VII.3.3 Sponsor's Proposed Labeling

VII.3.4 Calculation of Dose Ratio

For the labeling purpose, this section estimated the dose ratios between the reproductive toxicity studies in animals and the recommended daily dose in humans. Table 7.5 summarizes the calculated dose ratios that the current review used in the labeling review.

Table 7.5 Dose Ratios Used in Labeling

Species	CF	Study No	Route	Dose		Ratio (animal/human)	
				(mg/kg)	g/m^2	Calculated	Rounded to
Human	37		IH	0.00036	0.01332		
Rat	6	U93-0239,	IH	0.0008	0.0048	0.4	< 1
		U92-0622,		0.007	0.042	3.2	3
		U96-2493		0.139	0.834	62.6	60
		U90-0687	PO	1	6	450.5	450
				25	150	11261.3	11,000
				500	3000	225225.2	225,000
Rabbit	12	U92-0623	IH	0.001	0.012	0.9	1
				0.11	0.132	9.9	10
				0.05	0.6	45.0	45
		U91-0340	PO	1	12	900.9	900
				10	120	9009.0	9,000
				100	1200	90090.1	90,000

VIII. SPECIAL TOXICOLOGY STUDIES:

Previously Reviewed Studies

Study Description	Report #	Vol.	Review #
Guinea pig skin sensitization study (maximization test)	U91-0858	90	1
Special study in beagle dogs to determine the lowest oral dose causing inhibition of tear flow, using Schirmer's test	U92-0293	90	1
Mechanism of sudden death in Fischer rats	U94-0029	90	1
Acute local tolerance of injectable solution of Ba 679 BR 0.06 mg\ml (0.006 %) in rats after paravenous injection	U95-0136	91	2
Acute intravenous local tolerance test with Ba 679 BR injectable solution 0.06 mg\ml (0.006 %) in rabbit ear	U95-0137	91	2
Acute intraarterial local tolerance test with Ba 679 BR injectable solution 0.06 mg\ml (0.006 %) in rabbit ear	U95-0138	91	2
In vitro haemolytic effect of injectable solution of Ba 679 BR (0.006 %)	U95-0177	90	2
Investigation of Ba 679 BR antagonism against Pilocarpine induced salivation following treatment by inhalation	U95-0222	17	2
Effects of tiotropium bromide (Ba 679 BR), administered by inhalation on pilocarpine induced salivation in male CD1 mice	U95-0471	91	2
Validation of Ba 679 BR antagonism against Pilocarpine induced salivation following treatment by inhalation	U95-0221	17	2

Studies Reviewed in This Review

Study Description	Report #	Vol./p
Preliminary acute eye irritation test in the rabbit	U96-2247	90
14-day local ocular tolerance study of Ba 679 BR by instillation into the conjunctival sac of rabbits	U98-2198	91

Study Title: Preliminary acute eye irritation test in rabbits (Study No. 96-2247)

A preliminary Draize Test was conducted in two albino rabbits. Twenty mg of tiotropium bromide was instilled into conjunctival sac of one eye and the other eye serves control. Of these two rabbits, rinsing was performed 30 seconds post dosing for one rabbit but not the other. Irritation was evaluated according to OECD guidelines. No evidence of irritation was found.

Study title: 14-day local ocular tolerance study of Ba 679 BR by instillation into the conjunctival sac of rabbits

Key study findings: Tiotropium is not irritant to the eye in rabbits.

Study no: U98-2198
Volume #, and page #: Vol. 91
Conducting laboratory and location: Laboratory of Pharmacology and Toxicology, BI, Redderweg 8, D-21147 Hamburg
Date of study initiation: 4-NOV-1997 – 18-NOV-1997
GLP compliance: Yes

QA reports: yes (x) no ( ):
Drug, lot #, radiolabel, and % purity: Batch 970726
Formulation/vehicle: 0.001% and 0.003% tiotropium solution, placebo (ingredients not identified), and 0.9% NaCl
Animal species: New Zealand White Rabbits

Methods: Tiotropium was instilled into the conjunctival sac and degree of irritation was graded.

Dosing: Right conjunctival sac of 17-week-old rabbits (3/sex/group) was instilled with 50 µl of tiotropium, placebo, or 0.9% NaCl three to 6 times a day for fourteen days. The time interval was 90 (6 instillations/day) or 180 minutes (3 instillations/day) between two consecutive instillations. Table presents the study design.

Table Study Design of the Draize Test in Rabbits

Group	1	2	3	4	5
	Saline	Placebo	Tiotropium		
Treatment			0.001%	0.001%	0.003%
Treatment Frequency	6	6	3	6	6
Time interval between treatments	90	90	180	90	90
Total volume (µl/ left eye)	540	540	270	540	540

Observations and times:
Mortality and clinical signs: daily
Body wieght: weekly

Food consumption: daily
Ophthalmic examinations: 1) daily for signs of redness, chemosis, discharge and ulceration; and 2) detailed examination (with — ophmalmoscope, slit lamp, fluorescence staining and — camera) on days 1, 5, 10, 14 and 15.

Results: Tiotropium-treated eyes animals showed mydriasis from day one and onward. The mydriasis was more severe on day 2 than day one. Mean pupil diameters were 9 mm and 7 mm for the treated (right) and untreated (left) eye, respectively. The saline and placebo treatment group did not show mydriasis. Conjunctival redness, chemosis and discharge was seen in some animals in Groups 2 and 5 on days 12 – 13. The changes were not considered tiotropium treatment-related.

Summary of individual study findings:
A Draize test was completed to evaluate the irritation potential of tiotropium to the eyes. Tiotropium at the concentration up to 0.003% was instilled into conjunctival sac for 14 days in rabbits. No irritation as observed.

Special Toxicity Conclusions:
Tiotropium tests negative in the Draize test. Tiotropium does not possess potentiate skin sensitization associated with Freud's complete adjuvant in Guinea pigs. The drug is non-irritating after paravenous and intraarterial injections, but slightly irritating to local tissues after intravenous administration. Tiotropium is hemolytic to human blood *in vitro* at concentrations of ≤ 0.006% but not at 0.003%.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

IX.1 Conclusions:

This application has adequately evaluated the toxicity profile of tiotropium bromide and an approval is recommended for the active ingredient from the non-clinical viewpoint. The application contains study reports evaluating general toxicity, genetic toxicity, carcinogenicity, reproductive toxicity and special toxicity of tiotropium bromide. These studies have demonstrated that tiotropium possesses a toxicity profile of a typical muscarinic cholinergic antagonist and the drug is non-genotoxic, non-carcinogenic and non-teratogenic in animals. However, tiotropium is embryo/fetocidal at high doses when rats and rabbits are exposed to it during pregnancy. The drug also delays the sexual maturity of the pups exposed to it maternally.

General toxicity of tiotropium has been evaluated in mice, rats and dogs for the treatment duration of up to one year. The route of administration includes intravenous, oral, and inhalation. Inhalation is the route of administration for majority of toxicity studies. The formulation of inhalation studies includes dry power (in lactose) and aqueous solutions. The aqueous solution contains 0.01% benzalkonium chloride and 0.01% EDTA (pH = 3.0 – 3.5) and is the formulation for most inhalation studies. No mortality occurs at inhalation tiotropium doses of greater than 6.5 and 21 mg/kg in mice and rats, respectively. Repeat-dose inhalation toxicity studies up to

one year in treatment duration at doses up to 392 and 133 µg/kg/day in rats and dogs, respectively, have revealed a toxicity profile of a typical anticholinergic agent. The target organs of toxicity include the respiratory tract, gastrointestinal tract, secretory glands, eye, heart and urinary bladder. The NOAEL value of the drug depends upon the treatment duration: the longer the treatment duration, the lower the NOAEL. The one-year NOAEL value is < 7 and 0.4 µg/kg/day in rats and dogs, respectively.

Five genetic toxicity assays of tiotropium have revealed no evidence of genotoxicity. These assays are Bacterial mutation in *S. typhimurium* and *E. coli*, V79 CHO mammalian gene mutation *in vitro*, human lymphocyte chromosomal aberration *in vitro*, unscheduled DNA synthesis in primary rat hepatocytes *in vitro* and mouse micronucleus formation *in vivo*.

Three 19- to 24-month inhalation carcinogenicity studies in mice and rats have revealed no evidence of tiotropium tumorigenicity. These studies are a 104-week study in rats, a 83-week study in female mice, and a 101-week study in male mice. Their respective tiotropium are up to 5.3, 9.1 and 0.31 µg/kg/day. Each of the studies has achieved the maximum tolerated dose of the drug.

Six (4 inhalation and 2 oral) reproductive toxicity studies in rats and rabbits have revealed that tiotropium was embryo/fetal toxic, but non-teratogenic. All findings were limited to the inhalation studies only. In rats, a pre- and post-natal development study showed a total litter loss and a decrease in mean pup weights at tiotropium inhalation doses of ≥ 7 µg/kg/day. A fertility study showed fetal resorption and decreases in the number of corpora lutea, the percentage of implants, and the number of live pups at the same doses. The fertility index, however, was not affected. These two studies, along with a teratogenicity study, also showed a delay (1-3.5 days) in sexual maturation in pups exposed to the drug maternally at the same dose level. The sexual maturation is measured by vaginal opening in the female and occurrence of balanoprepuital skinfold in the male. In rabbits, an increase in post implantation loss was observed at an inhalation dose of 50 µg/kg/day. No such effects were noted at inhalation doses of 0.8 and 11 µg/kg/day in rats and rabbits, respectively.

The two oral Segment II reproductive studies showed no evidence of fetal development effect at respective tiotropium doses up to 500 and 100 mg/kg/day in rats and rabbits, respectively. However, oral bioavailability of the drug was poor while the inhaled drug is readily absorbed in both species.

IX.2 Recommendations:

1. Approval of tiotropium bromide is recommended from the non-clinical point of view.
2. Revise the sponsor's proposed labeling. The text of the proposed labeling is presented in the sections of the Executive Summary and Labeling Review. See these sections for details.

IX.3 Labeling with basis for findings:

1. The sponsor's proposed dose ratios between animals and humans should be revised. The sponsor's dose ratios for inhalation toxicity studies were calculated based on the total body burden in animals although it acknowledged that pulmonary deposits were better estimates of actual exposure. In fact, the application used pulmonary deposits as the exposure level for general inhalation toxicity studies, but not for carcinogenicity and reproductive toxicity studies. The current review has estimated pulmonary tiotropium doses for the carcinogenicity and reproductive toxicity studies and has derived dose ratios accordingly.

2. Tiotropium bromide should be given a Category C designation. The currently recommended labeling for tiotropium should include findings in the reproductive toxicity studies. Tiotropium caused dose-related decreases in the number of ovulation and implant, increases in post implantation loss and litter loss in the dam, and a delayed sexual maturity in pups. These findings occurred mostly in rats at inhalation tiotropium doses of $\geq$ 7 µg/kg/day, but not at 0.8 µg/kg/day. These findings warrant a Category C classification in the Pregnancy section.

X. APPENDIX/ATTACHMENTS:

Addendum to review: None.

