9. Histopathology: The stomach and liver were the target organs of toxicity. Inflammatory cell infiltrate in the cardiac and fundic region occurred at doses ≥15 mg/kg/day. Dilated crypts were noted in the stomach, duodenum, cecum, colon and rectum at doses ≥15 mg/kg/day. Brown pigment accumulation was found in the liver of the 90/60 mg/kg/day group and was still present at end of recovery period. Inspissated bile was observed in all drug treated groups.

10. Serum Drug Levels: At weeks 14 and 26, AUC values for pantoprazole and the sulfone metabolite increased in a manner generally proportional to increasing dose. AUC values for pantoprazole and the sulfone metabolite on day 1 increased with ascending dose; although increases were greater than proportional to ascending dose. Non-linearity on day 1 may have been due to a lack of steady state levels. The AUC value for 5 mg/kg/day on day 1 was significantly lower than that observed at weeks 14 and 26. The pantoprazole half-life on day 1 for the dose of 90 mg/kg/day was greater than that observed at lower doses. Slower drug absorption may have resulted in a longer rate of elimination or liver enzymes may have been saturated.

Median (min/max) pharmacokinetic characteristics of pantoprazole-Na in the dog following an oral dose on Day 1 of the 6-month-toxicity test

target dose (mg/kg)	actual	AUC(0-Inf.) (µgxh/mL)	Cmax (µg/mL)	tmax (h)	t½ (h)
90	90	305.7	78.30	1.5	2.3
45	45	86.59	46.85	1.25	0.89
15	15	21.59	15.0	0.5	0.69
5	5	1.73	1.01	1.0	0.64

Median (min/max) pharmacokinetic characteristics of pantoprazole-Na in the dog following an oral dose in week 14 of the 6-month-toxicity test

target dose (mg/kg)	actual	AUC(0-Inf.) (µgxh/mL)	Cmax (µg/mL)	tmax (h)	t½ (h)
90	--	-----	-----	----	----
45	30	51.58	38.60	0.75	0.63
15	15	32.74	16.50	1.25	0.59
5	5	7.66	5.93	0.50	0.47

Median (min/max) pharmacokinetic characteristics of pantoprazole-Na in the dog following an oral dose in week 26 of the 6-month-toxicity test

target dose (mg/kg)	actual	AUC(0-Inf.) (µgxh/mL)	Cmax (µg/mL)	tmax (h)	$t_{\frac{1}{2}}$ (h)
90	60	143.4	65.45	1.5	0.77
45	30	86.18	28.00	0.75	0.72
15	15	32.69	22.95	1.0	0.63
5	5	8.87	4.11	1.25	0.45

Median (min/max) pharmacokinetic characteristics of the sulphone metabolite in the dog following an oral dose on day 1 of the 6-month-toxicity test

target dose (mg/kg)	actual	AUC(0-8 h) (µgxh/mL)	Cmax (µg/mL)	tmax (h)	$t_{\frac{1}{2}}$ (h)
90	90	224.8	41.35	5.00	4.50
45	45	82.44	14.80	3.00	5.40
15	15	20.23	4.25	1.75	3.10
5	5	2.47	0.53	1.50	2.90

Median (min/max) pharmacokinetic characteristics of the sulphone metabolite in the dog following an oral dose in week 14 of the 6-month-toxicity test

target dose (mg/kg)	actual	AUC(0-8h) (µgxh/mL)	Cmax (µg/mL)	tmax (h)	$t_{\frac{1}{2}}$ (h)
90	--	-----	-----	----	----
45	30	47.17	10.20	2.00	2.60
15	15	21.74	4.37	3.00	2.80
5	5	7.22	1.82	1.75	2.10

Median (min/max) pharmacokinetic characteristics of the sulphone metabolite in the dog following an oral dose in week 26 of the 6-month-toxicity test

target dose (mg/kg)	actual	AUC(0-8h) (µgxh/mL)	Cmax (µg/mL)	tmax (h)	$t_{\frac{1}{2}}$ (h)
90	60	116.4	21.65	3.0	3.2
45	30	52.32	10.79	2.5	2.4
15	15	21.61	5.08	2.5	2.4
5	5	5.32	1.17	1.5	2.1

In a 6-month oral toxicity study, beagle dogs received pantoprazole at doses of 0, 5, 15, 45, and 90 mg/kg/day. Additional dogs were included in the high dose groups for a 4-week recovery period following treatment. The recovery portion of the study was flawed due to a lack of a corresponding control group. In order to minimize lung toxicity at doses of 45 and 90 mg/kg/day that had been observed in earlier studies, doses were elevated in an incremental fashion. When the final dose was reached, the 6-month treatment period was started. Due to clinical signs of toxicity observed at doses of 45 and 90 mg/kg/day, dosages were reduced to 30 and 60 mg/kg/day, respectively. Ataxia, vomiting, tremor and loss of appetite were observed in the 90/60 mg/kg/day group and loss of appetite and tremor were observed in the 45/30 mg/kg/day group before the dosing was reduced. Increased serum gastrin levels were found in all treated male and female dogs; however, due to the large standard deviations and small number of animals used, significant increases ($p \leq 0.05$) were only observed for the female 5, 45/30, and 90/60 mg/kg/day groups. Increased cholesterol and triglyceride levels were observed in the 45/30 and 90/60 mg/kg/day groups. The stomach and liver were the target organs of toxicity. Inflammatory cell infiltrate in the cardiac and fundic region occurred at doses ≥15 mg/kg/day. Dilated crypts were noted in the stomach, duodenum, cecum, colon and rectum at doses ≥15 mg/kg/day. Brown pigment accumulation was found in the liver of the 90/60 mg/kg/day group and was still present at end of recovery period. Inspissated bile was observed in all drug treated groups.

1-Year Oral Toxicity Study in Dogs (GTR-32000).

