A 14-17% reduction of Ba 679 was observed at 4 hours in the rat study control and a 64-68% reduction of Ba 679 was observed at 24 hours in the human study control. Thus, a significant portion of the observed Ba 679 metabolism does not appear to be human hepatocyte

related. Approximately 2% and 1% of total radioactivity remained in rat and human hepatocytes, respectively.

Table 6. In vitro Ba 679 metabolism by hepatocytes after incubation (rats: 4 hrs, humans: 24 hrs)

	1 μM		10 μM	
Rats @ 4hrs incubation	Males	Females	Males	Females
Ba 679*	6.5	6.4	7.9	27
Dithienylglycolic acid*	9.4	12.6	15.4	19.1
Other metabolites*	50.5	55	60	44.9
Humans @ 24 hrs incubation				
Ba 679*	20.2		25	
Dithienylglycolic acid*	35		46.7	
Other metabolites*	17.8		18.2	

* Values are in % of total HPLC run.

Excretion: Renal clearance and excretion balances were determined in rats following a single bolus IV or IT dose (8 mg/kg Ba679). Excretion was distributed similarly between urine and feces following IV administration while intratracheal administration resulted in primarily fecal excretion (Table 7). No gender differences were observed in renal clearance or elimination half-life in urine following IV dosing. Enterohepatic cycling did not play a major role as only 0.5% of total radioactivity was excreted in bile following ID injection of bile containing drug-related radioactivity. The drug related radioactivity did not partition into blood cells extensively in samples from either two male rats or a human volunteer (C_c/C_p ratios between 0.004 and 0.332 in rats; 0.004 and 0.065 in human sample).

Table 7. Excretion of Ba 679 following IV or IT dosing in rats.

Parameter	8 mg/kg Ba679, IV peripheral ^{14}C position	8 mg/kg Ba679, IT peripheral ^{14}C position	8 mg/kg Ba679, IV central ^{14}C positior
Cl_R (ml/min/kg)	28.1		
t½ urine (hr)	20.9		
Urine (%)	M: 54.6 F: 58.4	M: 39.6 F: 46.0	M: 45.7 F: 44.7
Feces (%)	M: 45.7 F: 44.7	M: 59.5 F: 54.1	M: 59.5 F: 54.1
$^{14}CO_2$ exhalation (%)	-	-	M: 9.6 F: 9.9

Excretion of radioactivity in female rabbits administered an IV dose of 0.8 mg/kg Ba 679 was nearly equally distributed into urine (55.8%) and feces (41.7%; Table 8). After oral administration, most of the radioactivity was excreted into the feces (101.5%). In mice, radioactivity was primarily excreted into the urine (68-79%) with 20-32% excreted into the feces following IV dosing. The opposite was observed following oral administration with 95% of radioactivity excreted into the feces.

Table 8. Excretion of Ba 679 following IV or IT dosing in mice and rabbits.

Parameter	Mouse 8 mg/kg		Rabbit 0.8 mg/kg	
	IV	oral	IV	oral
Urine (%)	68-79	13-14	56	6.2
Feces (%)	20-32	95	42	101.5

Radioactivity was detected in the milk of lactating rats following IV administration of 10 mg/kg ^{14}C-BA 679 BR on the 12th or 13th day after delivery. The maximum concentration of radioactivity was 1054.85 ng.eq/ml at 3.5 hours after administration. The ratio of the radioactivity concentration in milk to plasma ranged from 0.4 after 30 minutes to 18 after 24 hours. The elimination half-lives for milk and plasma were comparable (19.5 and 17.1 hours, respectively). There was no indication of drug accumulation in the milk. The amount of radioactivity transferred to each pup after 24 hours was approximately 0.1% of the dose.

PK/TK summary: PK parameters of Ba 679 were assessed at single doses in rats, dogs, mice and rabbits following IV and oral administration as well as IT administration in rats. Following bolus IV dosing, the drug was rapidly cleared with systemic levels declining to less than 5% within 1 hour. Clearance was greatest in mice followed by rats, rabbits and dogs; elimination half-life was similar in dogs and rabbits (2.3-2.5 hours) and increased in rats and mice (6.4-10.4 hours). In rats, absolute bioavailability was significantly greater following IT administration (132%) than following oral administration (0.7%). Absolute bioavailability in dogs, mice and rabbits was ~ 6%, 0.02% and 0.5%, respectively, following oral administration. Following IT administration to male rats, radioactivity was well distributed and levels peaked at 15 minutes after administration. Maximum concentrations were observed in the trachea, lung, liver, kidney, pancreas and digestive tract. Extensive distribution was indicated by a steady state volume of 4-10 l/kg in rats and mice decreasing to 1-2 l/kg in rabbits and dogs. A single IV dose to pregnant rats demonstrated that Ba 679 Br and/or its metabolites are transferred to the fetus at lower levels than observed in maternal plasma. Maximum tissue concentrations in the dam were noted in liver and kidney. In vitro metabolism of Ba 679 by rat and human metabolites indicated that dithienylglycolic acid was the primary identified metabolite; various unidentified metabolites were observed. Human hepatocyte metabolism was quantitatively similar to that of rats although the rate was slower in humans. Excretion of drug-related material was primarily through urine (54-58%) following IV administration and feces (54-59%) following IT administration. Excretion in mice and female rabbits was also primarily distributed in urine (56-79%) following IV administration and in feces following oral administration (95-101%). Drug-related radioactivity (0.1% of the administered dose after 24 hours) was detected in the milk of lactating rats following IV dosing.