Other relevant materials (Studies not reviewed, appended consults, etc.):

Previous Pharmacology and Toxicology reviews in IND 46, 687:

1. Dr. Satish Tripathi's review dated August 26, 1996.
2. Dr. Satish Tripathi's review dated September 17, 1997.
3. Dr. Satish Tripathi's review dated December 10, 1997.
4. Dr. Satish Tripathi's review dated January 8, 1998.
5. Dr. Timothy McGovern's review dated November 2, 2001

Any compliance issues: None.

ATTACHMENT 1

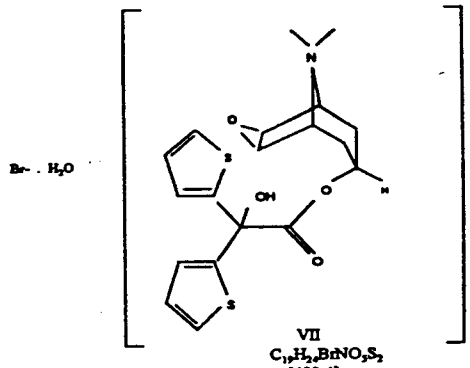
Pharmacology and Toxicology Review

By

Dr. Satish Tripathi

August 28, 1996

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL, REVIEW NO. 1

IND No.:	46,687
Serial No(s).:	000
Date(s) of Submission:	11/30/94
Information to be Conveyed to Sponsor:	Yes (X), No ( )
Reviewer:	Satish C. Tripathi, Ph.D.
Date Review Completed:	08/26/96
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:	**A 679 BR**
Other Names:	Tiotropium bromide
Chemical Name:	[7(S)-(1α,2β,4β,5α,7β)]-7-[(hydroxydi-(2-thienyl)acetyl)oxy]-9,9 dimethyl-3-oxa-9-azonia-tricyclo[3.3.1.$0^{2,4}$]nonane bromide hydrate.
CAS Number:	Not Available Yet
Structure:	 VII $C_{19}H_{22}BrNO_4S_2$ [490.4]

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: hard gelatin capsules containing a white powder for inhalation. The composition of these capsules is as follows:

Route of Administration: Oral inhalation using an FO_2 inhaler.

Label Claim	5.5 mg	11 mg	22 mg	44 mg	Placebo
Component		mg/Capsule			
Ba 679 BR	0.0055	0.0110	0.0220	0.0440	-
Lactose Monohydrate					
Powder Blend					
Hard Gelatin Capsule					
Ba 679 BR Capsule					

Proposed Clinical Protocol:

Objective: To determine optimal dose of Ba 679 BR for future phase II and III safety and efficacy studies.

Dose: 0.0 (placebo), 5.5, 11.0, 22.0, and 44.0 µg Ba 679 BR in lactose monohydrate, NF. Dose for a 50 kg individual: 0.11, 0.22, 0.44, and 0.88 µg/kg.

Frequency: Once daily

Duration: Four weeks

Patients: One hundred sixty out-patients of either sex $\geq$ 40 years with a diagnosis of COPD.

Previous Review, Date, and Reviewer: **Not Applicable.**

Studies Reviewed in this IND:

Study	Boehringer-Ingelheim #	Vol.	Page
PHARMACOLOGY			
Pharmacodynamics specific to indication:			
Affinity and binding kinetics	U93-0225	1.4	035
Binding to animal muscarinic receptor subtypes	90-0561	1.4	
002			
Mch-antagonism in isolated guinea pig trachea	U91-0455		
Bronchospasmolytic and cardiovascular effects: inhalation	U92-0621		
	U91-0455		
Bronchoprotective effects	U94-0070		
	U94-0005		
	U94-0069		
Other Pharmacodynamics:			
Receptor binding profile	**U90-0561**		
Inhibition of MAO activity	**U90-0561**		
Inhibition of transmitter uptake	**U90-0561**		
Influence on transmitter release	**U90-0561**		
Safety Pharmacology:			
Cardiovascular effects	U91-0408		
Antimiotic and antisalivatory effect in dogs, i.v.	U90-0668		
Antimiotic and antisalivatory effect in dogs, p.o.	U90-0669		
Lacrimation in dogs, p.o.	U92-0293		
Influence on EEG in rabbits, i.v.	U91-0469		
Gastric secretion in rats, s.c.	U91-0469		
Antisalivatory effect in rats, i.v. and p.o.	U91-0322		
Mydriatic and antimiotic effects in rats, p.o.	U90-0670		
Diuresis in rats, p.o.	U91-0468		
Nocturnal locomotor activity in mice, s.c.	U91-0466		
Pupil size in mice, p.o.	U93-0710		
Antisalivatory effect in mice, p.o. and i.v.	U91-0409		
Intestinal passage in mice, p.o.	U91-0410		
Interaction studies:			
Ba 679 BR and albuterol in dog, inhalation	U93-0657		
Ba 679 BR in dog eye: comparison with carbachol, timolol	U93-0910		

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Rat Distribution of drug: i.v., i.t., p.o.	*U90-0448*	*1.4*	*091*
Rat Cytochrome P-450 induction potential	U91-0117	1.4	126
Ba 679 BR in human plasma by HPLC and	U93-0887		
Affinity of metabolites to Hm_1 and Hm_5 receptors	U93-0507	1.4	067
Mice 13-wk Inhalation MTD: plasma levels	U93-0512	1.6	159
Mice 13-wk Inhalation MTD: plasma levels (Study II)	U93-0514	1.6	197
Mice Biochemical investigations	U91-0704	1.4	396
Rat 4-wk i.v. toxicity: satellite biochemistry	U92-0380	1.5	002
Rat 4-wk Oral toxicity: biochemistry	U91-0482	1.4	314
Dog: Preliminary oral and i.v. toxicity: biochemistry	U91-0491	1.4	349
Rat 13-wk Inhalation MTD: plasma levels	U92-0678	1.5	203
Rat 13-wk Inhalation: plasma levels	U92-0381	1.5	115
Rat 13-wk Inhalation (aq. Aerosol): plasma levels:	U93-0059	1.6	092
Rat 13-wk Inhalation toxicity: plasma levels of 2 species	U93-0905	1.7	059
Rat 13-wk Inhalation toxicity: plasma levels of 2 species	U93-0934	1.26	002
Rat 13-week Oral toxicity PK	U93-0740	1.6	242
Rat Biochemical investigations	U91-0236	1.4	177
Dog 2-wk Inhalation toxicity: plasma levels	U93-0933	1.7	092
Dog 4-wk Preliminary inhalation toxicity: plasma levels	U92-0716	1.5	232
Dog 13-wk Inhalation toxicity: plasma levels	U92-0784	1.6	028
Dog 13-wk Inhalation toxicity: plasma levels (powder with lactose)	U93-0948	1.7	136
Dog 13-wk Oral toxicity: plasma levels	U93-0747	1.6	309
Dog 52-wk Inhalation toxicity: plasma levels	U94-0086	1.7	268
Dog Biochemical investigations	U92-0476	1.5	139
Rat Inhalation: plasma levels in a reproductive toxicity study	U94-0030	1.7	208
Plasma protein binding	U92-0728	1.6	002

TOXICOLOGY

Mice: Aute oral and i.v. toxicity	U90-0494	1.9	122
Mice Acute i.v. toxicity: degradation product	U91-0844	1.17	079
Mice Acute i.v. toxicity: degradation product	U91-0845	1.17	105
Mice Acute i.v. toxicity: degradation product	U91-0860	1.17	208
Mice Acute i.v. toxicity: degradation product	U92-0680	1.20	405
Mice Single dose inhalation toxicity	U91-0812	1.17	046
Rat: Aute oral and i.v. toxicity	U90-0493	1.9	082
Rat: Acute inhalation toxicity	U90-0517	1.9	163
Dog Single dose inhalation	U91-0224	1.10	167
Mice 13-wk Inhalation MTD	U92-0717	1.21	066
Mice 13-wk inhalation MTD Amendment 1	U92-0271	1.18	002
Rat 4-wk preliminary inhalation toxicity	U90-0691	1.10	002
Rat 4-wk Oral range finding	U92-0477	1.19	002

Rat 13-wk Oral toxicity	*U91-0492*	*1.11-*	*002*
		1.12	002
Rat 3-wk i.v. range finding	U93-0632	1.23	002
Rat 4-wk i.v. toxicity	U93-0808	1.24	002
		1.25	002
Rat 2-wk Inhalation toxicity: powder with lactose	U93-0943	1.29	002
Rat 13-wk Inhalation toxicity	U91-0493	1.13	002
Rat 4-wk Inhalation toxicity:mechanism of sudden death	U94-0029	1.34	298
Rat 13-wk Inhalation toxicity	U93-0944	1.30	002
		1.31	002
Rat 13-wk Inhalation MTD Amendment 1	U92-0295	1.18	311
Rat 13-wk Inhalation MTD	U92-0765	1.22	002
Rat 52-wk Inhalation toxicity (aq. Aerosol)	U93-0945	1.32	002
Dog: 1-4 wk Preliminary oral and i.v. toxicity	U90-0614	1.9	193
Dog 13 wk	U91-0510	1.15-	002
		1.16	002
Dog 4-wk i.v. toxicity	U91-0494	1.14	002
Dog Inhalation feasibility	U93-0729	1.23	261
Dog 4-wk Inhalation torerability	U93-0766	1.23	301
Dog 4-wk Preliminary inhalation toxicity	U91-0306	1.10	216
Dog 13-wk Inhalation toxicity	U91-0511	1.16	160
Dog 2-wk Inhalation toxicity (powder)	U93-0941	1.28	002
Dog 13-wk Inhalation toxicity (powder)	U93-0942	1.28	153
Dog 52-wk Inhalation toxicity	U93-0938	1.27	002

SPECIAL TOXICITY

Guinea Pig skin sensitization (maximization test)	U91-0858	1.17	175

REPRODUCTIVE TOXICITY

EXPLORATORY STUDIES:			
Rat preliminary Oral segment II	U90-0079	1.9	023
Rat Inhalation Segment I Range Finding: mature ♂, ♀, offsprings	U92-0679	1.20	294
Rat Inhalation Range Finding: mature ♂, ♀, and offsprings: plasma levels	U94-0030	1.7	208
Rat Preliminary Inhalation Toxicity Segment II	U92-0684	1.21	002
Rabbit Oral preliminary segment II	U90-0301	1.9	061
Rabbit Preliminary Inhalation Toxicity, Segment II	U92-0484	1.19	342
DEFINITIVE STUDIES:			
Rat Oral segment II	U90-0687	1.9	253
Rat Inhalation Toxicity, Segment I	U93-0239	1.22	255
Rat Inhalation Toxicity, Segment II	U92-0622	1.20	002
Rabbit Oral segment II	U91-0340	1.10	339
Rabbit Inhalation Toxicity Segment II	U92-0623	1.20	194

GENETIC TOXICITY

Bacterial reverse mutation (Ames): activity	U90-0077	1.9	002
Bacterial point mutation (Ames): test	U92-0074	1.17	226
Bacterial point mutation (Ames): activity	U92-0498	1.19	397
Micronucleus test in mice	U91-0096	1.10	147
V79 gene mutation assay for HGPRT mutants	U91-0331	1.10	317
In vitro rat hepatocyte unscheduled DNA synthesis test	U91-0637	1.17	002
In vitro chromosomal aberrations in human lymphocytes	U91-0855	1.17	131

Studies Not Reviewed in this IND: None

Studies Previously Reviewed: ***None***

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

PHARMACOLOGY

The occurrence of muscarinic acetylcholine receptors throughout the body and their functional characteristics have contributed significantly to pulmonary medicine. It is well established that in the lung, the parasympathetic nervous system is the dominant neural bronchoconstrictor pathway and plays an important role in regulatory airway tone and secretions. Anticholinergic/antimuscarinic drugs have been used to reverse chronic obstructive disease. The sponsor has proposed that the introduction of quaternary ammonium congeners of atropine and local administration of the compound by inhalation instead of systemic use can significantly improve the therapeutic index of antiobstructive antimuscarinic therapy. The sponsor conjectured that slow and low systemic absorption from the lungs and the low systemic absorption potential of quaternary ammonium drugs from the G.I. tract for the portion of the drug that is to be swallowed should help minimize typical systemic anticholinergic side effects such as tachycardia, dry mouth or blurred vision at therapeutic doses.

