# Dog: 13-wk Inhalation Toxicity (Powder) Study

**Boehringer Ingelheim Study U93-0942, February 11, 1994, Vol. 1.28, Page 153**

*Study Dates:* May 24, 1993 to September 28, 1993.
*Testing Lab:* [redacted]
*Test Article:* Ba 679 BR/lactose blend powder: Low (0.16 mg/ [redacted] Batch Ch-B:301103) and high (1.6 mg/ [redacted] Batch Ch-B:301104) concentration. Control lactose powder batch Ch-B: 301102.
*GLP:* No toxicologically significant treatment-related effect.

## METHODS

*Species/Strain:* Beagle dogs.
*Animals:* 18/Sex; 3/Sex/group (control, treatments, recovery).
*Route:* Inhalation via oropharyngeal tube.
*Dosage:* 0 (Air Control), 0 (Lactose Powder Control), 0.01 (LD), 0.1 (MD), and 1.0 (HD) mg/kg/day.
*Duration of Exposure:* Once daily for 91 days; additional 28 days for recovery group.
*Clinical Observations:* Daily.
*Body Weights:* Weekly.
*Food Consumption:* Daily.
*Ophthalmoscopy:* Pre-trial, during Weeks 7 and 13 of treatment, and at the end of 4 week recovery period.
*Electrocardiography:* Twice pretrial and on Day 1, during Weeks 7 and 13 of treatment, and at the end of 4 week recovery period.
*Respiratory Function:* Twice pretrial, during Weeks 7 and 13 of treatment, and at the end of 4 week recovery period.
*Hematology:* Once pretrial, during Weeks 7 and 13 of treatment, and at the end of 4 week recovery period.
*Clinical Chemistry:* Once pretrial, during Weeks 7 and 13 of treatment, and at the end of 4 week recovery period.
*Urinalysis:* Once pretrial, during Weeks 7 and 13 of treatment, and at the end of 4 week recovery period.
*Drug Levels:* During Weeks 1, 7, and 13 of dosing (from treatment groups only) at the following time points: pre-dose, immediately after dose administration and at +30 min., +2 h, and +6 h post dose.
*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:* Mean achieved dose levels were 0.0121, 0.1416, and 1.3282 mg/kg/day. Particle size distribution measurements indicated that 40.5, 45.7 and 73.5% of the drug aerosol was [redacted] for groups LD, MD, HD respectively.

*Clinical Signs:* Dilated pupils and dry nose and mouth were noted for all MD and HD animals. Dry nose and mouth were also seen in recovery animals during the first 2 weeks of the recovery period while dilated pupils were noticed only during the first week.

*Body Weights:* Reduced bodyweight gains were seen in the males from HD (46%) and females from all treatment groups (LD 63%, MD 81%, HD 69%).

*Food Consumption:* Reduced food consumption in MD and HD of both sexes (up to 5% in males and 7.6% in females) and LD females (8.6%).

*Ophthalmoscopy:* High incidence of dry cornea in MD and HD animals at Week 7. A recovery from this effect at Week 13 was possibly due to veterinary treatment.

*Electrocardiography:* Significantly increased heart rate (tachycardia) seen at MD (♂ 47%, ♀ 10%) and HD (♂ 129%,♀ 59%) at +1 h post exposure on Day 1. Heart rates remained elevated at Week 13 (up to 58%↑) as well as Week 4 of the recovery period (♂ 51% ♀ 30%).

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

*Hematology:* No toxicologically significant treatment-related effect.

*Clinical Chemistry:* AST values were reduced in all Ba 679 BR treated males (13 to 24%) and MD (32%) and HD (40%) females.

*Urinalysis:* No toxicologically significant treatment-related effect.

*Organ Weights:* Heart weights were reduced in HD males (23%; $p<0.01$) and MD (13%) and HD (10%) females. Adrenal weights were reduced in HD males (29%) and in MD (41%, $p<0.05$) and HD (38%) females.

*Gross Pathology:* No toxicologically significant treatment-related effect.

*Histopathology:* Adrenal changes (zona fasciculata atrophy) were seen at MD (♂: 2/3, ♀: 3/3) and HD (♂: 3/3; ♀: 3/3), thymic atrophy at MD (♂: 2/3; ♀: 3/3) and HD

(♂: 3/3; ♀: 2/3). No other toxicologically significant treatment-related effects.

*Toxicokinetics:* Plasma drug levels did not increase proportionate to dose. There was no accumulation of drug at Week 7 and Week 13. See Table 25.

*NOAEL:* 0.01 mg/kg/day.

**Table 25. Effects of Ba 679 BR in dogs in a 13-wk inhalation Study: Toxicokinetics.**

| Dose | Geometric mean plasma concentrations (ng/mL) | | | |
|---|---|---|---|---|
| | Minutes postdose | Week 1 | Week 7 | Week 13 |
| 0.1 mg/kg/day | 0 | 0.03 | 0.02 | 0.02 |
| | 1 | 12.80 | 0.11 | 0.63 |
| | 30 | 4.65 | 0.30 | 1.39 |
| | 120 | 0.87 | 0.13 | 0.68 |
| | 360 | 0.05 | 0.38 | 0.01 |
| 1.0 mg/kg/day | 0 | 0.03 | 0.02 | 0.04 |
| | 1 | 22.10 | 22.97 | 2.60 |
| | 30 | 19.65 | 27.12 | 2.21 |
| | 120 | 6.72 | 5.70 | 0.77 |
| | 360 | 1.43 | 0.68 | 0.24 |

## Dog: 52-wk Inhalation Toxicity (Aqueous Formulation) Study

Boehringer Ingelheim Study U93-0938, November 17, 1993, Vol. 1.27, Page 002

*Study Dates:* October 16, 1991 to November 12, 1992
*Testing Lab:* [redacted]
*Test Article:* A 100 mL of O.8 mg/mL Ba 679 BR stock dosing solution consisted of 80 mg drug, 10 mg Benzalkonium chloride, 50 mg disodium edetate, 8.4 mg citric acid monohydrate, 0.8 mL 0.1 N NaOH, 0.6 mL 0.1 N HCl, and 100 mL water for injection.
*GLP:* Signed GLP Statement was included.

### *METHODS*

*Species/Strain:* Beagle dogs
*Animals:* 16/Sex; 4/Sex/group (exception: 1 additional HD ♀ was introduced into the study at the beginning of treatment Week. Due to poor skin condition, one HD ♀ already in the group had to be excluded.
*Route:* Inhalation via nebulization ( [redacted] nebulizer used).
*Dosage:* 0 (vehicle control), 0.004, 0.04, and 0.4 mg/kg/day at concentrations 0, 0.008, 0.08, and 0.8 mg/mL respectively. The doses were selected on the basis of a 13-week inhalation toxicity study (U91-0511, Vol. 1.16, Page 160) in dog. The lowest dose (0.004 mg/kg/day) represents 4.5 times the estimated maximum human dose of 0.00088 mg/kg/day. While the highest dose (0.4 mg/kg/day) chosen was higher than MD (0.1 mg/kg/day) used in the 13-week study, it was about one-third of the highest dose (1.235 mg/kg/day).
*Duration of Exposure:* 52 weeks (8 to 15 minutes daily).
*Clinical Observations:* Twice daily.
*Body Weights:* Weekly commencing 2 weeks pretrial.
*Food Consumption:* Daily commencing 2 weeks pretrial.
*Ophthalmoscopy:* Pre-trial and during Weeks 7, 13, 25, and 51 of treatment.
*Respiratory Function:* Pretrial and during Weeks 7, 13, 25, and 51/52 of treatment.
*Electrocardiography:* Twice pretrial and during Weeks 7, 13, 27, and 51 of treatment. During the treatment period, ECG recordings were done pre-dose, immediately post dose and at +1 h, +2 h, +4 h, +7 h, and +24 h post dose.
*Hematology:* Pretrial and during Weeks 6, 12, 25, 38, and 51 of dosing.
*Clinical Chemistry:* Pretrial and during Weeks 6, 12, 25, 38, and 51 of dosing.
*Urinalysis:* Pretrial and during Weeks 6, 12, 25, 38, and 51 of dosing.

*Drug Levels:* Day 1 and during Weeks 7, 13, 27, and 51 of dosing at the following time points: pre-dose, immediately after dose administration, and at +30 min, +2 h and +6 h post dose.
*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:* Achieved dose levels were 0, 0.0052, 0.0451, and 0.4483 mg/kg/day.

*Mortality:* One HD animal killed prematurely due to poor health.

*Clinical Signs:* Dry nose and mouth observed in most animals of MD and HD groups. Dry eyes among HD animals led to their appropriate veterinary treatment. Some LD and MD animals also required eye treatment.

*Body Weights:* In HD females, there was 10.6% bodyweight loss during the first week of dosing and 12.2% bodyweight loss at the end of the study.

*Food Consumption:* All animals showed reduction in food consumption during the first 7 weeks of treatment. In addition, HD females had reduced food intake during Weeks 8 to 13.

*Ophthalmoscopy:* Dilation of pupil seen in HD animals from Week 1 to Weeks 7, 13, 25, and 51. In addition, HD animals (3/9) also showed symptoms of keratoconjunctivitis sicca.

*Electrocardiography:* Increased heart rate (up to about 100%) was consistently observed in MD and HD animals following treatment on Day 1 to Weeks 7, 13, 27, and 51.

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

*Hematology:* No toxicologically significant treatment-related effect.

*Urinalysis:* No toxicologically significant treatment-related effect.

*Clinical Chemistry:* No toxicologically significant treatment-related effect.

*Organ Weights: Reduction in mean heart weights (♂: LD 10%*, MD 4.3%, HD* 14.4%*; ♀: LD 8.3%*, MD 14.6%*, HD 26.4%*). Data with asterisk (*) were statistically significant.

*Gross Pathology:* No toxicologically significant treatment-related effect.

*Histopathology:* Incidences of epithelial hyperplasia of the eyelid (MD:♂ 1/4; HD:♂ 2/4, ♀ 2/4). No other toxicologically significant treatment-related effect.

*Toxicokinetics:* Plasma drug levels increased with dose. However, there was no accumulation of drug at Weeks 7, 13, 27, and 51. See Table 26.

*NOAEL:* 0.004 mg/kg/day.

**Table 26. Effects of Ba 679 BR in dog in a 52-wk Inhalation Study: Toxicokinetics.**

| Dose mg/kg/day | Geometric Mean Plasma Concentration (ng/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Min. Postdose | Wk.1 | Wk.7 | Wk.13 | Wk.27 | Wk.51 |
| 0.04 | 0 | 0.11 | 0.17 | 0.19 | 0.16 | 0.13 |
| | 1 | 0.13 | 0.26 | 0.28 | 0.52 | 0.57 |
| | 30 | 0.13 | 0.17 | 0.14 | 0.24 | 0.25 |
| | 120 | 0.16 | 0.12 | 0.14 | 0.11 | 0.15 |
| | 360 | 0.14 | 0.31 | 0.17 | 0.13 | 0.18 |
| 0.40 | 0 | 0.10 | 0.12 | 0.11 | 0.16 | 0.10 |
| | 1 | 5.52 | 2.37 | 2.42 | 4.20 | 1.73 |
| | 30 | 2.77 | 1.20 | 2.07 | 2.02 | 1.83 |
| | 120 | 0.47 | 0.31 | 0.43 | 0.64 | 0.20 |
| | 360 | 0.11 | 0.16 | 0.16 | 0.13 | 0.11 |

# *SUMMARY OF TOXICOLOGY*

Single dose lethality oral (700, 1000, 1400 mg/kg) and intravenous (♂: 12.5, 16, 20 mg/kg; ♀: 16, 20, 25 mg/kg) toxicity studies in mice resulted in reduced motility, tremor, sedation, dyspnea, hunched posture, and coprostasis. Convulsions occurred only in the animals treated via i.v. route at 16 and 20 mg/kg doses. $LD_{50}$ values were 1336.4 mg/kg (p.o.), and 15.5 (♂) and 20.6 (♀) mg/kg (i.v.). Single dose inhalation administration to mice (131 mg/kg) resulted in mortality (3/10).