Testing Laboratories: [redacted]

Study Started: November 20, 1989

Study Completed: August 25, 1992

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: 12-14 months old beagle dogs (7-15 kg). Beagle dogs were obtained from

Drug Batch:	2 mg tablets	Batch Dre1604
	20 mg tablets	Batch Dre1605
	Placebo	Batch Dre1464A

Methods: Groups of 5 male and 5 female beagle dogs were given orally (non-enteric coated tablets in capsules) —— 96022-Z at daily doses of 2.5, 15.0 and 60 mg/kg/day (expressed as free acid) for 1-year. The highest tested dose was achieved by dose escalation [i.e., dogs were given 15 mg/kg/day for 2 days, 30 mg/kg/day for the next 2 days, and 60 mg/kg/day thereafter (365 days)]. The control group dogs received capsules containing placebo tablets. The dose selection was based on 6-month oral toxicity study.

All dogs were observed daily for clinical signs and mortality. Body weights and food consumption were recorded weekly. Ophthalmoscopic examinations and ECG tracing were performed during weeks 26 and 52 of the study. Blood samples were collected from jugular vein of all dogs once pretest and during weeks 13, 26, 39 and 52 of the study just prior to drug administration for hematology and serum chemistry tests. Overnight urine samples were also collected at the above mentioned time period for urinalysis. At the end of study period, all surviving dogs were sacrificed and subjected to complete necropsy and histopathological examinations. Additionally, stomach slides were stained for mast cells (toluidine blue stain), neuroendocrine cells (immunocytochemical method for chromogranin — stain) and enteroglucagon cells (immunocytochemical method for determining glucagon-like immunoreactivity [GLI] in a subset of neuroendocrine cells).

Results:

1. Observed Effects: On dog (D89M-5911) in 60 mg/kg/day group had pulmonary rales on day 4, while still on the ascending dosage regimen. Increased incidence of loose feces was also seen in high dose treated dogs of both sexes.

2. Mortality: One dog (D89M-5901) died after receiving 3 daily doses of 15 mg/kg/day. Gross pathological findings included red stained fluid in the thoracic cavity, white froth in the trachea, and red discoloration of the lung, which were consistent with pulmonary edema induced by pantoprazole treatment. Histopathological findings included peribronchial and periarteriolar edema, dilation of lymphatics, eosinophilic material in the alveoii, foamy alveolar macrophages, alveolar hemorrhage, and alveolar neutrophil infiltrates. A thiourea-like metabolite of pantoprazole (— 97165) is thought to be responsible for induction of pulmonary edema following administration of pantoprazole. A pantoprazole dose as low as 15 mg/kg/day had the capacity to induce pulmonary edema. Pulmonary edema appears to be transient and tolerance develops with multiple dosing and has no relation to the animal supplier.

3. Body Weight/Food Consumption/Water Consumption: Final body weights on day 364 were suppressed by >10% for male and female treatment groups at doses ≥15 mg/kg/day. Final body weights for male dogs that received pantoprazole at doses of 2.5, 15, and 60 mg/kg/day were 93.85, 87.7, and 76.9% of the control (13 kg), respectively. Final body weights for female dogs that received pantoprazole at doses of 2.5, 15, and 60 mg/kg/day were 96.5, 87, and 84% of the control (11.5 kg), respectively.

4. Hematology/Coagulation/Bone Marrow: No biologically significant effects were seen except increased number of echinocytes (abnormal shaped erythrocytes) were observed in 2/5 males and 3/5 females of high dose group.

5. Blood Chemistry/Urinalysis: Cholesterol levels on days 92, 183, 274, and 365 for male dogs that received doses of 15 and 60 mg/kg/day were elevated from 161.2 to 187.3% of control values (154.4-181.8 mg/dL), respectively; although, dose response relationships were not evident. Cholesterol levels on days 92, 183, 274, and 365 for female dogs that received doses of 15 and 60 mg/kg/day were elevated from 144.4 to 196% of the control (188.8-206.2 mg/dL), respectively; although, dose response relationships were not evident.

This increase in cholesterol was associated with an increase in high density lipoprotein (HDL: shown by electrophoresis of serum fraction from high dose group). Serum triglyceride levels in male treatment group were elevated on day 365. Total protein levels on days 92, 183, 274, and 365 for male and female dogs that received 60 mg/kg/day were decreased from 88.9 to 95.8% of control values (5.68-6.14 g/dL), respectively. Albumin levels on days 183, 274, and 365 for male and female dogs that received 60 mg/kg/day were decreased from 78.7 to 92.4% of control values (2.86-3.10 g/ dL), respectively. Alkaline phosphatase activity on day 365 for male rats that received 60 mg/kg/day was elevated to 142% of the control (174.2 U/L). Alkaline phosphatase activities on days 92, 183, 274, and 365 for female rats that received 60 mg/kg/day were elevated to 156.45-166.8 of control values (99.2-127.4 U/L). Serum gastrin levels were increased by 26-285% and 117-875% in male and female treatment groups, respectively. Urinalysis parameters were normal.

6. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment-related effects were seen.

7. Organ Weights: Dose-related increases in relative stomach weights were seen in treated dogs (males: low dose 33%, mid dose 67%, and high dose 86%; females: low dose 53%, mid dose 93%, and high dose 99%). Relative liver weights were increased by 33% and 51% in high dose treated males and females. respectively, when compared to their respective control values. Additionally, relative adrenal weights were increased by 32% and 54% in mid and high dose-treated male dogs, respectively, and an increase of 33% was recorded in high dose-treated female dogs, when compared to their respective control values. In high dose treated dogs, relative weights of thyroid (males 23% and females 34%) and kidney (males 33% and females 34%) were also increased compared to control values.

8. Gross Pathology: At the end of study period, increased incidence of rugae folds in the stomach were evident in all treated dogs (sponsor did not provide summary table for gross findings).