Ba 679 BR is a quaternary ammonium molecule with prolonged duration of action (than that of Ipratropium bromide, an approved Boehringer-Ingelheim product) profile and high selectivity to the subtypes hm_1 and hm_3 of muscarinic receptors. The increased duration of action is likely to stabilize lung function of the patients with chronic obstructive pulmonary disease (COPD) and asthma.

The pharmacological profile of Ba 679 BR was studied in comparison to positive controls such as ipratropium bromide, atropine, or scopolamine.

PHARMACODYNAMIC EFFECTS RELATED TO PROPOSED INDICATIONS:

I. *IN VITRO* ANTAGONISM OF MUSCARINIC RECEPTORS:

a) Binding Studies: Receptor binding studies with tissues from rat hippocampus and heart
and lacrimal tissues from guinea pig showed high affinity to the subtypes of muscarinic receptors (Ki < 0.1 nM). Studies with membrane preparations from [redacted] Chinese hamster ovary-K_1 cells expressing the genes for the human muscarinic receptors (hm_1 to hm_5) showed higher affinity for the binding of ^{3}H-tiotropium iodide to hm3 receptors (the hm3 receptor subtype, because of its ability to mediate bronchoconstriction by bronchial smooth cell, is considered significant in the broncholytic activity of antimuscarinic drugs) than iprotropium (Table 1) and the dissociation of receptor-Ba 679 complex is slow in receptor subtypes hm_1 (another receptor that is closely associated with bronchoconstriction) and hm_3. Ba 679 BR dissociates from the respective receptor subtypes at >100 times slower than Ipratropium (Table 2). Slow dissociation of Ba 679 BR was also seen in human **lung tissues.**

Table 1: K_D-values from kinetic parameters expressed as means [nM] of 3-4 determinations.

Table 2: Dissociation half-lives of receptor-ligand complexes at 23 °C

	Hm_1	Hm_2	Hm_3	Hm_1/Hm_2 ratio	Hm_3/Hm_2 ratio
Ba 679 iodide	0.041	0.021	0.014	2.0	0.7
Ipratropium iodide	0.183	0.195	0.204	0.9	1.1

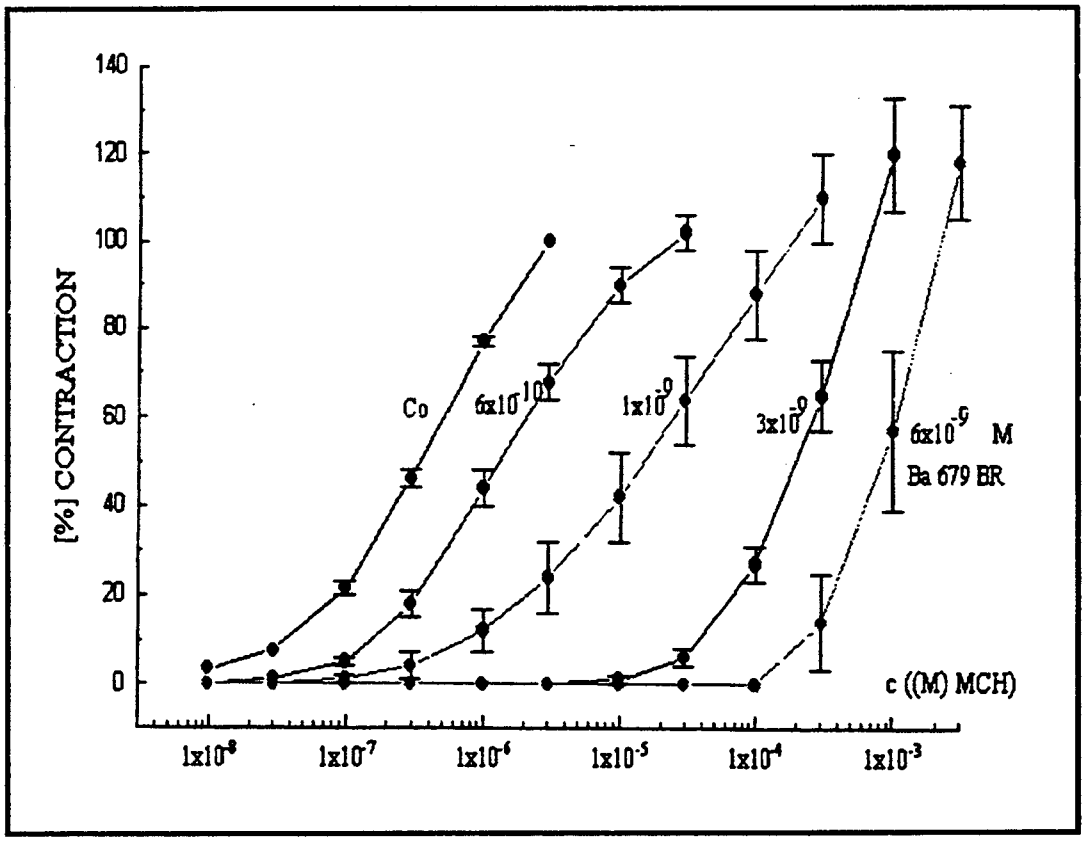
Expressed as means [h] of 3-4 determinations.

	Hm_1	Hm_2	Hm_3	Hm_1/Hm_2 ratio	Hm_3/Hm_2 ratio
Ba 679 iodide	14.6	3.6	34.7	4.1	9.6
Ipratropium iodide	0.11	0.035	0.26	3.1	7.4

b) Receptor-mediated Actions: Ba 679 BR inhibited (IC_{50}=0.24 nM) contractions of human bronchi induced by electrical field stimulation (EFS). The onset of action was slow and the duration of action was long in comparison to ipratropium bromide. Ba 679 BR facilitated acetylcholine release evoked by EFS suggesting that this antagonist also occupies prejunctional muscarinic M_2-receptors. The facilitation of Ach-release by Ba 679 BR was washed out after two hours when there was still complete blockade of cholinergic contractile responses evoked by EFS (effect on airway smooth muscle M_3-receptor).

The presence of drug (Ba 679 BR at 6 x 10^{-10} to 6 x 10^{-9}M concentrations) inhibited methacholine-induced contraction of isolated tracheal rings from guinea pigs (Fig 1):

Figure 1: Ba 679 BR antagonism of methacholine-induced contraction of



Guinea pig trachea smooth muscle rings.

Activation of muscarinic receptors stimulates the beat frequency of tracheal ciliated cells (J. Appl. Physiol. 14:901-904, 1959; Amer. Rev. Respir. Dis. 137: (Suppl.): 527, 1988). Treatment with Ba 679 BR and ipratropium bromide did not influence the base line frequency, but it blocked the frequency-response to methacholine (Life Sciences 52:537-544, 1993). The wash out of the two antagonists by perfusion of cell culture chamber containing the isolated cells with a methacholine-containing medium lasted significantly longer (5-times) after Ba 679 BR (82 min) than after ipratropium bromide (16 min). The antagonistic effects were reversible with both compounds as following washout, full stimulation of tracheal ciliated cell beating was seen with methacholine.

II. *IN VIVO* ANTAGONISM OF MUSCARINIC RECEPTORS:

a) Inhibition of Ach-induced bronchoconstriction:

Studies supporting the use of Ba 679 BR as a bronchodilator or bronchoprotective agent were conducted in anesthetized rabbits (i.v.); in anesthetized dogs, guinea pigs, and rats (inhalation); and in conscious guinea pigs (inhalation); Ipratropium bromide was used as positive control. These data showed either protection against bronchospasms induced by i.v. acetylcholine or protection against bronchospastic collapse induced by inhaled acetylcholine. The dose-response curves of the antimuscarinic drugs were steep with a factor of 10 or less from the no effect level to a 100%-protective dose. The ED values are shown in Table 3.

Table 3: The bronchoprotective effect of Ba 679 BR or ipratropium bromide against acetylcholine-induced bronchospasm.

Species/Condition	Characteristic Value	Ba 679 BR mg/kg	Ipratropium mg/kg	Route
Rabbit/Anesthetized	ED_{50}	0.0003-0.003	0.001-0.003	i.v.
Guinea Pig/ Anesthetized	ED_{50}	0.001-0.003	0.0003-0.003	inhal.
Guinea Pig/Conscious	ED_{50}	1.25-2.5	0.5-2.0	inhal.
Dog/Anesthetized	ED_{50}	0.03-0.3	0.1-10.0	inhal.
Dog/Anesthetized	ED_{70-80}	0.00036	0.0007	inhal.
Rat (Wistar)/ Anesthetized	Dose to double the PD15 of Ach (respiratory flow=-15%)	0.00039-0.0037		inhal.
Rat (Fischer)/ Anesthetized	Dose to double the PD15 of Ach (respiratory flow=-15%)	0.00023-0.00664		inhal.

Ba 679 BR was 2 to 4 times more potent than ipratropium bromide in rabbits, dogs, and anesthetized and conscious guinea pigs. The duration of action (= time elapsed from maximal protection to decline to half-maximal protection) in anesthetized dogs was >6 hr with inhaled Ba 679 BR (Fig 2 B) and about 1 and ½ hours with inhaled ipratropium
(Fig 2 A).

