Results: Overall effects of the drug on body weight, food consumption, hematology, organ weight, gastric mucosa and histology (stomach and spleen) were similar to that seen in earlier study. Effects on serum gastrin levels were as follows: blood samples were collected at 4 and 24 hr after drug administration on days 3 and 31 of the study. Blood samples were also collected at the end of 1, 4 and 8 weeks of recovery period. Serum gastrin levels increased with increasing dosages, however, the increases were not dose-related. After 4 and 8 weeks of the recovery period, serum gastrin levels had returned to normal. At the end of the first week of the recovery period, serum levels of gastrin were still significantly higher in high dose treated females compared to control values. Dose-related hypergastrinemia was seen at doses ≥ 5 mg/kg. The hypergastrinemic effect at 1 mg/kg was marginal.

Serum Gastrin Levels (ng/L)

						Recovery Period		
		Day 3		Day 31		Day 37	Day 57	Day 86
Treatment Group	Sex	Post 4 hr	Post 24 hr	Post 4 hr	Post 24 hr			
Control	M	207±18	262±25	276±22	237±23	240±36	235±43	240±57
	F	102±17	126±10	214±32	238±46	205±23	162±24	193±29
Pantoprazole (1 mg/kg)	M	194±13	255±12	521±73	398±44	ND	ND	ND
	F	138±36	166±31	574±90	342±64	ND	ND	ND
Pantoprazole (5 mg/kg)	M	644±48	494±51	854±80	720±110	ND	ND	ND
	F	509±30	361±40	919	619±86	ND	ND	ND
Pantoprazole (500 mg/kg)	M	635±43	843±43	>1167	>1372	293±52	154±27	144±12
	F	563±45	827±31	>1561	>1796	712±144	209±38	185±21

Data are presented as mean and standard error of the mean.
ND = not done

4-Week Oral Toxicity of the Thiol Metabolite of Pantoprazole (B 8401-026) in Rats (GTR-32263).

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

Study Started: September 25, 1991

Study Completed: May 25, 1992

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

Animals: Six weeks old Sprague Dawley rats (males: 213-264 g and females: 159-205 g).

Drug Batch No.: 200085

Methods: Groups of rats (10/sex/group) received the thiol metabolite of pantoprazole (B8401-026) by oral gavage at doses of 15, 50 and 150 mg/kg/day for 4 weeks. The control group animals were given vehicle (Polypropylene glycol and methocel E 15 in water) in similar fashion. The volume of administration was 10 mL/kg. All animals were observed for clinical signs and mortality at 0.5, 3, 6 and 24 hr after drug administration (Monday - Friday). Body weights and food intakes were recorded twice weekly. On day 29 of the treatment, just before drug administration, blood samples were collected from retro-orbital venous plexus for hematological and serum chemistry tests. On day 28/29 of the study urinalysis was also performed. Ophthalmoscopic examinations were performed on all rats once pretest and on day 24 of the study. ECG recordings were done on 6 rats/sex/group on day 21 of the study. At the end of the study period all rats were sacrificed and subjected to complete necropsy. Only control and high dose group animals were examined histopathologically. Additionally, liver, kidneys, thyroid, pituitary and adrenal from low and mid dose groups were also examined microscopically.

Results:

1. Observed Effects: Piloerection, polyuria, swollen soft abdomen, ptosis and reduced activity were seen in most of the high dose treated rats (both sexes). Piloerection and polyuria were also seen in some of mid dose treated males.

2. Mortality: One low dose treated male died during the study period. The death was not considered to be treatment-related.

3. Body Weight/Food Consumption/Water Consumption: At the end of treatment period, body weight gains were reduced by 33% in high dose treated males and this was accompanied with 13-29% reduction in food intakes when compared to the control values. Treatment had no significant effect on females body weight gains. However, during the last week of treatment females from high dose group consumed about 30% less food than the control rats. From second week onwards, all treated males and high dose treated females consumed about 26% more water than control rats.

4. Hematology/Coagulation/Bone Marrow: Leukocyte counts were significantly reduced in treated males (18-44%) and females (27-34%). Prothrombin time and APTT were significantly increased in high dose treated rats (PT: males: control = 13.2 ± 1.2 sec and test = 19.2 ± 5.3 sec, females: control = 11.4 ± 0.6 sec and test = 14.6 ± 3.0 sec; APTT: males: control = 17.0 ± 1.8 sec and test 26.2 ± 5.0, females: control = 17.5 ± 3.0 and test = 22.9 3.5 sec).

5. Blood Chemistry/Urinalysis: In high dose treated males, increases in serum total protein (13%), serum albumin (15%), serum globulin (9%), serum urea (23%), serum creatinine (12%) and serum ALAT activity (28%) were seen. In high dose treated females, increases in serum total protein (14%), serum albumin (18%) and serum ALAT activity (53%) were seen. Increases in urinary volumes (17-35%), and excretion of K^+ (21% only in females) and Ca^{2+} (845-908%) were seen in high dose treated rats of both sexes. Increases in urinary excretion of Ca^{2+} were also seen in mid dose treated rats (males: 235% and females: 217%). Dose-related increase in urinary Cl^- was seen in treated males (36-170%). In females, urinary Cl^- excretion was increased by 72% and 137% at mid and high doses, respectively.

6. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment-related effects were seen except significant reductions in heart rate and significant increases in the Q-T intervals in all high dose treated rats (both sexes). The P-Q interval was significantly higher in high dose treated males.