Single dose lethality (i.v.) studies on degradation products of Ba 679 BR in mice gave the following results: a) treatment with (100, 125, 160 mg/kg) resulted in dyspnea, tachypnea, and tremor at middle and high dose while, convulsions only at high dose. The $LD_{50}$ was 148 mg/kg; b) effects due to the treatment with (100, 125, 160 mg/kg) were similar to those of except that there were no incidences of convulsion at high dose and only 1 at middle dose. The $LD_{50}$ was 156 mg/kg. c) treatment with (20 mg/kg) resulted in low (10%) mortality. d) treatment with at 200 mg/kg (i.v.) showed no significant effects.

In rat, single dose oral administration of Ba 679 BR at 4000 mg/kg and i.v. administration at 16, 20, and 25 mg/kg resulted in incidences of reduced motility, dyspnea, tachypnea, tremor, and convulsions. $LD_{50}$ (p.o.) could not be established while, $LD_{50}$ (i.v.) was 21.5 and 19.5 mg/kg for males and females, respectively. In rats, single dose administration via inhalation (133.5 mg/kg) resulted in pupil dilation and follicular hemorrhage of thymus.

In dog, single dose administration of Ba 679 BR via inhalation (0.7, 3.6 mg/kg) resulted in drastic reduction (70% in males and 93% in females) in food consumption, a two-fold increase in heart rate (both sexes) and focal Kupffer-cell proliferation (males only).

In a 13-week MTD inhalation study in mice, treatment (0.04, 0.2, 1.0, 5.0 mg/kg/day) resulted in mortality (MD, MHD, HD), a decrease in body weight gain (50 to 67%) which was accompanied by a corresponding decrease in food consumption, about 70 to 80% increase in neutrophil counts, a slight but dose-related increase in the levels of ALT (13-21%), decrease in absolute and relative kidney weights, and increased incidences of epithelial hyaline droplets (LD 7/20, MD 9/17, MHD 12/19, HD 9/16) and focal acute inflammation in the nasal cavity (Control 3/20, LD 3/20, MD 2/17, MHD 4/16, HD 6/16). The NOAEL could not be determined, the MTD was 0.04 mg/kg/day, and the target organs of toxicity were kidney and liver. In another 13-week inhalation (dose ranging) study, treatment with the drug (0.08, 0.25, 0.75, 2.0 mg/kg/day) resulted in mortality in all groups, reduced bodyweight gains (40-60%); reduction in the relative weights of kidneys (15%), liver (12%), heart (21%) in both sexes and decrease in absolute weight of spleen (25%) in females; increase in relative rectum weights (up to 60%) in both sexes; and

incidences of ovarian cysts in females. Histopathological changes (drug-related) in both males and females were thymic atrophy and lymphoid depletion of splenic white pulp; changes restricted to females were squamous metaplasia of laryngeal epithelium and increased lymphocytolysis. The NOAEL could not be established and the MTD was <0.08 mg/kg/day. The target organs of toxicity were G.I. tract, lymph nodes, and thymus.

In rats, 4-week multiple dose oral administration of drug (0.1, 10, 200, 500 mg/kg/day) resulted in reduced bodyweight gains (25-75% in males and 27-90% in females) which was accompanied by reduced food consumption. Drug-related effect in both sexes was increased creatinine level, while effects in males were: increased creatinine levels; reduction in the weights of kidneys, liver, thymus, testes, and pituitary; and an increase in lung weights. Incidences of coprostasis were higher (up to 100%) in males than in females (33%). The NOAEL was 0.1 mg/kg/day and G.I. tract was the target organ of toxicity. Oral administration for 13-weeks (0.1, 5.0, 300 mg/kg/day) resulted in the following treatment-related effects: up to 80% higher incidences of chromodacryorrhea, reduced bodyweight gains accompanied by reduced food consumption, increased heart rate, coprostasis, white deposits in the urinary bladder, harderian gland red-brown-black discoloration, and pathologic lesions in lymph nodes. The NOEL was 0.1 mg/kg/day, the MTD was <5.0 mg/kg/day, and the target organs of toxicity was G.I. tract.

In a 3-week i.v. range finding study in rat, treatment with Ba 679 BR (0.01, 0.4, 16 mg/kg/day) resulted in chromodacryorrhea (eye), dyspnea, and epistaxis; reduced bodyweight gains; increased GOT levels (males only); and gross pathological changes such as white protein content in urinary bladder, harderian gland discoloration, and coprostasis. Incidences of bronchopneumonia with vicarious emphysema and per vascular edema of lung, urinary bladder cystitis and lymphofollicular hyperplasia in rectum were drug-related histologic changes. The NOAEL was 0.01 mg/kg/day and the target organs of toxicity were G.I. tract, harderian gland, and lung. When the drug was administered over a period of 4 weeks, additional clinical signs were sedation, pallor of tail, sternal recumbency, and apnoea. Reduction in bodyweight gains accompanied with reduction in food consumption. There was significant increase in heart rate. Gross- and histo-pathologic changes were comparable to the 3-week i.v. study. The NOAEL was 0.01 mg/kg/day and G.I. tract was the target organ of toxicity.

A 4-week inhalation study in rats (2.3 and 4.5 mg/kg/day) using aqueous formulation of Ba 679 BR resulted in mild reduction of lipids in liver cells. The NOAEL was 2.3 mg/kg/day. Treatment of rats with Ba 679 BR (0.1, 0.9, 3.8 mg/kg/day) dry powder with lactose for 2 weeks (inhalation route) resulted in dilation of pupil and hypersecretion of harderian glands, decreased bodyweight gains accompanied by a moderate (15%) reduction in food consumption by males, increased heart rate. Gross pathological changes that could be confirmed histologically were white flocculent precipitate in urinary bladder in males and discoloration of harderian gland in both sexes. The

*NOAEL was 0.1 mg/kg/day and the target organs of toxicity were eye and harderian* gland.

In a 13-week inhalation study in rat with aqueous aerosol of the drug (0.07, 0.6, 5.0 mg/kg/day), major clinical signs were increased pupil diameter (both sexes) and piloerection in MD and HD males. There was one mortality that the sponsor attributed to an accidental compression in the restraining tube during Week 13. Reduction in bodyweight gains was accompanied by lowered food consumption. Incidences of cataract and significant serum levels of alkaline phosphatase were drug-related. The greyish white flocculent precipitate was seen in the urinary bladder of all males but only MD and HD females. The NOAEL for this study was 0.07 mg/kg/day, the MTD was 0.6 mg/kg/day, and the target organs of toxicity was eye. In another 13-week inhalation study (0.09, 0.6, 5.6 mg/kg/day of Ba 679 BR powder with lactose) in rats, drug-induced changes included dilation of pupil, reduction in bodyweight gains accompanied by decreased food consumption, increased heart rate, incidences of cataracts, white flocculent precipitate in urinary bladder, discoloration of harderian gland, and coprostasis. The NOAEL and MTD were <0.09 mg/kg/day and, the target organs of toxicity were eye, G.I. tract, and harderian gland.

In a 13-week inhalation (MTD) study in F-344 rats, treatment with the aqueous formulation of drug (0.08, 0.25, 0.75, 4.0 mg/kg/day) resulted in red staining around head/snout/eyes with partially closed eyelids, incidences of noisy/wheezing respiration, and piloerection but no dilation of pupil. Other drug-related findings were reduced bodyweight gains, decrease in % of lymphocytes, increased AST in males, decreased AP in females, increased rectum weight in both sexes, increased adrenal weight in females, and reduced weights of kidney, liver, heart, brain, spleen, and thymus in males. Pathological findings consisted of sublingual acinar shrinkage in salivary gland, local inflammation in nasal cavity, and bilateral diffuse vacuolation in Adrenals. Due to mortality at the lowest dose, the NOAEL and the MTD could not be established. The target organs of toxicity were G.I. tract, nose, and salivary gland in both sexes and Adrenals in males. In another 13-week inhalation (MTD) study in F-344 rats, administration of 0.04, 0.2, 1.0, and 5.0 mg/kg/day of Ba 679 BR (aqueous formulation) resulted in incidences of exaggerated scratching of the snout and sneezing but no effect on pupil. There was reduction in bodyweight gains in males but not in females. Other drug-related effects were reduced WBC counts in males; increased rectum weights and diameter in both sexes; increased incidence of zona fasciculata vacuolation in Adrenals in males; incidences of inflammatory cell infiltrate, foci of goblet cell hyperplasia, and increased amounts of inflammatory exudate in the lumen of nasal cavity; and incidences of reduced eosinophils and dilated lumen of harderian gland in females and males respectively. The NOAEL was <0.04 mg/kg/day, the MTD was 0.04 mg/kg/day, and the target organs of toxicity were G.I. tract, nose, and harderian gland in both sexes and adrenals in males.

Administration of aqueous formulation of the drug (0.01, 0.06, 0.4 mg/kg/day) via inhalation in Wistar rats for 52 weeks showed the following toxicologically significant treatment-related effects: reduced body weights accompanied by decreased food consumption; incidences of cataracts; decreased minute volume (MV) and flow rate maximum during inspiration (PIFR); increased levels of alkaline phosphate; decreased levels of triglycerides and inorganic phosphates; increased lung weight and decreased weights of kidney, liver, heart, pituitary, thyroid, salivary gland, prostate, and Adrenals; increased incidences of flocculent material, occurrence of cystitis and hyperplasia of epithelium in urinary bladder in males; increased incidences of rhinitis, enlarged cells of exocrine pancreatic acini, epithelial plaques of anterior lens pole, and accumulation of secretory products in harderian glands in both sexes. The occurrence of coprostasis was low (♂: 1/24 at MD). The NOAEL was 0.01 mg/kg/day and the target organs of toxicity were eye, nose, pancreas, and harderian gland.

Based on 13-week toxicity studies in the two strains of rat, the drug was lethal to F-344 rats at or above 0.08 mg/kg/day while there was no mortality in Wistar rats up to 5.6 mg/kg/day. In addition, there was no lethality seen in Wistar rats even at 52 weeks at doses up to 0.4 mg/kg/day.

In 1-4 week exploratory oral (1, 3, 10 mg/kg/day) and i.v. (0.03, 0.1 mg/kg/day) studies in dog, treatment with the aqueous formulation of the drug resulted in reduction in bodyweight gain accompanied by reduced food consumption, inhibition of salivary secretion, tachycardia, dilation of pupil and catarrhal purulent keratoconjunctivitis. The NOAEL was not established. A 13-week oral administration of the drug (0.005, 0.03, 0.2, 1.0 mg/kg/day; powder formulation) resulted in incidences of dry mucosa of mouth and nose, loose feces, emesis, tachycardia, and dilation of pupil. Bodyweight gains were decreased in HD males and MD, MHD, and HD females and increased in LD, MD, MHD males and LD females. Other toxicologically significant drug-related effects were: incidence of keratoconjunctivitis sicca; increased total cholesterol levels; decreased tear flow and increased pupil diameter; decreased weights of heart, prostate, and testes; and no incidence of mortality. The NOAEL was 0.005 mg/kg/day. Target organs of toxicity were heart, eyes, salivary gland, and G.I. tract. In a 4-week i.v. study in dog with 0.004, 0.02, and 0.1 mg/kg/day of the drug (aqueous formulation), toxicologically significant drug-related changes were decreased bodyweight gains, tachycardia, decreased tear flow rate, increased pupil diameter, keratoconjunctivitis sicca, decreased thrombin time and increased prothrombin time. The NOAEL was <0.004 mg/kg/day and the target organs of toxicity were heart and eyes.