FIGURE 2

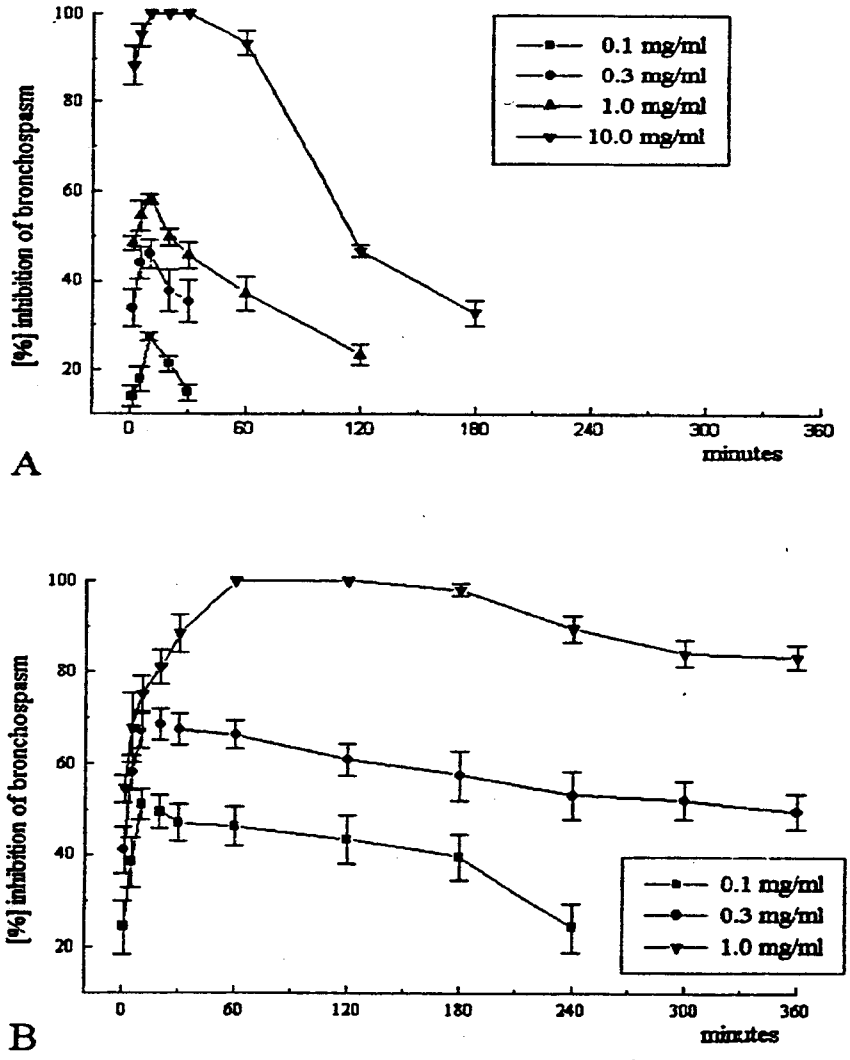
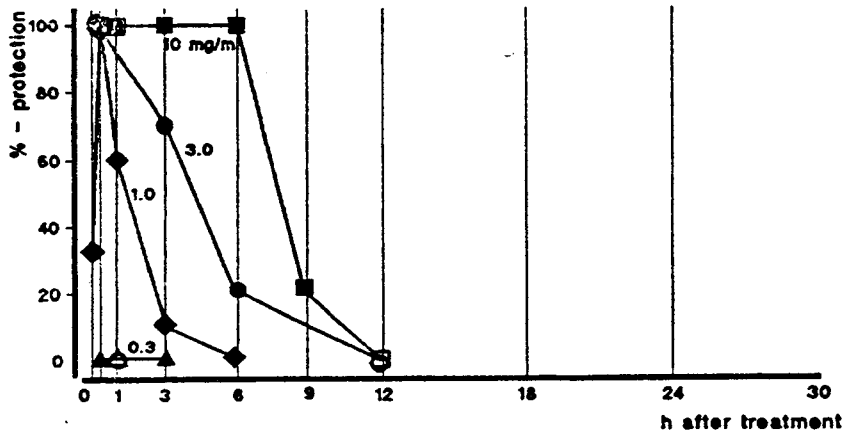


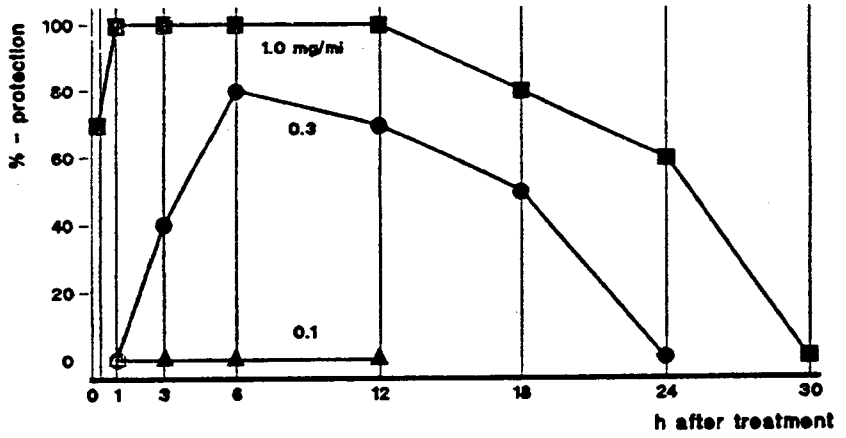
Figure 8.2.2: Time course of the protection against acetylcholine-induced bronchospasms by inhaled ipratropium bromide (panel A) or Ba 679 BR (panel B) in dogs. Means ± SEM, n = 6.

In conscious guinea pigs, a dose of 1 mg/mL nebulized concentration of Ba 679 BR was protective to 100% animals at 12 hours and declined to 60% protective at 24 hours (Fig 3 B). At equal dose, Ba 679 BR protected 100% of animals at 6 hours whereas ipratropium bromide protected only 20% of animals. Even a high dose of 10 mg/mL of nebulized ipratropium bromide offered less than 12 hours duration of drug effect (Fig 3 A).

In another study on guinea pigs, multiple doses of Ba 679 BR were administered (via inhalation) for 5 and 14 days and protection against bronchospastic collapse and inhibition of pilocarpine-stimulated salivation was measured. Results showed that a



A



B

Figure 8.2.3: Protective effect of inhalation of ipratropium bromide (panel A) or Ba 679 BR (panel B) against asphyctic collapse caused by exposure of conscious guinea pigs to acetylcholine aerosol, n = 10/time point.

non-protective dose of 0.1 mg/mL after single administration escalated to partial protection after 5 days and to full protection after 14 days of administration. ED_{50} decreased after multiple dosing as shown in Table 4.

Table 4

OTHER PHARMACODYNAMICS

1. Receptor Binding Profile: Ba 679 BR showed high affinity to muscarinic receptors

Table 8.2.6: Bronchoprotective effect and inhibition of stimulated salivation in guinea pigs following the first dose and up to 14 days of once daily treatment with inhaled Ba 679 BR.

Inhalation [mg/ml] solution	First dose	5 days	14 days	EC first dose / EC multiple dose
Bronchoprotection				
NOEL	0.1	<0.03	<0.03	
EC_{50}	0.34	0.045	<0.1	>7.6
Inhib. Salivation				
NOEL	1.0	0.3	0.3	
EC_{50}	1.82	0.54	0.48	3.8
EC salivation / EC bronchoprotection	5	12	>5	

(Ki<0.1 nM), low affinity for H_1-receptors (81 nM), and no affinity for α-,β-, serotonin-, dopamine-, histamine, nicotine-, benzodiazepine-, MK801- or adenosine-1-receptors.

2. Inhibition of MAO Activity: MAO type A or B was not inhibited by 100,000 nM of Ba 679 BR.

3. Inhibition of Transmitter Uptake: In a freshly prepared synaptosome suspensions from rat brain cortex, Ba 679 BR (10 μM) did not inhibit noradrenaline, dopamine, serotonin or choline uptake. The electrically evoked release of noradrenaline from rat brain hypothalamic slices was stimulated only at a high concentration (EC_{50}=36 μM).

4. Influence on Transmitter Release: In freshly prepared rat brain slices, there was no significant influence on electrically evoked release of dopamine, serotonine or acetylcholine up to a concentration of 100 μM.

5. Protection against Pilocarpine-stimulated salivation: A study was conducted in

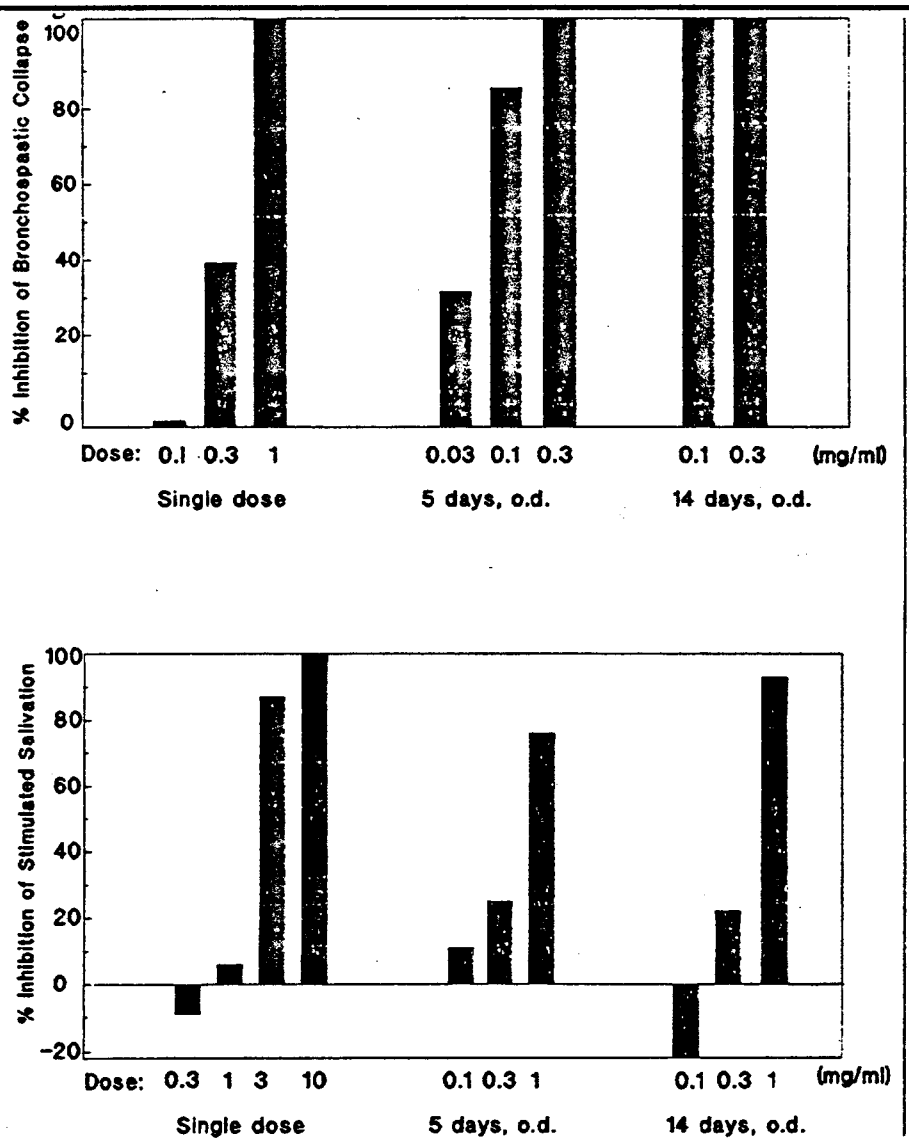


Figure 8.2.4: Protection against acetylcholine induced bronchospastic collapse in conscious guinea pigs and inhibition of pilocarpine-stimulated salivation in the same anesthetized animals (n=6). Ba 679 BR was administered by inhalation (whole body exposure; o.d.=once-a-day).

which protection against acetylcholine induced bronchospastic collapse in conscious guinea pigs and inhibition of pilocarpine-stimulated salivation in anesthetized guinea pigs was compared. Results (Figure 4) showed that inhibition of salivation required 5-12 times higher dose than for bronchoprotection. This therapeutic ratio had similar trend for single or multiple dose(s).

