Table 10. 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	0.08	0.25	0.75	2.0	0	0.08	0.25	0.75	2.0
Body weight gain (g)	5	3*	3*	2*	2*	5	4	4*	3*	2*
Clinical Chemistry										
Glucose (mmol/L)	7.1	6.6	6.0	6.4	6.2	7.7	6.7	5.9*	5.9*	6.1
Organ Weights (g)										
Kidney, abs	0.61	0.57	0.53*	0.46*	0.5*	0.42	0.36*	0.36*	0.35*	0.34*
rel	0.57	0.56	0.54	0.48	0.52	0.41	0.36*	0.36*	0.35*	0.35*
Liver, abs	1.58	1.64	1.46	1.35*	1.37*	1.45	1.22*	1.24*	1.25*	1.24*
rel	1.46	1.60	1.49	1.43	1.45	1.39	1.23*	1.23*	1.26*	1.30
Spleen, abs	0.09	0.09	0.08	0.08	0.07	0.12	0.10	0.09*	0.09*	0.09*
rel	0.08	0.09	0.08	0.08	0.07	0.11	0.10	0.09	0.09	0.10
Rectum, abs	1.22	1.41	1.40	1.47	1.43	1.14	1.33	1.44*	1.61*	1.55*
rel	1.13	1.38*	1.42*	1.53*	1.50*	1.04	1.35*	1.42*	1.63*	1.67*
Heart, abs	0.19	0.19	0.19	0.18	0.18	0.19	0.15*	0.17*	0.15*	0.15*
rel	0.18	0.18	0.20	0.19	0.19	0.19	0.15*	0.17*	0.15*	0.15*
Gross Pathology										
Ovarian Cysts								1/10	3/10	1/10
Histopathology										
Squam. metaplasia of Laryngeal Epithel.	3/10		1/10		2/10				1/10	4/10
Epithel. ulceration with necrosis		1/10								
Thymic atrophy			2/2 †	2/3 †						3/4 †
Lymph. depletion of splenic white pulp			1/2 †	2/3 †						3/4 †
Lymph node: ↑ lymphocytolysis			2/2 †	1/3 †						1/4 †
Toxicokinetics										
			Week 2					Week 12		
Plasma Levels (ng/mL)										

† These tissues were examined in only 2-4 animals.

Rat: 4-weeks Oral Toxicity Range Finding Study

Boehringer Ingelheim Study U92-0477, February 27, 1992, Vol. 1.19, Page 002

Study Dates: May 02 to September 03, 1990.
Testing Lab: Boehringer Ingelheim Dep. Experimental Pathology and Toxicology.
Test Article: Ba 679 BR (batch G, 05/02/1990) solubilized in water (pH 2.6).
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb:THOM Wistar rats.
Animals: 35/Sex; 7/Sex/group.
Route: Oral
Dosage: 0 (Control: water at pH 2.6), 0.1 (LD), 10 (MD), 200 (MHD), and 500 (HD) mg/kg/day. Concentration of drug solutions were 0.001% (LD), 0.1% (MD), 2% (MHD), and 5% (HD).
Duration of Exposure: Daily for 4 weeks.
Clinical Observations: Twice daily Monday to Friday; Daily on Saturdays, Sundays, and holidays.
Body Weights: Weekly.
Food Consumption: Weekly.
Hematology: Weeks -2, 1, and 4 (4 animals/group).
Clinical Chemistry: Weeks -2, 1, and 4 (4 animals/group).
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 11):

Dosage Levels: Within 15% of intended levels.

Clinical Signs: No toxicologically significant treatment-related effect.

Mortality: None.

Body Weights: Treatment resulted in dose-related reduced bodyweight gains in both males (MD 25%, MHD 60%, HD 75%) and females (MD 27%, MHD 36%, HD 90%).

Food Consumption: Reduced food consumption at Week 4 in males at MHD (13%) and HD (11%).

Hematology: No toxicologically significant treatment-related effect.

Clinical Chemistry: Statistically significant increase in creatinine in LD (21%) and MHD (29%) males and MHD (19%) females.

Organ Weights: No significant effect in females. In males, statistically significant reduction in the weights of kidneys (HD 22%), liver (HD 26%), thymus (HD 45%), testes (MHD and HD 15%), and pituitary (HD 28%) and increase in the weight of lungs (HD 13%).

Gross Pathology: Higher incidence of coprostasis in males (MD and MHD 100%, HD 33%) than in females (MD and MHD 33%).

Histopathology: No toxicologically significant treatment-related effect.

NOAEL: 0.1 mg/kg/day.

Table 11. Effects of Ba 679 BR in rats in a 4-week Oral Toxicity (Range Finding).

* Significant at P <0.05	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0.1	10	200	500	0	0.1	10	200	500
Body wt. gain (g)	60	68	45	24	15	11	10	8	7	1
Food consump. (g)	148	162	144	129	131	99	103	96	94	94
Clinical Chemistry										
Creatinine, 4wk	52	63*	57	67*	62	54	57	60	64*	58
Organ Weights										
Kidney, g	2.44	2.45	2.36	2.17	1.9*	1.7	1.65	1.62	1.53	1.56
Liver, g	10.3	10.2	10.3	9.7	7.6*	6.4	6.4	6.2	6.0	6.3
Lung, g	1.11	1.13	1.19	1.25*	1.09	0.95	0.94	0.92	0.99	0.91
Thymus, mg	313	337	331	335	171*	190	276	226	241	220
Testes, g	4.6	4.2	4.3	3.9*	3.9*					
Pituitary, mg	11.5	10.2	10.0	10.0	8.3*	10.0	11.0	10.5	10.2	10.5
Gross Pathology										
Rectum (coprostasis)			4/4	3/3	1/3			1/4	1/4	

Rat: 13-week Oral Toxicity Study
Boehringer Ingelheim Study U91-0492, August 08, 1991, Vol. 1.11-1.12, Page 002

Study Dates: 29 March, 1990 to 19 September, 1990.
Testing Lab: Boehringer Ingelheim, Dep. of Experimental Pathology and Toxicology.
Test Article: Ba 679 BR (Batch G) in 0.1% aqueous solution of methylhydroxyethyl cellulose 300 (pH 2.6). Concentrations of LD, MD, and HD dosing solutions were 0.01, 0.5, and 30.0 mg/mL.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb:THOM (Wistar) rats.
Animals: 60/Sex; 20/Sex/group for Control and HD and 10/Sex/group for LD and MD groups.
Route: Oral via esophageal tube.

Dosage: 0 (Vehicle Control), 0.1 (LD), 5.0 (MD), and 300.0 (HD) mg/kg/day.
Duration of Exposure: 13 weeks; 7 days/week.
Clinical Observations: Twice daily on Mondays to Fridays, Once daily on Saturdays, Sundays, and holidays.
Body Weights: Weekly.
Food Consumption: Weekly.
Blood Pressure and Heart Rate: Prior to, during, and after the period of treatment. On first 5 animals (both sexes) of all four groups; In case of Control and HD groups, the first 5 recovery animals were used. Systolic blood pressure and heart rate were recorded at Weeks -1 (once); 3, 7, and 12 (90 min. and 24 h after administration); and once during the recovery period (week 20) in Control (R) and HD (R) groups (R stands for recovery).
Ophthalmoscopy: The last 10 male and female rats in Control group and all animals in HD group were examined at Weeks -1, 4, 13, and 21.
Hematology: Weeks -2, 1, 6, 13, and 21.
Clinical Chemistry: Weeks -2, 1, 6, 13, and 21.
Urinalysis: Weeks 5, 6, and 12 (for only 10 animals/Sex of Control and HD groups).
Drug Levels: A satellite experiment was conducted using same doses as those used in the present study. Drug levels were determined on samples taken 1, 4, and 8 hours after the administration of the drug.
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 12):

Dosage Levels: Stability deviations over treatment period were within 1% of dose levels.

Clinical Signs: Significantly high Incidences of chromodacryorrhea at HD (♂ 18/20; ♀ 18/20) than at LD (♂ 1/10; ♀ 0/10) or MD (♂ 2/10; ♀ 0/10).

Mortality: Four decedents consisting of 1 Control (♀) and 3 HD (2 ♂, 1♀).

Body Weights: Reduced bodyweight gains were dose dependent in both sexes (statistically significant values were 32% and 59% for MD and HD males respectively and 40% for HD females).
Food Consumption: Reduction in bodyweights was accompanied by reduced food consumption (statistically significant values were 11% and 19% for LD and MD males respectively and 22% for HD females.

Blood Pressure and Heart Rate: There was significant increase in heart rate at HD after 1.5 h (47%) and 24 (37%) hours post treatment (both values statistically significant).

Ophthalmoscopy: No toxicologically significant treatment-related effect.

Hematology: There were statistically significant increases in hemoglobulin, hematocrit, and MCV and decrease in reticulocytes in HD males and females. The difference was biologically not significant.

Clinical Chemistry: Slight but statistically significant decreases in GPT, total bilirubin, tryglycerides, urea, inorganic phosphates, and potassium, calcium, and chloride ions and increase in sodium ions mostly at HD were present. Total cholesterol increased in males while decreased in females. The differences are not biologically significant.

Urinalysis: No toxicologically significant treatment-related effect.

Organ Weights: There was statistically significant reduction in the weights of kidney (HD), liver (MD, HD), heart (MD, HD), thymus (MD, HD), and spleen (HD) in males and kidney (HD), liver (HD), and spleen (MD, HD) in females. These differences were not biologically significant.

Gross Pathology: Coprostasis was seen at MD (♂ 60%, ♀ 30%) and HD (♂ 38%, ♀ 44%). Other major changes were the occurrence of white deposits in urinary bladder at MD (♂ 80%, ♀ 10%) and HD (♂ 50%), harderian gland dark red-brown-black discolorations at MD (♂ 90%, ♀ 40%) and HD (♂ 88%, ♀ 33%), lymph node lesions at various dose levels (♂: MD 20%, HD 38%; ♀: Control 10%, MD 20%). The sponsor indicated these lesions to be pathological lesions/abnormal changes. However, at the end of the recovery period, the incidence of these lesions/abnormal changes at HD (♂: 4/10; ♀: 5/10) was comparable to that in control (♂: 7/10; ♀: 4/9).

Histopathology: No toxicologically significant treatment-related effect.

Toxicokinetics: Drug plasma levels increased in dose-dependent manner.

NOEL: 0.1 mg/kg/day. *MTD:* <5.0 mg/kg/day.

Table 12. Effects of Ba 679 BR in rats in a 13-wk Oral Toxicity Study.