In a feasibility study, the effects of Ba 679 BR (0.01, 0.1, 1.0 mg/kg/day; powder formulation) via inhalation (2 exposures/animal) were studied in dogs. Toxicologically significant drug-related effects were: pupil dilation, dry nose and mouth, and reduced food consumption in females. In a 4-week tolerability study in dogs, 0.04 and 0.24 mg/kg/day of the drug (powder formulation) administration via inhalation resulted in

dilation of pupil, keratoconjunctivitis sicca (eye), emesis, tachycardia, multi focal lymphoid follicles in the mucosa and submucosa in larynx, and focal or multi focal mixed cell infiltrates in trachea and lungs. The NOAEL was 0.04 mg/kg/day and the target organs of toxicity were eye, heart, larynx, trachea, and lung. In a 4-week exploratory study in dogs, inhalation administration of the drug (0.2, 1.0 mg/kg/day; aqueous formulation) resulted in tachycardia; dry mouth and nose; pupil dilation; and inflammation in lungs, trachea, and carina. The NOAEL was 0.2 mg/kg/day and the target organs of toxicity were heart, salivary gland, nose, eye, lungs, trachea, and carina. In a 13-week inhalation study with the aqueous formulation of the drug (0.01, 0.1, 1.235 mg/kg/day), toxicologically significant treatment-related effects were: incidences of dry mouth and nose, keratoconjunctivitis sicca, photophobicity (visual intolerance of light), and reduced palpebral (eye lid) opening; decreased bodyweight gains (males only); tachycardia; and inflammatory changes in the eyelids (females only). The NOAEL was 0.01 mg/kg/day and the target organs of toxicity were salivary gland, heart, and eyes.

*In a 2-week inhalation study in dog, treatment with drug (0.01, 0.1, 1.0 mg/kg/day;* powder formulation) resulted in dry nose and mouth; dilated pupil, enlarged nictitans membrane (MD and HD treated animals required veterinary eye treatment); reduced bodyweight gains in females from all dose groups and in HD males; reduced food consumption (HD only), tachycardia (MD, HD); and inflammatory lesions on the conjunctival surface of the eyelids (HD). The NOAEL was 0.01 mg/kg/day and the target organs of toxicity were heart and eye. Using similar dose regimen and formulation, in another inhalation toxicity study with extended duration (13-weeks), the following toxicologically significant drug-related effects were seen: dry nose and mouth and dilated pupils and dry cornea; reduced bodyweight gains (HD males and females of all treatment groups); tachycardia (MD, HD); decreased values of AST; reduced weights of heart and Adrenals; and adrenal (zona fasciculata) and thymic atrophy. The NOAEL was 0.01 mg/kg/day and the target organs of toxicity were heart, eye, thymus, and adrenals. When the drug (0.004, 0.04, 0.4 mg/kg/day; aqueous formulation) was given to dog via inhalation for 52 weeks, toxicologically significant drug-related effects were: dry nose, mouth, and eyes; reduced body weights accompanied with decreased food consumption (HD females only); dilation of pupil; keratoconjunctivitis sicca (HD only); tachycardia; reduction in heart weights; and incidences of epithelial hyperplasia in the eyelid. One animal was killed prematurely due to poor health. The NOAEL was 0.004 mg/kg/day and the target organs of toxicity were eyes, salivary gland, and heart.

Based upon data from 13-week inhalation toxicity studies in mice, rats (F-344), and dogs, it can be concluded that that systemic toxicity of Ba 679 BR has been fully investigated.

# CARCINOGENICITY

## Status of Rat: 104-wk Inhalation Toxicity (Carcinogenicity) Study
**Boehringer Ingelheim Study U93-0946, November 22, 1993, Vol. 1.34, Page 002**
**Pathology Report U94-0087, January 20, 1994, Vol. 1.34, Page 324**

F-344 rats (360/Sex; 60/Sex/group for air control, LD, MD, and HD; 50/Sex/group for Vehicle Controls I and II; 20/Sex for Cage Control) were administered 0.012 (0.08 mg/mL), 0.030 (0.15 mg/mL), and 0.075 (0.35 mg/mL) mg/kg/day. The study was intended to proceed for 104 weeks. However, due to excessive mortality by Week 16, the study was terminated. The sponsor attributed excess mortality to particular sensitivity of F-344 rats to muscarinic effects of Ba 679 BR. Cause of death could not be established by histopathology except that congestion of food particles in proximal oesophagus (79/92) and focal or multi focal hemorrhages in the thymus (80/89) might suggest cause of death to be asphyxia.

The sponsor indicated that another 104-wk carcinogenicity study in Wistar rats was scheduled to begin January, 1994. The sponsor planned to employ doses of —— —— mg/kg/day for this new study.

## Rat: 4-week Inhalation Toxicity (Mechanistic) Study

**Boehringer Ingelheim Study U94-0029, Vol. 1.34, Page 298**

This 4-wk inhalation toxicity study was conducted in an attempt to address the issue of excess mortality in F-344 rat carcinogenicity study. A 1% (or 10 mg/mL) aqueous solution of Ba 679 BR was administered to rats via nose only inhalation. All rats were administered 1.0 mg/kg/day. The rats in this study were divided in three groups: a) rats (20/Sex) were fed pellets, every second day, after treatment and during the night; b) rats (20/Sex) were fed liquid food during the night; and c) rats (20/Sex) were fed pellets every day, after treatment and during the night. The extent of mortality in this study was as follows: group (a): ♂: 4/20, ♀: 11/20; group (b) ♂: 0/20, ♀: 0/20; and group (c) ♂: 5/20, ♀: 6/20. The sponsor indicated that mortality among the animals fed with dry pellets was due to obstruction of nasopharyngeal region by feed mash of an abnormal, sticky consistency. The reason why only Fischer rats are affected is not known.

## Status of Mice: 104-wk Toxicity (Carcinogenicity) Study

The sponsor is presently conducting a 104 weeks carcinogenicity study in mice. The sponsor discussed issues pertaining to survival of animals in this study with Division of Pulmonary Drug Products (Dr. Satish Tripathi, Pharmacology/Toxicology Reviewer; Dr. Joseph Sun, Acting Team Leader; Minutes of telecons of August 21 and 31 have been included with this review) and with the Chairman of CDER's Carcinogenicity Assessment Committee (See attached electronic communication from Dr. Joseph DeGeorge to Dr. Joseph Sun; January 11, 1996). Complete data were not submitted.

## SPECIAL TOXICITY

### Guinea Pig: Skin Sensitization Study
### Boehringer Ingelheim Study U91-0858, August 28, 1996, Vol. 17, Page 175

*Study Dates:* 19 November, 1990 to 14 December, 1990.
*Testing Lab:* Boehringer Ingelheim Dep. Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR, Batch G
*GLP:* Signed GLP Statement was included.

This "Guinea Pig Maximization Test" was conducted on 30 female guinea pigs (Strain: Pirbright White). The animals were divided in two groups: a negative control (Vehicle only) group and a test group. Test group animals were sensitized in two stages: On first day of the study, the guinea pigs received three pairs of intradermal injections with a 5% Ba 679 BR solution, Freud's Complete Adjuvant, and 0.9% NaCl solution (1:1:1). On the 8th day of study, 0.4 mL paraffin oil containing filter paper (group a) or 0.4 mL of 25% Ba 679 BR suspension in paraffin oil ' — ; (group b) was placed on the shaved skin. The filter paper was covered with Occlusive plaster. The occlusive system was removed 48 hours after application. On the 22nd day, animals of both groups were treated on left and right flanks with filter papers soaked with paraffin oil or paraffin oil containing 0.2 mL of Ba 679 BR. The filter papers were covered with occlusive plaster. The occlusive system was removed 24 hours after application and both areas of application were evaluated for level of irritation. After a period of another 24 hours, the areas of application were evaluated again. Clinical observations were conducted once daily during non treatment days and hourly during treatment days. Body Weights were recorded on Days 1, 8, 15, 22 and 25. Necropsy and histopathological examination were done on the animal that died during the study.

Results showed that the animal that died had focal necrosis of the colon with ulceration and hemorrhages and peritonitis. Ba 679 BR did not alter body weight gains in a dose dependent way, nor it revealed sensitization potential under the stated experimental conditions.

## GENETIC TOXICITY

### Bacterial Reverse Mutation (Ames) Test: Ba 679 BR
### Boehringer Ingelheim Study U90-0077, January 23, 1990, Vol. 1.9, Page 002

*Study Dates:* 21 June, 1989 to 15 August, 1989.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR, Batch 2.
*GLP:* Signed GLP Statement was included.

**Method:** Ba 679 BR was tested in bacterial strains *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA100 and TA98) and *Escherichia coli* (WP2uvrA) with and without S9 (derived from liver homogenates from Aroclor 1254-treated male rats). The bacterial cultures (in triplicates) were incubated with 0 (no add control), 0 (solvent control i.e. DMSO 100 µL/plate), 0.01, 0.1, 0.5, 1.0, 3.0, and 5.0 mg per plate of test article for a period of 48 hours. Two independent experiments were conducted to determine reproducibility of the assays. The positive controls were 2-animoanthracene, 1-ethyl-3-nitro-1-nitrosoguanidine, 1-methyl-3 nitro-1-nitrosoguanidine, 2-nitrofluorene, and 4-nitroquinoline-1-oxide. Negative controls were untreated and solvent-treated cultures. A test is considered positive if the increase in the reversion rate in 2 independent assays is at least two-fold over the value of negative control and is dose dependent.

**Results:** Treatment of bacterial cultures with Ba 679 BR did not result in an increase in mutation frequencies with or without S9. The negative and positive controls were within the historical control range. Therefore, Ba 679 BR was non mutagenic in the Ames Test.

### Bacterial Point Mutation (Ames) Test: Degradation Products of Ba 679 BR
### Boehringer Ingelheim Study U92-0074, November 18, 1991, Vol. 1.17, Page 226

*Study Dates:* 18 September, 1991 to 24 October, 1991.

*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* —, Batch I; — Batch I; — Batch I.
*GLP:* Signed GLP Statement was included.

**Method:** — degradation products of Ba 679 BR ( — , were tested for mutagenic potential in five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA100 and TA98). The bacterial cultures (in triplicates) were incubated with 0 (no add control), 0 (solvent control i.e. DMSO 100 µL/plate), 0.01, 0.1, 0.5, 1.0, 3.0, and 5.0 mg per plate of test articles for a period of 48 hours. Two independent experiments were conducted to determine reproducibility of the assays. The positive controls were 9-amino acridine, 2-animoanthracene, 1-methyl-3-nitro-1-nitrosoguanidine, and 2-nitrofluorene. Negative controls were untreated and solvent-treated cultures. A test is considered positive if the increase in the reversion rate in 2 independent assays is at least two-fold over the value of negative control and is dose dependent.

**Results:** Treatment of bacterial cultures with test substances did not result in an increase in mutation frequencies with or without S9. The negative and positive controls were within the historical control range and hence, the degradation products of Ba 679 BR were considered non-mutagenic in the Ames Test.

**Bacterial Point Mutation (Ames) Test: Degradation Products of Ba 679 BR**
**Boehringer Ingelheim Study U92-0498, July 01, 1992, Vol. 1.19, Page 397**

*Study Dates:* 5 May, 1992 to 4 June, 1992.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* — degradation product of Ba 679 BR).
*GLP:* Signed GLP Statement was included.

**Method:** A degradation product of Ba 679 BR ( — ) was tested for mutagenic potential in five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA100 and TA98). The bacterial cultures (in triplicates) were incubated with 0 (no add control), 0 (solvent control i.e. DMSO 100 µL/plate), 0.01, 0.1, 0.5, 1.0, 1.5, and 5.0 mg per plate of test article for a period of 48 hours. The two highest doses 1.5 and 5.0 mg/plate precipitated in the medium. Two to three independent experiments were conducted to determine reproducibility of the assays. The positive controls were 9-amino acridine, 2-animoanthracene, 1-methyl-3-nitro-1-nitroso-guanidine, and 2-

nitrofluorene. Negative controls were untreated and solvent-treated cultures. A test is considered positive if the increase in the reversion rate in 2 independent assays is at least two-fold over the value of negative control and is dose dependent.