SAFETY PHARMACOLOGY

CNS Effects:

Mice: A 2.5 mg/kg (s.c.) dose of Ba 679 BR had no effect on nocturnal locomotion of mice (scopolamine was active at this dose).

Rabbit: An i.v. dose of 0.1 mg/kg did not induce changes in the EEG-pattern.

Cardiovascular System:

Dog: No cardiovascular effects (heart rate, ventricular systolic pressure and systolic and diastolic aortic pressure) were induced when dogs inhaled 100 times of bronchospasmolytic EC_{50} dose (1% Ba 679 BR in distilled water). Information on volume of test article and duration of experiment was not provided hence, dose (mg/kg) could not be estimated.

Renal System:

Rat: Oral dosages of 3-10 mg/kg did not influence urine or electrolyte excretion.

Gastro-intestinal Effect:

Mice: The intestinal passage was delayed with an ED_{50} of 2.3 mg/kg, p.o. (Table 5). Pilocarpine-stimulated salivation inhibited at ED_{50} 0.0042 mg/kg, i.v. (Table 5).

Rat: Inhibition of pilocarpine-stimulated salivation with an ED_{50} of 0.005 mg/kg, i.v. (Table 5); inhibition of gastric juice secretion at an ED_{50} of 0.0032 mg/kg, s.c. (Table 5).

Rabbit: Meal-induced salivation inhibited at an ED_{50} of 0.0015 mg/kg, i.v. (Table 5).

Dog: Meal-induced salivation inhibited at an ED_{50} of 0.0015 mg/kg, i.v. (Table 5).

Ocular Effect:

Mouse: Pupil dilation was seen at ED_{50} of 0.0136 mg/kg, s.c. (Table 5).

Rat: Pupil dilation occurred at ED_{200} (dose that produces doubling of pupil diameter) of 0.0017 mg/kg, i.v. while antimiotic activity was found at ED_{50} 0.0034 mg/kg (Table 5).

Rabbit: Antimiotic activity was not noticed at 0.01 mg/kg, i.v. (Table 5).

Dog: One hour topical administration of 0.005 mg Ba 679 BR, 0.005 mg ipratropium bromide or 0.01 mg atropine into the eyes resulted in maximal pupil dilation (mydriasis) within one hour (Table 5). The pupillary reflex to light was completely blocked. While high dosages of carbachol (0.6 mg) temporarily induced miosis for 2 to 3.5 hours in the ipratropium or atropine exposed dogs, the Ba 679 BR-induced mydriasis was not influenced by dosages of carbachol up to 3.6 mg. Mydriasis could not be reversed by timolol (β blocker). Mydriasis lasted for longest period in the Ba 679 BR-treated dogs (7 days as opposed to 4-5 days for ipratropium bromide or 2 days for atropine).

TABLE 5: *In vivo* antagonistic effects of systemic Ba 679 BR at muscarinic acetylcholine receptors of different organs of various animal species.

Species/ Condition	Effect	Characteristic Value	Unit	Ba 679 BR Doses	Ipratropium Doses	Route
Rabbit/ Conscious	Antimiotic (light)	NOEL	mg/kg	>0.01	>0.03	i.v.
	Meal-induced salivation	ED_{50}	mg/kg	0.0015	<0.03	i.v.
Rat/ Anesthetized	Pilocarpine stimulated salivation	ED_{50}	mg/kg	0.005	0.009	i.v.
Rat/ Conscious	Gastric juice secretion (pylorus lig./unstimulated)	ED_{50}	mg/kg	0.0032	0.175	s.c.
	Antimiotic (light)	ED_{50}	mg/kg	0.0034	0.0149	i.v.
	Pupil dilation	ED_{200}**	mg/kg	0.0017	0.0022	i.v.
Mice/ Anesthetized	Pilocarpine stimulated salivation	ED_{50}	mg/kg	0.0042	0.0125	i.v.
Mice/ Conscious	Pupil dilation	ED_{50}*	mg/kg	0.0136	0.0364	s.c.
Dog	Meal-induced salivation	ED_{50}	mg/kg	0.0015		i.v.
	Pupil dilation	ED_{100}	mg	0.005	0.005	Topical to eyes

* ED_{50} = 50% inhibition of maximum drug effect (Only this study); Other studies: ED_{50}=50% of the total effect.

** ED_{200} = Dose that produced doubling of pupil diameter.

Impact of Swallowed fraction of inhaled drug: To assess the impact of swallowed fraction of inhaled drug on antimuscarinic effects, studies were conducted in dogs, rats, and mice. Results (Table 6) showed that orally effective doses of Ba 679 BR were 100 times higher than systemic dosages in dogs, 400 times higher in rats, and 1000 times higher in mice.

Table 6. Antagonistic effects of Oral Ba 679 BR at muscarinic acetylcholine receptors of different organs of several species.

Species/ Condition	Effect	Characteristic Value	Dose (mg/kg)		F (Oral/ Systemic)**	
			Ba 679	Ipra.	Ba 679	Ipra.
Dog/Conscious	Antimiotic (light) Meal-induced Salivation	NOEL ED_{50}	0.3 0.13	3.0 5.0	87	>167
Dog/Conscious	Lacrimal secretion (Schirmer test)	NOEL ED_{50}	>0.02 0.196			
Rat/Anesthetized	Pilocarpine-stimulated salivation	ED_{50}	0.71	0.68	142	76
Rat/Conscious	Antimiotic (light) Pupil diameter	ED_{50} ED_{200}	2.47 0.686	2.12 0.901	726 404	142 409
Rat/Conscious	Diuresis (water-loaded)	NOEL	3-10	10-30		
Mice/Anesthetized	Pilocarpine-stimulated salivation	ED_{50}	8.2	13.8	1952	1104
Mice/Conscious	Pupil diameter	ED_{50}*	14.0	48.3	1029	1327
Mice/Conscious	Intestinal passage	ED_{50}*	2.3	9.1		

* Only in these studies, ED_{50} refers to 50% inhibition of the maximal drug effect. In the other studies, 50% means 50% of the total effect.
** Ratio of orally effective dose to parenteral (i.v., s.c.) effective dose to produce the effect.

These results suggest that the proportion of the dose which is likely to be swallowed after inhalation (> ½ of the dose) will not produce pharmacodynamic effects.

Interaction Studies:

Ba 679 BR and albuterol were inhaled by dogs to achieve 50% protection against acetylcholine bronchospasms. While bronchoprotection by Ba 679 BR (0.0025 mg) resulted in delayed onset and a half life of 260 minutes with no cardiovascular effects, a 0.2 mg salbutamol dose acted rapidly with a half life of only 12 minutes and resulted in increase in heart rate and dp/dt_{max}. Combination of both drugs at the same doses resulted in additive effects such as fast onset, up to 80% bronchoprotection, and long duration of action attributable to Ba 679 BR. However, cardiovascular effects of albuterol were retained. Thus, the positive pulmonary effects of both classes of compounds of a long duration of antimuscarinic effect and a fast onset of β-adrenergic effect, respectively, were present in the combination. However, none of the two drugs had potentiation effect on each other.

SUMMARY OF PHARMACOLOGY

Ba 679 BR is an *in vitro* (M1, M2, and M3 muscarinic receptor binding; inhibition of methacholine-stimulated beat frequency in ciliated tracheal cells) and *in vivo* (protection against acetylcholine induced bronchospasm in rats, rabbits, guinea pigs, and dogs) muscarinic receptor antagonist (MRA). It shows pharmacodynamic properties at low doses (ED_{50} range for inhalation: 0.03-6.64 μg/kg; ED_{50} range for i.v.: 0.3-3.0 μg/kg). Its anticholinergic characteristics suggest role in chronic obstructive pulmonary disease (COPD). General pharmacodynamic studies (receptor binding profile, inhibition of MAO activity and transmitter uptake, influence on transmitter release, protection against acetylcholine-induced bronchospastic collapse) showed its activity as an MRA to be selective.

Ba 679 BR had no significant effect on CNS in mouse and rabbit, cardiovascular system in dog, and renal system in rat. There were typical anticholinergic effects. There was a delay in intestinal passage in mice. Topical administration of small quantity of drug resulted in mydriasis (pupil dilation) in dog and, therefore, an inadvertent contamination from inhalable Ba 679 BR has a potential for a risk to eyes of Subjects enrolled in the clinical study. The risk would be greater to patients with small angle glaucoma and, therefore, such patients should be excluded from entering the study. Administration of Ba 679 BR to rat (i.v.) and mice (s.c.) also resulted in pupil dilation. Ba 679 BR 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. Combined administration of specified doses of Ba 679 BR and albuterol resulted in additive effects.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Drug Absorption: (See Table 7 on page 24 for data on pharmacokinetic parameters). The oral bioavailability of Ba 679 BR is low (dog=4%; mouse and rat=1%). Plasma drug concentrations were time dependent. The disappearance of the drug from plasma was biphasic after administration via i.v. or inhalation. There was an early rapid phase with $t_{1/2}$ of less than half-hour which was followed by a slower terminal phase with a $t_{1/2}$ 2 to 8 hours. Very little (if any) accumulation of the drug was observed by inhalation, i.v. or p.o. The exposure of the drug (AUC) via oral and i.v. route was higher in mice than in dog or rat. The mean residence time of the drug in dog was shorter via inhalation (1.1 hours) than via i.v. (3.8 hours) or p.o. (5.5 hours). Based upon AUC (0-6 h) values from a dose of 0.1 mg/kg administered to dogs via i.v. (42.4 ng.h/mL) and inhalation (6.6 ng.h/mL), the amount of drug which will reach the deeper airways (the assumed site of absorption following inhalation administration) can be estimated to be about 16%.