ECG	Sex	Control	Low Dose	Mid Dose	High Dose
Heart rate (beats/min)	M	400	390	390	310
	F	380	400	420	290
P-Q intervals (m.sec)	M	50	53	57	62
	F	56	53	53	56
Q-T intervals (m.sec)	M	79	80	86	108
	F	84	86	84	119

7. Organ Weights: The relative weights of liver and lungs were increased by 16-47% and 7-19%, respectively, in treated rats of both sexes. Absolute adrenal weights were increased by 19% and 36% in mid and high dose treated males, respectively.

8. Gross Pathology: Discolored adrenal glands were seen in some of low and mid dose treated male rats and in most of the high dose treated rats (both sexes). Thyroid glands were discolored in 1/10 male of low dose group, 5/10 males and 2/10 females of mid dose group, and 10/10 males and 6/10 females of high dose group. Additionally, enlarged thyroid glands were seen in 1/10 females of control group, 8/10 males of mid dose group and 8/10 males and 5/10 females of high dose group. Discoloration of the pituitary gland was seen in 1/10 females of control group and 7/10 males and 3/10 females of high dose group.

9. Histopathology: Histopathological examinations revealed centrilobular hepatocellular hypertrophy in the liver, diffuse follicular hyperplasia of thyroid, hyperplasia and hypertrophy of "throtroph cells" of the pituitary gland, and increased incidences (and severity) of vacuolation and degeneration of adrenal cortex in mid and high dose treated rats. In high dose group, increased incidence (and severity) of tubular regeneration in the kidneys, pelvic uroliths and chronic pyelitis were also seen. The incidences of these findings were as follows:

Histopathological Findings in Rats					
Organs	Sex	Control	5 mg/kg	50 mg/kg	150 mg/kg
Liver					
Centril. Hypertrophy	M	0/10	0/10	3/10	10/10
	F	1/10	0/10	1/10	8/10
Thyroid					
Follicular Hyperplasia	M	0/10	0/10	10/10	10/10
	F	0/10	0/10	7/10	9/9
Pituitary					
Hyperpl./Hypertrophy	M	0/10	0/10	6/10	10/10
	F	0/10	0/10	2/10	10/10
Adrenal Glands					
Vacuolation	M	1/10	1/10	7/10	9/10
	F	0/10	0/10	0/10	4/10
Cortical Degeneration	M	0/10	0/10	0/10	0/10
	F	0/10	0/10	0/10	2/10
Kidneys					
Tubular Regeneration	M	4/10	3/10	3/10	5/10
	F	3/10	1/10	0/10	10/10
Uroliths	M	0/10	0/10	0/10	4/10
	F	0/10	0/10	0/10	5/10
Pyelitis	M	0/10	0/10	0/10	2/10
	F	0/10	0/10	0/10	2/10

The data indicate that the liver, thyroid gland, pituitary gland, adrenal glands, and kidney were the target organs of toxicity and the lowest tested dose (15 mg/kg/day) was the no effect dose in this study. Thiol metabolite of pantoprazole (150 mg/kg/day) produced histopathological changes in liver, kidney and thyroid, while no such changes were evident in 4-week oral toxicity of pantoprazole (1, 5, 20 and 500 mg/kg/day) in rat. Hence thiol metabolite is more toxic than pantoprazole.

Addendum:

Water Consumption: Water consumption for female rats that received 150 mg/kg/day was increased to 109.1-118.2% of the control (39.4 mL/animal/day) during the 4-week treatment period.

Hematology: For female rat that received 150 mg/kg/day, erythrocyte counts, hematocrit, and hemoglobin levels on day 29 were decreased to 85, 87, and 84.3% of control values (7.69×10^{12}/L, 0.46, and 10.8 mmol/L), respectively. Segmented neutrophils on day 29 for male and female rats that received 150 mg/kg/day were increased to 254.6 and 237.5% of control values (0.11 and 0.08), respectively.

Blood Biochemistry: Serum potassium levels on day 29 for male and female rats that received 150 mg/kg/day were decreased to 69.1 and 67.4% of control values (4.37 and 3.99 mmol/L), respectively. Urinary pH values on day 29 for male treatment groups were decreased to 6.6-6.7 as compared to a control value of 7.2. Urinary sodium excretion on day 29 for male and female rats that received 150 mg/kg/day were increased to 131.9 and 141.1% of control values (2.10 and 2.58 mmol/kg), respectively.

3-Month Oral Toxicity Study in Aged Rats (GTR-31984).

Testing Laboratory: Byk Gulden Pharmaceuticals.

Date of the Study: Oct. 24 1988 to Feb. 7, 1989.

GLP Requirement: This is a preliminary study.

Animals: Female Sprague-Dawley, Wistar and Fischer rats weighing 230 -410 g and ages of 52-57 weeks were used.

Methods: Three groups of each strain of rats consisting of 10 female rats were given pantoprazole (batch no. 189-025) dissolved in water by gavage at acid dose levels of 0, 0.8 and 4 mg/kg/day for three months. The endocrine cells in a section of the fundus of the stomach were stained according to GRIMELIUS. The objective of the study was to show possible differences among these stains in response to the treatment. Histological examination of the stomach, liver, lungs and thyroids of all groups were performed. All animals dying before termination of the study as well as abnormalities in all groups were also examined.

Results:

Clinical Signs (daily): There were no treatment-related changes.

Mortality: One Sprague-Dawley rats in the 0.8 mg/kg/day group died on day 12. Cause of death was not determined.

Body Weight: Normal.

Food Consumption: Normal.

Hematology: Normal.

Serum Gastrin Level (days 0, 3, 31 and 93): Basal serum gastrin levels were similar in the three strains. It caused an increase (84-1055%) in gastrin levels in all 4 mg/kg/day groups.

Organ Weight: Increase in stomach weight (13-22%) was noted in all 4 mg/kg/day groups.