9. Histopathology: The stomach, thyroid, gallbladder, and lung (discussed under Mortality) were the target organs of toxicity. Histopathological examination of the stomach revealed increased mucosal height, increased mucosal folding, dilation of the glands and cellular debris in the lumen of dilated gland in almost all treated dogs at the fundic region. Additionally, parietal cell vacuolation (multifocal) was seen in 3/5 and 3/5 treated males at mid and high doses, respectively, and 4/5 high dose-treated females. Increased incidence of chromogranin positive cells at fundic region were seen in most of the treated dogs (both sexes) and none in the control group. The drug had no effect on density of glucagon-like immunoreactivity positive cells in the mucosa nor on mucosal mast cells. Hypertrophy of the thyroid follicular cells were seen in mid dose treated males (1/5) and high dose treated males (3/5) and females (3/5). Crypt dilation in the gall bladder were seen in mid dose treated males (1/5), and in 1/5, 1/5 and 2/5 low, mid and high dose treated females. The incidence of above abnormalities were as follows:

Histopathological Findings in 1-Year Oral Toxicity Study in Dogs					
Organs	Sex	Control	2.5 mg/kg	15 mg/kg	60 mg/kg*
# Examined	(M/F)	5	5	5	5
Stomach					
Increased height of fundic mucosa	M	0	2	4	4
	F	0	5	3	5
Increased fundic mucosal folding	M	0	3	4	4
	F	0	4	4	4
Dilation of fundic gland	M	0	4	4	5
	F	0	5	5	5
Cellular debris in lumen of dilated gland	M	0	4	4	5
	F	0	4	4	5
Vacuolation of parietal cell (multifocal)	M	0	0	3	3
	F	0	0	0	4
Increased chromogranin-positive cells (fundic)	M	0	1	3	3
	F	0	3	3	3
Thyroid					
Follicular cell hypertrophy	M	0	0	1	3
	F	0	0	0	3
Gall Bladder					
Crypt dilation (multifocal)	M	0	0	1	0
	F	0	1	1	2

* 2 days at 15 mg/kg/day, 2 days at 30 mg/kg/day then 60 mg/kg/day thereafter.

In a 1-year oral toxicity study, beagle dogs received pantoprazole (non-enteric coated tablets in capsules) at doses of 0, 2.5, 15.0 and 60 mg/kg/day. The highest tested dose was achieved by dose escalation. The dose of 2.5 mg/kg/day could be considered a tolerated dose given that stomach changes, described below, are likely due to the pharmacological action of the drug. One male dog at 60 mg/kg/day had drug-induced pulmonary edema, which was resolved by the 7th day of the study. One male dog at 15 mg/kg/day died on day 4 of the study due to drug-induced pulmonary edema. Histopathological findings for this dog included peribronchial and periarteriolar edema, dilation of lymphatics, eosinophilic material in the alveoli, foamy alveolar macrophages, alveolar hemorrhage, and alveolar neutrophil infiltrates. A thiourea-like metabolite of pantoprazole (— 97165) is thought to be responsible for induction of pulmonary edema following administration of pantoprazole. A pantoprazole dose as low as 15 mg/kg/day had the capacity to induce pulmonary edema. Pulmonary edema appears to be transient and tolerance develops with multiple dosing and has no relation to the animal supplier. Final body weights on day 364 were suppressed by >10% for male and female treatment groups at doses ≥15 mg/kg/day. At the end of treatment period, serum cholesterol levels were increased at all dose levels. Serum triglyceride levels were increased for all male treatment groups. Serum gastrin levels were elevated in all male and female treatment groups. The stomach, thyroid gland, gall bladder, and lungs were the target organs of toxicity. Histopathological findings for the stomach at doses ≥2.5 mg/kg/day were as follows: increased height of fundic mucosa, increased fundic mucosal folding, dilation of fundic gland, cellular debris in lumen of dilated

gland, and increased chromogranin-positive cells in the fundic region. Additional findings at doses ≥15 mg/kg/day included vacuolation of parietal cells (multifocal). Pantoprazole had no effect on density of glucagon-like immunoreactivity positive cells in the mucosa or on mucosal mast cells. For the thyroid gland, hypertrophy of the follicular cells was seen for male dogs at doses ≥ 15 mg/kg/day and female dogs at 60 mg/kg/day. For the gall bladder, crypt dilation was observed for all female treatment groups and one male dog at 15 mg/kg/day.

CARCINOGENICITY

Carcinogenicity studies with pantoprazole have been conducted as follows:

1. Two-year carcinogenicity study in B6C3F1 mice.
2. Two-year carcinogenicity study in Sprague Dawley rats.
3. Two-year carcinogenicity study in Fischer 344 rats.
4. Tumor promotional study in the stomach and forestomach of Sprague Dawley rats.
5. Tumor promotional study in the liver and thyroid gland of Sprague Dawley rats.

The carcinogenicity studies with pantoprazole in B6C3F1 mice and Sprague Dawley rats

The sponsor raised the possibility that pantoprazole might have tumor promoting potential. In response, the Division requested that the sponsor assess its tumor promoting potential in liver, thyroid, stomach, and forestomach in rats as per design/protocols that are enclosed below.

3 PAGE(S) REDACTED

Design/Protocols for assessing the tumor promoting potential of pantoprazole in liver, thyroid, stomach, and forestomach in rats.

A. Protocol for Assessing Tumor Promoting Activity in Liver and Thyroid: This protocol is based on the experimental model described by Diwan et al (JNCI, 75: 1099-1105, 1985). Groups of rats should be treated according to following regimen:

1) Control (untreated).

2) N-nitroso-N-methylurea (NMU: 0.05 mmol/kg i.v., 1 injection/week) for 4 weeks. Two weeks after the last NMU injection, rats should be given daily administration of Pantoprazole [dose level(s) which produced liver and thyroid tumor in the bioassay] for 52 weeks.

3) N-nitroso-N-methylurea (NMU: 0.05 mmol/kg i.v., 1 injection/week) for 4 weeks. Two weeks after the last NMU injection, rats should be given daily administration of vehicle of Pantoprazole (alkaline solution) for 52 weeks.

4) Vehicle of N-nitroso-N-methylurea intravenously (1 injection/week) for 4 weeks. Two weeks after the last injection, rats should be given daily administration of Pantoprazole [dose level(s) which produced liver and thyroid tumor in the bioassay] for 52 weeks.