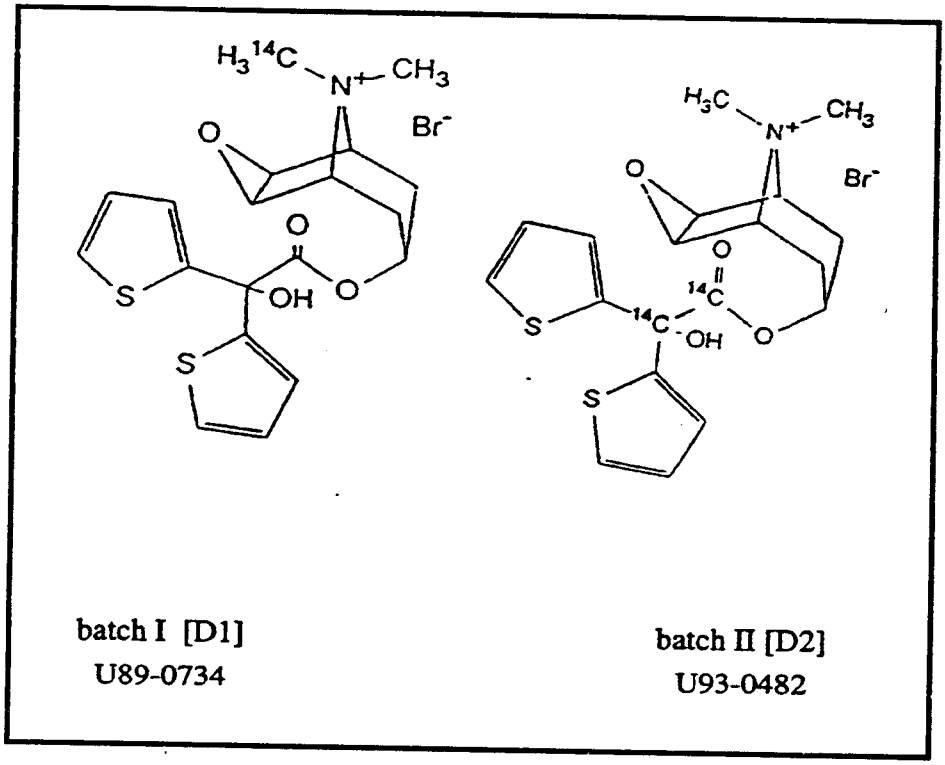
TABLE 7. Ba 679 BR: PHARMACOKINETIC PARAMETERS.

Parameter	Mouse	Rat	Dog
ORAL			
Dose (mg/kg)	10.0	10.0	1.0
C_{max} (ng/mL)	740 ng/g	17.0	3.4
T_{max} (hours)	0.08	4.5 (♂), 15 (♀)	2.0
F (%)	1%	1%	4%
Mean residence time (hours)	----	----	5.5
AUC (ng.hr/mL): 0-24 hr.	4300	----	15.4
0-96 hr.	----	418 (♂), 557 (♀)	35.0
INTRAVENOUS			
Dose (mg/kg)	10.0	10.0	0.1
C_{max} (ng/mL)	----	----	27 (♂), 11 (♀)
$t_{1/2}$: 0-1 hr.	0.13-0.18	----	0.25
8-24 hr.	6.0	8.0	----
Mean residence time (hours)	----	----	3.8
AUC (ng.hr/mL): 0-8 hr.	9500	----	----
0-24 hr.	----	7429	42.4
INHALATION			
Dose (mg/kg)	0.2	0.07, 0.6, 5.0	0.1
$t_{1/2}$: 0-1 hr.	0.13	0.28	----
0-24 hr.	----	8.0	2.2
Mean residence time (hours)	----	----	1.1
AUC (ng.hr/mL) 0-7 hr.	----	----	6.6

---- Data not prvided.

Methods: Studies on drug distribution, metabolism, and excretion were conducted using radioactively labeled drug. Ba 679 BR was labeled with ^{14}C at two different positions in the molecule (as shown in Figure): peripheral (batch I) and central (batch II). Radioactive labelling of the drug at peripheral and central positions enables the study of the metabolism of whole drug molecule as well as N-methylscopine and dithienylglycolic acid after a postulated cleavage by base or enzymatic catalysis.

Distribution: In mice, following i.v. administration, the rank order of drug levels within the first 5 minutes was (all values in µg/g organ weight): kidney (144.9)>liver



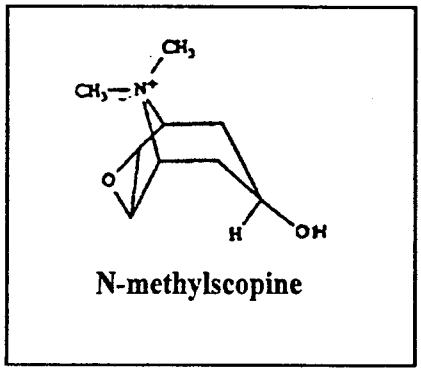
(44.9)>plasma (12.3)>lung (6.3)> blood (6.6)>heart (4.0):. At 8 hr., the drug level in the liver was the highest (5.1 µg/g organ weight). By the p.o. route also, the drug level was highest in the liver (0.8 µg/g organ weight). In rats, whole body autoradiographical investigations showed that within 10 minutes of drug administration via i.v. route, most of the drug was distributed in liver, kidneys, stomach, and intestine. When the drug was given p.o., it could be autoradiographically detected in the G.I. tract only. Following intratracheal administration, the drug concentrations were: lungs (658.5)>kidney (79.3)>liver (25.8)>plasma (16.3)>heart (8.3); even after 24 hrs, 1% of the dose was present in the lungs.

Metabolism: A combination of HPLC [redacted] was used to identify and validate Ba 679 BR content in plasma. The limit of quantitation of the assay was [redacted] The content as well as the metabolic profile of the drug in individual biological samples was determined by [redacted] HPLC detection. Degree of metabolism is not known.

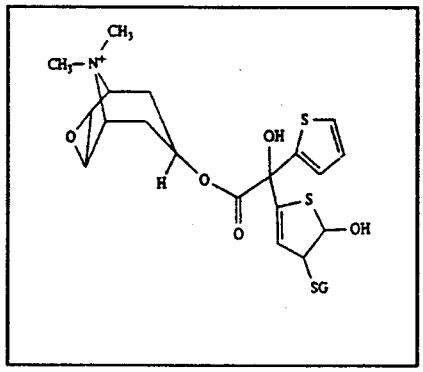
MICE:

Metabolic Profile in the Urine: In a single dose i.v. study, N-methylscopine was the major metabolite and a glutathione conjugate of Ba 679 BR was the minor metabolite in the urine. However, when the drug was given via p.o., an additional (and slightly more polar) minor metabolite appeared which could not be identified. Information on metabolites for intratracheal route was not provided.

Metabolic Profile in the Plasma: In the single dose study via i.v. route, glutathione conjugate accounted for about 50% of the drug substance while two



N-methylscopine



minor metabolites were not identified.

Metabolic Profile in the Bile: Glutathione conjugate was the main metabolite in the single dose i.v., intratracheal (data not provided), and p.o. studies. A second metabolite present in samples from i.v. and p.o. studies was not identified.

Metabolic Profile in the Liver, Lung, Kidneys, and Heart: In single dose studies via i.v. and p.o., glutathione conjugate of Ba 679 was the main metabolite in the liver, lung, kidney, and heart. Same finding was reported for intratracheal route but data were not provided. The quantities of metabolite in kidney and heart were relatively low.

RAT:

Metabolic Profile in the Urine: In single dose study via i.v. with peripherally labeled drug molecule, the main metabolite was N-methylscopine. However, with centrally labeled drug molecule, N-methylscopine was not seen due to the position of the label but 4 other metabolites were detected. In a single dose study via p.o., the drug completely metabolized to N-methylscopine at 90 mg/kg while at 543 mg/kg, some parent compound was also observed.

Metabolic Profile in the Plasma: In the single dose intratracheal administration of peripherally labeled drug, N-methylscopine was formed. However, with the administration of centrally labeled drug, N-methylscopine was not produced and only small quantities of other metabolites were produced.

Metabolic Profile in the Bile: The metabolites that were present in small quantities in urine were the major components in the bile but were unidentified.

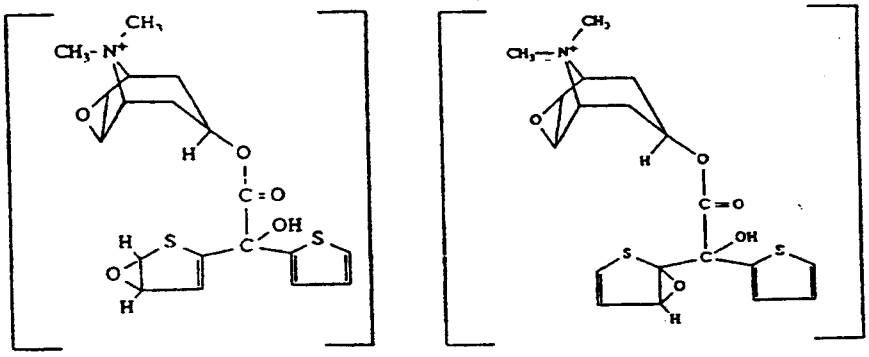


Figure 8.3.7: Postulated epoxides as the first metabolic attack of Ba 679 BR

Metabolic Profile in the Liver, Lung, Kidneys, and Heart: In an intratracheal single dose study, major metabolites in the liver, heart, and kidney were the same as reported for bile. No metabolite was seen in lung samples.

The metabolic pathway elucidated from the chemical structures of the compounds is divided into two steps:

(i) The first (main) metabolic pathway was the epoxidation (Figure 6) of one or both thiophene rings of the esterified di-(2-thienyl) glycolic acid.

If only one of the two thiophene rings are oxidized, two isomeric epoxides may be formed; if both thiophene rings are oxidized, an additional three isomeric di-epoxide may be formed. These epoxides have not been isolated yet.

(ii) The subsequent metabolic pathway consisted of a phase II reaction with glutathione. The metabolic mixture of the glutathione conjugates is further degraded by gamma-glutamyl transferase and glycine dipeptidase. The major detoxification reaction is the conjugation with glutathione.

A complex mixture of metabolites resulted from the possible isomers of the oxidative attack and subsequent phase II metabolic reactions (by glutathione-S-transferase, by epoxide hydrolase reaction, and by aromatization to hydroxylated thiphene rings). The major portion of these metabolites were directly eliminated from liver (site of origin) to the bile and only a small fraction of these metabolites reached the systemic circulation.

Cytochrome P-450 Enzyme Induction: Multiple i.v. administration of 10 mg/kg drug in male rats over a period of 5 days did not induce cytochrome P-450 isozymes.

Glutathione Content of the Liver: At 0.01, 0.04 and 16 mg/kg doses of Ba 679 BR administered via i.v., there was no decrease in the glutathione content of the liver.

DOG:

Metabolic Profile in the Urine: When the drug was administered via i.v. route, major metabolite was N-methylscopine while small quantities of glutathione conjugated metabolites were also present. When the drug was given p.o., only the metabolite N-methylscopine was seen.

Metabolic Profile in the Plasma: When the drug was given via i.v., only parent compound and no metabolites were detected.

Metabolic Profile in the Bile: When the drug was given via i.v., the metabolite that was detected in feces was N-methylscopine. When the drug was administered p.o., no metabolite was detected in feces.

Excretion: In mice, renal excretion was about 64% and fecal excretion about 29% for i.v. and about 16% renal excretion and 73% fecal excretion for p.o. routes. In rats, following i.v. administration, the drug excreted about 40% (females) to 50% (males) renally and 40 to 50% (either sex) through bile. In dogs, about 75% of the drug was renally excreted within 9 days and remaining 25% excreted fecally following i.v. administration; following p.o. administration, only about 35% of the drug excreted renally in 9 days while, about 62% excreted fecally.