Gross Pathology: There were no treatment-related changes.

Histopathology: Inflammation and bile duct proliferation in livers were found in the 4 mg/kg/day groups. Increases in follicular cell and C-cell in the thyroid were detected in all 4 mg/kg/day groups and in the 0.8 mg/kg/day groups of Fischer and Wistar strains. Increased mucosal thickness were noted in all three strains receiving 4 mg/kg/day. Parietal cell, chief cell, and foveolar hyperplasia were observed in all 4 mg/kg/day groups. They were also

noted in the Wistar and Fischer rats receiving 0.8 mg/kg/day. A section of the gastric fundus stained according to GRIMELIUS showed a dose-related increasing trend and significantly higher cell numbers in the 4 mg/kg/day groups. The Sprague-Dawley rats had the highest basal number of these cells.

In conclusion, pantoprazole given orally to three strains of aged rats produced histopathological changes in liver and stomach at 4 mg/kg/day dose level. The objective of the study was to determine any differences in response to the treatment among three strains of rats. Target organs of toxicity of liver, thyroid and stomach were identified. In general, there were no differences in response to the treatment in all three strains. Increase in ECL cell was observed at 4 mg/kg/day dose.

Addendum: Some additional detail regarding the histopathological data has been added below.

Histopathology: The stomach, liver, and thyroid gland were the target organs of toxicity. In the gastric fundus, parietal cell hyperplasia, foveolar hyperplasia, chief cell hyperplasia, and atypical eosinophilic chief cells were observed at a dose of 4 mg/kg/day for all rat strains. Foveolar hyperplasia and chief cell hyperplasia were also evident for Wistar and Fischer rats at a dose of 0.8 mg/kg/day. The number of Grimelius-positive cells increased dose-dependently for all rat strains. For the liver, peribiliary inflammation and bile duct proliferation were observed in Sprague Dawley and Wistar rats that received 4 mg/kg/day. For the thyroid gland, the follicular epithelium was slightly increased in Wistar rats that received 4 mg/kg/day. Proliferation of c-cells was evident in all strains at 4 mg/kg/day and for Fischer rats at 0.8 mg/kg/day.

Histopathological changes for Sprague Dawley, Wistar, and Fischer female rats that received pantoprazole by oral gavage all doses of 0, 0.8, and 4 mg/kg/day for 3 months. Values in parentheses indicate severity.

Organ/Tissue	Sprague Dawley Rat			Wistar Rat			Fischer Rat		
	0	0.8	4	0	0.8	4	0	0.8	4
Gastric fundus									
-foveolar hyperplasia	0	0	3	0	4	3	0	3	2
-atypical eosinophilic chief cells	0	0	4	0	0	6	0	0	6
-parietal cell hyperplasia	0	0	5	0	3	6	0	5	8
-chief cell hyperplasia	0	0	4	0	3	4	0	2	4
Gastric antrum									
-mucosal hyperplasia	1	0	2	3	5	3	0	0	2
Liver									
-peribiliary inflammation	4	2	6	5	5	9	5	2	3
-bile duct proliferation	3	3	6	4	6	9	2	4	4
Thyroid gland									
-epithelial height	10(1.90)	9(1.70)	10(1.90)	10(1.80)	10(2.00)	10(2.20)	10(1.40)	10(1.00)	10(1.10)
-C-cells, diff.	10(1.10)	9(1.40)	10(2.10)	10(1.20)	10(2.10)	10(1.80)	10(1.40)	10(2.00)	10(2.00)

Grimelius-Positive cells (cells/mm^2) in Sprague Dawley, Wistar, and Fischer female rats that received pantoprazole by oral gavage all doses of 0, 0.8, and 4 mg/kg/day for 3 months.

Dose, mg/kg/day	Grimelius-Positive Cells (cells/mm^2)		
	Sprague Dawley Rat	Wistar Rat	Fischer Rat
0	994	564	659
0.8	1312	735	859
4	1688	1165	1102

90-Day Dose Range Finding Study with the Thiol Metabolite (B8401-026) in Rats: Comparison with Pantoprazole (GTR-33264).

Testing Laboratory: [redacted]

Date Started: July 15, 1992

Date Completed: December 20, 1993

GLP Compliance: A statement of compliance with GLP regulations and the quality assurance unit was included.

Animals: Fischer 344 rats were used in this study. At the start of treatment, animals were 6 weeks of age and body weight ranges were 108-132 g for male rats and 83-104 g for female rats.

Drug Batch: B8401-026 (5-difluoromethoxy-1H-benzimidazole-2-thiol), batch no. 200 085; Pantoprazole, batch no. 500 205.

Methods: A 90-day oral dose range finding study with the thiol metabolite of pantoprazole (B8401-026) was conducted in Fischer 344 rats to identify suitable doses for a 2 year carcinogenicity study in rats. Rats received B8401-026 by oral gavage at doses of 0, 20, or 50 mg/kg/day. For comparison, rats were treated pantoprazole by oral gavage at a dose of 200 mg/kg/day. For toxicology studies, there were 8 rats/sex/group. The vehicle for B8401-026 was propylene glycol in a 3% suspension of methocel E15 in distilled water. The vehicle for pantoprazole was distilled water with the pH adjusted to 10.7-10.9. The dosing volume was 10 mL/kg. Electron microscopic examination of the tissues was performed with 4 female rats/group after a 90 day treatment period as follows: vehicle group for B8401-023, 50 mg/kg/day B8401-023, vehicle group for pantoprazole, 50 mg/kg/day pantoprazole, and 200 mg/kg/day pantoprazole. Further, electron microscopic examination of tissues following an 8 week recovery period was performed with 4 female rats/group in the 50 mg/kg/day B8401-026 and 200 mg/kg/day pantoprazole groups.