* Significant at P <0.05	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.1	5.0	300	0	0.1	5.0	300
Body weight gain (g)	148	130	101*	60*	52	46	39	31*
Food consumption, (g)	149	132*	140	120*	123	121	115	96
Chromodacryorrhea	0/20	1/10	2/10	18/20	0/20	0/10	0/10	18/20
Hematology								
HB g/100mL	16.5	16.4	16.3	17.0*	15.7	15.8	15.8	16.2*
HCT vol. %	45.5	45.6	45.7	47.6*	43.8	43.9	44.2	45.1*
MCV Um^3	54.5	53.9	55.0	55.6*	55.3	56.2	55.4	56.9*
Reticulocytes (% of eryth.)	25.4	21.5	24.4	18.1*	25.7	20.2	19.1	15.2*
Clinical Chemistry								
GPT UMol/L	28.7	30.8	30.5	24.5*	25.4	24.0	27.0	20.0*
Total Bili UMol/L	2.9	2.3*	2.5*	2.3*	2.8	2.4	2.7	2.5
Triglyc. mmol/L	2.4	1.8	1.7	1.5*	2.0	2.2	2.2	1.8
Urea mmol/L	7.6	7.3	8.0	6.2*	7.7	7.6	7.6	6.3*
Na mmol/L	144	144	146*	145	144	145	145	145
K mmol/L	4.7	4.5	4.6	4.4*	4.1	3.9	4.0	4.1
Ca mmol/L	2.5	2.4*	2.4*	2.4	2.5	2.4	2.4	2.4
Cl mmol/L	100	100	99	98*	102	102	99*	99*
Inorg. Ph. mmol/L	1.9	1.8	1.9	1.7*	1.6	1.6	1.5	1.5
Total Chol. mmol/L	1.8	1.9	1.7	1.9	2.1	1.8*	1.9*	1.6*
Organ Weights								
Kidney, g	2.5	2.5	2.3	2.1*	1.6	1.7*	1.6	1.5*
Liver, g	11.5	11.0	10*	8.8*	6.7	6.8	6.4	5.9*
Heart, g	1.2	1.2	1.1*	1.1*	0.8	0.8	0.8	0.8
Thymus, mg	283	245	214*	147*	192	161	169	155
Spleen, g	0.9	0.8	0.8	0.7*	0.7	0.6	0.5*	0.5*
Gross Pathology								
Rectum, coprostasis	0/10	0/10	6/10	3/8	0/10	0/10	3/10	4/9
Urinary Bladder: white deposits	0/10	0/10	8/10	4/8	0/10	0/10	1/10	0/9
Harderian gland: Red-brown-black	0/10	0/10	9/10	7/8	0/10	0/10	4/10	3/9

discolorations									
Lymph node: path. lesions/ abnormality	0/10	0/10	2/10	3/8		1/10	0/10	2/10	0/9

Toxicokinetics	Dose mg/kg/day	Mean Concentrations- hours postdose (ng/mL) 1 hour	4 hours	8 hours
Plasma Levels	0.1	0.03	0.02	0.03
	5.0	0.63	0.21	0.27
	300.0	17.95	3.24	2.36

Rat: 3-week Intravenous Range Finding Study

Boehringer Ingelheim Study U93-0632, June 28, 1993, Vol. 1.23, Page 002

Study Dates: 02 August to 18 September, 1990
Testing Lab: Boehringer Ingelheim, Dep. of Experimental Pathology and Toxicology.
Test Article: Ba 679 BR (Batch G) prepared in 0.9% NaCl solution. Concentrations of LD, MD, and HD dosing solutions were 0.01, 0.4, and 16.0 mg/mL.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb: THOM Wistar rats.
Animals: 16/Sex; 4/Sex/group.
Route: Intravenous.
Dosage: 0 (Control, 0.9% saline), 0.01 (LD), 0.4 (MD), and 16.0 (HD) mg/kg/day.
Duration of Exposure: 3 weeks.
Clinical Observations: Twice daily on Mondays to Fridays and once daily on Saturdays, Sundays, and holidays.
Body Weights: Weekly.
Food Consumption: Weekly.
Hematology: Week -2 and 2.
Clinical Chemistry: Week -2 and 2.
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 13):

Dosage Levels: Analysis of solutions/suspensions for concentration and stability of test article showed standard deviations < 1.4%.

Clinical Signs: Drug-related clinical signs were seen in males [Chromodacryorrhea (MD 1/4, HD 2/4), dyspnea (HD 4/4), and epistaxis (HD 1/4)] as well as females [chromodacryorrhoea (HD 1/4), dyspnea (HD 4/4), eplistaxis (HD 1/4), and poor health status (HD 1/4).
Mortality: One HD ♀ was sacrificed due to poor health.

Body Weights: Reduced bodyweight gains were seen in MD (30%) males while there was bodyweight loss in HD (9.5%) males and HD females (2.8%).

Food Consumption: No toxicologically significant treatment-related effect.

Hematology: Minor changes in hemoglobulin, hematocrit, and MCHC were significant statistically but not biologically.

Clinical Chemistry: There was a statistically significant decrease in the values of GOT in males (LD 25%, MD 27%, HD 35%) and Sodium in both sexes (♂: 1.4% MD and HD; ♀: 1.4% MD.

Organ Weights: Statistically significant decrease in the weights of kidneys (HD), liver (HD), heart (HD), spleen (MD, HD), and prostate (MD, HD) in males and spleen (all doses) and ovaries (MD, HD) in females were not biologically significant.

Gross Pathology: Dilated urinary bladder (LD 3/4, MD 1/4) in males, presence of white protein content in urinary bladder in males (LD 4/4, MD 3/4) and females (HD 1/4); and harderian gland discoloration (♂: LD 1/4, MD 4/4, HD 3/4; ♀: LD 1/4, MD 4/4, HD 2/4) and coprostasis (♂: LD 1/4, MD 4/4, HD 3/4; ♀: LD 1/4, MD 4/4, HD 2/4) were drug related.

Histopathology: At HD, incidences of bronchopneumonia with vicarious emphysema and per vascular edema of lung (♂ 2/4, ♀ 1/4), urinary bladder cystitis (♂ 1/4, ♀ 2/4), lymphofollicular hyperplasia in rectum (♀ 1/4), and harderian gland artefact (♀ 1/4) constituted toxicologically significant findings.

NOAEL: 0.01 mg/kg/day.

Table 13. Effects of Ba 679 BR in rats in a 3-wk Intravenous (Range Finding) Study.

* Significant at P <0.05	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.01	0.4	16.0	0	0.01	0.4	16.0
Body weight gain (g)	20	19	6	-27	13	14	12	-6
Hematology								
HB g/100 mL	17.0	16.3*	16.5*	17.3	16.1	15.6	15.5	16.1
HCT vol. %	48.6	45.3*	45.7*	47.7	43.4	42.5	42.5	45.2
MCHC g/100 mL	34.9	35.9*	36.2*	36.2*	37.1	36.7	36.5	35.6*
Clinical Chemistry								
GOT U/L	47.5	35.6*	34.7*	30.9*	34.8	32.2	34.5	29.3
Na mmol/L	148	147	146*	146*	146	145	144*	147
Organ Weights								
Kidneys, g	2.0	2.0	2.0	1.7*	1.7	1.5	1.4	1.5
Liver, g	8.6	8.3	8.5	7.0*	7.0	6.2	6.1	6.1
Heart, g	1.0	0.9	0.9	0.8*	0.7	0.7	0.8	0.7
Spleen, g	0.8	0.7	0.6*	0.6*	0.7	0.5*	0.5*	0.5*
Prostate, g	0.8	0.7	0.5*	0.5*				
Ovaries, mg					99.2	89.0	77.7*	69.3*
Gross Pathology (4 animals/group)								
Urinary Bladder: Dilated White prot. cont.	 0 0	 3 4	 1 3	 0 0	 0 0	 0 0	 0 0	 0 1
Rectum: coprostasis	0	2	3	2	0	0	2	1
Harderian gland: Dark discoloration	 0	 1	 4	 3	 0	 1	 4	 2
Histopathology								
Urin. Bladder: Cystitis				1/4				2/4
Rectum: Lympho-follicular hyperpla.								1/4
Lung: broncho-pneumonia, vicar. emphysema, peri-vascular oedema				2/4				**1/4**
Harderian gland artefact								**1/4**

Rat: 4-week Intravenous Toxicity Study

Boehringer Ingelheim Study U93-0808, September 24, 1993, Vol. 1.24-1.25, Page 002

Study Dates: 06 December, 1990 to 27 March, 1991
Testing Lab: Boehringer Ingelheim, Dep. Experimental Pathology and Toxicology.
Test Article: Ba 679 BR (Batch I) was a 0.16% solution which was further diluted to obtain various concentrations necessary for this study: LD 0.01 mg/mL, MD 0.4 mg/mL, and HD 16.0 mg/mL.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb:THOM Wistar rats.
Animals: 60/Sex; 20/Sex/group for Control and HD and 10/Sex/group for LD and MD.
Route: Intravenous.
Dosage: 0 (Control), 0.01 (LD), 0.4 (MD), and 16.0 (HD) mg/kg/day.
Duration of Exposure: 4 weeks. Ten males and 10 females of Control and HD groups were observed for a recovery period of 6 weeks after the end of dosing.
Clinical Observations: Twice daily.
Body Weights: Weekly.
Food Consumption: Weekly.
Blood Pressure and Heart Rate: Systolic blood pressure and heart rate were recorded in weeks -3 and in weeks 1 and 4 (1 and 24 hours after drug administration) on 5 males and 5 females from all groups.
Ophthalmoscopy: The last 10 male and female rats of Control and all animals of HD group were examined in weeks -1 and 4, and the recovery animals of Control and HD groups were examined in week 9.
Hematology: Weeks -3, 1, 4, and 10 (recovery group).
Clinical Chemistry: Weeks -3, 1, 4, and 10 (recovery group).
Urinalysis: Week 3 (only on 10 animals/Sex of Control and HD groups).
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 14):

Dosage Levels: The concentration and stability of the test article varied up to 3.1% during the period of the study.

Clinical Signs: Drug-related signs were chromodacryorrhea (eye), dyspnea, sedation, pallor of tail, respiratory sounds, sternal recumbency, apnoea (data are presented in Table 14), and increased urine output.

Mortality: 16 (Control 5♀; LD 1♂; HD 2♂, 8♀).

Body Weights: There was a decrease in bodyweight gains in LD (10%) and MD (77%) males and MD females (26%). However, there was bodyweight loss in HD (23 g) males and and HD (1 g) females.

Food Consumption: There was statistically significant reduction in food consumption at MD (11%) and HD (23%) in males and HD (14%) in females.

Ophthalmoscopy: No toxicologically significant treatment-related effect.

Blood Pressure and Heart Rate: Statistically significant and biologically relevant increase in heart rate in all treatment group after 1 hour of treatment (LD 12%, MD 19%, HD 23%) and only in HD group (22%↑) 24 hours after treatment.

Hematology: Statistically significant decrease in MCV, TPT, and percentages of SEG-CE, lymphocytes, and hematocrit were not biologically significant.

Clinical Chemistry: No toxicologically significant treatment-related effect.

Urinalysis: No toxicologically significant treatment-related effect.

Organ Weights: There was statistically significant decreases in the weights of kidneys (HD), liver (HD), heart (HD), spleen (MD, HD), thymus (HD), testes (HD), Adrenals (MD, HD) in males and kidneys (HD) and pituitary (MD, HD) in females. However, the actual change in the weights of these organs was not large enough to be of biological significance.

Gross Pathology: Grey white deposits in urinary bladder, discoloration of harderian gland, and coprostasis were main drug-related changes.