PROTEIN BINDING

Plasma protein binding of Ba 679 BR was higher in human (65.3%) than in mouse (21.7%), dog (21.0%), rat (19.7%) or rabbit (15.5%).

TOXICOLOGY

ACUTE, SINGLE DOSE, TOXICITY:

Mice: Acute Oral and i.v. Toxicity
Boehringer Ingelheim Study U90-0494, 18 June, 1990, Vol 1.9, Page 122

METHODS

Species/Strain: Albino mice, strain Chbb:NMRI
Animals: 30/Sex and 5/Sex/group
Route: Oral and intravenous
Dosage: 700 (LD), 1000 (MD), and 1400 (HD) mg/kg, p.o.; Details of i.v. dosing are as follows:

i.v.	LD	MD	HD
♂	12.5 mg/kg	16 mg/kg	20 mg/kg
♀	16.0 mg/kg	20 mg/kg	25 mg/kg

Duration of Exposure: Single dose with 14 days observation period
Clinical Observations: Twice daily
Body Weights: One day prior to dosing and on Day 8 and Day 14
Gross Necropsy: Day 14

RESULTS (p.o.):

Clinical Observations: **See Table 8.**
Mortality: 3/10 MD and 5/10 HD.
Body Weights: No toxicologically significant treatment-related effect.
Gross Pathology: Coprostasis (HD, 4/10); Lung: emphysema (LD 1/10, MD 2/10). No other toxicologically significant treatment-related effect.
Histopathology: Conducted on only one animal. No toxicologically significant treatment-related effect.
LD_{50}: 1336.4 mg/kg (Probit analysis).

Table 8. Clinical Observations in Acute Lethality Study in mice , p.o.

Toxic Symptom		INCIDENCE Male LD	MD	HD	Female LD	MD	HD
First Day:	Reduced motility	-	-	-	-	1	-
	Tremor	1	-	4	1	2	2
Second Day:	Sedation	1	-	2	-	-	1
	Dyspnea	1	-	2	-	-	-
	Hunched posture	-	-	-	-	-	1
Third Day:	Abdominal or lateral position	-	-	1	-	-	1
Sedation		1	-	2	-	-	-
	Dyspnea	1	-	2	-	-	3
	Hunched posture	-	-	2	-	-	3
Fourth Day:	Sedation	-	-	3	-	-	1
	Dyspnea	1	-	3	-	-	2
	Retention of faeces	-	-	3	-	-	2
Fifth Day:	Retention of faeces	-	-	-	-	-	2

RESULTS (i.v.):

Clinical Observations: Dyspnea (♂: 1/5 LD, 4/5 MD, 4/5 HD; ♀: 2/5 LD, 5/5 MD & HD), Clonic convulsions (♂: 1/5 MD, 5/5 HD; ♀: 1/5 MD). No other toxicologically significant treatment-related effect.
Mortality: ♂: 3/5 at 16 mg/kg and 5/5 at 20 mg/kg; ♀: 3/5 at 20 mg/kg and 4/5 at 25 mg/kg.
Body Weights: No toxicologically significant treatment-related effect.
Gross Pathology: No toxicologically significant treatment-related effect.
LD_{50}: ♂: 15.5 mg/kg (Karber method); ♀: 20.6 mg/kg (Probit analysis). According to the sponsor, use of Probit (a parametric method) or Karber (a non-parametric method) was based upon the nature of data analyzed.

Mice Acute i.v. Toxicity Study: —— (degradation product of Ba 679 BR)
Boehringer Ingelheim Study U91-0844, 30 August, 1991, Vol. 1.17, Page 079

15 animals/sex (5 ♂ and 5 ♀ per group) were administered 100, 125, and 160 mg/kg of — via i.v. Toxicologically important Clinical findings included dyspnea (3/10 MD, 10/10 HD), tachypnea (1/10 MD), tremor (2/10 MD, 2/10 HD), clonic convulsions (4/10 HD) and mortality (3/10 MD, 6/10 HD). LD_{50} was 148 mg/kg.

Mice Acute i.v. Toxicity Study: — (degradation product of Ba 679 BR)
Boehringer Ingelheim Study U91-0845, 20 August, 1991, Vol. 1.17, Page 105

15 animals/sex (5 ♂ and 5 ♀ per group) were administered 100, 125, and 160 mg/kg of Ba 679 BR — via i.v. Toxicologically important Clinical findings included dyspnea (1/10 LD, 4/10 MD, 8/10 HD), tachypnea (3/10 HD), tremor (1/10 MD and HD), saltatory convulsions (1/10 MD), and mortality (3/10 MD, 5/10 HD). LD_{50} was 156 mg/kg.

Mice Acute i.v. Toxicity Study: — (degradation product of Ba 679 BR)

Boehringer Ingelheim Study U91-0860, 10 January, 1991, Vol. 1.17, Page 208

5 animals/sex were administered 200 mg/kg of — via i.v. No toxicologically significant treatment-related effects. Due to no mortality, LD_{50} could not be established.

Mice Acute i.v. Toxicity Study: — (degradation product of Ba 679 BR)
Boehringer Ingelheim Study U92-0680, 26 October, 1992, Vol. 1.20, Page 405

20 animals/sex (5 ♂ and 5 ♀ per group) were administered 0, 16, and 20 mg/kg of — and 16 mg/kg of Ba 679 BR via i.v. Mortality was observed at 20 mg/kg (1/10) — and at 16 mg/kg (8/10) Ba 679 BR treated animals. High dose of degradation product employed was not high enough for estimating LD_{50}. No other toxicologically significant treatment-related effects.

Mice: Acute Inhalation Toxicity

Boehringer Ingelheim U91-0812, 17 October 1991, Vol. 1.17, Page 046

CD-1 mice (5/Sex) were administered Ba 679 BR aerosol at 20 mg/mL (per sponsor, 20 mg/mL was the maximum concentration of the drug that could be obtained) via inhalation (snout only) for a period of 4 hours and observed for 14 days. About 50% of test aerosol contained particle size of — Total volume of drug used was 120.5 mL. The nominal and pulmonary dosages were 131 and 63 mg/kg respectively. There were 3 mortalities (1♂ and 2♀) and no other toxicologically significant effects.

Rat: Acute Oral and i.v. Toxicity

Boehringer Ingelheim U90-0493, 22 June, 1990, Vol. 1.9, Page 082

METHODS

Species/Strain: Rat Chbb:THOM
Animals: 25/Sex and 5/Sex/group
Route: Oral and intravenous
Dosage: 2000 (LD) and 4000 (HD) mg/kg, p.o. and 16, 20, and 25 mg/kg, i.v.
Duration of Exposure: Single dose with 14 days observation period
Clinical Observations: Day of treatment and twice daily post-treatment
Body Weights: One day prior to dosing and on Days 8 and 14
Gross Necropsy: Day 15. Autopsy findings were recorded only on animals that died. Histopathology conducted on 1 (p.o.) or 2 (i.v.) animals.

RESULTS (p.o.):

Clinical Observations: Reduced motility (1/5♂, 3/5♀), tremor (1/2♀), ataxia (1/5♀), dyspnea (1/5♂, 2/5♀), and emaciation (1/5♀): all toxic symptoms in HD group only.
Mortality: 1/5 HD ♂ and 3/5 HD ♀.
Body Weights: No toxicologically significant treatment-related effects.
Gross Pathology: No toxicologically significant treatment-related effects.
Histopathology: No toxicologically significant treatment-related effects.
LD_{50}: About 4000 mg/kg.

RESULTS (i.v.):

Clinical Observations: Reduced motility (♂: 2/5 LD, 2/5 MD; ♀: 2/5 MD), dyspnea (♂: 3/5MD, 4/5HD; ♀: 1/5LD, 2/5MD, 4/5HD), tachypnea (♂: 2/5 LD, 1/5 MD and HD; ♀: 3/5 LD, 2/5 MD), tremor (♂: 1/5 MD; ♀: 1/5 LD, 2/5 MD), and convulsions (♂:1/5 MD).
Mortality: ♂: 2/5 MD, 4/5 HD; ♀: 3/5 MD, 5/5 HD.
Body Weights: No toxicologically significant treatment-related effects.
Gross Pathology: All 14 dead animals showed blood congestion in liver and kidneys while 2/14 dead animals showed petechial hemorrhage.
Histopathology: Both dead animals whose thymus was investigated showed mild to moderate, multi focal venous congestion and multi focal hemorrhages.
LD_{50}: ♂: 21.5 mg/kg (Probit analysis); ♀: 19.5 mg/kg (Karber method). Per sponsor, nature of data dictated which method to use: a parametric (Probit) or a non-parametric (Karber) method.

Rat: Acute Inhalation Toxicity
Boehringer Ingelheim U90-0517, 27 April, 1990, Vol. 1.9, Page 163

Chbb:THOM rats (7/Sex) were administered Ba 679 BR aerosol (concentration of airborne drug: 2.7 mgL^{-L}) via inhalation (nose only) for a period of 4 hours and observed for 1 (2/Sex) or 14 (5/Sex) days. The animals inhaled 334.5 mg/kg of drug. Based upon aerodynamic diameter of particles ([redacted] , 40% of the effective inhaled dose was considered to represent deposition factor. Therefore, the actual drug intake was 133.5 mg/kg. Treatment resulted in pupil dilation which was reversible. Follicular hemorrhage in thymus (♂: 3/7) was histologically confirmed. There were no other toxicologically significant effects including mortalities.

Dog: Single Dose Inhalation Toxicity

Boehringer Ingelheim U91-0224, 02 August, 1991, Vol. 1.10, Page 167

Study Dates: 23 March, 1990 to 06 April, 1990
Testing Lab: [redacted]
Test Article: Ba 679 BR, White powder (Batch G).
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Beagle dogs.
Animals: 2/Sex, 1/Sex/group.
Route: Inhalation via [redacted] ultrasonic nebulizer.
Dosage: 0.5 and 3.0 mg/kg.
Duration of Exposure: 13 to 15 minutes. Following treatment, the animals were observed for a period of 14 days.
Clinical Observations: During and up to 4 hours after dosing.
Body Weights: Twice weekly commencing 2 weeks pre-trial.
Food Consumption: Daily commencing 2 weeks pre-trial.
Electrocardiography: Twice pre-trial (Weeks -2 and -1), pre-dose, immediately after dosing, and at +30, +90, +120, +180 and +240 min post dose.
Hematology: Once pre-trial, 24 h after dosing, and on Day 14 of observation period.
Clinical Chemistry: Once pre-trial, 24 h after dosing, and on Day 14 of observation period.
Drug Levels: Blood samples were obtained pre-dose and post dose at +5 min, +15 min, +30 min, +1 h, +2 h, +4 h, and +8 h.
Necropsy: Terminal.
Organ Weights and Gross- and Histo-Pathology: Absolute and relative weights of selected organs were determined and gross- and histo-pathological examinations conducted.

RESULTS

Dosage Levels: The dose levels that the animals of two groups received were estimated to be 0.7 and 3.6 mg/kg.

Clinical Signs: Two-fold increase in heart rate on the day of treatment.