Vehicle and treatment groups are shown in the table below. Animals were observed for clinical signs of toxicity and morbundity/mortality 3 times/day from Monday through Friday and 1 time/day on Saturday and Sunday. Body weights and food consumption were measured twice per week for the first five weeks of the study, and once per week for the remaining period of the study. Blood for determination of hematological, coagulation, and clinical chemistry parameters was collected from the retro-orbital venous plexus at weeks 5/6 and 11/12. Blood for determination of thyroid stimulating hormone levels was collected at week 11/12. Blood for determination of serum drug levels was collected from 3 male and 2 female rats from groups that received B8401-026 at 20 and 50 mg/kg/day on days 1 and 90 at 1 hr prior to treatment and at 15, 30, 90, and 180 minutes after drug administration. Urine specimens were analyzed at weeks 5/6 and 11/12 following a 16-hr diuresis period. Animals were sacrificed and subjected to gross examination during week 13/14 (days 91-94). Absolute and relative organ weights were determined for the following: heart, liver, kidneys, brain, thyroid glands, adrenal glands, testes, ovaries, uterus (with vagina), thymus, spleen, lungs, pituitary (fixed), seminal vesicles (fixed), and prostate (fixed). Female rats for electron microscopic evaluation of tissues were sacrificed at week 13/15 (days 91, 92, 98, or 99 at 24 hr after the last administration) and week 21 (days 147 or 148 after an 8 week recovery period). For determination of hepatic cytochrome P450 enzyme activities, the liver of each animal was weighed, the left lateral and caudate lobes were removed for histopathology, and the remaining portion of the liver was homogenized and the 10,000 x g supernatant was collected for measurement of enzyme activities. Activities of lonazolac hydroxylase, 7-ethoxycoumarin dealkylase, ethylmorphine demethylase, and uridyldiphosphoglucuronyl transferase were determined with 0.1-1.0 mg rat liver microsomal protein. Histopathological of the liver, kidney, stomach, intestine, lungs, heart, spleen, and thyroid glands (with parathyroids) were examined for animals that received water, B8401-026 at 50 mg/kg/day, or pantoprazole at 200 mg/kg/day. Only the liver, stomach, and thyroid glands (with parathyroids) were examined for animals that received propylene glycol or B8401-026 at 20 mg/kg/day. Livers were prepared for electron microscopic examination from female rats that received pantoprazole at 50 or 200 mg/kg/day. The colon was prepared for electron microscopic examination from 2 female rats/group that received water or pantoprazole at 50 or 200 mg/kg/day. Results from electron microscopic examinations of the liver and colon were not reported in the present study.

Test Groups and Doses.

Group	Treatment	Dose mg/kg/day	Animals (n) Male/Female in Toxicology Study	Animals (n) Male/Female for Electron Microscopy
1	Water	0	8/8	0/4
2	Propylene Glycol	0	8/8	0/4
3	B8401-026	20	8/8	0/0
4	B8401-026	50	8/8	0/4 0/4[A]
5	Pantoprazole	50	0/0	0/4
6	Pantoprazole	200	8/8	0/4 0/4[A]

A. Tissues from these animals were animals were evaluated by electron microscopy following an 8 week recovery period after drug treatment.

Results:

1. Observed Effects: Polyuria was observed for 75% (6/8) of rats that received B8401-026 at 50 mg/kg/day during the treatment period. There were no treatment-related observed effects for rats that received pantoprazole at 200 mg/kg/day.

2. Mortality: No treatment-related mortality.

3. Body Weight and Food Consumption: There were no treatment-related effects on body weight gain or food consumption for rats that received either B8401-026 or pantoprazole. Body weights for the male propylene glycol group on days 1 and 90 were 123 and 280 g, respectively. Body weight gains for the male rats that received B8401-026 at 20 or 50 mg/kg/day were 107.1 and 116.2% of the control, respectively. Body weights of the male water control group on days 1 and 90 were 119 and 271 g, respectively. Body weight gain for male rats that received pantoprazole at 200 mg/kg/day were 104.7% of the control. Body weights for the female propylene glycol group on days 1 and 90 were 95 and 161 g, respectively. Body weight gains for the female rats that received B8401-026 at 20 or 50 mg/kg/day were 102.2 and 126.9% of the control, respectively. Body weights of the female water control group on days 1 and 90 were 95 and 160 g, respectively. Body weight gain for female rats that received pantoprazole at 200 mg/kg/day were 107.7% of the control.

4. Hematology and Blood Coagulation: Alterations in reticulocyte counts were observed for male and female rats that received either B8401-026 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day during the treatment period. Small decreases in leukocyte counts were observed for male rats that received B8401-026 at 20 or 50 mg/kg/day during the treatment period. Variations in other hematological parameters (i.e., red blood cell counts, hematocrit, hemoglobin levels, mean corpuscular volume, prothrombin time, APTT) were observed during the treatment period; however, changes were <5-10% and appeared to have little biological significance.