Histopathology: Incidences of moderate cystitis was present in the urinary bladder in both sexes.

NOAEL: 0.01 mg/kg/day.

Table 14. Effects of Ba 679 BR in rats in a 4-wk Intravenous Toxicity Study.

* Significant at P < 0.05	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0.01	0.4	16.0		0	0.01	0.4	16.0	
Body weight gain (g)	40	36	9	-23		13	25	10	-1	
Food consumption (g)	148	144	131*	114*		105	104	99	90*	
Chromodacryorrhea			2	5				0	2	
Dyspnea			1	13				0	13	
Sedation				0					3	
Pallor of tail	0	3	9	19		1	1	2	13	
Respiratory sounds				17					14	
Sternal recumbency				14					12	
↑ Urine Output			1	7				1	5	
Apnoea: 1-3 min post admin during anesth.			 0 1	 1 1				 0 0	 3 2	
Hematology										
MCV UM^3	57.0	57.1	56.2	55.9*		59.0	58.7	58.6	57.5*	
SEG-CE %	8.6	7.9	8.4	13.9*		11.6	9.2	11.7	22.2*	
Lympho %	87.3	89.1	87.1	82.2*		83.7	86.4	84.7	72.0*	
HCT vol. %	44.0	43.5	44.7	43.6		45.5	43.5*	44.6	42.6*	
TPT Sec.	17.2			17.4		18.5			20.1*	
Clinical Chemistry										
GOT U/L	40.3	34.0*	39.4	35.1*		33.3	31.6	35.4	40.9*	
AP U/L	202	187	185	175*		155	143	149	139	
Tot-Bili UMol/L	2.1	1.9	2.2	2.6*		2.2	1.8	2.4	2.0	
Na mmol/L	145	143*	143*	144*		143	143	143	143	
Creatinine Umol/L	54.8	55.1	60.4*	53.2		52.0	57.4*	61.2*	61.0*	
Organ Weights										
Kidneys, g	2.2	2.2	2.1	1.9*		1.6	1.6	1.5	1.4*	
Liver, g	9.3	9.7	8.8	7.9*		6.3	6.6	6.5	5.8	
Heart, g	1.1	1.0	1.0	0.9*		0.8	0.8	0.8	0.7	
Spleen, g	0.8	0.8	0.6*	0.6*		0.5	0.5	0.5	0.5	
Thymus, mg	292	272	235	217*		232	248	197	172	
Testes, g	4.7	4.4	4.5	4.3*						
Adrenals, mg	78.2	72.4	65.7*	63.9*		92.2	74.5	78.1	76.0	
Pituitary, mg	10.8	9.9	9.9	9.4		12.6	11.7	10.4*	9.2*	
Gross Pathology (10 animals/group)										
Urin. Bladder: Grey white deposits		6	5	5			1	2	1	
Harderian gland: Discoloration		1	8	8			3	3	3	
Rectum: coprostasis		2	1	3			0	1	2	
Histopathology (10 animals/group)										
Moderate cystitis		1	1	0			1	2	2	

Rat: 4-wk Inhalation Toxicity (Preliminary) Study
Boehringer Ingelheim Study U90-0691, October 01, 1990, Vol. 1.10, Page 002

Chhb:THOM (Wistar) rats (21/Sex; 7/Sex/group) were administered Ba 679 BR aerosol at 0 (Control), 2.3 (LD), and 4.5 (HD) mg/kg/day using aqueous solutions (LD 1%; HD 2%). The animals were exposed to Test article once daily (7 days/week for 4 weeks) for 120 (LD) or 180 (Control, HD) minutes. Treatment resulted in a reduction in body weight gains in Week 1 (LD 8%, HD 10%) but not in Weeks 2, 3 or 4. Histopathological examination revealed a mild reduction of lipids in liver cells. Based on results from this study, the sponsor selected 4 mg/kg/day as the high dose for toxicologic evaluation of Ba 679 BR via inhalational route. Signed GLP Statement was included. The NOAEL was 2.3 mg/kg/day.

Rat: 2-week Inhalation Toxicity (Powder with Lactose) Range Finding Study
Boehringer Ingelheim Study U93-0943, January 05, 1994, Vol. 1.29, Page 002

Study Dates: 27 February, 1993 to 10 March, 1993.
Testing Lab: Boehringer Ingelheim Dep. Experimental Pathology and Toxicology.
Test Article: Ba 679 BR, [redacted], mixed with lactose [redacted]. Batches: Drug: 301 103; Placebo: 301 102.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb:THOM Wistar rats.
Animals: 56/Sex; 14/Sex/group.
Route: Inhalation (breathing of respirable powder inside the exposure chamber).
Dosage: 0 (Control, lactose), 0.1 (LD), 0.9 (MD), and 3.8 (HD) mg/kg/day.
Duration of Exposure: 2 weeks, 80 min./day.
Clinical Observations: Daily.
Body Weights: Once prior to study initiation and weekly during the study.
Food Consumption: Once prior to study initiation and weekly during the study.
Heart Rate: Week -1 and Week 2 before and after drug administration.
Hematology: Week 2.
Clinical Chemistry: Week 2.
Drug Levels: Twice in Week 2 (Once before and once after the inhalation session).
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 15):

Dosage Levels: Achieved dose levels were 0.11, 0.94, and 3.8 mg/kg/day.

Clinical Signs: Dilation of pupil in all treatment groups and hypersecretion of harderian glands in MD and HD groups.

Mortality: None.

Body Weights: Reduction in bodyweight gains occurred in both sexes at all doses. However, statistically significant decrease in bodyweight gains was seen only in MD (70%) and HD (61%) males.

Food Consumption: There was statistically significant (non dose dependent) reduction in food consumption by males (15%) at all doses.

Heart Rate: There was biologically relevant and statistically significant increase in the heart rate in HD males (19%) and females of all dose groups (LD 19%, MD 15%, HD 18%).

Hematology: Statistically significantly increased mean corpuscular hemoglobin concentration (MCHC) in males (LD and MD: 2.6%; HD: 3.4%) and thromboplastin time (TPT) in females (HD: 7%) are too small to be of biological significance.

Clinical Chemistry: Statistically significant changes in many parameters. However, none of them were toxicologically significant treatment-related effects.

Organ Weights: Statistically significant changes are listed in Table 16. None of these changes are biologically significant.

Gross Pathology: Discoloration of harderian gland (both sexes) and white flocculent precipitate in urinary bladder in males were drug related.

Histopathology: No toxicologically significant treatment-related effect.
Toxicokinetics: Drug plasma levels in Week-2 (10 min. after end of dosing) increased in a dose dependent manner.
NOAEL: 0.1 mg/kg/day.

Table 15. Effects of Ba 679 BR in rats in a 2-wk Inhalation Toxicity Study

* Significant at P < 0.05	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.1	0.9	3.8	0	0.1	0.9	3.8
Body weight gain (g)	44.4	28.4	13.5*	17.3*	15.7	15.6	12.8	11.4
Food consumption (g)	137	116*	116*	116*	96	94	95	98
Hematology								
MCHC g/100 mL	35.3	36.2*	36.2*	36.5*	36.3	36.5	35.9	36.1
TPT (Sec.)	19.9	20.4	21.7	20.8	20.1	20.9	20.9	21.5*
Clinical Chemistry								
GOT U/L	44.9	39.5	40.3	36.2*	43.7	39.3	42.1	38.3
AP U/L	204	199	192	167*	136	139	141	127
Tot. Chol. mmol/L	1.4	1.6	1.8*	1.8*	1.8	2.0*	1.7	1.7
Creatinine Umol/L	56.7	56.4	51.8	51.2*	57.0	56.0	54.0	55.8
Glucose mmol/L	7.9	6.7*	6.4*	6.5*	7.1	6.5	6.2*	6.8
Na mmol/L	144	143*	142*	142*	142	140*	144*	144*
K mmol/L	5.2	5.1	4.7*	4.8*	4.6	4.5	4.8	4.8
Mg mmol/L	0.96	0.93	0.87*	0.82*	0.98	0.95	0.88*	0.86*
Fe mmol/L	39.1	36.4	36.2	37.8	63.9	62.9	45.4*	47.3*
Ca mmol/L	2.5	2.6	2.5	2.5	2.4	2.5	2.5	2.5*
Organ Weights								
Kidneys, g	2.3	2.0*	1.9*	2.0*	1.7	1.5*	1.5*	1.6*
Liver, g	13.3	8.5*	7.9*	10.7*	8.8	5.9*	6.0*	7.9*
Heart, g	1.0	0.9*	0.9*	0.9*	0.74	0.68*	0.65*	0.69*
Spleen, g	0.8	0.6*	0.7*	0.7*	0.6	0.6	0.5	0.5
Prostate, g	0.8	0.6*	0.6*	0.6*				
Pituitary, mg	10.0	8.3*	7.4*	9.0	11.0	9.9	9.7	10.7
Thyroid, mg	18.4	12.9*	14.7*	18.5	13.7	13.0	11.6	15.0
Adrenals, mg	65.1	63.1	61.3	62.8	91.0	78.1*	75.0*	75.1*
Gross Pathology								
Harderian gland: dark discolored		1	5	4		2	7	2
Urinary Bladder: White floccul. ppt.		4	4	5		0	0	0
Toxicokinetics								
Plasma Levels	**Mean Plasma Concentrations (ng/mL)**							
	Dose		**Pre-dose**		**10 min. postdose**			
	0.1 mg/kg/day		**0.02**		**1.32**			
	0.9 mg/kg/day		**0.16**		**6.86**			
	3.8 mg/kg/day		**0.64**		**20.22**			

Rat 13-week Inhalation Toxicity (Aqueous Aerosol) Study
Boehringer Ingelheim Study U91-0493, January 31, 1991, Vol. 1.13, Page 002

Study Dates: 18 April, 1990 to 19 July, 1990
Testing Lab: Boehringer Ingelheim Dep. Experimental Pathology and Toxicology.
Test Article: Aerosol of Ba 679 BR were prepared using aqueous solutions of 2% (HD), 0.5% (MD), and 0.05% (LD) drug. The pH of water used for making these solutions was 3.0.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb:THOM Wistar rats.
Animals: 76/Sex; 24/Sex for Control and HD and 14/Sex for LD and MD groups.
Route: Inhalation (breathing in the exposure chamber)
Dosage: 0 (Control), 0.07 (LD), 0.6 (MD), and 5.0 (HD) mg/kg/day.
Duration of Exposure: 13 weeks; 160 (Control, HD) or 60 minutes daily; 10/Sex from Control and HD groups were observed for a recovery period of 8 weeks following last day of treatment.
Clinical Observations: Daily.
Body Weights: Once prior to study initiation and weekly during treatment period.
Food Consumption: Once prior to study initiation and weekly during treatment period.
Ophthalmoscopy: Only for HD group: Weeks -1, 5, and 13; Recovery Week 7.
Heart Rate: Electrocardiogram taken (5 animals/Sex/group) on the last day of Weeks -1, 3, 8, and 12 before and immediately after drug administration and once during recovery period.
Hematology: Weeks -2, 1, 6, 13, and 20 (recovery group).
Clinical Chemistry: Weeks -2, 1, 6, 13, and 20 (recovery group).
Urinalysis: Weeks 4 and 12 (10 animals/Sex: Control and HD only).
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 16):

Dosage Levels: The inhalational doses were same as targeted ones.