5) N-nitroso-N-methylurea (NMU: 0.05 mmol/kg i.v., 1 injection/week) for 4 weeks. Two weeks after the last NMU injection, rats should be given daily administration of phenobarbital [positive control: 0.05% in drinking water] for 52 weeks.

6) N-nitroso-N-methylurea (NMU: 0.05 mmol/kg i.v., 1 injection/week) for 4 weeks. Two weeks after the last NMU injection, rats should be given daily administration of vehicle of phenobarbital (water) for 52 weeks.

7) Vehicle of N-nitroso-N-methylurea intravenously (1 injection/week) for 4 weeks. Two weeks after the last injection, rats should be given daily

administration of phenobarbital [positive control: 0.05% in drinking water] for 52 weeks.

B. Protocol for Assessing Tumor Promoting Activity in Stonach and Forestomach: This protocol is based on the experimental model described by Newberne et al (Cancer Letters, 38: 149-163, 1987). Groups of rats should be treated according to following regimen:

1) Control (untreated).

2) N-methyl-N-nitro-N-nitroso-N-guanidine (MNNG: 75 mg/l in drinking water for 3 month. Additionally, rats should be given daily dose of Pantoprazole [dose level(s) which produced tumors in stomach and forestomach in the bioassay] for 52 weeks.

3) N-methyl-N-nitro-N=nitroso-N-guanidine (MNNG: 75 mg/l in drinking water for 3 month. Additionally, rats should be given daily dose of vehicle of Pantoprazole (alkaline solution) for 52 weeks.

4) Daily administration of Pantoprazole [dose level(s) which produced tumors in stomach and forestomach in the bioassay] for 52 weeks.

In the above protocols, you should use 4 to 5 week-old Sprague Dawley rats and there should be at least 20 rats/sex/group.

At the end of the treatment period, all animals should be sacrificed and examined histologically.

MOUSE

Oral Dose Range Finding Studies

4-Week Dose-Range Oral Toxicity Study in Mice (GTR-31373).

Testing Laboratories: Byk Gulden Pharmaceuticals
Konstanz, Germany

Study Started: March 29, 1989

Study Completed: July 24, 1990

Animals: CD1 (7 weeks old, 23-28 g) and B6C3F1 (7 weeks old, 19-23 g) females.

Drug Batch No.: 289035

Methods: In the 4-week oral toxicity study with pantoprazole, two different strains of mice (i.e., CD1 and B6C3F1) were used. The sponsor compared the effects of pantoprazole on expected target organs (i.e., stomach, liver, lung, and thyroid gland) and the serum gastrin levels in two strains of mice in order to select the most appropriate strain for a future carcinogenicity study. Groups of mice (10/strain/group) were given orally (gavage) 96022-Z at daily doses of 1 and 150 mg/kg/day for 4 weeks. The control group animals (5/strains) received the vehicle (purified water pH 10.0) in similar fashion. The volume of administration was 10 mL/kg and pH of the drug solution was adjusted to 10.4 before administration. Additionally, two groups were also included in this study, one group (5/strain) received the vehicle and the other group (10/strain) received high dose of the drug and were used for a 4-week recovery study. All mice were observed 3 times a day for 5 days a week (Monday-Friday) for clinical signs and mortality. Body weights and food intakes were recorded twice weekly during treatment period and once a week during the recovery period. Blood samples were collected from retro-orbital venous plexus at 24 hr after drug administration on day 29 and 57 of the study for measuring serum gastrin levels. At the end of treatment/recovery period mice were sacrificed and subjected to complete necropsy. Only the liver, stomach, thyroid and lungs were examined microscopically.

Results:

1. Observed Effects: Some of the high dose treated mice showed reduced motility, piloerection, and sat in a hunched position.

2. Mortality: There were 6 deaths (5 belonged to CD1 mice [1 at 1 mg/kg and 4 at 150 mg/kg], and 1 in high dose treated B6C3F1 mice). Sponsor attributed cause of deaths to accidents.

3. Body Weight/Food Consumption: No treatment-related effects were seen.

4. Serum Gastrin Levels: At high dose, serum gastrin levels were increased by 51% and 161% in B6C3F1 and CD1 strains of mice respectively, when compared to their respective control values (serum gastrin levels in controls: B6C3F1 = 221 ng/L and CD =105 ng/L). The 1 mg/kg dose level had no effect on serum gastrin levels, and at the end of recovery period serum gastrin levels were comparable in all groups.

5. Organ Weights: Absolute stomach weights were increased by 20% (relative wt: 22%) and 42% (relative wt: 38%) in high dose treated CD1 and B6C3F1 mice, respectively.

6. Gross Pathology: According to sponsor no treatment-related effects were seen.

7. Histopathology: Histological examination of gastric fundus revealed parietal cell degeneration, glandular dilation, chief cell hyperplasia, and parietal cell hyperplasia in treated mice. Additionally, in liver, eosinophilic cell swelling and/or vacuolization were also seen in high dose treated groups. The incidence of above mentioned findings were as follows:

Histopathological Findings in Female Mice						
	CD1 Mice			B6C3F1 Mice		
Organs	Control	Low Dose	High Dose	Control	Low Dose	High Dose
# Examined	10	10	10	10	10	10
Stomach (fundus)						
Parietal cell degeneration	2	0	4	0	1	1
Glandular dilation	0	1	4	0	0	3
Chief cell hyperplasia	0	0	2	0	0	2
Parietal cell hyperplasia	0	1	7	0	0	7
Liver						
Eos. cell swelling	0	0	3	0	1	4
Vacuolization	4	0	4	1	1	5

At the end of 4 weeks of recovery period, histopathological abnormalities in the control and treated mice were comparable.

This study was conducted to select a maximum tolerated dose (MTD) dose in mice (CD1 or B6C3F1). Unfortunately, the highest tested dose only produced pharmacological effects [i.e., increase in serum gastrin and some reversible changes in stomach (fundus region)]. No significant drug-induced toxicities were evident in this study. Sponsor selected B6C3F1 strain of mice for repeat dose-range study (see below).