PK/TK conclusions: Assessment of rats, dogs, mice and rabbits demonstrated high clearance of drug-related material following IV administration, low bioavailability following oral administration and extensive tissue distribution. Rat and human metabolism was comparable following in vitro hepatocyte metabolism and excretion was primarily through the urine following IV administration and through the feces following IT or oral administration. Ba 679 Br and/or its metabolites was transferred to the fetus at low levels following IV dosing and drug-

related radioactivity was detected in the milk of lactating rats following IV dosing indicating a potential for infant exposure.

III. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: The studies evaluating potential undesirable pharmacodynamic effects were performed at doses exceeding expected clinical doses with the exception of the study on pulmonary effects in conscious dogs. Studies of pulmonary, CNS, GI and cardiovascular effects revealed no significant findings except for drug-related increases in heart rate. Thus, clinical subjects should be monitored for cardiac effects, as heart rate effects were observed in dogs at doses approximately 5-fold greater (on mg/kg basis) than those administered clinically. Previously reviewed studies demonstrated no significant effects on the CNS in the mouse and rabbit, cardiovascular system in the dog, and renal system in the rat. Topical administration resulted in mydriasis in dogs. Thus, Ba 679 Br has the potential to adversely affect patients and sensitive subjects with small angle glaucoma should be excluded from clinical investigations. Pupil dilation was also observed in rats and mice following IV and SC administration, respectively. Ba 679 Br also inhibited pilocarpine-induced salivation in rats and guinea pigs, meal-induced salivation in rabbits and dogs, gastric juice secretion in rats, and miotic activity in rats and rabbits. Ba 679 Br also inhibited GI transit in mice following SC administration. Combined administration with albuterol resulted in additive effects. Typical anticholinergic effects were also observed.

PK parameters of Ba 679 were assessed at single doses in rats, dogs, mice and rabbits following IV and oral administration as well as IT administration in rats. Following bolus IV dosing, the drug was rapidly cleared with systemic levels declining to less than 5% within 1 hour. Clearance was greatest in mice followed by rats, rabbits and dogs; elimination half-life was similar in dogs and rabbits (2.3-2.5 hours) and increased in rats and mice (6.4-10.4 hours). Drug accumulation was not observed after IH, IV or PO administration. Plasma protein binding was highest in humans (65.3%) than in rats, mice, rabbits and dogs (15-22%). Systemic exposure of Ba 679 was greater in mice than in dogs or rats after PO or IV administration. In rats, absolute bioavailability was high following IT administration (132%). Following oral administration, absolute bioavailability was low in dogs, rats, mice and rabbits (~0.7%, 6%, 0.02% and 0.5%, respectively). Drug distribution is extensive with highest levels observed in the lung, liver, kidney, pancreas and digestive tract. Extensive distribution was indicated by a steady state volume of 4-10 l/kg in rats and mice decreasing to 1-2 l/kg in rabbits and dogs. Ba 679 Br and/or its metabolites were transferred to the fetus at low levels following a single IV dose. Maximum tissue concentrations in the dam were noted in the liver and kidney. N-methylscopine was identified as the major in vivo metabolite in rats, dogs and mice. In vitro metabolism of Ba 679 by rat and human metabolites indicated that dithienylglycolic acid was the primary identified metabolite. Various unidentified metabolites were observed in vivo and in vitro. Human hepatocyte metabolism was quantitatively similar to that of rats although the rate was slower in humans. Excretion of drug-related material in rats, dogs, mice and rabbits was primarily through urine following IV administration and through the feces following IT or oral administration. Drug-related radioactivity (0.1% of the administered dose after 24 hours) was detected in the milk of lactating rats following IV dosing indicating a potential for infant exposure.

General Toxicology Issues: None at this time.

Recommendations:

Clinical subjects should be monitored for cardiac effects should the sponsor propose clinical protocols utilizing the IV route, as increased heart rate was observed in dogs at doses approximately 5-fold greater (on mg/kg basis) than those administered clinically. This finding is not expected to be a concern for the IH route since bioavailability is ~ 6-16% by IH, resulting in a safety factor of ~ 25-fold.

Reviewer signature: /S/

Supervisor signature: Concurrence - /S/

IND 46,687
cc: list: CJ Sun
TJ McGovern
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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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I concur.