Male Rats: Reticulocyte counts at week 5/6 for male rats that received B8401-026 at 20 and 50 mg/kg/day were decreased to 65 and 60% of the control (0.020/1000 erthyrocytes), respectively. Reticulocyte counts at week 5/6 for male rats that received pantoprazole at 200 mg/kg/day were increased to 135% of the control (0.012/1000 erythrocytes). No alterations in reticulocyte counts were observed at week 11/12. Mean corpuscular hemoglobin levels for at week 11/12 for male rats that received pantoprazole at 200 mg/kg/day were decreased to 88.9% of the control (1.17 fmol). Leukocyte counts for male rats at week 5/6 for male rats that received B8401-026 at 20 and 50 mg/kg/day were decreased to 85.3 and 84% of the control (7.5 10^9/L), respectively. Leukocyte counts for male rats at week 11/12 for male rats that received B8401-026 at 20 and 50 mg/kg/day were decreased to 89.3 and 84% of the control (7.5 10^9/L), respectively. Segmented neutrophil counts at weeks 11/12 for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 122.2 and 155.6% of the control (0.09/1000 white cells), respectively. Segmented neutrophil counts for male rats that received pantoprazole at 200 mg/kg/day were decreased to 80% of the control (0.15/1000 white cells).

Female Rats: Reticulocyte counts at week 5/6 for female rats that received B8401-026 at 20 and 50 mg/kg/day were decreased to 69.2 and 53.8% of the control (0.013/1000 erthyrocytes), respectively. Reticulocyte counts at week 11/12 for female rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 171.4 and 200% of the control (0.007/1000 erthyrocytes), respectively. Reticulocyte counts at week 11/12 for female rats that received pantoprazole at 200 mg/kg/day were decreased to 66.7% of the control (0.012/1000 erythrocytes).

5. Blood Biochemistry and Urinalysis: Alterations in serum levels of bilirubin, thyroid stimulating hormone (TSH), bile acids, and cholesterol were observed for treatment groups at week 11/12. Variations in serum K^+ levels were observed for treatment groups at weeks 5/6 and 11/12; however, changes were < 5-10% and appeared to have little biological significance. There were no treatment-related urinalysis changes.

Blood Biochemistry: Bilirubin levels at week 11/12 for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 125 and 133.3% of the control (1.2 μmole/L), respectively. Bilirubin levels at week 11/12 for male rats that received pantoprazole at 200 mg/kg/day were increased to 141.6% of the control (1.2 μmole/L). TSH levels at week 11/12 for male rats that received B8401-026 at 50 mg/kg/day were increased to 331.6% of the control (0.73 mU/L). TSH levels at week 11/12 for female rats that received B8401-026 at 20 or 50 mg/kg/day were decreased to 80.6 and 57.8% of the control (2.42 mU/L), respectively. TSH levels at weeks 11/12 for male and female rats that received pantoprazole at 200 mg/kg/day were decreased to 29.6 and 17.8% of the control (1.89 and 5.61 mU/L), respectively. Bile acid levels at week 11/12 for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 127.7 and 148.1% of the control (13.7 μmole/L), respectively. Bile acid levels at week 11/12 for male rats that received B8401-026 at 50 mg/kg/day were increased to 140% of the control (20 μmole/L), respectively. Bile acid levels at week 11/12 for male rats that received pantoprazole at 200 mg/kg/day were increased to 120.8% of the control (14.9 μmole/L). Bile acid levels at week 11/12 for female rats that received pantoprazole at 200 mg/kg/day were decreased to 71.3% of tne control (22.3 μmole/L). Cholesterol levels at week 11/12 for female rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 139.9 and 175% of the control (2.48 mmole/L), respectively. Cholesterol levels at week 11/12 for female rats that received pantoprazole at 200 mg/kg/day were increased to 146.6% of the control (2.51 mmole/L).

6. Physical Examinations: Hepatic cytochrome P450 activities (i.e., lonazolac hydroxylase, 7-ethoxycoumarin dealkylase, ethylmorphine demethylase, and uridyldiphosphoglucuronyl transferase) were measured following treatment with B8401-025 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day. B8401-026 at 20 or 50 mg/kg/day increased uridyldiphosphoglucuronyl transferase activity for both male and female treatment group. B8401-026 at 20 and 50 mg/kg/day decreased ethylmorphine demethylase activity for male treatment groups in a dose-related manner; although, activities for female treatment groups were unchanged. Effects of B8401-026 at 20 or 50 mg/kg/day on the activities of lonazolac hydroxylase and 7-ethoxycoumarin dealkylase for both male and female treatment groups were relatively small. In contrast, pantoprazole at 200 mg/kg/day increased the activities of lonazolac hydroxylase, 7-ethoxycoumarin

dealkylase, and uridyldiphosphoglucuronyl transferase in both male and female treatment groups. Pantoprazole increased ethylmorphine demethylase activity for the female treatment group; although, it was unchanged for the male treatment group. Histopathological observations of centrolobular swelling in the liver appear to correlate with increased hepatic cytochrome P450 activity.

Changes of hepatic cytochrome P450 activities for rats that received either B8401-026 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day for 90 days (n = 8).

Group	LONAH		ECOD		EMDM		UDPGT	
	Male	Female	Male	Female	Male	Female	Male	Female
Vehicle of B8401-026 (nmoles/mg protein)	32.1 nmoles/ mg	19.7 nmoles/ mg	10.37 nmoles/ mg	4.88 nmoles/ mg	52.61 nmoles/ mg	16.83 nmoles/ mg	27.79 nmoles/ mg	32.30 nmoles/ mg
20 mg/kg/day B8401-026 (% of control)	133.6%	91.4%	77.1%	90.8%	69.4%	108.7%	257%	199.9%
50 mg/kg/day B8401-026 (% of control)	127.4%	104.6%	86.5%	107.4%	40.4%	99.2%	245.8%	194.4%
Vehicle of pantoprazole (nmoles/mg protein)	33.8 nmoles/ mg	18.0 nmoles/ mg	8.66 nmoles/ mg	4.88 nmoles/ mg	50.65 nmoles/ mg	16.83 nmoles/ mg	22.82 nmoles/ mg	32.30 nmoles/ mg
200 mg/kg/day pantoprazole (% of control)	184.8%	231.7%	268.6%	227.1%	83.7%	143.5%	252.7%	156.2%

Lonazolac hydroxylase, LONAH; 7-ethoxycoumarin dealkylase, ECOD; ethylmorphine demethylase, EMDM; and uridyldiphosphoglucuronyl transferase, UDPGT.