Repeat 4-Week Dose-Range Oral Toxicity Study in Mice **(GTR-31375).**

Testing Laboratories: Byk Gulden Pharmaceuticals
Konstanz, Germany

Study Started: August 22, 1989

Study Completed: July 24, 1990

Animals: B6C3F1 Female Mice (7 weeks old, 18-23 g).

Drug Batch No.: 289035

Methods: Groups of mice (10 females/group) were given orally (gavage) —— 96022-Z at daily doses of 5 (low), 200 (mid) and 500 (high) mg/kg/day for 4 weeks. The control group mice received the vehicle (distilled-water pH 10.0) in a similar fashion. This study was intended as a dose range finding study for the mouse carcinogenicity study. The volume of administration was 10 mL/kg and pH of the drug solution was adjusted to 10.4 before administration. One additional group (supplementary group) of 5 females was included, which received the low dose in a similar fashion. All mice were observed 3 times a day for 5 days a week for clinical signs and mortality. Body weights and food consumption were recorded twice weekly. Blood samples were collected from retro-orbital

venous plexus at 24 hr after drug administration (at 3 hr after drug administration from mice of supplementary group) on day 29 of the study for measuring serum gastrin levels. At the end of treatment period all surviving mice were sacrificed and subjected to complete necropsy. Only the lung, liver and stomach were weighted and histopathological examinations of only the liver, lung, stomach and thyroid were performed.

Results:

1. **Observed Effects:** All of the high dose treated mice showed reduced motility, ptosis, piloerection, and sat in hunched position.

2. **Mortality:** Three high dose treated mice died during study period. Two of the 3 deaths were drug related, and 1 death was accidental.

3. **Body Weight/Food Consumption:** No treatment related effects were seen.

4. **Serum Gastrin Levels:** At 24 hr after drug administration, the serum gastrin levels were increased by 111% and 471% in mid and high dose treated group, when compared to the control values (mean gastrin levels = 176 ng/L). At 24 hr after drug administration, the serum gastrin level in low dose group was comparable to that seen in control group. However, at 3 hr after low dose administration, the serum gastrin level was increased by 117% when compared to the control values.

5. **Organ Weights:** Stomach weights were increased by 41% and 51% in mid and high dose treated mice, respectively, when compared to the control values.

6. **Gross Pathology:** According to sponsor, no treatment-related effects were seen.

7. **Histopathology:** Histopathological examination of gastric fundus revealed mucosal thickening, parietal cell degeneration, glandular dilation, parietal cell hyperplasia, chief cell atrophy, yellow secretion, and submucosal edema in treated mice. In addition, hyperkeratosis in the forestomach and hyperplasia of gastric antrum were also seen in some of the mid and high dose treated mice. Squamous cell metaplasia was evident in 1/5 high dose treated mice. In liver, centrilobular hypertrophy was seen in all dose groups, and 1/7 high dose treated mice had hepatic necrosis. The incidence of above mentioned findings were as follows:

Histopathological Findings in Female B6C3F1 Mice				
Organs	Control	5 mg/kg	200 mg/kg	500 mg/kg
Stomach (fundus)				
# Examined	7	13	10	7
Mucosal thickening	0	0	10	5
Parietal cell degeneration	0	0	4	6
Glandular dilation	0	3	3	2
Parietal cell hyperplasia	0	6	8	4
Chief cell atrophy	0	0	0	3
Yellow secretion	0	5	3	6
Submucosal edema	0	0	0	3
Forestomach				
# Examined	0	14	10	7
Hyperkeratosis	0	0	10	6
Gastric Antrum				
# Examined	10	13	7	5
Squamous cell metaplasia	0	0	0	1
Hyperplasia	0	0	1	1
Liver				
# Examined	10	15	10	7
Necrosis	0	0	0	1
Hypertrophy	0	2	2	5

In this study, the maximum tolerated dose was not identified. The data indicated that the high dose level (500 mg/kg/day) was lethal. The mid dose (200 mg/kg/day) produced only pharmacodynamic effects (i.e., changes in the stomach and increased serum gastrin levels) along with liver hypertrophy without hepatocellular necrosis. This liver hypertrophic effect could be related to the effect of drug as a marginal hepatic metabolizing enzyme inducer.

COVERSHEET FOR CARCINOGENICITY STUDY IN MOUSE

1. Study No.: KH8929 (Report # 42/92)

2. Name of Laboratory: Byk Gulden
Inst. for Pathology & Toxicology
Hamburg, Germany

3. Strain: B6C3F1

4. No./sex/group: 50

5. Doses (O, L, M, H): 0 (cage control), 0 (vehicle control), 5, 25 and 150 mg/kg/day

6. Basis for dose selection stated: Yes

7. Interim sacrifice: No

8. Total duration (weeks): 104 Weeks

9. Week/site for first tumor:

	Male	Female
O	58/Hemangiosarcoma (skin)	67/Malignant Lymphoma (hemolymphoret. sys.)
O	72/Hepatocellular Carcinoma (liver)	54/Malignant Lymphoma (hemolymphoret. sys.)
L	85/Hepatocellular Carcinoma (liver)	62/Hemangiosarcoma (liver)
M	86/Hepatocellular Carcinoma (liver)	68/Malignant Lymphoma (hemolymphoret. sys.)
H	55/Malignant Lymphoma (hemolymphoret. sys.)	64/Malignant Lymphoma (hemolymphoret. sys.)

10. No. alive at termination:

	Male	% Survival	Female	% Survival
O (cage control)	42/50	84	34/50	68
O (vehicle control)	41/50	82	44/50	88
L	41/50	82	46/50	92
M	45/50	90	39/50	78
H	25/50	50	31/50	62

11. Statistical Methods Used: Peto etal (International Agency for Research on Cancer Monograph 1980 [Suppl. 2]; 311-426

12. Attach tumor and non-tumor data for each tissue (i.e., benign; malignant; hyperplastic): See Appendix 1

Two-Year Oral (gavage) Carcinogenicity Study in Mice (GTR-31899).