**Results:** Treatment of bacterial cultures with [redacted] at certain levels ($\geq$ 1.0 mg/plate in the absence of and $\geq$ 0.01 mg/plate in the presence of S9) was bacteriotoxic. A the concentrations used, treatment of bacterial cultures with the test substances did not result in an increase in mutation frequencies with or without S9. The negative and positive controls were within the historical control range and hence, the degradation products of Ba 679 BR were considered non-mutagenic in the Ames Test.

***In Vivo* Mouse Micronucleus Test**
**Boehringer Ingelheim Study U91-0096, October 31, 1990, Vol. 1.10, Page 147**

*Study Dates:* 12-14 March, 1990.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR, Batch F.
*GLP:* Signed GLP Statement was included.

**Method:** A preliminary dose finding study was conducted in which mice were intravenously administered the drug Ba 679 BR at 8 mg/kg (2 ♂, 3 ♀) and 10 mg/kg (5 ♂, 4 ♀). The doses 8 (LD) and 10 mg/kg represented about 50% of $LD_{50}$ (15.5 to 20.6 mg/kg with a maximum non-lethal dose of 12.5 mg/kg). Treatment at these doses (LD, HD) resulted in toxicities such as increased respiratory rate, sedation, dyspnoea, and ptosis. There were 2 mortalities at HD and none at LD; therefore, LD was chosen as the highest dose for the mouse micronucleus assay.

Mice (25/Sex; 5/Sex: 24 hr negative control or saline, 24 hr positive control or 30 mg/kg cyclophosphamide, and 8, 24, 48 hr Ba 679 BR) were administered 8 mg/kg test article (volume 10 mL/kg) via i.v. into a lateral tail vein. The animals were sacrificed and femoral bone marrow samples were taken at appropriate periods for the test groups (8, 24, 48 hours). One thousand polychromatic erythrocytes (PE) from each animal were evaluated for the presence of micronuclei. In addition, the observed number of micro nucleated normochromatic erythrocytes (NE) was also recorded and PE/NE ratio determined. Criterion for a positive response is an occurrence of 2 significant responses from different sampling times in the same experiment.

**Results:** The PE/NE ratio were 0.89 for negative and 0.52 for positive controls and 0.75, 0.70 and 0.59 for 8, 24, and 48 hr treatment groups respectively. These data suggest that Ba 679 BR caused bone marrow toxicity. The drug did not cause

*significant effect on the frequencies of micro nucleated NEs. Thus, Ba 679 BR was* non-clastogenic in this test.

**CHO/HGPRT Mammalian Mutation Assay**
**Boehringer Ingelheim Study U91-0331, February 22, 1991, Vol. 1.10, Page 317**

*Study Dates:* 31 July, 1990 to 13 December, 1990.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR, Batch F.
*GLP:* Signed GLP Statement was included.

**Method:** The mutagenic potential of Ba 679 BR was assessed in V79 Chinese hamster lung cells using HGPRT (or resistance to 6-thioguanine) as a marker. The cells were exposed to the test substance in the presence as well as absence of S9 (metabolic activation system from Aroclor 1254 pre-treated rats) for 4 hours. A preliminary dose ranging study indicated that 5 mg/mL was not toxic in the presence or absence of S9 and, therefore, chosen as the highest concentration for this assay. Two independent experiments were conducted in which 0.1, 1.0, 3.0, and 5.0 mg/mL concentrations of the drug were tested, in triplicates, in the presence and in the absence of S9 mix. Ethyl methane-sulfonate was used as a positive control "without metabolic activation" while 9, 10-Dimethyl-1,2-benzanthracene (DMBA dissolved in DMSO; final concentration in growth medium 1% v/v) served as positive control "with metabolic activation." The criterion for a positive control for this test is a reproducible concentration-related increase in mutation frequency.

**Result:** Ba 679 BR was non-mutagenic under the conditions of this assay. Positive control yielded a typical result.

***In Vitro* Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Test**
**Boehringer Ingelheim Study U91-0637, April 08, 1991, Vol. 1.17, Page 002**

*Study Dates:* 17 December, 1990 to March 28, 1991.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR, Batch G.
*GLP:* Signed GLP Statement was included.

**Method:** The ability of Ba 679 BR to induce unscheduled DNA synthesis (UDS) was determined in the primary cultures of rat hepatocytes. The cell cultures (64.5-70% viability), in triplicates, were exposed to various concentrations of Ba 679 BR (0.02, 0.1, 0.5, 1.0, 2.5, and 5.0 mg/mL) in the presence of radio labeled thymidine (10 μCi/mL) for 18 hours and processed for autoradiography to assess UDS activity. Culture medium without drug represented negative control while 2-AAF served as positive control. The sponsor's criterion for evaluation of this test was a concentration dependent (for at least 2 concentration levels) increase in the net grain count as compared with the negative control. A $\pm$ 5 increase in the mean net grain count for any dose group was regarded a positive result.

**Results:** No dose dependent increase in the net grain count in the treatment groups indicates that treatment with Ba 679 BR may not induce unscheduled DNA repair in rats. Thus, Ba 679 BR is non-genotoxic in this test. Positive control yielded a typical result.

## In Vitro Chromosomal Aberrations in Human Lymphocytes

**Boehringer Ingelheim Study U91-0855, September 10, 1991, Vol. 1.17, Page 131**

*Study Dates:* 10 December, 1990 to June 18, 1991.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR, Batch G.
*GLP:* Signed GLP Statement was included.

**Method:** Asynchronous population of lymphocytes was treated with Ba 679 BR in the presence and the absence of metabolically active liver homogenates from Aroclor 1254-induced male rats (S9 mixture). The dosing range was chosen from an exploratory study with 7 different drug concentrations ranging from 0.025 to 5.0 mg/mL. Lymphocyte cultures (in duplicates) were exposed to 0 (negative control), 0.625, 2.5, and 5.0 mg/mL of drug for a period of 4 (cultures with S9) and (cultures without S9) 24 hours; the cultures containing S9 were continued for another 20 hours in medium free of S9. Cells in all the cultures were harvested at 24 hours, stained and scored for structural chromosomal aberrations. The experiment was repeated once with a delayed harvesting at 48 hours included in the top dose. Adriamycin treated (0.05-0.1 μg/mL) cultures served as positive control.

The clastogenic potential of Ba 679 BR was evaluated by an increase in the percentage of cells showing structural chromosomal aberrations. A response was considered positive if it resulted in a reproducible and concentration dependent increase in the aberration frequency over that in negative controls.

**Result:** Ba 679 BR did not induce chromosomal aberrations in human lymphocytes and, therefore, was non-clastogenic. Positive control yielded a typical result.

## SUMMARY OF GENETIC TOXICITY

At the dose ranges used in these studies, Ba 679 BR was neither genotoxic (Ames battery, CHO HGPRT test, *in vitro* rat hepatocyte unscheduled DNA synthesis test) nor clastogenic (cytogenetic assays: *in vivo* mouse micronucleus test, *in vitro* chromosomal aberrations in human lymphocytes). The degradation products of the drug were nongenotoxic in Ames test.

## REPRODUCTIVE TOXICITY

**Rat: Range Finding Segment-I Inhalation Toxicity Study**
**Boehringer Ingelheim Study U92-0679, September 28, 1992, Vol. 1.20, Page 292**
**Testing Laboratory:** [redacted]

**Method:** Effects of Ba 679 BR on fertility and pregnancy were studied by administering 0 (Control), 0.05 (LD), 0.5 (MD), and 5.0 (HD) mg/kg/day to Crl: CD (SD) BR rats (Main Study: 10/Sex/group; Satellite Study: 5 males/group and 5 or 8 females/group (Control: 5/group; Treatment: 8/group) via snout only inhalation. The male and female rats were exposed for 1 hour each day for 4 and 2 weeks prior to mating, respectively and then through mating, pregnancy, and lactation. For pregnant females, treatment was stopped after Day 20 of pregnancy for the birth of young and recommenced on Day 1 post partum. Blood samples from the Satellite group animals were taken during pre-mate, pregnancy, and lactation periods for determination of toxicokinetic parameters.

**Result:** In the males, treatment with Ba 679 BR resulted in pupil dilation (LD 44%, MD & HD 100%), reduction in bodyweight gain from Weeks 0 to 1 (LD 47%, MD 95%; at HD, there was bodyweight loss: -21%) as well as Weeks 0 to 10 (LD 5%, MD 20%, HD 25%), and reduction in food consumption at Week 1 (LD 10%, MD and HD: 19% each). Mortalities were in the Control (1/10) as well as high dose groups (MD 2/10, HD 1/10). Only fewer animals (MD 1/10, HD 1/10) failed to induce pregnancy.

In the females, treatment with the drug resulted in pupil dilation (LD 33%, MD and HD: about 82%). From Week 2 to Week 3, there was bodyweight loss (and no bodyweight gain) in all groups including Controls (bodyweight loss in LD, MD, and HD groups was 1.5X, 1.75X, and 3.5X times of that in the Control group). During Week 2 to 10, there was reduction in bodyweight gains (LD 30%, MD 27%, HD 77%). Food consumption decreased from 10% at LD to 22% and 14% at MD and HD. Mating was not successfully completed from Day 1 to Day 20 only in a few animals (MD and HD: 1/10 each). There were 2 mortalities at HD which occurred at Day 8 of pairing (1/10) and Day 13 post partum (1/10). Other toxicities were as follows: one MD female being non pregnant, 1 HD female having a resorption, 1 litter loss at HD on Day 2 post partum, and reduced number of rearing youngs to weaning at MD (9/10), and HD (6/10).

The reduction in the values of mean litter parameters (implantation sites, total young born, Live young at birth and at Day 21, and mean pup weights at birth and at Day 21) was toxicologically not significant.

*Toxicokinetics:* Drug plasma levels increased with dose in the samples 60 and 300 min. postdose (Table 27).

Table 27. Effects of Ba 679 BR in rats in a Segment-I Inhalation Study: Toxicokinetics.

| Dose mg/kg/day | Mean Plasma Concentration (ng/mL) | | | |
|---|---|---|---|---|
| | Minutes Postdose | Males | Mated Females | Unmated Females |
| 0.5 | 0 | 0.13 | 0.13 | 0.19 |
| | 60 | 0.36 | 0.34 | 0.27 |
| | 300 | 0.17 | 0.22 | 0.19 |
| 5.0 | 0 | 0.42 | 0.43 | 0.38 |
| | 60 | 4.77 | 5.25 | 6.38 |
| | 300 | 1.60 | 1.92 | 2.54 |

This study was used in the selection of doses (0.01, 0.1, and 2.0 mg/kg/day) in a definitive Segment-I inhalation toxicity study in rats.

**Rat: Fertility and Reproductive Performance (Segment-I) Study by Inhalation Boehringer Ingelheim Study, U93-0239, Vol. 1.22, Page 255**

*Study Dates:* 4 October, 1991 to 26 May, 1992

*Testing Lab:*

*Test Article:* Ba 679 BR Batch II; The aqueous solution of test article was prepared in a vehicle that contained benzalkonium chloride (10 mg), disodium edetate (50 mg), citric acid monohydrate (8.4 mg), 0.1N NaOH (0.8 mL), 0.1N HCl (0.6 mL), and water for injection (100 mL). The pH was adjusted to 3.0. Three different strength solutions (0.0025%, 0.025%, 0.5%) were prepared in order to achieve the expected doses. The pH of final dosing solutions was also adjusted to 3.0.

*GLP:* Signed GLP Statement was included.