Clinical Signs: All drug-treated animals showed increased pupil diameters while piloerection was seen only in males (MD and HD).

Mortality: One female (Control group) rat died apparently due to an accidental compression in the restraining tube during week 13.

Body Weights: Reduction in bodyweight gains was statistically significant and dose dependent (♂: LD 40%, MD 67%, HD 78%; ♀: LD 28%, MD 48%, HD 71%).

Food Consumption: Reduction in bodyweight gains was accompanied by lowered food consumption (♂: LD 8.4%, MD and HD 16.4%; ♀: LD 10%, MD 15.8%, HD 19.6%).

Ophthalmoscopy: Incidences of cataract were drug-related.

Heart Rate: There was statistically significant increase in heart rate in male rats on the last day last day of Week 12 before (8% at MD or HD) and after (MD 9%) drug administration; in female rats however, there was decrease in heart rate (HD 7%) after drug administration on the last day of Week 12 while, there was no change in heart rate before drug administration.

Hematology: Statistically significant changes are listed in Table 16. No toxicologically significant treatment-related effect.

Clinical Chemistry: Statistically significant increased serum levels of AP were drug-related. Other parameters that changed statistically significantly but are not biologically significant are given in Table 16.

Urinalysis: No toxicologically significant treatment-related effect.

Organ Weights: Statistically significant changes in organ weight (listed in Table) are not biologically significant.

Gross Pathology: There was greyish white flocculent precipitate present in the urinary bladders of males of all treatment groups and females of MD and HD groups.

Histopathology: No toxicologically significant treatment-related effect.

NOAEL: 0.07 mg/kg/day. *MTD:* 0.6 mg/kg/day.

Table 16. Effects of Ba 679 BR in rats in a 13-wk Inhalation Toxicity.

* Significant at P <0.05	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.07	0.6	5	0	0.07	0.6	5
Body weight gain (g)	108	64.1*	36.0*	24.2*	45.2	32.5	23.6*	13.3*
Food consumption (g)	142	131*	122*	122*	110	100*	95*	92*
Hematology								
SEG-CE %	7.5	11.0	15.5*	18.1*	11.7	16.5	17.1	18.5*
Mono %	4.8	7.0*	3.2	7.3*	4.3	4.0	6.6	6.3
Lympho %	83.4	77.8*	77.3*	70.1*	79.4	74.3	72.1	69.8*
TPT Sec.	22.5			22.9	19.2			20.2*
Clinical Chemistry								
GOT U/L	33.2	32.9	34.2	37.7*	37.8	30.7	37.0	36.8
AP U/L	155	162	156	173*	113	124	133*	133*
Urea mmol/L	8.6	7.8*	7.0*	7.0*	8.4	8.0	7.5	8.2
Creatinine Umol/L	66.8	60.1*	61.3*	61.6*	63.8	65.2	68.3	65.4
Na mmol/L	146	146	145*	146	144	145	146	144
Ca mmol/L	2.45	2.43	2.4	2.36*	2.4	2.4	2.5	2.3*
Cl mmol/L	103	100*	100*	102*	101	102	100	101
Inorg. Phos. mmol/L	1.8	1.6*	1.6*	1.6*	1.7	1.6	1.8	1.6
Tryglyc. mmol/L	1.4	1.4	1.2	1.2	1.6	1.8	1.7	1.2*
Total Chol. mmol/L	1.8	1.9	1.7	1.9	1.8	2.0	2.2*	1.8
Glucose mmol/L	6.9	6.4	6.1	6.6	6.5	6.0	5.8*	6.5
Organ Weights								
Kidneys, g	2.3	2.2	2.1*	1.9*	1.7	1.6	1.6*	1.5*
Liver, g	9.5	9.3	8.1*	7.3*	6.6	6.5	6.1	5.9*
Heart, g	1.1	1.0*	0.9*	0.9*	0.8	0.7	0.7	0.7
Lung, g	2.2	2.0*	2.0*	2.0*	1.8	1.7	1.6*	1.6*
Thymus, mg	198	185	190	134*	173	190	167	148
Spleen, g	0.74	0.65	0.64	0.55*	0.58	0.57	0.45*	0.48*
Prostate, g	0.93	0.76*	0.69*	0.60*				
Brain, g	2.0	2.0	2.0	1.9*	1.8	1.9	1.8	1.8
Gross Pathology								
Urin. Bladder: Greyish white floccul. ppt	0	4	3	2	0	0	1	1

Rat: 13-week Inhalation Toxicity (Powder with Lactose) Study
Boehringer Ingelheim Study U93-0944, January 19, 1994, Vol. 1.30-1.31, Page 002

Study Dates: 20 April, 1993 to July 20, 1993.
Testing Lab: Boehringer Ingelheim Dep. Experimental Pathology and Toxicology.
Test Article: Ba 679 BR, [redacted] mixed with lactose, [redacted] Batch 301 103; Lactose as placebo: Batch 301 102.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb:THOM Wistar rats.
Animals: 106/Sex; 20/Sex for Sham Control, 24/Sex for Placebo Control and HD, and 19/Sex for LD and MD.
Route: Inhalation (breathing of respirable powder inside the exposure chamber).
Dosage: 0 (Sham Control; filtered air only), 0 (Placebo Control; lactose), 0.09, 0.6, and 5.6 mg/kg/day.
Duration of Exposure: 13 weeks; 100 minutes daily.
Clinical Observations: Daily.
Body Weights: Once before study initiation and weekly during the study.
Food Consumption: Once before study initiation and weekly during the study.
Heart Rate: Week -1 (once), weeks 5 and 12 immediately and 24 hours after drug administration, and during week 18 of recovery period (once).
Ophthalmoscopy: Weeks -1 (Sham Control and HD), 6 (HD), 13 (all groups), and 20 (recovery group).
Hematology: Weeks 1, 6, 13, and 19 (recovery group).
Clinical Chemistry: Weeks 1, 6, 13, and 19 (recovery group).
Urinalysis: Weeks 4, 12 (Placebo Control and HD), and 18 (recovery)
Drug Levels: Blood samples drawn on Week 1 (Day 2), 6, and 13 before the inhalation session and 10 min. thereafter.
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 17):

Dosage Levels: Inhalational doses of the drug were 0.09, 0.6, and 5.6 mg/kg/day.
Clinical Signs: Dilation of pupil was seen in both sexes at all dose levels.

Mortality: None.

Body Weights: There was statistically significant reduction of body weight gains (♂: LD 31%, MD 59%, HD 60%; ♀: LD 29%, MD and HD 57%).

Food Consumption: Reduction in bodyweight gains was accompanied by decreased food consumption.

Heart Rate: There was statistically and biologically significant increase in heart rate at MD and HD immediately after administration (12% and 14% respectively) and after 24 hours of dosing (11% and 17% respectively).

Ophthalmoscopy: Incidences of cataract (♂: Placebo Control 7%, LD 20%, MD 47%, HD 80%; ♀: Placebo Control 7%, LD 13%, MD 33%, HD 47%) were drug-related.

Hematology: Statistically significant changes in hematology parameters are listed in Table 17. These were not biologically significant.

Clinical Chemistry: Statistically significant changes in hematology parameters are listed in Table 17. No toxicologically significant treatment-related effect.

Urinalysis: No toxicologically significant treatment-related effect.

Organ Weights: No toxicologically significant treatment-related effect.

Gross Pathology: White flocculent precipitate in urinary bladder; black, brown discoloration of harderian gland, and coprostasis were consistent with other toxicologic studies.

Histopathology: No toxicologically significant treatment-related effect.

Toxicokinetics: Drug plasma levels were more than proportional to the increase in dose. There was accumulation of drug at Weeks 6 and 13 especially at LD and MD.

NOAEL and MTD: <0.09 mg/kg/day.

Table 17. Effects of Ba 679 BR in rats in a 13-wk Inhalation Toxicity.

* Significant at P < 0.05	Males (mg/kg/day)					Females (mg/kg/day)				
	Air	Lact	0.09	0.6	5.6	Air	Lact	0.09	0.6	5.6
Body weight gain (g)	139	121*	83*	50*	49*	55	56	40*	24*	24*
Food consumption (g)	142	129*	121*	120*	112*	99	107*	95	92*	84*
Cataract		1/15	3/15	7/15	12/15		1/15	2/15	5/15	7/15
Hematology										
Ery. Mill/mm^3	7.8	7.9	8.1	8.0	8.5*	7.7	7.8	8.0*	8.1*	8.0*
HB g./100 mL	15.9	16.0	16.5*	15.9	16.9*	15.9	16.1	16.2	16.5*	16.1
HCT vol. %	44.0	44.4	45.4*	44.5*	45.8*	43.6	43.6	45.0*	45.6*	45.1*
MCV UM^3	56.6	56.3	56.0	55.7	53.9*	56.5	56.0	56.0	56.4	56.2
MCHC g./100 mL	36.1	36.0	36.3	35.8	36.8*	36.5	36.8	36.0	36.2	35.6*
Reticulocytes (% of Erythrocytes)	66.5	52.3	50.6	50.0	19.4*	20.6	20.6	18.0	18.1	17.0*
Thromb 1000/mm^3	1137	1143	1137	1123	984*	1021	1041	1109	1081	1123
SEG-CE %	8.2	10.8	11.2	13.7*	15.8*	12.0	12.0	12.5	15.4	14.6
Lympho. %	86.6	84.6	83.1	79.9*	76.9*	81.3	82.9	81.1	77.5	78.9
TPT Sec.	18.8	19.7			19.3	18.7	16.1*			19.5*
Clinical Chemistry										
GPT U/L	37.5	34.8	37.3	36.7	32.2*	30.2	28.0	24.1*	22.5*	22.6*
Tryglyc. mmol/L	2.1	2.4	2.1	1.7	1.4*	1.9	1.7	1.2*	1.1*	1.2*
Urea mmol/L	7.7	7.6	7.3	6.7*	6.4*	7.8	7.5	7.1	6.1*	5.9*
Glucose mmol/L	7.6	6.4*	6.9*	6.6*	6.3*	6.0	6.3	5.9	6.3	6.1
Na mmol/L	144	146*	144	146*	145	143	143	144*	143	144*
Fe U mol/L	41.0	38.7	39.4	35.9*	30.7*	55.4	54.1	53.9	51.9	52.7
Mg mmol/L	0.92	0.94	0.87	0.79*	0.83*	1.06	0.97*	0.95*	0.94*	0.96*
AP U/L	154	167	164	166	164	164	176	166	153	137*
K mmol/L	4.9	4.9	4.7	4.7	4.6	4.6	4.7	4.9*	5.0*	5.0*
Organ Weights										
Kidneys, g	2.6	2.2*	2.3*	2.1*	2.0*	1.7	1.9*	1.7	1.6*	1.7
Liver, g	13.4	10.0*	9.8*	9.0*	10.9*	7.2	7.5	6.8	6.5*	8.0*
Heart, g	1.24	1.23	1.05*	1.05*	0.99*	0.82	0.86	0.78*	0.75*	0.72*
Lung, g	2.4	2.3	2.2*	2.0*	2.1*	1.9	1.9	1.8	1.8	1.8
Thymus, mg	267	242	210*	196*	191*	192	214	199	169	181
Spleen, g	0.92	0.80*	0.69*	0.72*	0.66*	0.58	0.59	0.55	0.50*	0.56
Prostate, g	0.93	0.87	0.78*	0.73*	0.65*					
Adrenals, mg	70.3	62.8	62.1	63.1	54.1*	82.5	81.7	77.4	74.3	68.1*
Pituitary, mg	11.1	11.2	10.3	9.9	8.5*	13.1	14.2	11.9	11.2*	10.7*
Salivary gland, g	0.88	0.81	0.84	0.82	0.75*	0.57	0.57	0.58	0.61	0.56
Gross Pathology										
Urinary Bladder: white floccul. ppt.			6	0	7			0	0	0
Harderian gland: black, brown discol			2	7	5			2	4	12
Rectum: coprostasis			0	0	1			1	0	0
Toxicokinetics	$t_{1/2}$ = 17 min. for interval 2-22 min. after end of exposure									
Plasma Level.(ng/mL), Wk 1										
Wk 6										