Testing Laboratories: Byk Gulden
Inst. for Pathology and Toxicology
Hamburg, Germany

Date Started: November 6, 1989

Date Completed: January 6, 1993

GLP Requirements: A Statement of Compliance with GLP regulation was included.

Testing Species & Strain: B6C3F1 Mice (7-8 weeks old, males: 21-28 g and females: 18-24 g).

Drug Batch No.: 399175

Methods: In this study dose selection was based on two preliminary 4-week dose range studies (see above). Sponsor has selected 150 mg/kg/day as the top dose for 2-year oral carcinogenicity study in mice. The selection of 150 mg/kg/day as the high dose is not appropriate since it only produced some clinical signs and pharmacodynamic effects (changes in the stomach, increased serum gastrin level and liver hypertrophy without hepatocellular necrosis) of the drug. This dose level is not the maximum tolerated dose. The remaining dose levels selected for carcinogenicity study were 25 and 5 mg/kg/day. Groups of mice (50/sex/group) were given pantoprazole (pH 10.0) orally via gavage at daily doses of 5, 25 and 150 mg/kg/day for 24 months. Two additional groups (50/sex/group) were also included, one group was given vehicle (distilled water pH 10.4) and the other group was used as cage control. The volume of administration was fixed at 10 mL/kg. All mice were observed twice daily for clinical signs and mortality. Physical examination of all animals were performed weekly. Body weights and food intakes were recorded weekly during the first 13 weeks then every 4 weeks thereafter. Just before sacrifice, blood samples were collected from the retro-orbital venous plexus for hematological tests. Additionally, blood samples were also collected from 6 males and 6 females each from mid and high dose groups at 5, 15, 30 and 120 minutes after the last dose for measuring serum drug levels. All surviving mice were sacrificed at the end of the study period and subjected to complete necropsy and histopathological examinations. Stomach samples from 5 mice/sex/group were also examined under electron microscope. Additionally, endocrine cells in a section of fundus of the stomach of all mice sacrificed at term were stained with silver based stain (Grimelius method) and examined microscopically. Statistical evaluation of neoplastic lesions was performed according to Peto et al. (International Agency for Research on Cancer Monograph 1980 [Suppl 2]; 311-426).

Results:

1. Observed Effects: Piloerection, reduced activity, hunchbacked posture, weight loss and ataxia were seen in high dose treated males.

2. Mortality: Significant increase in intercurrent mortality rates were seen in high dose treated males and the survival rate at termination was reduced in high dose treated mice (cage control; 84%, vehicle control: 82%, low dose: 82%, mid dose: 90% and high dose: 50%). In females treatment had no effect on intercurrent mortality rates when compared to cage control. However, intercurrent mortality rates as well as survival rate at termination in high dose treated females were significantly decreased compared to vehicle control (survival rates: vehicle control = 88%, low dose = 92%, mid dose = 78% and high dose = 62%).

Intercurrent Mortality Rates										
Male Mice										
Days	CC	%	VC	%	Low Dose	%	Mid Dose	%	High Dose	%
1-364	0/50	0.0	1/50	2.0	1/50	2.0	1/50	2.0	4/50	8.0
365-546	4/50	8.0	3/49	6.1	0/49	0.0	1/49	2.0	11/46	23.9
547-766	4/46	8.7	5/46	10.9	8/49	16.3	3/48	6.2	10/35	28.6
Terminal	42	--	41	--	41	--	45	--	25	--
Survival rate	--	84	--	82	--	82	--	90	--	50
Female Mice										
1-364	0/50	0.0	1/50	2.0	0/50	0.0	3/50	6.0	4/50	8.0
365-546	6/50	12.0	1/49	2.0	1/50	2.0	2/47	4.2	6/46	13.0
547-766	10/44	22.7	4/48	8.3	3/49	6.1	6/45	13.3	9/40	22.5
Terminal	34	--	44	--	46	--	39	--	31	--
Survival rate	--	68	--	88	--	92	--	78	--	62

CC = cage control
VC = vehicle control

3. Body Weight/Food Consumption/Water Consumption: No treatment-related effects were seen.

Body Weight (g) of Male Mice					
Dose (mg/kg/day)					
Weeks	CC	VC	5	25	150
1	24 ± 1	25 ± 1	25 ± 1	25 ± 1	24 ± 1
13	31 ± 2	31 ± 2	30 ± 2	31 ± 2	30 ± 2
25	36 ± 3	35 ± 3	34 ± 3	35 ± 3	34 ± 2
53	41 ± 4	39 ± 4	39 ± 3	40 ± 4	38 ± 4
105	39 ± 4	38 ± 5	38 ± 4	39 ± 4	37 ± 3

CC = Cage control
VC = Vehicle control

Food Consumption (g/animal/day) in Male Mice					
Dose (mg/kg/day)					
Days	CC	VC	5	25	150
1-8	5.7 ± 0.4	5.8 ± 0.4	5.6 ± 0.5	5.7 ± 0.5	5.5 ± 0.5
29-85	6.0 ± 0.3	5.7 ± 0.3	5.7 ± 0.4	5.9 ± 0.4	5.6 ± 0.4
85-197	6.2 ± 0.3	5.9 ± 0.3	5.9 ± 0.4	6.0 ± 0.3	5.9 ± 0.4
197-365	6.2 ± 0.4	5.7 ± 0.3	5.7 ± 0.4	5.8 ± 0.4	5.8 ± 0.3
365-533	6.3 ± 0.6	5.8 ± 0.3	5.9 ± 0.4	6.1 ± 0.5	5.9 ± 0.4
533-701	7.2 ± 1.0	6.7 ± 0.7	6.8 ± 0.7	6.9 ± 0.7	6.8 ± 0.8
701-729	7.1 ± 1.1	6.7 ± 0.7	6.8 ± 0.6	6.8 ± 0.7	6.9 ± 0.9