## METHODS

*Species/Strain:* Rats VAF/Plus (Crl: $CD^R$ (SD) BR strain.
*Animals:* 120/Sex; 30/Sex/group.
*Route:* Snout-only inhalation.
*Dosage:* 0 (Control, Vehicle), 0.01 (LD; Concentration: 0.025 mg/mL), 0.1 (MD; 0.25 mg/mL), and 2.0 (HD; 5.0 mg/mL) mg/kg/day
*Duration of Exposure:* Male and female rats were exposed for one hour daily for 9 and 2 weeks, respectively prior to mating and then throughout mating, gestation, and lactation. Exposure of pregnant females was suspended after Day 20 of pregnancy to allow the birth of their young but recommenced on Day 1 post partum.

*Clinical Observations: Fo Generation:*
*1. Adult Animals:*

*Clinical Signs:* Daily.
*Body Weights:* Week-1 (♂), Week 6 (♀); then, Weekly (♂, ♀).
*Food Consumption:* Daily during: ♂: Weeks -1, 1, 8; ♀: Weeks 7, 8.
*Pregnancy Rate:* Percentage of surviving paired females that became pregnant.
*Mating Performance:* Daily: 7 days prior to- and during 20-days mating period.
*Duration of Pregnancy:* Time between the day of successful mating and the day on which pups were first seen.

*2. Litter data for rats withdrawn from treatment after Day 19 and sacrificed on Day 20 of Pregnancy:*

On Day 20 of pregnancy, selected number of females from all groups were sacrificed and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteruses were examined to determine: a) number of corpora lutea; b) number and distribution of live young; c) number and distribution of embryo fetal Deaths; d) fetal weights from which litter weight was calculated; and d) fetal abnormalities.

*3. Litter data for rats rearing young:*

All litters were examined daily. Pups were weighed on Days 4, 8, 12, 16, and 21 post partum. During the pre-weaning period, all offspring in all litters examined to determine age at which the following developmental stages attained:

*Surface righting reflex:* Day 1 post partum to 100% success.
*Startle reflex:* Day 11 post partum to 100% success.
*Air righting reflex:* Day 14 post partum to 100% success.
*Pupil reflex:* Day 20 post partum.

*Clinical Observations: $F_1$ Generation:*

*1. Adult Animals:*

*General Aspects:* Animals were observed for abnormal behavior and obvious changes in temperament. Animals were weighed weekly.

*Sexual Maturation:* Onset of vaginal opening monitored in all females from 28 days post partum. Occurrence of cleavage of the balanopreputial skin fold monitored in all males from 35 days post partum.

*Developmental/Behavioral Examinations:* Confined to the animals of Batch 2:

*Accelerating rotarod test:* Performed at 4 weeks of age.
*Actimat test:* Performed at 5 weeks of age.
*One trial passive avoidance test:* Performed at 7-8 weeks of age.
*Activity monitoring* [redacted]: Three consecutive afternoons.

Following the completion of above behavioral tests and assessment of results, batch 2 animals were sacrificed and examined externally and internally for abnormalities.

*Assessment of Reproductive Capacity:* Was confined to animals of Batch 1. When the animals were 84 days of age, they were mated on a one male and one female basis for 20 days. During mating period, all females were weighed daily and daily weighing continued until parturition. Dams that littered were weighed on Days 0, 7, 14, 21 post partum. Vaginal smears were taken 7 days prior to mating and daily during 20-day mating period.

*2. Litter Data:* Pups were weighed on Days 4, 8, 12, 16, and 21 post partum. On or shortly after Day 21 post partum, all $F_2$ pups and $F_1$ adults were sacrificed and examined for abnormalities

**RESULTS**

*Dosage Levels:* Measured concentrations of the drug were 0.030, 0.28, and 5.46 mg/mL. Thus, the dose levels were 0.012, 0.112, and 2.184 mg/kg/day, respectively.

*$F_0$ Generation:*

*1. Adult Animals:*

*Clinical Signs:* Pupil dilation was seen in all animals exposed to MD and HD and most LD exposed (53/60) animals. Incidences of brown stained head, peri-orbital nasal crusting (♂: Control 1/30, LD 7/30, MD 15/30, HD 25/30; ♀: 7/30, 10/30, 9/30, 21/30) and dry eyes (♂: MD 2/30, HD 7/30;♀: HD 17/30) were drug-related.

*Mortalities:* Two MD (♂) and 6 HD (4 ♂, 2 ♀) animals died.

*Body Weights:* Bodyweight gains were statistically significantly reduced at higher doses in both males (Wk 0-1: MD 39%, HD 100%; Wk 1-9: MD 10%, HD 24%; Wk 0-15: MD 15%, HD 33%) and females [Wk 7-8: MD 86%, (body weight loss at HD was about 0.01%); Wk 8-9: HD 57%; Wk 7-12: MD 13%, HD 38%). In females, statistically significantly reduced bodyweight gains at HD were consistent during pregnancy (Day 20: 31%) and lactation (Day 21 post partum: 70%).

*Food Consumption:* Reduction in bodyweight gains was accompanied by slight but statistically significant reduction in food consumption at Week 9 in both males (HD 10%) and females (MD 8%, HD 5%).

*Mating Performance and Pregnancy Rate:* No toxicologically significant treatment-related effect.

*Duration of Pregnancy:* No toxicologically significant treatment-related effect.

*2. Litter Data:*

*Total Litter Loss:* Total resorptions at HD (2) and MD (1) were drug-related.

*Litter Values at Day 20 of Pregnancy:* At higher doses, significantly lower number of corpora lutea (MD 16%, HD 13%) and implants (MD 20%, HD 15%) and higher post implantation loss/early deaths at HD (13.1%) resulting in fewer live young (HD 12%) compared to control. At higher doses, there was also a significant reduction in mean litter weight (MD 22%, HD 26%).

*Fetal Examination:* No toxicologically significant treatment-related changes in the number of fetuses with malformations or visceral and skeletal abnormalities.

*Litter Values to Day 21 Post Partum:* No toxicologically significant treatment-related effect.

*Pre-weaning development:* No toxicologically significant treatment-related effect.

*3. Terminal Autopsy:* No toxicologically significant treatment-related effect.

*$F_1$ Generation:*

*1. Adult Animals:*

*Clinical Signs:* No mortalities. No other toxicologically significant treatment-related effects.

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

*Sexual Maturation:* No toxicologically significant treatment-related effect.

*Post Weaning Behavioral Tests:*

*Accelerating Rotarod:* No toxicologically significant treatment-related effect.

*Actimat:* Mean amount of time spent by HD males ($F_1$) was statistically significantly lower (16%) than Control.
*Passive Avoidance:* No toxicologically significant treatment-related effect on parameters of this test (pre-shock: Day 1; Post shock: Day 2; retrival performance on Day 22) in either sex.

*Activity Monitoring (* [redacted] No toxicologically significant treatment-related effects on mean total activity path length, mean percentage time spent in activity, pattern of activity over time, and mean percentage time spent in the area of low level illumination.

*Mating Performance and Pregnancy Rate:* No toxicologically significant treatment-related effect.

*Duration of Pregnancy:* No toxicologically significant treatment-related effect.
*2. Litter Data:*

*Total Litter Loss:* There was one total litter loss of 18 pups by Day 14 post partum at MD.

*Litter Values:* Lower number of implantation sites and higher post implantation loss resulted in small but statistically significant decrease in litter size (LD 11%, MD 10%, HD 14%) and weight (LD 14%, MD 8%, HD 11%) at birth

*Terminal Autopsy:* No toxicologically significant treatment-related effect.

**Rat: Preliminary Segment-II Oral Toxicity Study**
**Boehringer Ingelheim Study U90-0079, February 28, 1990, Vol. 1.9, Page 23**

**Method:** Five mated rats/group (Chbb:THOM, Wistar) were administered 0 (0.5% tylose-treated negative control), 100 (LD), 500 (MD), and 1000 (HD) mg/kg/day of Ba 679 BR from Day 7 to the Day 16 of gestation. On Day 22 of gestation, cesarian section was performed in all the dams and the uterine contents were examined.

**Results:** Treatment with Ba 679 BR resulted in mortalities at HD (3/5 mothers) but it did not cause significant embryo lethal or fetotoxic effect at any dose. At MD and HD, there were drug-related incidences of chromodacryorrhoea, piloerection, decreased body weight gain (LD, MD: 7%↓; HD 23%↓), and food consumption (HD 5%↓). Mean fetal birth weights were significantly reduced at HD while mean placental weights did not. Based upon these data, MD (500 mg/kg/day) seemed to be close to MTD and, therefore, chosen as top dose for definitive segment-II study in rat via p.o.

**Rat: Segment-II Oral Toxicity Study**
**Boehringer Ingelheim Study U90-0687, October 25, 1990, Vol. 1.9, Page 253**

*Study Dates:* 5 February, 1990 to 23 April, 1990.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR (Batch F) in 0.5% aqueous solution of tylose.
*GLP:* Signed GLP Statement was included.

## METHODS

*Species/Strain:* Rat Chbb:THOM.
*Animals:* 144 Females (36/group: 24 cesarian and 12 normal delivery).
*Route:* Oral by gavage.
*Dosage:* 0 (Control, 0.5% tylose), 1 (LD), 25 (MD), and 500 (HD) mg/kg/day.

*Duration of Exposure:* Day 7 to Day 16 of gestation.
*Clinical Observations:* Daily.
*Body Weights:* Daily.
*Food Consumption:* Weekly.

*Observations: Fo Generation:*

*1. Adult Females:*

*Clinical Signs and Mortality:* Daily.
*Body Weights:* On Days 1, 7 through 16 and 22 of gestation and weekly during the lactation period.
*Food Consumption:* Weekly during the periods of gestation and lactation.

*2. Litter data and fetal examinations for animals sacrificed on Day 22 of gestation:*

On Day 22 of gestation, animals from Control and all treatment groups were sacrificed and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine number of corporate lutea, number and distribution of live young, number and distribution of embryo fetal deaths, individual fetal weights, and fetal malformations and skeletal and visceral abnormalities.

*3. Litter data for animals rearing young:*

All litters were examined daily. Pups were weighed and sexed. The litters were examined for litter size, still-births, live births, and skeletal and visceral abnormalities. On Day 4 after delivery, the litters were reduced to 8 pups (4/Sex). Bodyweight was recorded on days 7, 14 and 21 after delivery as the loss of the young was calculated during the lactation period (Days 1-21). Dead pups were autopsied.

The growth, maturation, and behavior of the youngs was evaluated using the development of the following physiological landmarks: erection of pinnae, start of fur growth, running with raised venter, eruption of maxillar incisors, opening of eyelids, negative geotaxis, descensus of testes, and opening of vagina. During the fourth week of life, pupillary reflex, righting reflex and preyer reflex were tested in the young:

## RESULTS

*Dosage Levels:* The achieved daily doses were within 5% of expected doses.

*Fo Generation:*

*1. Adult Females:*

*Clinical Signs:* At HD, 25/36 females showed chromodacryorrhoea a few days after dosing. Sixteen of the 144 mated females did not get pregnant.

*Mortalities:* No intercurrent deaths and one miscarriage.
*Body Weights:* There was a decrease in bodyweight gains at HD (about 10% in cesarian and 11% in spontaneous delivery group) from Day 1-22 of gestation. There was about 27% increase in bodyweight gains between Day 1 and 21 of lactation in the spontaneous delivery group.

*Food Consumption:* No toxicologically significant treatment-related effect.

*2. Litter Data on Fetuses in Cesarian Section Group:*

There was one resorption at LD. None of the following litter parameters showed toxicologically significant effect: corpora lutea, pre- and post-implantation loss, number of viable fetuses, % of male and female (pups), resorptions, % of early and later in, and malformations.