7. Organ Weights: Organ weight changes were observed for liver, lungs, thyroid gland, and kidney for rats that received either B8401-026 or pantoprazole that appeared to correlate with gross and histopathological abnormalities. Changes in absolute adrenal gland weight were observed for male and female rats that received B84010-026 at 50 mg/kg/day and female rats that received pantoprazole at 200 mg/kg/day were increased; however, there were no corresponding gross and histopathological correlations.

Liver: Absolute liver weight for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 114.9 and 137.8% of the control (10.60 g), respectively. Relative liver weight for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 110.8 and 129% of the control (3.78%), respectively. Absolute liver weights for female rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 112.2 and 152.4% of the control (5.34 g), respectively. Relative liver weights for female rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 109 and 135.6% of the control (3.40%), respectively. Absolute liver weights for male and female rats that received pantoprazole at 200 mg/kg/day were increased to 115.3 and 128.5% of the control (10.41 and 5.23 g), respectively. Relative liver weights for male and female rats that received pantoprazole at 200 mg/kg/day were increased to 116 and 123.6% of the control (3.80 and 3.30%), respectively.

Lungs: Absolute lung weights for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 122.2 and 124.6% of the control (1.26 g), respectively. Relative lung weights for male rats that received B8401-026 at 20 and 50 mg/kg/day were both increased to 117.8% of the control (0.45%). Absolute lung weight for female rats that received B8401-026 at 50 mg/kg/day was increased to 129.9% of the control (0.87 g). Relative lung weights for female rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 107.1 and 114.3% of the control (0.56%), respectively. Absolute lung weight for female rats that received pantoprazole at 200 mg/kg/day was increased to 119.3% of the control (0.88 g). Relative lung weight for female rats that received pantoprazole at 200 mg/kg/day was increased to 114.5% of the control (0.55%).

Thyroid Gland: Absolute thyroid gland weight for male rats that received B8401-026 at 20 and 50 mg/kg/day were increased to 140 and 320% of the control (0.010 g), respectively. Absolute thyroid gland weight for male rats that received pantoprazole at 200 mg/kg/day was increased to 130% of the control (0.010 g). Absolute thyroid gland weight for female rats that received B8401-026 at 50 mg/kg/day was increased to 150% of the control (0.010 g).

Kidney: Absolute kidney weights for male and female rats that received B8401-026 at 50 mg/kg/day were increased to 112.1 and 115% of the control (1.82 and 1.13 g), respectively. Absolute kidney weights for male and female rats that received pantoprazole at 200 mg/kg/day were increased to 105.7 and 133% of the control (1.74 and 1.12 g), respectively.

8. Gross Pathology: An increased incidence of enlarged kidneys were observed for rats that received either B8401-026 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day. An increased incidence of enlarged thyroid glands were observed for male rats that received either B8401-026 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day. Thickened areas of the glandular stomach were observed for rats that received pantoprazole at 200 mg/kg/day.

Gross pathological changes for rats that received either B8401-026 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day for 90 days (n = 8).

Tissue	Water		Propylene glycol		B8401-026				Pantoprazole 200 mg/kg/day	
					20 mg/kg/day		50 mg/kg/day			
	M	F	M	F	M	F	M	F	M	F
Kidney -enlarged	0	0	2	0	4	2	4	6	4	2
Thyroid gland -enlarged	0	0	0	0	2	0	5	0	2	0
Glandular stomach -thickened areas	0	0	0	0	0	0	0	0	2	1

9. Histopathology: Target organs of toxicity for both B8401-026 and pantoprazole included the stomach, thyroid gland, lungs, and liver. Thyroid gland activation was observed for male and female rats that received B8401-026 at 20 and 50 mg/kg/day. In contrast, the incidence of the thyroid gland activation for male and female rats that received pantoprazole at 200 mg/kg/day was lower. Changes in the glandular stomach in the fundic region for male and female that received B8401-026 at 50 mg/kg/day included glandular ectasia and/or parietal cell swelling in the gastric mucosa. Changes in the glandular stomach in the fundic region for male and female rats that received pantoprazole at 200 mg/kg/day included parietal cell swelling, parietal cell degeneration/vacuolation, chief cell hyperplasia, eosinophilic chief cells, glandular ectasia, inspissated secretory products and a mild lymphocytic infiltration of the submucosa. Grimelius-positive cell (GPC) hyperplasia was observed in the stomach for male and female rats that received pantoprazole at 200 mg/kg/day. Centrolobular swelling was observed for male and female rats that received either B8401-026 at 50 mg/kg/day or pantoprazole at 200 mg/kg/day. Round/mixed cell infiltration and alveolar histiocytosis were observed in the lung for male and female rats that received either B8401-026 at 50 mg/kg/day or pantoprazole at 200 mg/kg/day.

Histopathological changes for rats that received either B8401-026 at 20 or 50 mg/kg/day or pantoprazole at 200 mg/kg/day for 90 days (n = 8).