CC = Cage control
VC = Vehicle control

Body Weight (g) of Female Mice					
	Dose (mg/kg/day)				
Weeks	CC	VC	5	25	150
1	20 ± 1	21 ± 1	21 ± 1	20 ± 1	20 ± 1
13	25 ± 1	25 ± 1	25 ± 2	25 ± 1	26 ± 1
25	28 ± 2	27 ± 2	27 ± 2	27 ± 2	28 ± 1
53	32 ± 4	30 ± 3	31 ± 4	31 ± 3	31 ± 2
105	36 ± 4	35 ± 5	34 ± 5	35 ± 5	33 ± 3

CC = Cage control
VC = Vehicle control

Food Consumption (g/animal/day) in Female Mice					
	Dose (mg/kg/day)				
Days	CC	VC	5	25	150
1-9	5.1 ± 0.3	4.7 ± 0.6	5.0 ± 0.3	4.8 ± 0.2	4.8 ± 0.2
30-86	5.1 ± 0.2	4.7 ± 0.1	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2
86-199	4.8 ± 0.2	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.2	4.7 ± 0.1
199-367	5.1 ± 0.2	4.8 ± 0.2	4.8 ± 0.1	4.9 ± 0.3	5.0 ± 0.2
367-535	5.1 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	4.9 ± 0.3	5.1 ± 0.2
535-703	5.9 ± 0.7	5.4 ± 0.3	5.5 ± 0.2	5.7 ± 0.4	6.0 ± 0.4
703-729	6.2 ± 0.6	5.7 ± 0.5	5.6 ± 0.2	5.8 ± 0.4	6.5 ± 0.7

CC = Cage control
VC = Vehicle control

4. Hematology/Coagulation/Bone Marrow: No treatment-related effects were seen.

5. Gross Pathology: At termination increased incidence of thickened gastric glandular mucosa were seen in treated mice (Males: cage control 2.4%, vehicle control 0%, low dose 22%, mid dose 100% and high dose 100%; Females: cage control 0%, vehicle control 0%, low dose 13%, mid dose 100% and high dose 100%). Additionally, increased incidence of pale liver were also seen in mid and high dose treated male mice when compared to the control livers.

6. Histopathology:

Non-neoplastic Findings: Dose dependent increased incidence of hyperplasia in the fundic region of stomach were seen in treated mice (both sexes). In liver, statistically significant increase incidence of Kupffer cell proliferation, patchy necrosis, single cell necrosis and fatty change in high dose treated mice of both sexes, centrilobular necrosis in mid and high dose treated males, hepatocellular hyperplasia in high dose treated females were seen. Additionally, increased incidence of distended heart chamber in high dose treated mice (both sexes), distention of gallbladder and renal tubular dilation in high dose treated males were also seen. One high dose treated male had osseous metaplasia in the kidney. The incidence of above mentioned findings were as follows:

Number of Mice With Non-Neoplastic Findings

# Examined	Sex (M/F)	CC 50	VC 50	L 50	M 50	H 50	P-Value* Trend (FDA)
Stomach							
Fundic Hyperplasia	M	0	0	9	49	50	0.0000
	F	0	0	24	50	50	0.0000
Heart							
Distended Chamber	M	3	5	5	2	10	0.0113
	F	4	3	0	4	10	0.0012
Gallbladder							
Distension	M	1	1	2	3	5	0.0238
	F	2	4	3	5	1	0.8665
Kidneys							
Tubular Dilation	M	0	0	0	0	5	0.0003
	F	0	0	0	0	0	---
Osseous Metaplasia	M	0	0	0	0	1	0.2000
	F	0	0	0	0	0	---
Liver							
Kupffer Cell Prolif.	M	6	9	10	9	26	0.0000
	F	13	16	9	1	22	0.0061
Patchy Necrosis	M	3	3	3	1	9	0.0043
	F	2	1	2	4	8	0.0025
Fatty Change	M	1	1	3	0	8	0.0007
	F	4	1	1	3	9	0.0015
Centrilobular Necrosis	M	0	0	0	1	5	0.0004
	F	0	1	0	0	1	0.3606
Single Cell Necrosis	M	2	6	9	7	8	0.1876
	F	6	8	13	10	21	0.0003
Hepatoc. Hyperplasia	M	11	8	10	13	13	0.1823
	F	3	3	2	2	7	0.0255

CC = cage control
VC = vehicle control
L = low dose
M = mid dose
H = high dose
* = p values were provided by Dr. Chen (HFD-715)

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Semiquantitative Evaluation of Grimelius Positive Cells in the Gastric Fundus:

The number of Grimelius positive cells (GPC) in the stomach was assessed in all mice killed at term.

Sponsor's table, Page 11336, Vol. 1.11

The number of Grimelius positive cells (GPC) was evaluated semiquantitatively according to the following scheme:

Finding	Degree	Definition
1) GPC diffuse	1	= normal condition of the controls, single GPC in the distal (lower) half of the musoca
	2	= single GPC lying in up to two-thirds of the mucosal height, slightly increased number
	3	= single GPC lying in up to two-thirds of the mucosal height, moderately increased number; cell groups but with no more than 8 cells per group
2) GPC focal	1	= groups with more than 8 cells, but no more than 2 groups per histologic section
	2	= groups with more than 8 cells, more than 2 groups per histologic section
3) GPC, chains:		single cell chains of GPC
4) GPC, micronodules:		nodules wider than 2 glandular necks
5) GPC, submucosal growth:		GPC in submucosal, hyperplastic mucosa

Significant dose related increase in focal/chain GPC were seen in treated males and submucosal growth was seen in 8% of high dose treated males. Focal, chains and micronodules GPC were also seen in high dose treated females (13%, 23% and 3% respectively). However no endocrine cell tumors were seen.