At LD, there were 3 malformed fetuses: cleft vertebra in fetus, anophthalmia plus hydrocephaly in fetus, and cleft vertebrae plus synostosis of ribs. At LD, there was 8% increase in the number of fetuses with short 13th rib. At MD, there was 28% increase in the number of fetuses with short 13th rib. At HD, there was about 7% decrease in birth weight of fetuses, 86% increase in the number of fetuses with short 13th rib, and one fetus had cleft vertebra. The drug was neither fetotoxic nor teratogenic.

*3. Litter Data on Pups in Spontaneous Delivery Group:*

There was no toxicologically significant treatment-related effect on mean gestation period of dams, mean body weight of pups at birth and at day 4 before and after litter reduction. Mean body weight of pups increased on Day 21 (LD 12.9%, MD 9.2%, HD 7.5%). There were two still-born pups in HD group.

During the 21-day rearing period, pup mortality was slightly higher in the treatment groups (Control: 3, LD and MD: 5 each, HD: 4) but the difference was not significant especially considering that cannibalism and unknown reasons were stated as the cause of deaths. No toxicologically significant treatment-related effect was found in the parameters of pup development during lactation

period and in the results from functional tests (righting reflex, pupillary reaction, hearing test) in the $F_1$ offsprings.

Autopsy of pups at Day 4 as well as of young at Day 28 (males) or Day 42 (females) showed no malformations or drug-related effect in gross pathological examination. The drug was not teratogenic in this study.

**Rat: Preliminary Segment-II Inhalation Toxicity Study**
**Boehringer Ingelheim Study U92-0684, September 28, 1992, Vol. 1.21, Page 002**
**Laboratory.** [redacted]

**Method:** Female time-mated rats [VAF/Plus Crl:CD (SD) BR strain; 10/group] were administered 0 (Control), 0.05, 0.5, and 5.0 mg/kg/day of Ba 679 BR via snout only inhalation (1 hour daily) from Day 6 to Day 17 post coitum. On Day 20, all females were sacrificed and subjected to post mortem examination. Litter parameters were studied and fetuses examined for gross pathological changes. Blood samples were taken from animals of the Satellite group on Days 6 and 17 post coitum for toxicokinetic evaluations.

**Result:** Treatment with Ba 679 BR resulted in pupil dilation (LD 71%, MD 87%, HD 100%), decreased bodyweight gains from Day 7 to Day 20 (16% at HD), and reduced food consumption on Days 6 to 7 (LD 22%, MD 37%, HD 48%) which was recovered thereafter. There was one mortality in the HD Satellite group on Day 15. Litter parameters such as numbers of corpora lutea and implantations, % post implantation loss, number of live young, and the weights of litter and foetus did not show toxicologically significant treatment-related effects. The sponsor selected 0, 0.01, 0.1, and 2.0 mg/kg/day for definitive Segment-II inhalation toxicity study in rats.

**Rat: Inhalation Effects on Pregnancy (Segment-II) Study**

**Boehringer Ingelheim Study, U92-0622, September 4, 1992, Vol. 1.20, Page 002**

*Study Dates:* 26 August, 1991 to 30 January, 1992
*Testing Lab:* [redacted]
*Test Article:* Ba 679 BR Batch II; The aqueous solution of test article was prepared in a vehicle that contained benzalkonium chloride (10 mg), disodium edetate (50 mg), citric acid monohydrate (8.4 mg), 0.1N NaOH (0.8 mL), 0.1N HCl (0.6 mL), and water for injection (100 mL). The pH was adjusted to 3.0. Three different strength solutions (0.0025%, 0.025%, 0.5%) were

prepared in order to achieve the expected doses. The pH of final dosing solutions was also adjusted to 3.0.

*GLP:* Signed GLP Statement was included.

## METHODS

*Species/Strain:* VAF/Plus rats (Crl: $CD^R$ (SD) BR strain.
*Animals:* 40 Females/group (15 for batch A and 25 for batch B).
*Route:* Snout-only inhalation via ——— nebulizer ( ———
*Dosage:* 0 (Control, Vehicle), 0.01 (LD; Concentration: 0.025 mg/mL), 0.1 (MD; 0.25 mg/mL), and 2.0 (HD; 5.0 mg/mL) mg/kg/day.
*Duration of Exposure:* One hour daily between Days 6 and 17 post coitum.
*Observations: Fo Generation:*

*1. Adult Females:*

*Clinical Signs and Mortality:* Daily.
*Body Weights:* On Days 1 (batch A only), 2, 3, 6, 7, 8, 10, 12, 14, 16, 18, and 20. Dams allowed to litter weighed on Days 0, 7, 14, and 21 post partum.
*Food Consumption:* On each weigh day between Day 3 and 20 post coitum.

*2. Litter data and fetal examinations for animals sacrificed on Day 20 of Pregnancy:*

On Day 20 post coitum, subgroup B animals from Control and all treatment groups were sacrificed and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine number of corporate lutea, number and distribution of live young, number and distribution of embryo fetal deaths, individual fetal weights, and fetal abnormalities.

*3. Litter data for animals rearing young:*

All litters were examined daily. Pups were weighed on Days 4, 8, 12, 16, and 21 post partum. One week after the birth of litters, non-pregnant females were sacrificed and their uteri examined for evidence of implantation. During the pre-weaning period, all offsprings in all litters were examined to determine the age at which the following developmental stages were attained:

a) Surface righting reflex: Day 1 post partum to 100% success.
b) Startle reflex: Day 11 post partum to 100% success.
c) Air righting reflex: Day 14 post partum to 100% success.
d) Pupil reflex: Day 20 post partum.

*Observations: $F_1$ Generation (post weaning):*

*1. Adult Animals:*

*General Aspects:* Animals were observed for abnormal behavior and obvious changes in temperament. Animals were weighed weekly.
*Sexual Maturation:* Onset of vaginal opening in females: 28 days post partum; Occurrence of cleavage of balano preputial skinfold: 35 days post partum.

*Developmental/behavioral examinations:*
*Accelerated rotarod test:* 4 weeks of age.
*Actimat test:* 5 weeks of age.
*One trial passive avoidance test:* 7-8 and 10-11 weeks.
*Tail withdrawal test:* 11 weeks of age.

*Assessment of Reproductive Capacity:* On 84th day of their birth, the offsprings were mated on a one male to one female basis for 20 days. All females were weighed daily until parturition. Body Weights were reported for Days 0, 3,7, 10, 14, 17, and 20 of pregnancy. Dams that littered were weighed on Days 0, 7, 14, and 21 post partum.

*2. Litter Data:* Clinical examination was performed daily. The pups were weighed on Days 4, 8, 12, 16, and 21 post partum. On Day 21 post partum, all $F_2$ pups and $F_1$ parents were sacrificed and examined for abnormalities. Uterus of each female which gave birth was inspected for number of implantation sites.

**RESULTS**

*Dosage Levels:* The achieved daily doses were 0.0106, 0.1, and 1.838 mg/kg/day.

*$F_0$ Generation:*

*1. Adult Females:*

*Clinical Signs:* Dilation of pupil (LD 95%, MD and HD 100%), red/brown peri-orbital crusting (MD 10%, HD 55%), and dry eyes (HD 18%).

*Mortalities:* One HD female was humanely sacrificed of Day 23 of pregnancy following signs of dystocia. Post mortem examination showed 14 full term but recently dead fetuses within the uterus. Main finding in the dam was severe serosanguineous fluid distension of the uterus, grey/blue content of G.I. tract and pale liver, kidneys, and spleen.

*Body Weights:* Between Day 2 to 20 of pregnancy, there was small but statistically significant decrease in bodyweight gains (MD 8%, HD 17%).

*Food Consumption:* From Day 6 to Day 17 post coitum, there was a dose dependent decrease in food consumption (LD 7%, MD 15%, HD 18%). Treatment with Ba 679 BR had no effect on food intake during lactation.

*2. Litter Data at Day 20 of Pregnancy:* There was no incidence of total litter loss (total resorption) at the Day 20 sacrifice.

*Litter Values:* HD group showed statistically significant reduction in litter weight (13%).

*Fetal Examination:* Incidence of total number of malformed fetuses was as follows: Control: 1 (umbilical hernia), LD: 1 (diaphragmatic hernia), MD: 2 (one fetus with hydrocephaly, palatine irregularity, interventricular septal defect, malrotated heart, displaced umblical vein; one fetus with double/incomplete aortic arch), HD: 3 (one fetus with microphthalmia/anophthalmia and two fetuses with flattened/reduced interparietal cranium). No toxicologically significant treatment-related other skeletal or visceral abnormalities. The drug was neither fetotoxic nor teratogenic.

Duration of Pregnancy: HD rats showed tendency of reduction in gestation period from 22 to 21 days resulting in 33% more animals with 21 days of gestation (than Controls).

*3. Litter Data to Weaning at Day 21 Post Partum:*

*Litter Values:* At HD, there was one incidence of total resorption. The sponsor attributed it to the occurrence of only a single implantation (implantation occurs prior to treatment). Also at HD, there was a statistically significant decrease in mean pup weights (Day 4: 13%; Day 8: 11%; Day 12: 9%).

*Pre-weaning development:* No toxicologically significant treatment-related effect.

*4. Terminal Autopsy:* No toxicologically significant treatment-related effects were observed in $F_0$ adults and their offsprings.

*$F_1$ Generation:*

*1. Adult Animals:*

*Clinical Signs:* There were no mortalities and no other toxicologically significant treatment ($F_0$)-related effect.

*Body Weights:* During Weeks 4-10, there was a slight but statistically significant decrease (10%) in bodyweight gains. From Day 0 of pregnancy to Day 21 post partum, there was a decrease in bodyweight gains in all the treatment groups (LD 14%, MD 13%, HD 35%).

*Sexual Maturation:* There was a 2 days delay in the balano preputial cleavage (LD, MD, HD) and 1 to 3 days delay in vaginal opening (LD=1 day; MD=3 days; HD=2 days).

*Post Weaning Behavioral Tests:*

*Accelerating Rotarod:* No toxicologically significant treatment ($F_0$)-related effect.

*Actimat:* No toxicologically significant treatment ($F_0$)-related effect.

*Passive Avoidance:* No toxicologically significant treatment ($F_0$)-related effect in the median pre-shock entry times (Day 1). However, median post shock performance (Day 2) time in HD group was 74% faster (in seconds) than that in Control group. Also at HD, median total time spent in safe chamber was slightly but statistically significantly reduced (9%). On Day 22, the values of median total time to first entry and median total time in safe chamber (both in the HD group) were 45% and 21%, respectively, to those for Controls.

*Tail Withdrawal:* No toxicologically significant treatment ($F_0$)-related effect.

*Mating Performance and Pregnancy Rate:* No toxicologically significant treatment ($F_0$)-related effect.

*2. Litter Data:*

*Duration of Pregnancy:* No toxicologically significant treatment ($F_0$)-related effect.

*Litter Values:* No toxicologically significant treatment ($F_0$)-related effect.

*3. Terminal Autopsy:* No toxicologically significant treatment ($F_0$)-related effect.

**Rabbit: Preliminary Segment-II Oral Toxicity Study**
**Boehringer Ingelheim Study 90-0301, May 04, 1990, Vol. 1.9, Page 061**

**Method:** Nulliparous Himalayan (strain Chbb:HM) rabbits (4/group) were administered 0 (Control, 0.5% aqueous solution of tylose), 100 (LD), 250 (MD), and 500 (HD) mg/kg/day (all dosing solutions prepared in 0.5% aqueous solution of tylose) of Ba 679 BR via oral (gavage) route from Day 6 to Day 18 of gestation. On Day 29 of gestation, all animals were sacrificed and subjected to post-mortem examination.

**Result:** All treatment groups showed signs of maternal effects, e.g., reduced bodyweight gains (LD 4%, MD 6%, HD 25%) and reduced food consumption (LD 5%, MD 10%, HD 66%). The higher doses (MD 3/4, HD 3/3) caused abortion in most of the dams while there were no macroscopic abnormalities present in fetuses. Incidences of resportion were: Control 2/20, LD 5/20, and MD 1/10. The sponsor selected 100 mg/kg/day as the top dose and 1.0 and 10.0 mg/kg/day as lower doses for a definitive Segment-II oral toxicity study in rabbits.