Body Weights: **No toxicologically significant treatment-related effect.**

Food Consumption: Treatment resulted in drastic reduction (♂: 70%↓; ♀: 93%↓) in food consumption on the day of the treatment.

Hematology: No toxicologically significant treatment-related effect.

Clinical Chemistry: There was a 50% (1 day after treatment) to 100% (14 days after treatment) increase in lactate dehydrogenase (LDH) and hydroxybutyric dehydrogenase (HBDH) levels (increase in HD as compared to LD).

Organ Weights: No toxicologically significant treatment-related effect.

Gross Pathology: No toxicologically significant treatment-related effect.

Histopathology: Focal Kupffer-cell proliferation was seen in the livers of both males while, no such effect was noticed in females.

Toxicokinetics: Data were not provided.

SUBCHRONIC AND CHRONIC, MULTIPLE DOSE TOXICITY:

Mice: 13-wk Inhalation toxicity MTD Study
Boehringer Ingelheim Study U92-0717, 11 September, 1992, Vol. 1.21, Page 066

Study Dates: 16 September, 1991 to 17 December, 1991
Testing Lab:
Test Article: Ba 679 BR Batch 6187; Vehicle constituents: benzalkonium chloride (10 mg), disodium edetate (50 mg), citric acid monohydrate (8.4 mg), 0.1 N NaOH (0.8 mL), and 0.1 N HCl (0.6 mL) prepared in 100 mL water for injection.
GLP: GLP Statement signed by Study Director; Quality assured by Q.A. Unit.

METHODS

Species/Strain: Mouse CD-1
Animals: 90/Sex and 10/Sex/group (main study) or 8/Sex/group (Satellite study)
Route: Inhalation (nose only)
Dosage: 0 (vehicle only), 0.04 (LD), 0.2 (MD), 1.0 (MHD), and 5.0 (HD) mg/kg/day
Duration of Exposure: 13-weeks
Clinical Observations: Prior to, during, and one hour after the exposure.
Body Weights: Weekly
Food Consumption: Weekly
Ophthalmoscopy: Pre-study (all animals) and on Weeks 6 and 13 (Control and HD)
Hematology: Weeks 6 and 12
Clinical Chemistry: Weeks 7 and 13
Drug Levels: Blood samples were obtained 15 minutes after the end of administration of drug at the end of Weeks 2 and during Week 12.
Necropsy: Terminal. Necropsy was not done on animals in satellite groups.
Organ Weights and Gross- and Histo-Pathology: Absolute and relative weights of selected organs were determined and gross- and histo-pathological examinations conducted.

RESULTS (Table 9):

Dosage Levels: Up to 25% less than proposed dose levels.

Clinical Observations: No toxicologically significant treatment-related effects.

Mortality: Reported for Control (2), MD (3), MHD (5), and HD (4) in main study and control (1), MHD (2), and HD (1) in satellite study. Of these, 1 MD, 3 MHD, and 2 HD animals died during blood sampling. The mortality was drug-related (because of mortality, the sponsor lowered dose in the next 13-week toxicity study in mice).

Body Weights: Body weight gain was decreased in HMD and HD males (50% and 67%) and HD females (50%).

Food Consumption: The decrease in body weight gain was accompanied by decrease in food consumption (♂: about 1-3%; ♀: about 9-13%).

Ophthalmoscopy: No toxicologically significant treatment-related effects.

Hematology: At Week 6, there was about 80% increase in neutrophil counts for HD females. At Week 12, there was a 70% increase in neutrophil counts for HD males.

Clinical Chemistry: At Week 13, there was a dose-dependent increase in the levels of ALT (MD 13%, MHD 20%, HD 21%) and an increase in BUN (MD 25%, HMD 17%, HD 19%; females only). No other toxicologically significant treatment-related effects.

Organ Weights: There was a small but statistically significant decrease in absolute weights of kidney (♂: MHD 27%, HD 33%; ♀: MD 15%, MHD 11%, HD 22%), liver (♂: MHD 11%, HD 16%; ♀: MD 10%, MHD 12%, HD 19%), and spleen (♂: MHD 10%, HD 38%; no effect on spleen in females). Relative kidney weights slightly decreased (♂: LD 11%, MHD 16%, HD 18%; ♀: MD 12%, HD 12%) while there was no effect on relative liver or spleen weights.

Gross Pathology: No toxicologically significant treatment-related effects.

Histopathology: In males, effects on nasal cavity such as occurrence of epithelial hyaline droplets (Control 0/10, LD 3/10, MD 3/7, MHD 3/6, HD 3/8; Decedents: MHD 1/3) and focal acute inflammation (Control 0/10, LD 2/10, MD 1/7, MHD 2/6, HD 1/8) were drug-related but not dose dependent. In addition, 1 MD decedent animal had squamous metaplasia. In females, focal acute inflammation was obvious only at HD (5/8 HD vs. 3/10 controls) while, epithelial hyaline droplets were all the groups (Control 4/10, LD 6/10, MD 6/10, MHD 7/9, HD 6/8).

Toxicokinetics: Drug plasma levels increased in a linear dose dependent manner. There was accumulation of drug at Week 12.

NOAEL: Could not be established. *MTD:* 0.04 mg/kg/day.

Table 9. Effects of Ba 679 BR in mice in a 13-wk Inhalation Toxicity MTD Study.

* Significant at P <0.05	Males (mg/kg/day)					Females (mg/kg/day)				
	0.0	0.04	0.2	1.0	5.0	0.0	0.04	0.2	1.0	5.0
Body weight gain (g)	6	7	5	3	2	6	5	5	5	3
Food Consumption (g)	442	445	437	430	434	459	412	401	418	412
Hematology										
Neutrophils, 10^9/L,										
6 weeks	0.56	0.67	0.49	0.66	0.80	0.36	0.47	0.36	0.46	0.26*
12 weeks	0.52	0.69	0.52	0.79	0.89*	0.48	0.46	0.42	0.46	0.63
Clinical Chemistry										
AST (i.u./L) week 13	70	53	59	67	67	56	56	63	67*	68*
BUN (mmol/L) wk 7	6.2	6.1	6.3	6.5	6.6	5.5	5.5	6.8*	7.3*	6.9*
wk 13	6.7	6.6	7.5	7.2	7.4	6.3	6.7	7.9*	7.4*	7.5*
Glucose (mmol/L)	10.2	8.4*	8.9*	9.0*	8.4*	7.8	6.8	6.7	7.5	6.6
Organ Weights										
Kidney (g), abs.	0.61	0.56	0.57	0.48*	0.46*	0.39	0.4	0.34*	0.35*	0.32*
rel.	0.59	0.53*	0.56	0.51*	0.50	0.38	0.39	0.34*	0.36	0.34*
Liver (g), abs.	1.78	1.75	1.81	1.6*	1.53*	1.58	1.52	1.44*	1.41*	1.33*
Spleen (g), abs.	0.11	0.11	0.1	0.1*	0.08*	0.14	0.13	0.12	0.11	0.11
Uterus (g), abs.						0.26	0.17*	0.21	0.17*	0.23
rel.						0.26	0.17*	0.21	0.17*	0.23
Histopathology										
Nasal Cavity:										
Epi. Hyaline droplets	0/10	3/10	3/7	4/9	3/8	4/10	6/10	6/10	8/10	6/8
Focal acute inflamma.	0/10	2/10	1/7	2/6	1/8	3/10	1/10	1/10	2/10	5/8
Toxicokinetics										
		Week 2					Week 12			
Plasma Levels (ng/mL)										

Mice: 13-weeks Inhalation Toxicity Dose Ranging Study

Boehringer Ingelheim Study U92-0271, 7 April, 1992, Vol. 1.18, Page 002

Study Dates: 26 March to 26 June, 1991
Testing Lab: —
Test Article: Ba 679 BR Batch II; Vehicle constituents: benzalkonium chloride (10 mg), ethylene diamine tetra acetic acid (Na salt) (50 mg), sodium chloride (900 mg), citric acid monohydrate (8.4 mg), 0.1 N NaOH (0.8 mL), and 0.1 N HCl (0.6 mL) prepared in 100 mL water for injection.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: CD-1 mice
Animals: 90/Sex; 10/Sex/group for main groups and 8/Sex/group for satellite groups.
Route: Inhalation via snout — ultrasonic nebulizer).
Dosage: 0 (Control vehicle solution), 0.08 (LD), 0.25 (MD), 0.75 (LMD), and 2.0 (HD) mg/kg/day.
Duration of Exposure: 91 days (1 hour exposure/day).
Clinical Observations: Daily.
Body Weights: Weekly commencing 1 week pretrial.
Food Consumption: Weekly commencing 1 week pretrial.
Ophthalmoscopy: Pretrial (all animals) and during Weeks 6 and 13 (Control and HD only) of treatment.
Hematology: Weeks 6 and 12.
Clinical Chemistry: Weeks 7 and 13.
Drug Levels: At the end of Weeks 2 and 12 about +15 min. post exposure from satellite animals.
Necropsy: Terminal.
Organ Weights and Gross- and Histo-Pathology: Absolute and relative weights of selected organs were determined and gross- and histo-pathological examinations conducted.

RESULTS (Table 10):

Dosage Levels: Calculated dose levels were 0, 0.0764, 0.2388, 0.8187, and 1.7184 mg/kg/day.
Clinical Signs: No toxicologically significant treatment-related effect.

Mortality: Excluding animals that were killed in extremis or during blood sampling, 10 animals died (LD, MD, MHD: 2 each; HD: 4).

Body Weights: There was consistent reduction in body weight gains (♂: 40% LD and MD and 60% MHD and HD; ♀: 20% LD and MD; 40% MHD, 60% HD).

Food Consumption: No toxicologically significant treatment-related effect.

Ophthalmoscopy: No toxicologically significant treatment-related effect.

Hematology: No toxicologically significant treatment-related effect.

Clinical Chemistry: Glucose levels were reduced in MD (23%), MHD (23%), and HD (21%) females. No other toxicologically significant treatment-related effect.

Organ Weights: Relative kidney weights in females reduced by about 15% at all doses (statistically significant but no dose dependence). Relative liver weights in females were reduced up to 12% (LD, MD, MHD statistically significant but no dose dependence). Absolute spleen weights were reduced in females by 25% at MD, MHD, and HD while there was no effect on relative spleen weights. Relative rectum weights were increased in both sexes at all doses by up to 60% (dose dependent and statistically significant). Relative heart weights were increased in females by about 21% at all doses (statistically significant but no dose dependence).

Gross Pathology: Incidences of ovarian cysts in females were drug-related.

Histopathology: In males, thymic atrophy (MD 2/2, MHD 2/3) and lymphoid depletion of splenic white pulp (MD 1/2, MHD 2/3) are probably drug related. In females, squamous metaplasia of laryngeal epithelium (MHD 1/10, HD 4/10), thymic atrophy (HD 3/4), lymphoid depletion of splenic white pulp (HD 3/4), and increased lymphocytolysis (HD 1/4) are drug-related.

Toxicokinetics: Drug plasma levels increased linearly in a dose-dependent manner. There was no accumulation of drug from Week 2 to Week 12 of exposure period.

MTD: <0.08 mg/kg/day.