Tissue	Water		Propylene glycol		B8401-026				Pantoprazole 200 mg/kg/day	
					20 mg/kg/day		50 mg/kg/day			
	M	F	M	F	M	F	M	F	M	F
Glandular stomach/fundus										
-chief cell hyperplasia	0	0	0	0	0	0	0	0	8	8
-parietal cell degen/vacuolation	0	0	0	0	0	0	0	0	5	8
-glandular (cystic) ectasia	0	0	0	0	0	0	3	2	5	6
-eosinophilic chief cells	0	0	0	0	0	0	0		8	7
-inspissated secretory products	1	0	0	0	1	0	4		7	3
-parietal cell swelling	0	0	0	0	0	0	0	2	2	5
-submucosal lymphocytic infiltration	0	0	0	0	0	0	0	1	3	7
-increasing mucosal height	0	0	0	0	0	0	0	0	7	1
-erosion	0	0	0	0	0	0	0	1	0	1
Glandular stomach/pylorus										
-increasing mucosa height	0	0	0	0	0	0	0	0	1	1
-neutrophilic infiltration	0	0	0	0	0	0	0	0	1	0
-erosion	0	0	0	0	0	0	0	0	1	1
-mixed cell infiltration	0	0	0	0	0	0	1	0	1	0
-mucosal hyperplasia	0	0	0	0	0	0	0	0	0	0
-superficial inflammation	0	0	0	0	0	0	0	0	2	0
Stomach/Grimelius stain										
-GPC hyperplasia	0	0	0	0	3	1	2	4	7	8
- GPC hyperplasia, chains	0	0	0	0	0	0	0	0	3	4
Lungs										
-leukocytic infiltration	0	0	0	0	0	0	1	0	0	0
-round/mixed cell infiltration	0	2	0	1	2	1	4	5	6	7
-alveolar histiocytosis	0	4	0	1	2	2	6	7	2	5
Thyroid 1 + 2 activation	0	0	0	0	3	0	6	1	2	1
Liver -centrolobular swelling	0	0	2	0	1	0	2	5	7	3

10. Plasma Drug Levels: Blood for determination of plasma drug levels was collected; however, no data was reported.

A 90-day oral dose range finding study with the thiol metabolite of pantoprazole (B8401-026) was conducted in Fischer 344 rats to identify suitable doses for a 2 year carcinogenicity study in rats. Rats received B8401-026 by oral gavage at doses of 0, 20, or 50 mg/kg/day. For comparison, rats were treated pantoprazole by oral gavage at a dose of 200 mg/kg/day. B8401-026 at 50 mg/kg/day appeared to be well tolerated. There were no treatment-related effects on body weight gain for male or female rats that received either B8401-026 or pantoprazole. Target organs of toxicity for both B8401-026 and pantoprazole included the stomach, thyroid gland, lungs, and liver. Thyroid gland activation was observed for male and female rats that received B8401-026 at 20 and 50 mg/kg/day. In contrast, the incidence of the thyroid gland activation for male and female rats that received pantoprazole at 200 mg/kg/day was lower. Changes in the glandular stomach in the fundic region for male and female that received B8401-026 at 50 mg/kg/day included glandular ectasia and/or parietal cell swelling in the gastric mucosa. Changes in the glandular stomach in the fundic region for male and female rats that received pantoprazole at 200 mg/kg/day included parietal cell swelling, parietal cell degeneration/vacuolation, chief cell hyperplasia, eosinophilic chief cells, glandular ectasia, inspissated secretory products and a mild lymphocytic infiltration of the submucosa. Grimelius-positive cell (GPC) hyperplasia was observed in the stomach for male and female rats that received pantoprazole at 200 mg/kg/day. Centrolobular swelling was observed for male and female rats that received either B8401-026 at 50 mg/kg/day or pantoprazole at 200 mg/kg/day. Round/mixed cell infiltration and alveolar histiocytosis were observed in the lung for male and female rats that received either B8401-026 at 50 mg/kg/day or pantoprazole at 200 mg/kg/day. B8401-026 at 20 or 50 mg/kg/day increased uridyldiphosphoglucuronyl transferase activity for both male and female treatment group. B8401-026 at 20 and 50 mg/kg/day decreased ethylmorphine demethylase activity for male treatment groups in a dose-related manner; although, activities for female treatment groups were unchanged. Effects of B8401-026 at 20 or 50 mg/kg/day on the activities of lonazolac hydroxylase and 7-ethoxycoumarin dealkylase for both male and female treatment groups were relatively small. In contrast, pantoprazole at 200 mg/kg/day increased the activities of lonazolac hydroxylase, 7-ethoxycoumarin dealkylase, and uridyldiphosphoglucuronyl transferase in both male and female treatment groups. Pantoprazole increased ethylmorphine demethylase activity for the female treatment group; although, it was unchanged for the male treatment group. Histopathological observations of centrolobular swelling in the liver appear to correlate with increased hepatic cytochrome P450 activity. For pantoprazole at 200 mg/kg/day, based upon no mortality, no impairment of body weight gain, and observed histopathological changes, this dose could be considered a maximum tolerated dose in Fischer 344 rats.

Electron Microscopical Evaluation of Female Rat Liver After 3-Month Oral Treatment With Pantoprazole (B 8610-023) or B8401-026 (a Thiol Metabolite of Pantoprazole) (GTR-31821).

Animals: Female Fischer 344 rats.

Methods: Groups of female rats (n=4/group) were given oral (gavage) doses of vehicle 1 (distilled water, pH 10), vehicle 2 (propylene glycol 150 mg/kg), pantoprazole (50 or 200 mg/kg/day) or B 8401-026 (50 mg/kg/day) for 3 months. Two additional groups were also included which received 200 mg/kg/day of pantoprazole or 50 mg/kg/day of B 8401-026 for same duration of time and used for 8-weeks of recovery study. At the end of treatment/recovery period, rats were killed, livers were removed and processed for electron microscopical examinations.