**Rabbit: Segment-II Oral Toxicity Study**
**Boehringer Ingelheim Study, U91-0340, January 18, 1991, Vol. 1.10, Page 339**

*Study Dates:* 21 May, 1990 to 5 July, 1990.
*Testing Lab:* Boehringer Ingelheim Dep. of Experimental Pathology and Toxicology.
*Test Article:* Ba 679 BR (Batch I) was prepared in 0.5% aqueous solution of tylose.
*GLP:* Signed GLP Statement was included.

## METHODS

*Species/Strain:* Nulliparous rabbits, Chbb:HM strain.
*Animals:* 18 inseminated (female) rabbits/group.
*Route:* Oral by gavage.
*Dosage:* 0 (Control, 0.5% tylose), 1.0 (LD), 10.0 (MD), and 100 (HD) mg/kg/day. The concentration of Ba 679 BR in these dosing solutions was 0, 1.0, 10.0, and 100 mg/mL, respectively.
*Duration of Exposure:* Daily from Day 6 through Day 18 of gestation.
*Body Weight:* Weekly.
*Food Consumption:* Weekly.

## RESULTS

*Dosage Levels:* Achieved dose levels were 0.975, 9.284, and 95.507 mg/kg/day.

*1. Effect on Dams:*

*Clinical Signs:* Incidences of dilation of pupil (HD 18/18), coprostasis (MD 1/18, HD 15/18), and abortion (Control 1/18, LD 1/18, MD 1/18, HD 3/18) were drug related. Six of the 72 mated females (Control, LD, MD: 1 each; HD:3) did not become pregnant.

*Mortalities:* Death of one LD female was drug related. The sponsor attributed death of two females of the Control group to defense movements in the restraining cage.

*Body Weight:* There was bodyweight loss in the MD dams between Days 8 and 12 (about 1%) of gestation and in the HD dams between Days 7 and 18 of gestation (about 3%). At the end of NO TREATMENT (recovery) period (Day 29), mean body weight of HD females was 5% less than that of control group.

*Food Consumption:* Reduced food consumption was reported in the MD (47% between Days 7 and 12 of gestation) and the HD dams (80% between Days 7-12 and 46% between Days 13 to 18 of gestation).

*Autopsy Results:* No toxicologically significant treatment-related effect.

2. *Effect on Fetuses:* No toxicologically significant treatment-related effect on fetal mortality and mean numbers of corpora lutea, implantations/dam, viable fetuses, post-implantation loss, fetal body weights, placental weights. There were 5 low birth weight fetuses (Control 1, MD 2, HD 2) whose body weights were below 65% of mean fetal weight of the Controls group and, therefore, qualified to be "runts." Developmental toxicity consisted of 1 HD dam with total resorption. Five fetuses had malformations: 1 fetus with finger malformation such as lack and reduction of the distal and middle phalanges 2-4 on the forepaws (LD), 3 fetuses with synostosis of sternebrae (MD), and one fetus with no gall bladder (HD). Minor variations were seen in 11 fetuses and included flexures of fore- or hind paws, additional 13th or missing 12th rib, asymmetric sternebrae, and absence of the accessory lobe of the lung but these were not significant. The percentage of unossified and poorly ossified sternebra V in the fetuses from treatment groups were not significantly different from those in controls. The drug was neither fetotoxic nor teratogenic in this study.

**Rabbit: Preliminary Segment-II Inhalation Toxicity Study**
**Boehringer Ingelheim Study 92-0484, July 16, 1992, Vol. 1.19, Page 342**
**Laboratory:** [redacted]

**Method:** Female time-mated New Zealand White rabbits (6/group) were treated with 0 (Control), 0.1, 0.3, and 1.0 mg/kg/day of Ba 679 BR via snout-only inhalation exposure for 15 minutes from Day 6 to Day 18 post coitum inclusive. On Day 29, all females were sacrificed and subjected to post mortem examination. Blood samples were taken from 3 animals/group for toxicokinetic evaluations.

**Result:** Treatment with Ba 679 BR resulted in 1 mortality at HD (Day 28), 1 non-pregnant MD female, absent pupil reflex (LD 6%, MD 26%, HD 55%), reduced bodyweight gains (HD 48%, Day 0 to 29), and decreased food consumption over a period of Day 6 to Day 18 (LD 35%, MD 32%, HD 68%). Significant changes in litter parameters consisted of 2-fold increase in post implantation loss (MD, HD), a 10% decrease in the number of live young (HD), a 20% decrease in litter weight (HD), and a 13% decrease in fetal weights (HD). There were no significant changes in the number of corpora lutea and number of implantations. Based upon toxicities associated with HD, the sponsor chose 0.5 mg/kg/day as the top dose and 0.01 and 0.1 as lower doses for a definitive Segment-II inhalation toxicity study in rabbits.

Rabbit: Inhalation Effects on Pregnancy (Segment-II) Study
Boehringer Ingelheim Study, U92-0623, August 18, 1992, Vol. 1.20, Page 194

*Study Dates:* 9 September, 1991 to 9 October, 1991
*Testing Lab:* [redacted]
*Test Article:* Ba 679 BR Batch II; The aqueous solution of test article was prepared in a vehicle that contained benzalkonium chloride (10 mg), disodium edetate (50 mg), citric acid monohydrate (8.4 mg), 0.1N NaOH (0.8 mL), 0.1N HCl (0.6 mL), and water for injection (100 mL). The pH was adjusted to 3.0. Three different strength solutions (0.00375%, 0.0375%, 0.1875%) were prepared in order to achieve the expected doses. The pH of final dosing solutions was also adjusted to 3.0.
*GLP:* Signed GLP Statement was included.

## METHODS

*Species/Strain:* New Zealand White rabbits (time-mated).
*Animals:* Control: 17 females; Treatments: 16 females/group.
*Route:* Inhalation via [redacted] ultrasonic nebulizer [redacted]
*Dosage:* 0 (Control, Vehicle), 0.01, 0.1, and 0.5 mg/kg/day.
*Duration of Exposure:* Day 6 to Day 18 post coitum (13 days); 15 min./day.

*Observations:*

*1. Adult Females:*

*Clinical Signs and Mortality:* Daily.
*Body Weights:* Days 0, 2, 6, 8, 10, 14, 19, 23, and 29 of pregnancy.
*Food Consumption:* Days 0, 2, 6, 8, 10, 14, 19, 23, and 29 of pregnancy.
*Terminal Autopsy:* Day 29.

*2. Litter Parameters:* Day 29 post coitum. The ovaries and uteri were examined to determine: number of corpora lutea, number and distribution of live young, number and distribution of embryonic/fetal deaths, individual fetal weight, and fetal abnormalities (skeletal and visceral).

## RESULTS

*Dosage Levels:* The achieved dose levels were 0.009, 0.11, and 0.5 mg/kg/day.

*Adult Females:*

*Clinical Signs:* absence of pupil reflex post-dose (Control 2/17, LD 6/16, MD 12/16, HD 16/16), reduced fecal output (coprostasis): Days 7-19 (Control 4/17, LD 9/16, MD 12/16, HD 16/16), and soiled anogenital region: Days 7-9 (Control 1/17, LD 7/16, MD 7/16, HD 10/16).

*Mortalities:* None (during or after treatment).

*Body Weights:* There was slight bodyweight loss during Day 6 to Day 8 (LD 0.4%%, MD 2.4%, HD 3.2%) and slight bodyweight gain during Day 6 to 19 (Control 7.6%, LD 5%, MD 2%, HD: no effect). Bodyweight loss was regained during recovery period (Day 19-29).

*Food Consumption:* There was statistically significant decrease in total food consumption from Days 6 to 18 (MD 36%, HD 53%)

*Terminal Autopsy:* No toxicologically significant treatment-related effect.

*Litter Data:*

*Litter Values:* There was an increase in mean post implantation loss at HD due primarily to 3 litters with losses of 50 to 70%. This incidence is drug-related. According to the sponsor, an increase in post implantation losses at LD and MD was within historical control range. Mean fetal weight was significantly lower (by 10%) in HD group. There was slight decrease in the % of males at MD (9.5%) and HD (7.2%) which was statistically not significant. No significant treatment related effects on the numbers of corpora lutea, implantations, live youngs, and litter weights.

*Fetal Examination:* The incidence of malformed fetuses were as follows: Control: 3/125 (one with cranial and thoracic anomalies such as misshapen cranium, retinal dysplasia, misaligned cranial suture, and malformed cervico-thoracic arteries; one fetus with cranial anomaly microphthalmia; one fetus with appendicular anomaly, e.g., forelimb flexures/malrotated hind limbs), LD: 6/136 (one fetus with lenticular fiber degeneration, microphathalmia, and retinal dysplasia: all in cranium; one fetus with malforned systemic/pulmonary arteries and interventricular septal defect: both thoracic anomalies; four fetuses with malformed cervico-thoracic arteries), MD: 0/118, HD: 7/118 (one fetus with retinal dysplasia, two fetuses with malformed cervico-thoracic arteries, one fetus with scoliosis, one fetus without kidney, two fetuses with malformed systemic/pulmonary arteries: one of these also had malrotated heart, kyphosis,

distorted rib cage, and thoraco-gastrostachisis, and otocephaly, whle the other had interventricular septal defect. The number of litters affected was 2 (control), 6 (LD), 0 (MD), 7 (HD). An increase in the mean % of fetuses with extra (13) ribs was drug-related (LD 5.2%, MD 29.8%, HD 23.2%; MD and HD values were statistically significantly larger than Controls). The incidence of visceral anomalies was as follows: corneal/lenticular haze/opacity (1 HD), atelactic lungs (3 MD, 1 HD), abnormal lobation lungs (1 each for LD and MD, 2 HD), abnormal lobation of liver (1 MD, 4 HD), subcapsular cysts in liver (1 HD), subcapsular area in liver (1 HD), cystic ovaries (2 HD), and bilobed/bifurcated gall bladder (1 Control, 1 MD, 4 HD). The drug was not teratogenic.

## SUMMARY OF REPRODUCTIVE TOXICITY

Administration of Ba 679 BR to rats and rabbits orally or via inhalation route resulted in typical systemic anticholinergic toxic effects such as dry mouth and dilation of pupil. In addition, there were toxicologically significant drug-related decrease in bodyweight gains accompanied by reduced food consumption, and incidences of coprostasis in the $F_0$ parents. Drug-related findings on reproductive and developmental parameters are as follows:

In a Segment-I inhalation study in rat, treatment with drug (0.01, 0.1, 2.0 mg/kg/day) resulted in: $F_0$: statistically significant decrease in bodyweight gains in both parents, reduced mean litter weights, lowered numbers of corpora lutea and implants and higher post implantation loss leading to fewer live young, and total resorption (MD 1, HD 2); $F_1$: one total litter loss of 18 pups by day 14 post partum and decreased litter size and weight at birth. There was no toxicologically significant treatment-related effect on fertility and reproductive performance but the drug was embryo/fetotoxic at 2.0 mg/kg/day.

In the Segment-II oral study in rats, treatment with drug (1, 25, 500 mg/kg/day; Day 7-Day 16 of gestation) resulted in: $F_0$: There was decrease in bodyweight gain at HD (10-11%); $F_1$: There were 3 malformed (cleft vertebrae, fused ribs, and anophthalmia with hydrocephaly) fetuses in LD group and dose dependent increase in the number of fetuses with short 13th rib. There was one single incidence of resorption (LD) in the cesarian group. No maternotoxic or significant embryo/fetotoxic effects were seen at any dose. The drug was not teratogenic.