Wk 13

Rat: 13-week Inhalation Toxicity (MTD) Study
Boehringer Ingelheim Study U92-0295, April 7, 1992, Vol. 1.18, Page 311

Study Dates: 03 April, 1991 to 04 July, 1991
Testing Lab:
Test Article: Aerosol of Ba 679 BR generated from a drug solution prepared in an aqueous vehicle. Powder Ba 679 BR was dissolved in 100 mL of vehicle that contained: Benzalkoniumchloride (10 mg), Na salt of ethylene diamine tetra-acetic acid (50 mg), NaCl (900 mg), citric acid monohydrate (8.4 mg), 0.1 N NaOH (0.8 mL), 0.1 N HCl (0.6 mL), and water for injection (100 mL). Batch No. of test article: II.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: F-344 rats.
Animals: 50/Sex; 10/Sex/group.
Route: inhalation via ultrasonic nebulizer.
Dosage: 0 (Control vehicle), 0.08 (LD), 0.25 (MD), 0.75 (MHD), and 4.0 mg/kg/day. The concentration of drug solutions were as follows: LD=0.25 mg/mL, MD=0.75 mg/mL, MHD=2.0 mg/mL, and HD=10 mg/mL.
Duration of Exposure: 13-weeks; 1 hour daily.
Clinical Observations: Daily.
Body Weights: Once pretrial and weekly during treatment period.
Food Consumption: Once pretrial and weekly during treatment period.
Ophthalmoscopy: Once pretrial (all animals) and during Weeks 6 and 13 of treatment (Control and HD only).
Hematology: Weeks 7 and 13.
Clinical Chemistry: Weeks 7 and 13.
Drug Levels: At the end of Weeks 2 and 13 at 15 and 30 min post exposure.
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 18):

Dosage Levels: Achieved dose levels were 0.0707, 0.3165, 0.9352, and 4.5788 mg/kg/day.

Clinical Signs: Prolonged unkempt appearance, piloerection (MHD, HD), red staining around head/snout/eyes with partially closed eyelids (MD, MHD, HD), and incidences of noisy/wheezing respiration (all treatment groups).

Mortality: Excluding deaths following damage in the dosing tube or animals killed following blood sampling, there were 13 mortalities (LD: 2; MD: 4; MHD: 4; HD: 3).

Body Weights: There were statistically significant reductions in bodyweight gains in MD (35%), MHD (43%), and HD (49%) males and in HD (59%) females.

Food Consumption: No toxicologically significant treatment-related effect.

Ophthalmoscopy: No toxicologically significant treatment-related effect.

Hematology: Lower WBC counts were attributed to statistically significant reduction in the % of lymphocytes in both sexes (♂: LD 28%, MD 32%, MHD 24%, HD 36%; ♀: MD, MHD 22%, HD 26%).

Clinical Chemistry: There were statistically significant increases in AST in MHD (60%) and HD (14%) males and AP in MD (11%) , MHD (20%), and HD (12%) females.

Organ Weights: There was statistically significant increase in the weights of rectum in both sexes (♂: LD 46%, MD 26%, MHD 37%, HD 51%; ♀: LD 39%, MD 48%, HMD 51%, HD 57%) and Adrenals in females (MHD and HD (20%). Data on significant reductions in the weights of kidneys, liver, heart, brain, spleen, and thymus in males are listed in Table 18.

Gross Pathology: Sublingual acinar shrinkage in salivary gland, local inflammation in nasal cavity, and bilateral diffuse vacuolation in Adrenals were drug-related.

Histopathology: All gross pathology findings were confirmed histologically.

Toxicokinetics: Drug plasma levels increased with increase in dose. There was some accumulation of drug over a period from Week 2 to Week 13.

MTD: Due to mortality at LD, the MTD could not be determined.

Table 18. Effects of Ba 679 BR in rats in a 13-wk Inhalation (MTD) Toxicity.

* Significant at P <0.05	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0.08	0.25	0.75	4.0	0	0.08	0.25	0.75	4.0
Body weight gain (g)	82	68	53*	47*	42*	29	27	25	24	12*
Food consumption (g)	105	108	103	96	102	77	89	86	81	83
Hematology										
Lymph. (%)	2.5	1.8*	1.7*	1.9*	1.6*	2.3	2.2	1.8*	1.8*	1.7*
Clinical Chemistry										
AST (i.u./L)	84	88	86	134*	96*	103	93	97	97	104
AP (U/L)	377	374	394	498	395	368	395	410*	440*	412*
Organ Weights										
Kidneys, g	1.73	1.76	1.58	1.55*	1.54*	1.13	1.13	1.13	1.11	1.08
Liver, g	8.9	8.6	7.8*	7.6*	7.2*	4.9	5.2	5.1	4.9	4.6
Heart, g	0.84	0.80	0.76*	0.76*	0.75*	0.6	0.6	0.6	0.6	0.6
Brain, g	1.86	1.84	1.81*	1.75*	1.79*	1.7	1.7	1.7	1.7	1.7
Spleen, g	0.55	0.53	0.50*	0.47*	0.45*	0.4	0.4	0.4	0.3	0.3
Thymus, mg	0.15	0.12	0.11*	0.09*	0.09*	0.1	0.1	0.1	0.1	0.1
Rectum (g), abs. rel.	8.9 8.1	12.1* 11.8*	9.9 10.2*	10.7* 11.1*	11.9* 12.2*	6.2 6.1	8.9* 8.5*	9.1* 9.0*	9.0* 9.2*	9.1* 9.6*
Adrenals (g), rel.	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.06*	0.06*
Gross Pathology										
Salivary gland: Sublingual acinar shrinkage	0				5	2			2	7*
Nasal cavity: local inflammation	0				2	0			0	0
Adrenals: bilateral diffuse vacuolation	0	0	0	0	3	0	0	0	0	0
Histopathology (10/group)										
Adrenal cortex: vacuolation	0	0	0	0	3	0	0	0	0	0
Salivary gland: sub lingual mild atrophy muc. acini	0	0	0	0	4	2	0	0	2	7

Nasal cavity: local inflammation	1	0	1	0	4	0	0	0	2	4
Toxicokinetics	**Drug Plasma Levels (ng/mL); 3-4 animals/sex/group**									
Week 2										
Week 13										

Rat: 13-week Inhalation Toxicity (MTD) Study
Boehringer Ingelheim Study U-92-0765, October 19, 1992, Vol. 1.22, Page 002

Study Dates: 12 September, 1991 to 13 December, 1991.
Testing Lab:
Test Article: A stock solution of Ba 679 BR (8 mg/mL) was prepared using the following ingredients: Ba 679 BR (800 mg), Benzalkoniumchloride (10 mg), disodium edetate (50 mg), citric acid monohydrate (8.4 mg), 0.1 N NaOH (0.8 mL), 0.1 N HCl (0.6 mL), and water for injection (100 mL). Batch No. of test article: II.
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: F-344 rats.
Animals: 50/Sex; 10/Sex/group.
Route: Inhalation via ultrasonic nebulizer.
Dosage: 0 (Control vehicle), 0.04 (LD), 0.2 (MD), 1.0 (MHD) and 5.0 (HD) mg/kg/day. The concentrations of dosing solutions were: LD: 0.1 mg/mL; MD: 0.4 mg/mL; MHD: 2.0 mg/mL; HD: 8 mg/mL (a 20 mg/mL solution was used to increase HD from 5 mg/kg/day to 15 mg/kg/day.
Duration of Exposure: 13 weeks; 1 hour daily.
Clinical Observations: Twice daily.
Body Weights: Weekly commencing 1 week pretrial.
Food Consumption: Weekly commencing 1 week pretrial.
Ophthalmoscopy: Once pretrial on all animals; Weeks 6 and 13 of treatment: Control and HD only.
Hematology: Weeks 7 and 13 of treatment.
Clinical Chemistry: Weeks 7 and 13 of treatment.
Drug Levels: End of Week 2 and 12, +15 min. post exposure.
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 19):

Dosage Levels: Achieved dose levels were 0.0503 (LD), 0.2306 (MD), 1.17 (MHD), 4.2829 (HD), and 13.342 (HD dose that was increased to 15 mg/kg/day at Week 8) mg/kg/day.

Clinical Signs: All treatment animals had unkempt appearance immediately after each dosing session. In the weeks when the HD was increased to 15 mg/kg/day, incidences of exaggerated scratching of the snout and sneezing were seen.

Mortality: 21 (MD 3♂, 3♀; MHD 6♀; HD 2 ♂, 7♀). One LD female died during blood sampling.

Body Weights: Statistically significantly reduced bodyweight gains were seen in males (LD 18%, MD 33%, MHD and HD 36%) but not in females.

Food Consumption: No toxicologically significant treatment-related effect.

Ophthalmoscopy: No toxicologically significant treatment-related effect.

Hematology: Statistically significantly reduced WBC counts in males (LD 24%, MD 39%, MHD 13%, HD 21%) primarily due to a commensurate reduction in lymphocyte counts (LD 24%, MD 44%, MHD 20%, HD 30%) while in the females, there was a slight (but statistically significant) increase in RBC counts (MD 4%, MHD 5%, MHD 8%).

Clinical Chemistry: No toxicologically significant treatment-related effect.

Organ Weights: In males, weights of kidneys, liver, heart, spleen, thymus were statistically significantly reduced while weights of testes and rectum increased. In females, only rectum weights statistically significantly increased. These data are presented in Table 19. The extent of changes other than rectum weights is not biologically significant.

Gross Pathology: There was tendency of increased diameter of rectum in the animals of all treatment groups.

Histopathology: Significant changes were seen in Adrenals (↑ zona faciculata, vacuolation; males only), nasal cavity (inflammatory cell infiltrate, incidences of foci of goblet cell hyperplasia, ↑ amounts of inflammatory exudate in the lumen), and harderian gland (↓ eosinophils in females, dilated lumen in males). These data are presented in Table 19.