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Percent of GPC in the Stomach						
Findings	Sex (M/F)	CC	VC	L	M	H
Diffused	F	100	100	100	100	100
	M	100	100	100	100	100
Focal	F	3	0	0	0	13
	M	0	0	3	18	52
Chains	F	0	0	0	0	23
	M	0	2	3	27	40
Micronodules	F	0	0	0	0	3
	M	0	0	0	2	0
Submucosal Growth	F	3	0	0	0	0
	M	0	0	0	0	8

CC = cage control
VC = vehicle control
L = low dose
M = mid dose
H = high dose

Neoplastic Findings: Only in females statistically, significant increase incidence of hepatocellular adenomas (cage control 4%, vehicle control 10%, low dose 8%, mid dose 4% and high dose 14%; p=0.0257 [trend test done by FDA using combined control]), hepatocellular carcinomas (cage control 6%, vehicle control 0%, low dose 2%, mid dose 2% and high dose 16%; p=0.0004 [trend test done by FDA using combined control]) were seen. The combined incidence of hepatocellular adenomas + carcinomas in females were also significant (cage control 10%, vehicle control 10%, low dose 10%, mid dose 6%, and high dose 30%, p=0.0000 [trend test done b FDA using combined control]). When incidence rates in the high dose group were compared with the incidence rates in combined control, then results were still significant for hepatocellular adenomas and carcinomas (p=0.0007, Fisher exact test done by FDA).

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Number of Mice With Neoplastic Findings							
# Examined	Sex (M/F)	CC 50	VC 50	L 50	M 50	H 50	p-Value Trend Test (FDA)
Liver							
Hepatocellular Adenomas	M	10	11	7	13	6	0.5561
	F	2	5	4	2	7	0.0257*
Hepatocellular Carcinomas	M	9	12	12	12	13	0.2616
	F	3	0	1	1	8	0.0004*
Hepatocellular Adenomas + Carcinomas	M	19	23	19	25	29	
	F	5	5	5	3	15	0.0000*
Pancreas							
Islet Cell Adenoma	F	0	0	0	0	2	0.0508
Spleen							
Hemangiosarcomas	F	0	0	0	0	2	0.0284*
Mammary Gland							
Hemangiosarcomas	F	0	0	0	0	1	
Bone							
Osteochondrosarcoma	F	0	0	0	0	1	0.1714
Lymph nodes							
Hemangioma	F	0	0	0	0	1	

CC = cage control
VC = vehicle control
L = low dose
M = mid dose
H = high dose
* = statistically significant

In this study the dose selection was based on two 4-week dose range studies which were not very informative with respect to the maximum tolerated dose (see above). However, significant increase in intercurrent mortality rates were seen in high dose treated mice and the survival rates at termination were reduced in high dose treated mice (males: vehicle control = 82%, low dose = 82%, mid dose = 90% and high dose = 50% and females: vehicle control = 88%, low dose = 92%, mid dose = 78% and high dose = 62%) when compared to vehicle controls. Dose-dependent increased incidence of hyperplasia in the fundic region of stomach were seen in treated mice (both sexes). In liver, statistically significant increase incidence of Kupffer cell proliferation, patchy necrosis, single cell necrosis and fatty change in high dose treated mice of both sexes, centrilobular necrosis in mid and high dose treated males, hepatocellular hyperplasia in high dose treated females were seen. Only in females statistically, significant increased incidence of hepatocellular adenomas and carcinomas were seen (vehicle control = 10%, low dose = 10%, mid dose = 6% and high dose = 30%, p=0.0001; peto trend test). Furthermore, pairwise comparisons of incidence of hepatocellular adenomas + carcinomas in high dose treated females with the vehicle control gave a p value of 0.003. The incidence of liver tumor (adenomas + carcinomas) in high dose treated females was higher than the mean incidence in sponsor's historical controls (2 studies: 7.6% range, 6-8.2%) and in published literature (8.3%, range 0-20%, Haseman et al. JNCI 75: 975-984, 1985; NTP historical controls: 5%, range 0-15%; Maronpot et al., Arch. Toxicol. Suppl. 10: 10-26, 1987). The mean time to liver tumor (adenomas &/or carcinomas) was not affected by the treatment. Thus the drug produced hepatocellular adenomas + carcinomas in female mice.

Toxicokinetics of Pantoprazole in the Mouse After an Acute Dose or Following a 2-Year Treatment (GTR-31303).

Methods: In support of the 2-year carcinogenicity study with B6C3F1 mice, plasma toxicokinetic parameters for unchanged pantoprazole were determined after a single dose (study LM0215) and after the last dose following two years (days 730-758) of continuous daily dosing (study KM8929, batch 399175). Pantoprazole was administered as an aqueous solution at pH 10 by oral gavage at doses of 25 and 150 mg/kg/day. Blood samples were obtained at 0, 5, 15, 30, and 120 min after dosing. For each time point, blood was collected from 6 mice/sex. The dose volume was 10 mL/kg. Serum concentrations of pantoprazole were measured by

Results: Serum AUC values for pantoprazole on day 1 and after 2 years were similar. However, C_{max} values after 2 years were 2 to 5 times higher than values observed on day 1. Serum AUC values on day 1 or after 2 years increased in a manner proportional to increasing dose. C_{max} values on day 1 increased in a manner approximately proportional to dose. However, after 2 years, C_{max} values increased with ascending dose, although, observed increases were less than proportional to dose.

	25 mg/kg				150 mg/kg			
	day 1		after 2 years		day 1		after 2 years	
	males	females	males	females	males	females	males	females
AUC (µgxh/mL)	4.13	4.55	3.45	3.89	37.54	34.86	23.39	32.54
Cmax (µg/mL)	9.20	10.32	15.00	14.62	52.00	42.54	32.30	33.36
$t_{\frac{1}{2}}$ (h)	0.40	0.36	0.11	(.11)$)	0.52	0.44	0.32	0.32
tmax (h)	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083

$) λ_z (and t1/2) were taken from the male animals

In support of the 2-year carcinogenicity study with B6C3F1 mice, plasma toxicokinetic parameters for unchanged pantoprazole were determined after a single dose and after the last dose following two years of continuous daily dosing. Pantoprazole was administered at doses of 25 and 150 mg/kg/day. Serum AUC values for pantoprazole on day 1 and after 2 years were similar. Serum AUC values on day 1 or after 2 years increased in a manner proportional to increasing dose.

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