In a Segment-II inhalation study in rats, drug-induced (0.01, 0.1, 2.0 mg/kg/day) changes were: $F_0$: On Day 23 of pregnancy, 1 HD female showed signs of dystocia and was sacrificed; all 14 full-term fetuses were found dead in the uterus of one $F_0$ female; main effect on dams were severe serosanguineous fluid distension of the uterus, grey/blue content of G.I. tract and pale liver, kidneys, and spleen. At Day 20 of pregnancy, there was a decrease in litter weights (HD) and incidence of malformed fetuses (Control 1, LD 1, MD 2, HD 3). In one-third animals, gestation period decreased from 22 to 21 days. There was one incidence of resorption and decrease in mean pup weights. There was decrease in bodyweight gains at HD (17%) and decrease in food consumption at MD (15%) and HD (18%). $F_1$: Treatment resulted in 2 day delay in balano preputial cleavage and 1-3 day delay in vaginal opening. Toxicologically significant drug-related (HD) effects in a post weaning behavioral (passive avoidance) test were as follows: 74% faster median post shock performance time (Day 2), 55% and 79% reduced mean total time to first entry and median total time in safe chamber. The drug was neither fetotoxic nor teratogenic.

In a Segment-II oral study in rabbits, administration of Ba 679 BR (1.0, 10.0, 100 mg/kg/day; Day 6-18 of gestation) resulted in drug-related increase in the incidences of abortion at HD (HD: 3/18; Control, LD, MD: 1/18 each). The drug was abortifacient at 100 mg/kg/day. Treatment resulted in developmental toxicities: 1 dam (HD) with total resorption and 5 fetuses with malformations such as finger malformation (1 LD), complete absence of gall bladder (1 HD) and incidence of synostosis of sternebrae (3 MD). The drug was neither fetotoxic nor teratogenic.

In a Segment-II inhalation study in rabbits, drug (0.01, 0.1, 0.5 mg/kg/day)-induced changes in the litters were: reduced (10%) mean fetal weights (HD) and decrease in the number of males (MD 9.5%, HD 7.2%). The drug was embryocidal and fetotoxic at 0.5 mg/kg/day but not teratogenic.

# OVERALL SUMMARY AND EVALUATION

General Pharmacology, Pharmacodynamics, and Safety Pharmacology: Ba 679 BR is an *in vitro* and *in vivo* muscarinic receptor antagonist (MRA). It shows pharmacodynamic properties at low doses ($ED_{50}$ ranges for inhalation and i.v.: 0.00003-0.00664 and 0.0003-0.003 mg/kg). Its anticholinergic characteristics suggest role in chronic obstructive pulmonary disease (COPD). General pharmacodynamic studies showed its activity as an MRA to be selective. In safety pharmacology experiments, treatment with Ba 679 BR resulted in delay in intestinal passage (mice); dilation of pupil (topical-dog, i.v.-rat, s.c.-mice); inhibition of pilocarpine-induced salivation (rat, guinea pig), meal-induced salivation (rabbit, dog), gastric juice secretion (rat), and miotic activity (rat, rabbit). These are tpical cholinergic effect. In addition, combination administration of specified doses of Ba 679 BR and albuterol resulted in additive protection against acetylcholine-induced bronchospastic collapse. A prolonged duration of action of Ba 679 BR (>24 hours) than that of Ipratropium bromide (6 hours) is likely to stabilize the lung function and the well being of patients with COPD and asthma.

Absorption, Distribution, Metabolism, and Excretion: The oral bioavailability of Ba 679 BR is low (dog 4%; mouse and rat 1%). The disappearance of the drug from plasma was biphasic after administration via i.v. or inhalation. In mice (i.v., inhalation) and rats (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. Relatively very little (if any) accumulation of the drug was observed by inhalation, i.v. or p.o. The plasma protein binding of the drug is higher in human (65.3%) than in animal species (mouse 21.7%, rat 19.7%, dog 21%, rabbit 15.5%). 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).

The distribution of the drug in mice (i.v.) was as follows: kidney > liver > plasma > lung > blood > heart. At 8 hr., the drug level in the liver was the highest (5.1 μg/g organ weight). By the p.o. route, the drug level was highest in the liver. In rats (i.v.), most of the drug was distributed in liver, kidneys, stomach, and intestine. When the drug was given p.o., it was detected in the G.I. tract only. Following intratracheal administration, the drug was distributed to lungs > kidney > liver > plasma > heart.

Degree of metabolism is not known. In mice, N-methylscopine was major metabolite in urine. Glutathione conjugate of Ba 679 BR was a minor metabolite in the urine and major metabolite in plasma, bile, liver, lung, kidney, and heart. There was 1 unidentified metabolite in urine and bile and 2 unidentified metabolites in plasma. In

rat, there were 1 major (N-methylscopine) and 4 minor (unidentified) metabolites in urine; these minor metabolites were major metabolites in bile, liver, kidney, and heart; In plasma, N-methylscopine was the major metabolite while minor metabolites were in too small quantities to be quantified. In dog, major metabolite in urine was N-methylscopine and minor metabolite glutathione conjugate; there were no metabolites in plasma; in bile, N-methylscopine was detected.

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.

**Toxicology:** The most common effects of Ba 679 BR were decreased bodyweight gains and food consumption, and incidences of coprostasis (except that there was only 1/192 incidence in 52-week inhalation study in dog). Anticholinergic effects in mice, rat and dog consisted of mydriasis (excluding F-344 rats) and tachycardia; effects such as keratoconjunctivitis sicca, dry mucosa in mouth and nose were seen only in dogs. A comprehensive account of the toxicology profile of the drug is given in the section "Summary of Toxicology."

**a) Single Dose:** In single dose lethality studies, the $LD_{50}$ values of Ba 679 BR in rodents were as follows: Mice: 1336.4 mg/kg (p.o.) and 18.1 mg/kg (i.v.); Rats: 20.5 mg/kg (i.v.). Mice $LD_{50}$ values for degradation products were: [redacted] 148 mg/kg (i.v.); [redacted] 156 mg/kg (i.v.).

**b) Multiple Dose:** In mice, two 13-week inhalation studies resulted in a NOAEL of 0.08 mg/kg/day. The MTD was 0.04 mg/kg/day and the target organs of toxicity were G.I. tract, kidney, liver, thymus, and lymph nodes.

In rats, oral administration of the drug for 4 weeks resulted in a NOAEL of 0.1 mg/kg/day. When the drug was given for 13 weeks, the NOEL was 0.1 mg/kg/day and the MTD was <5.0 mg/kg/day. In both studies, G.I. tract was the target organ of toxicity. These studies did not show any duration dependent toxicity. When the drug was given intravenously for 3 to 4 weeks, the NOAEL was 0.01 mg/kg/day and the target organs of toxicity were G.I. tract, harderian gland, urinary bladder, and lung.

In rats, administration of the dry powder formulation of the drug via inhalation for 2 and 13 weeks resulted in NOAEL values of 0.1 and <0.09 mg/kg/day, respectively. The target organs of toxicity were G.I. tract (13 week only), eye, and harderian gland. In the

inhalation studies with Wistar rats (4 , 13, 52 weeks) using aqueous formulation of the drug, the NOAEL values were 2.3 mg/kg/day (4 weeks), 0.07 mg/kg/day (13 weeks), and 0.01 mg/kg/day (52 weeks) implying the occurrence of duration dependent toxicity. The MTD in the 13 week study (aqueous formulation) was 0.6 mg/kg/day. The target organ of toxicity was eye in the 13 weeks study. Target organs of toxicity in the 52 weeks study were eye, nose, pancreas, and harderian gland. Data from two 13 weeks studies (aqueous formulation) in F-344 rats could not establish NOAEL but the MTD was 0.04 mg/kg/day; the target organs of toxicity were G.I. tract, nose, harderian gland, and salivary gland. The sponsor used these studies for selecting doses (0.012, 0.030, and 0.075 mg/kg/day) for carcinogenicity study in F-344 rats.

In dogs, a 13 week (p.o.) study resulted in a NOAEL of 0.005 mg/kg/day and heart, eye, salivary gland, and G.I. tract were the target organs of toxicity. When the drug was given for 4 weeks via i.v., the NOAEL was <0.004 mg/kg/day and the target organs of toxicity were heart and eye. When the dry powder formulation of the drug was administered via inhalation (2, 4, and 13 week studies), the animals were able to tolerate 0.04 mg/kg/day (NOAEL) for 4 weeks without any significant toxicity while in the 13 week study, the animals could tolerate only 0.01 mg/kg/day (NOAEL) thus, implying duration dependent toxicity. In the 2 week study, the animals could tolerate only 0.01 mg/kg/day because the experimental design had 0.1 mg/kg/day as the next dose group and no dose between these two doses. The target organs of toxicity in the 2 week study were heart and eye. The target organs of toxicity in 4 and 13 week studies were heart, eye, larynx, trachea, lung, thymus, and adrenals. When the dogs were administered aqueous formulation of the drug via inhalation over 4, 13, and 52 weeks, NOAEL varied considerably (4 wk: 0.2 mg/kg/day; 13 wk: 0.01 mg/kg/day; 52 wk: 0.004 mg/kg/day) thus, implying duration dependent toxicity. The target organs of toxicity in the 4 week study were heart, eye, salivary gland, nose, lung, trachea, and carina. The target organs of toxicity in the 13 and 52 weeks study were heart, eye, and salivary gland.

A 104-wk carcinogenicity study in F-344 rats was terminated due to excess mortality. The sponsor argued that this species of rat is susceptible to the formulation of the drug used in the study. This study has been repeated in another species of rat and mice but the study reports have not been submitted yet (See attached telephone conversation with the sponsor).

**Special Toxicity:** In a guinea pig skin sensitization study, treatment with Ba 679 BR did not reveal sensitization potential under the stated experimental conditions.

**Genotoxicity:** Ba 679 BR was neither genotoxic nor clastogenic in a battery of tests. Also, the four degradation products of the drug were nongenotoxic in Ames test.

**Reproductive Toxicity:** Treatment with Ba 679 BR resulted in neither impairment of fertility in rats, nor it was teratogenic in rats and rabbits. However, the drug was

embryo- and feto-toxic in rats at an inhalation dose of 2.0 mg/kg/day (Segment I). In rabbits, the drug was abortifacient at an oral dose of 100 mg/kg/day and embryocidal and fetotoxic at an inhalation dose of 0.5 mg/kg/day.

**Dose Levels:** Doses used in all the inhalation studies are based upon the doses that were delivered. Deposition factor was not taken into account while determining doses. Therefore, actual drug intake in studies via inhalation would be less than the delivered dose.

**Clinical Dose:** The proposed maximum dose for the clinical study is 44 μg. Based on the NOAEL in rat (0.01 mg/kg/day) and dog (0.004 mg/kg/day) in the 52 weeks inhalation toxicology studies and with safety factors of 10 and 6 for rats and dogs, respectively, being considered, an estimated safe clinical dose would be 33.5-50 μg (on a 50 kg body weight basis). However, deposition factor was not considered. In previous clinical experience, 40 μg has been given for 2 weeks in healthy volunteers. Therefore, the proposed maximum daily dose of 44 μg in the clinical study is supported by preclinical data.

## RECOMMENDATION

The proposed clinical study is safe from a preclinical standpoint.

**a) Comments for further studies:** The sponsor should provide information on deposition factor for all pre-clinical inhalation studies. Such information will help in estimating actual doses that reached into the animals in these studies. Per information contained in the annual report of this IND (04/25/96, page 21), a Segment III study in rat is underway.

**b) Points discussed with Medical Officer:** Based upon preclinical data, inhalable Ba 679 BR, through an inadvertent contact, has potential of a risk to the eyes of Subjects enrolled in the clinical study.

Draft Letter to the Sponsor:

You should provide information on deposition factor for all pre-clinical inhalation studies. Such information will help in estimating actual doses that reached into the animals in these studies.

File Name: N:\IND\46687\PHARM\94-11-30.REV

/S/

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