Toxicokinetics: Drug plasma levels increased with dose; drug accumulation (3-5 times) was observed at Wk-13.

MTD: 0.04 mg/kg/day.

Table 19. Effects of Ba 679 BR in rats in a 13-wk Inhalation (MTD) Toxicity.

* Significant at P <0.05	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0.04	0.2	1.0	5.0	0	0.04	0.2	1.0	5.0
Body weight gain (g)	103	84*	69*	66*	66*	39	34	33	30	32
Hematology										
Lymph., %	4.1	3.1*	2.3*	3.3*	2.9*	4.3	4.2	4.5	4.6	3.8
RBC, 10^{12}/L	8.9	8.8	8.8	8.9	9.1*	8.5	8.5	8.8*	8.9*	9.2*
WBC, 10^{12}/L	6.2	4.7*	3.8*	5.4*	4.9*	6.0	5.9	6.3	6.8	5.7
Organ Weights (g)										
Kidneys	1.6	1.5*	1.3*	1.4*	1.4*	1.0	1.0	1.0	1.0	1.0
Liver	8.2	8.1	6.7*	6.6*	6.7*	4.7	4.5	4.4	4.8	4.7
Heart	0.8	0.7	0.7*	0.7*	0.7*	0.5	0.5	0.5	0.5	0.6
Spleen	0.50	0.45*	0.43*	0.39*	0.38*	0.4	0.3	0.3	4.0	0.3
Thymus	0.13	0.12	0.1*	0.09*	0.07*	0.1	0.1	0.1	0.1	0.1
Rectum, abs rel.	7.7 6.4	8.4 7.9*	8.9* 9.3*	8.9* 9.7*	9.4* 10.2*	5.5 5.4	7.3* 7.2*	7.4* 7.5*	8.2* 8.4*	8.3* 8.5*
Testes, rel.	3.2	3.5*	3.5*	3.5*	3.7*					
Histopathology										
Adrenals: increased zona faciculata vacuolation	0				6*	1				0
Nasal Cavity: Inflammatory cell infiltrate	0/10	4/10	2/7	9/10*	7/8*	0/10	0/9	3/7	4/4*	2/3*
Total incidence of foci of goblet cell hyperplasia	0/10	2/10	2/7	1/10	2/8	0/10	0/9	0/7	0/4	1/3*
Inflammatory exudate within the lumen (amount)	0/10	4/10	2/7	9/10*	8/8*	0/10	0/9	2/7	4/4*	3/3*
Harderian gland: reduced eosino. dilated lumens	0/10 2/10				0/8 8/8	1/10 0/10				**3/3** **0/3**
Toxicokinetics										
Plasma Level (ng/mL) Wk 2										
Wk 13										

Rat: 52-week Inhalation Toxicity (Aqueous Aerosol) Study
Boehringer Ingelheim Study U93-0945, January 14, 1994 Vol. 1.32, Page 002

Study Dates: 27 August, 1991 to 26 August, 1992.
Testing Lab: Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
Test Article: Aqueous aerosol of Ba 679 BR (2% Stock solution: Batch 107302; Placebo solution without Ba 679 BR: Batch 107301). The composition of 100 mL of 2% stock is as follows: Ba 679 BR (2000 mg), benzalkoniumchloride (10 mg), ethylene diamine tetra-acetic acid: Na salt (50 mg), NaOH 0.1 N (0.8 mL), citric acid monohydrate (8.4 mg), water for injection: 100 mL, and HCl 1 N to adjust pH to 3.0. Concentration of dosing solutions were 0.01% (LD), 0.06% (MD), and 0.4% (HD).
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Chbb: THOM Wistar rats.
Animals: 96/Sex; 24/Sex/group.
Route: Nose only inhalation.
Dosage: 0 (Control Vehicle), 0.01 (LD), 0.06 (MD), and 0.4 (HD) mg/kg/day.
Duration of Exposure: 52 Weeks.
Clinical Observations: Daily.
Body Weights: Before study initiation and weekly during the study.
Food Consumption: Before study initiation and weekly during the study.
Ophthalmoscopy: Weeks -1 (Control, HD), 4 (Control, HD), 14 (Control, HD), 25 (HD only), and 51 (Control and all treatment groups); 20 animals/Sex (10/Sex in Week 4).
Heart Rate and Respiratory Parameters: 5 animals/Sex (all 4 groups). Heart rate recorded in Week -1, and in Weeks 7, 14, 27, 40, and 51/52 before and after drug administration. Respiratory parameters were determined on Control and HD animals (10/Sex/group) in Weeks -1, 13, 26, and 52.
Hematology: On 10 animals/sex in Weeks -2, 7, 13, 26, 39, and 52.
Clinical Chemistry: On 10 animals/sex in Weeks -2, 7, 13, 26, 39, and 52.
Urinalysis: On 10 animals/sex (Control and HD only) in Weeks 12, 25, and 51.
Drug Levels: Determined on 4 animals/sex for control (placebo) and treatment (0.01, 0.06, and 0.4 mg/kg/day) groups; this should be considered as a separate satellite study.
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 20):

Dosage Levels: The effective inhaled doses were 0.013, 0.096, and 0.641 mg/kg/day.

Clinical Signs: All HD animals showed unkempt ruffled coat.

Mortality: 4 (2 Control ♂, 1 MD ♀, 1 HD ♂).

Body Weights: Treatment resulted in retardation of body weight in males (LD -9%, MD -22%, HD -29%) and females (MD -14%, HD -17%).

Food Consumption: Retardation in body weight at Week 52 was accompanied by decrease in food consumption (♂: LD 9%, MD 17%, HD 21%; ♀: MD and HD 14%).

Ophthalmoscopy: The incidences of anterior polar cataract in both males (MD 8/20, HD 16/19) and females (MD 4/20, HD 14/20) were drug-related and dose dependent.

Heart Rate and Respiratory Parameters: At Week 52, the values for minute volume (MV) and flow rate maximum during inspiration (PIFR) were statistically significantly decreased in both HD males (25% and 32% respectively) and HD females (15% and 20% respectively). In the same week, there was no toxicologically significant treatment-related effect on heart rate or blood pressure.

Hematology: Statistically significant reduction in WBCs due to reduced lymphocyte counts was seen in males (up to 13% at HD) and females (up to 12% at MD or HD).

Clinical Chemistry: There was a statistically significant increase in the levels of alkaline phosphate (up to 20% in MD ♂ and 47% in MD ♀) and decrease in triglycerides (up to 28% in HD ♂) and inorganic phosphates (up to 9% in HD ♂ and 21% in HD ♀).

Urinalysis: No toxicologically significant treatment-related effect.

Organ Weights: While Weight of lungs increased (up to 17% LD ♂ and 17% HD ♀), all other organ weights showed statistically significant decrease: kidneys (up to 18% in HD ♂ and 20% in HD ♀), liver (up to 35% in HD ♂ and 22% in HD ♀), heart (up to

22% in HD ♂ and 21% in HD ♀), spleen (up to 33% in HD ♂ and 17% in HD ♀), pituitary (up to 22% in HD ♂ and 26% HD ♀), thyroid (up to 21% in HD ♂ and 26% HD ♀), salivary gland (up to 13% in ♂), prostate (up to 22% in ♂), and Adrenals (up to 19% in ♀).

Gross Pathology: High incidences of flocculent material in urinary bladder (75% at LD and MD, 20% at HD) and occurrence of stones or urolithiasis (about 10%) in males (Control, LD, and MD: 1/24 each; HD 2/24) and none in females was the major finding. In addition, one single case of coprostasis was present in MD male.

Histopathology: Gross pathology findings of flocculent precipitate were confirmed histologically in males. In addition, there were cystitis (up to 60% at MD) and hyperplasia of urinary bladder epithelium (up to 30%) present in males. Incidences of rhinitis (nasal cavity; up to 55% in MD ♂ and 35% in LD ♀), enlarged cells of exocrine pancreatic acini (up to 95% in HD ♂ and 85% in HD ♀), small epithelial plaques of anterior lens pole (up to 20% in MD ♂), and accumulation of secretory products in harderian glands (up to 100% in HD ♂ and 45% in HD ♀) were also present.

Toxicokinetics: Plasma drug levels increased with increase in dose. There was accumulation of drug at Week 32 but less so at Week 52.

NOAEL: 0.01 mg/kg/day.

Table 20. Effects of Ba 679 BR in rats in a 52-wk Inhalation Toxicity.

* Significant at P < 0.05	Males (mg/kg/day)				Females (mg/kg/day)			
	0.	0.013	0.096	0.641	0	0.013	0.096	0.641
Body weight (g)	503	457*	395*	357*	272	264	235*	229*
Food consumption (g)	146	133	121	115	102	98	88*	88*
Hematology								
Lympho. %	79.3	75.6	66.9*	66.7*	79.8	72.4*	68.3*	68.4
Clinical Chemistry								
AP U/L	138	155	165*	155	90	123*	132*	126*
Tryglyc. mmol/L	1.8	1.5*	1.3*	1.3*	2.0	2.0	1.4	1.6
Inorg.Phos. mmol/L	1.64	1.46*	1.47*	1.50*	1.72	1.45*	1.31*	1.36*
Organ Weights								
Kidneys, g	2.8	2.7	2.4*	2.3*	2.0	1.9	1.7*	1.6*
Liver, g	12.3	11.3*	9.7*	8.0*	7.7	7.5	6.5*	6.0
Heart, g	1.34	1.24*	1.07*	1.04*	0.87	0.83	0.77*	0.77*
Lung, g	2.4	2.8*	2.6	2.4	1.8	2.2*	2.1*	2.1*
Spleen, g	0.9	0.8*	0.7*	0.6*	0.6	0.6	0.5*	0.5*
Pituitary, mg	13.3	12.2*	10.6*	10.4*	14.0	11.5*	10.9*	10.3*
Thyroid, mg	28.4	27.2	23.2*	22.4*	22.7	20.6	19.1*	16.8*
Salivary gland, g	0.94	0.92	0.85*	0.82*	0.6	0.6	0.6	0.6
Prostate, g	0.9	0.8	0.8	0.7*				
Adrenals, mg	60.0	62.7	59.1	55.2	78.3	71.8	67.4*	63.8*
Gross Pathology (20 animals/group)								
Urinary Bladder: Floccul. material Urolithiasis (stones)	 1 1	 15 1	 15 1	 2 2	 0 0	 0 0	 0 0	 0 0
Rectum:coprostasis	0	0	1	0	0	0	0	0
Histopathology (20 animals/group)								
Nasal Cavity: Rhinitis	1	4	11	5	2	7	3	4
Pancreas: Enlarged cells of exocrine pancreatic acini	11	11	10/19	19	13	13	13/19	17
Eyes: Small epithel. plaques of anterior lens pole			4	2				
Harderian glands: accumulation of secretory products	1	5	6	20	0	2	6	9
Urinary Bladder: Hyperplasia, transit, epithelium Cystitis Flocculent ppt.	 2 4 0	 3 6 6	 6 12 5	 1 4 3				
Toxicokinetics								
Plasma Levels:	**Mean Plasma Concentration (ng/mL)**							
	mg/kg/day:-	**0.013 (LD)**	**0.096 (MD)**	**0.641 (HD)**				
Wk 12		**0.02**	**0.25**	**4.45**				
Wk 32		**0.10**	**1.09**	**14.25**				
Wk 52		**0.04**	**0.57**	**9.99**				

Dog: 1-4 wk Exploratory Oral and i.v. Toxicity Study
Boehringer Ingelheim Study U90-0614, Vol. 1.9, Page 193

Beagle dogs (1/Sex/group) were administered Ba 679 BR via p.o. (10, 3, and 1 mg/kg/day) and i.v. (0.1 and 0.03 mg/kg/day using 0.1% and 0.03% concentration) for varying durations (p.o.: 9, 7, and 17 days respectively; i.v.: 25 and 20 days). Treatment via p.o. and i.v. resulted in a reduction in body weight gain and food consumption, inhibition of salivary secretion, tachycardia, mydriasis with subsequent catarrhal purulent keratoconjunctivitis. The ophthalmic effects are consistent with anticholinergic nature of the drug. The sponsor selected 1 and 0.1 mg/kg/day as high doses for 13-wk p.o. and 4-wk i.v. administration. The sponsor stated that low doses for the toxicology studies (p.o. and i.v.) could be small multiple of the prospective therapeutic dose for human (2 inhalations of 1-2 puffs of 0.001 mg drug = 0.0008 mg/kg/day). Signed GLP Statement was included.

Toxicokinetics: (See Table 21): For both p.o. and i.v. route, the increase in exposure (AUC) was proportional to the dose. C_{max} and T_{max} increased with dose.

Table 21. Effects of Ba 679 BR in dog (1-4 wk p.o., i.v.): Toxicokinetics.

Species (route) Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (ng•h/mL)	Other
Dog (p.o.)				
1	M [redacted] F:	2	33.5-36.4	
3	M [redacted] F	4	60.8-86.0	
10	M: [redacted] F:	8	219.6-755.9	
(i.v.) 0.03			4.73-5.45	
0.1	M: [redacted] F:	0.25	9.1-23.2	$t_{1/2}$ 10-15 min.

Dog: 13-wk Oral Toxicity Study
Boehringer Ingelheim Study U91-0510; Vol. 1.15, P 002 and Vol. 1.16, P 002

Study Dates: 15 January, 1990 to 24 April, 1990
Testing Lab: Boehringer Ingelheim KG, Dep. Exptl. Path. and Toxicology, Germany
Test Article: Ba 679 BR in gelating capsules obtained from ——— , Batch: D 3326/1
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Beagle dogs
Animals: 20/Sex; 3/Sex/group (0, 0.005, 0.03, 0.2 mg/kg/day), 6/Sex (1 mg/kg/day), 2/Sex (0.005 mg/kg/day chronic pharmacology group).
Route: p.o.
Dosage: 0 (empty gelatin capsule), 0.005 (LD), 0.03 (MD), 0.2 (MHD), and 1.0 (HD) mg/kg/day
Duration of Exposure: 13 weeks
Clinical Observations: Daily
Body Weights: Once before and weekly during the study
Food Consumption: Daily
Ophthalmoscopy: Once before and on weeks 8, 14, 22 (recovery animals) during the study. Shiemer tear test in weeks -1 and 1.5 and 24 hours after administration of drug at weeks 2, 8, and 12 (recovery group: week 20). Pupil diameter measured once in week -1 and then 1.5 and 24 hours after administration of drug at weeks 2, 8, and 12 (recovery group: week 20). Intra-ocular pressure was measured once at week -1, and 1.5 and 24 hours after drug administration in weeks 4 and 10 (recovery group: week 18).
Hematology: Weeks -2, 1, 6, 13 and 21 (recovery animals)
Clinical Chemistry: Weeks -2, 1, 6, 13 and 21 (recovery animals)
Heart Rate: Once during week -1, and afterwards at 1.5, 3, and 24 hours of completion of last dosing in weeks 2, 8, and 12. Systolic and diastolic blood pressure, amplitude of blood pressure and heart rate were measured in all (2/Sex) animals of chronic pharmacology group.
Drug Levels: Prior to and at 1, 4, and 8 h after administration during weeks 1, 4, and 12 on 6 animals (3/Sex).
Necropsy: End of study

Organ Weights and Gross- and Histo-Pathology: Absolute and relative weights of selected organs were determined and gross- and histo-pathological examinations conducted.

RESULTS

Clinical Observations: Dry mucosa of mouth and nose and incidences of loose feces, emesis, and mydriasis at all doses.

Mortality: None.

Body Weights: Bodyweights increased in LD (11.9%), MD (5.7%), and MHD (4.2%) males and LD (2.2%) females and decreased in HD (4%) males and MD (3.8%), MHD (11.3%), and HD (10.3%) females.

Food Consumption: No toxicologically significant treatment-related effect.

Ophthalmoscopy: Treatment with Ba 679 BR resulted in keratitis or keratoconjunctivitis sicca (KCS; 2/6 LD, 4/6 MD, 4/6 MHD, and 4/6 HD recovery) which was confirmed by histology and Schirmer's tear test. After 8-week recovery period, 3/6 HD animals showed ophthalmological and histopathological signs of previous KCS. There was statistically significant ↓of tear flow at MD, MHD, and HD and ↑ of the pupil diameter at MHD and HD. No any other toxicologically significant treatment-related effects.

Hematology: No toxicologically significant treatment-related effect.

Clinical Chemistry: There was dose dependent increase in total cholesterol (LD 17%, MHD 37%, HD 53%) at all doses except MD at which there was no effect.

Urinalysis: No toxicologically significant treatment-related effect.

Heart Rate: There was about 30% increase in heart rate at LD while, at MD, MHD, and HD, the increases in heart rate were about 40-100%. Increases in heart rates were higher for measurements taken at 1.5 and 3.0 hours than for 24 hours post-dose.

Organ Weights: There was decrease in the weights of heart (MD 21%, MHD 30%, HD 20%), prostate (LD 13%, MHD 15%, HD 43%), and testes (MD 21%, MHD 7%, HD 17%) in males.

Gross Pathology: No toxicologically significant treatment-related effect.

Histopathology: No toxicologically significant treatment-related effect.

Toxicokinetics: Drug plasma levels increased with increase in dose. See Table 22.

NOAEL: 0.005 mg/kg/day.

Table 22. Effects of Ba 679 BR in dogs in a 13-wk oral study: Toxicokinetics.

Species (route) Dose (mg/kg)	Mean Plasma Concentration (ng/mL)			
Dog (p.o.)	Geometric mean plasma concentrations - hr. postdose			
	Pre-dose	1 Hour.	4 Hour	8 Hour
200	M: 0.01	0.50	0.51	0.15
	F: 0.12	0.23	0.34	0.15
1000	M: 0.10	3.78	3.35	1.74
	F: 0.12	1.38	1.57	1.08

APPEARS THIS WAY
ON ORIGINAL

Dog: Subacute 4-wk i.v. Toxicity

Boehringer Ingelheim Study U91-0494, January 31, 1991, Vol. 1.14, Page 002

Study Dates: 15 May, 1990 to 12 June, 1990.
Testing Lab: Boehringer Ingelheim KG, Dep. Exptl. Path. and Toxicology, Germany.
Test Article: Ba 679 BR (0.002% aqueous solution for LD, MD, and chronic pharmacology groups; 0.02% aqueous solution for HD group).
GLP: Signed.

METHODS

Species/Strain: Beagle Dog.
Animals: 14/Sex; 3/Sex/group; 2/Sex/group for chronic pharmacology.
Route: i.v. (bolus injection in the fore limb vein).
Dosage: 0 (water), 0.004 (LD), 0.020 (MD), and 0.1 (HD) mg/kg/day.
Duration of Exposure: Daily (7 days/week) for a period of 4 weeks.
Clinical Observations: Daily.
Body Weights: Before study initiation and once weekly during the study.
Food Consumption: Once daily.
Ophthalmoscopy: Before the start of the study and at the end (Week 4).
Hematology: Weeks -2, 1, and 4.
Clinical Chemistry: Weeks -2, 1, and 4.
Urinalysis: Weeks -2, 1, and 4.
Heart Rate: Weeks 1 and 5 from 1 hour before until 2 hours after drug administration.
Drug Levels: Not done.
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

Clinical Signs: Typical anticholinergic effects such as keratoconjunctivitis sicca, ↓tear flow and ↑ pupil diameter were seen (see ophthalmoscopy section for details).

Mortalitly: None.

Body Weights: At LD, there was an increase (5%), while at MD, there was a decrease (28%) in bodyweight gains in males. At HD, there was bodyweight loss (5.3%) in males. In females, there was bodyweight loss in all treatment groups (LD 2.3%, MD 3.1%, HD 1.5%).

Food Consumption: Food consumption decreased significantly only at HD (♂ 72%, ♀ 55%) during week one.

Ophthalmoscopy: Ophthalmoscopy showed (and histopathology confirmed) that keratoconjunctivitis sicca was drug-related (3/6 MD, 5/6 HD). Treatment resulted in decrease in tear flow rate (about 1/3 to 7-fold, dose dependent), and an increase in pupil diameter (1 hr. post-dose: two-fold in MD and HD).

Hematology: There was drug related decrease in thrombin time (15% LD, MD; 21%HD) and increase in prothrombin time (23% MD, 41% HD).
Clinical Chemistry: No toxicologically significant treatment-related effect.

Urinalysis: No toxicologically significant treatment-related effect.

Heart Rate: There was significant increase in heart rate [about 100% after 1 minute and 1 hour post-dose at all doses; 37% (LD), 48% (MD), and 76% (HD) after 24 hours of the administration of drug.

Organ Weights: No toxicologically significant treatment-related effect.

Gross Pathology: No toxicologically significant treatment-related effect.

Histopathology: No toxicologically significant treatment-related effect.

NOAEL: <0.004 mg/kg/day.

Dog: Inhalation Toxicity (Lactose Powder Formulation) Feasibility
Boehringer Ingelheim Study U93-0729, August 02, 1993, Vol. 1.23, Page 261

Beagle dogs (1/Sex) were administered 0.01 (LD), 0.1 (MD) and 1 (HD) mg/kg/day of Ba 679 BR/lactose powder formulation. Each animal was dosed at each exposure on two separate occasions. The study was conducted by ——— ——— from 17 December, 1992 to 10 February, 1993. Signed GLP Statement was included.

The respirable fraction of drug aerosol (percentage below ——— were 47.7, 71.2, and 60.0% respectively. Clinical signs included pupil dilation at HD (both sexes) and at LD and MD in the female and, dry nose and mouth at MD and HD. There was no significant effect on body weight gain. Food consumption reduced in the female throughout the exposure period while in male only during the first week of treatment.

There was dose dependent increase in heart rate but no gross pathology changes. Hematology, clinical chemistry, urinalysis, and histopathology were not done. The NOAEL was not established.

Dog: 4-wk Inhalation (Lactose Powder Formulation) Tolerability
Boehringer Ingelheim Study U93-0766, August 23, 1993, Vol. 1.23, Page 301

Beagle dogs (8 ♂ and 4 ♀) were administered 0 (lactose only; 3♂ , 1♀), 0.04 (LD; 3♂, 1♀), and 0.24 (HD; 2/Sex) mg/kg/day of a lactose (5 mg) containing formulation of Ba 679 BR for a period of 4 weeks. The study was conducted between April 15, 1992 and May 14, 1992. Signed GLP Statement was included.

The achieved doses of the drug were 0.028 and 0.161 mg/kg/day for LD and HD respectively. Anticholinergic effects such as dry mucosa of mouth and nose were seen in all animals while pupil dilation was observed in 1 female in LD and all animals in HD group within one hour of drug administration and lasted until following administration next day. Keratoconjunctivitis sicca occurred in the female of LD group and 1 male and 1 female in HD group. Incidences of emesis were noticed. Treated animals showed slight reduction in body weight gains. Food consumption reduced by 69% in HD group at wk 1. Heart rate increased 24 to 59% in LD and 102 to 109% in HD group. No toxicologically significant treatment-related changes were observed in organ weights or gross pathology. Important histopathological findings included multi focal lymphocytic aggregates (lymphoid follicles) in the mucosa or submucosa in larynx (Control 2/4, LD 2/4, HD 4/4), focal or multi focal mixed cell infiltrates in trachea (LD 1/4, HD 2/4), and multi focal mixed cell infiltrates in the lungs (Control 2/4, LD 1/4, HD 4/4).

Toxicokinetics: Drug plasma levels increased with an increase in dose in a less than proportionate manner. See Table 23.

NOAEL: 0.04 mg/kg/day.

Table 23. Effects of Ba 679 BR in dog in a 4-wk Inhalation Study: Toxicokinetics.

Dose mg/kg/day	$AUC_{0\text{-}6\ h}$ (ng•h/mL)	MRT (h)	Other
			"Predicted" (mean) plasma concentrations (ng/mL)
			2 min / 30 min / 2 hr / 6 hr
0.04	2.4	1.1	1.40 / 0.68 / 0.08 / 0.02
0.24	5.8	1.1	4.24 / 2.72 / 0.32 / 0.06
			$t_{1/2}$ 1 h

Dog: 4-wk Exploratory Inhalation (Aqueous Formulation) Toxicity
Boehringer Ingelheim Study U91-0306, March 04, 1991, Vol. 110, Page 216

Beagle dogs (6/Sex; 3/Sex/group) were administered a nebulized aqueous aerosol of Ba 679 BR via inhalation route at 0.2 and 1.0 mg/kg/day for 4 weeks. The study was conducted by [redacted] from May 29, 1990 to June 26, 1990. Signed GLP Statement was included.

Mean achieved dosage levels were 0.287 and 1.24 mg/kg/day. Clinical signs included increased heart rate (100%), dry mouth and nose, pupil dilation, and occasionally dry eyes. Treatment did not alter body weight gain. There was reduction in food consumption at the end of week one (9 to 11%). No toxicologically significant treatment-related effects seen in hematology, clinical chemistry, urinalysis, organ weights, and gross pathology. Histopathological changes were: inflammation in lungs (♂: LD 1/3, HD 3/3 ♀: LD 0/3, HD 2/3), trachea (1/3 HD:♂ & ♀), and carina (1/3 HD: ♂ & ♀). $AUCs_{(0\text{-}7\ h.)}$ for LD and HD were 6.8 and 37.2 ng.h/mL, respectively. Half-lives of the drug ($t_{1/2}$) were 1.1 and 1.8 hours for LD and MD respectively, while mean resident time (MRT) was 1.6-1.9 hours. The NOAEL was 0.2 mg/kg/day.

Dog: 13-wk Inhalation Toxicity (Aqueous Formulation) Study
Boehringer Ingelheim Study U91-0511, May 23, 1991, Vol. 1.16, Page 160

Study Dates: August 16, 1990 to January 10, 1991

Testing Lab: —

Test Article: A 2 mg/mL aqueous solution of Ba 679 BR; A 100 mL solution contained 200 mg Ba 679 BR, 10 mg Benzalkonium chloride, 50 mg ethylene diamine tetra acetic acid (Na salt of EDTA), 900 mg NaCl, 8.4 mg Citric acid monohydrate, 0.8 mL of 0.1 N NaOH, 0.6 mL of 0.1 N HCl, and 100 mL of water for injection.

GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Beagle dogs
Animals: 15/Sex and 3/Sex/group (HD included 3/Sex/group for recovery study).
Route: Inhalation via nebulization.
Dosage: 0 (vehicle only), 0.01 (LD), 0.1 (MD), and 1.235 (HD) mg/kg/day.
Duration of Exposure: 10-15 min. daily for 91 days.
Clinical Observations: Twice daily.
Body Weights: Weekly.
Food Consumption: Daily.
Ophthalmoscopy: Once before treatment; Weeks 7 and 13 during treatment.
Respiratory Function: Prior to treatment and during weeks 7 and 13 of treatment.
Electrocardiography: Twice prior to initiating study and on Day 1 and during Weeks 7 and 13 of treatment. ECG recordings were obtained pre-dose, immediately post dose and at +1, +2, +4, +7, and +24 hour post dose.
Hematology, Clinical Chemistry, and Urinalysis: Prior to study and during Weeks 7 and 13 of dosing and at the end of 8 Week recovery period.
Drug Levels: Day 5 of Weeks and +30 min, 2 h and 6 h post dose during Weeks 6 and 12.
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: Up to 12% more than targeted dose levels.

Clinical Signs: Dry nose and mouth in all treatment groups (Occurrence of incidences: LD: 9 to 42/wk; MD: 39 to 42/wk; HD: 77 to 84/wk). Incidences of conjunctivitis increased with dose (Control 1/6, LD 2/6, MD 6/6, HD 12/12). Signs were recovered within 2 days of recovery period.

Body Weights: There was dose dependent decrease in body weight gains in males (LD 10%, MD 21%, HD 34%). In females, there was an increase in body weight gains (LD 109%, MD 55%, HD 18%) but it was not dose related.

Food Consumption: No toxicologically significant treatment-related effect.

Ophthalmoscopy: Drug-related partial and full dilation of pupil was observed at MD and HD respectively. Keratoconjunctivitis sicca in about 50% animals in MD and HD groups. Dogs in MD (1/6) and HD (3/6) groups became photophobic (abnormal visual intolerance to light) and showed reduced palpebral (eye lid) openings (MD 3/6, HD 4/6).

Respiratory Functions: No toxicologically significant treatment-related effect.
Electrocardiography: Tachycardia at MD and HD (heart rate increase up to 100%) lasted 24 hours post dose.

Hematology: No toxicologically significant treatment-related effect.

Clinical Chemistry: No toxicologically significant treatment-related effect.

Urinalysis: No toxicologically significant treatment-related effect.

Organ Weights: No toxicologically significant treatment-related effect.

Gross Pathology: No toxicologically significant treatment-related effect.

Histopathology: Inflammatory changes in the eyelids of females (LD 1/3, MD 1/3, HD 2/3). Corneal limbal inflammation seen in all groups (Control 2/6, LD 1/6, MD 2/6, HD 4/12), limbal basal swelling in 2/6 HD animals.

Toxicokinetics: $AUCs_{0\text{-}6\,h}$ were 1.64 (LD), 6.55 (MD), and 29.58 (HD) while $t_{1/2}$ was 2.2 hours at MD and 1.5 hours at HD.

NOAEL: 0.01 mg/kg/day.

Dog: 2-wk Inhalation Toxicity (Powder) Study
Boehringer Ingelheim Study U93-0941, January 28, 1994, Vol. 1.28, Page 002

Study Dates: March 03, 1993 to April 29, 1993
Testing Lab: [redacted]
Test Article: Ba 679 BR powder with lactose
GLP: Signed GLP Statement was included.

METHODS

Species/Strain: Beagle dogs
Animals: 8/Sex; 2/Sex/group
Route: Inhalation via an oropharyngeal tube.
Dosage: 0 (Control), 0.01 (LD), 0.1 (MD), and 1.0 (HD) mg/kg/day
Duration of Exposure: 14 days; 1 to 5 min. dosing period/day
Clinical Observations: Daily.
Body Weights: Weekly
Food Consumption: Daily
Electrocardiography: Pre-trial and on Days 1 and 13.
Respiratory Function: Twice pre-trial and prior to dosing on Days 1 and 13 of treatment.
Hematology: Pre-trial and on Day 14 of dosing.
Clinical Chemistry: Pre-trial and on Day 14 of dosing.
Urinalysis: Pre-trial and on Day 14 of dosing.
Drug Levels: On Day 7: pre-dose, and +0 min., +30 min., +2 h, and +6 h postdose.
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 achieved dose levels were 0.045, 0.214, and 1.219 mg/kg/day. Particle size distribution for these groups was 44.9, 23.5, and 59.1% of the drug aerosol being less than [redacted].

Clinical Signs: Dry nose and mouth and dilated pupils were noted in all animals of MD and HD groups. Enlarged nictitans membrane was seen in all HD animals and in the

females of MD group. Conjunctivitis and opacity of eyes were present in 1 MD female and 1 HD male. Veterinary eye treatment was given to all MD and HD animals.

Body Weights: In males, bodyweight gains increased at LD (14%) and MD (29%) and decreased at HD (70%). In females, there was decrease in bodyweight gains (LD 75%, MD 25%, HD 50%) but it was not dose-related.

Food Consumption: Reduced food consumption in HD groups (♂ 18%, ♀ 26%).

Electrocardiography: Significant increase (80-90%) in heart rate at MD and HD and only a slight increase at LD.

Respiratory Function: No toxicologically significant treatment-related effect.
Hematology: No toxicologically significant treatment-related effect.

Clinical Chemistry: No toxicologically significant treatment-related effect.

Organ Weights: No toxicologically significant treatment-related effect.

Gross Pathology: No toxicologically significant treatment-related effect.

Histopathology: Inflammatory lesions on the conjunctival surface of the eyelids in HD (3/4 animals: 2♂, 1♀).

Toxicokinetics: Drug plasma levels increased with increase in dose and duration postdose (1-30 min.) and tailed off at 120 and 360 min. See Table 24.

NOAEL: 0.01 mg/kg/day.

Table 24. Effects of Ba 679 BR 679 BR in dog in a 2-wk Inhalation study: **Toxicokinetics.**

Species (route) Dose	Mean Plasma Concentration (ng/mL)		
Dog (inhalation) Target Dose (Day 7)	Geometric mean plasma concentrations (ng/mL)		
	Min. Postdose	Males	Females
0.1 mg/kg/day	0	0.16	0.10
	1	0.59	0.33
	30	0.28	0.16
	120	0.38	0.64
	360	0.16	0.10
1.0 mg/kg/day	0	0.37	0.10
	1	36.45	30.12
	30	37.15	31.12
	120	8.86	8.59
	360	2.88	3.44