Results: Electron Microscopic examinations of the liver of pantoprazole or B8401-026 treated rats revealed increases in endoplasmic reticulum (related to induction of P450 isozymes), cell membrane turnover, intracellular cholesterol storage and bile secretion. These findings, but of milder nature, were also seen in vehicle 2 (propylene glycol) treated rats. These findings were mostly reversible after 8 weeks of recovery period in vehicle 2 and pantoprazole treated rats, while reversibility was not complete in B8401-026 treated rats. It should be noted here that there was no evidence of peroxisomal proliferation in the liver of any rat.

Chronic Toxicity

Oral Route of Administration

6-Month Oral Toxicity Study in Rats (GTR-31379, 31372, and GTR-31320).

Testing Laboratory: Byk Gulden Pharmaceuticals
Konstanz, Germany

Date of the Study: May 1988 to January 31, 1990.

GLP Requirement: A statement of compliance with OECD's principles of GLP was included.

Animals: Sprague-Dawley rats weighing 160-205 g were used.

Methods: Five groups of animals each consisting of 24 males and 24 females were given pantoprazole (batch no. 289-035) dissolved in water (the pH was adjusted to 10.7-10.9) by gavage at constant volume of 10 mL/kg and at acid dose levels of 0, 0.8, 4, 16 and 320 mg/kg/day for 6 months. An additional 8 animals per sex were assigned to the control and 320 mg/kg/day groups for a recovery period of 8 weeks. Histopathological examinations of all tissues were conducted in the control and the 320 mg/kg/day groups and the animals dying before termination. In the case of changes in the high dose group, the respective organs of the remaining groups were also examined. The stomach and

thyroid of all groups were examined. The spleens in the 16 mg/kg/day were evaluated. No staining for ECL cell was performed. Blood for measurement of serum levels of pantoprazole and its sulfone metabolites was collected at predose, 0.5 and 2 hour after drug administration on days 1, 85 and 176 of the study. Levels were measured by ——— (limits of detection ———.

Results:

1. Clinical Signs (daily): Salivation was observed in the 320 mg/kg/day group.

2. Mortality: There were nine deaths but none of them were treatment-related.

3. Body Weight (twice weekly or weekly): Final body weights for male rats that received 0.8, 4, 16, and 320 mg/kg/day were 98, 95.9, 97, and 94.8% of the control (557 g), respectively. Final body weights for female rats that received 0.8, 4, 16, and 320 mg/kg/day were 96.8, 92.6, 94.2, and 89.4% of the control (312 g), respectively. Food consumption for female rats that received 0.8, 4, 16, and 320 mg/kg/day were reduced to 95.8, 82, 95.4, and 91.2% of the control (21.6 g/animal/day), respectively.

4. Food Consumption (twice or once weekly): Reduction in food intake (4-9%) was noted in the 320 mg/kg/day group.

5. Hematology (weeks 0, 6, 12, 26, and 34): Lower hemoglobin (5-7%) and hematocrit (5%) were noted in the 320 mg/kg/day group. They returned to normal at recovery period.

5. Blood Chemistry (weeks 0, 6, 12, 26, and 34) and Urinalysis (weeks 6, 12, 26, and 34): Increases in protein (6%), albumin (10%) and AP (6%) were seen in the 320 mg/kg/day group. All returned to normal during recovery period. Serum gastrin levels were measured during the treatment period on days 7, 69, and 168 at 4 and 24 hr after dosing. Levels were also measured on days 188 and 240 during the recovery period. During the treatment period at all sampling times, gastrin levels were elevated at doses ≥4 mg/kg/day. During the recovery period, gastrin levels were elevated in the 320 mg/kg/day group on day 188 (week 1), but not on day 240 (week 8). Urinary calcium excretion was lowered (33%) in all dose groups during the 26-week treatment period. At the end of the 8-week recovery period, calcium excretion was similar between the control and 320 mg/kg/day groups.

6. Physical Examinations: Ophthalmic examinations during weeks 25 and 35 found no treatment-related changes. During weeks 13-16, there were no differences in the numbers of estrous cycles between the female control and treatment groups.

7. Organ Weights: Increases in liver (12-36%), lung (7-19%), thyroid (27-48%) and ovaries (16%) and decreases in thymus weights (39-40%) were observed in the 320 mg/kg/day group. Increases in stomach weights (7-18%) were noted in the 4 mg/kg/day and above groups. After an 8 week recovery period, higher liver, lung and thyroid weights were still present.

8. Gross Pathology: Increased thickness of the gastric mucosa was observed in all treated groups.

9. Histopathology: The stomach, liver, thyroid gland, and spleen were the target organs of toxicity.

Stomach: For the fundic region of the stomach, increased incidences of chief cell hyperplasia and inspissated secretory products occurred at doses ≥0.8 mg/kg/day. An increased incidence of chief cell atrophy was found at doses ≥0.8 mg/kg/day. An increased incidence of parietal cell degeneration/vacuolation and cell infiltrates was found primarily for rats that received 320 mg/kg/day. The incidence of parietal cell hyperplasia was increased for rats that received 4 and 16 mg/kg/day, but decreased at 320 mg/kg/day. Parietal cell hyperplasia and inspissated secretory products persisted through the recovery period. Other changes were not found at the end of the recovery period. For the forestomach, there was an increased incidence of hyperkeratosis for rats that received 320 mg/kg/day; however, this change was not observed at the end of the recovery period.

Liver: For male rats that received 0.8, 4, 16, and 320 mg/kg/day, the incidence of centrilobular swelling of hepatocytes was found to be 8/24, 4/24, 5/24, and 10/24, respectively. For female rats that received 0.8, 4, 16, and 320 mg/kg/day, the incidence of centrilobular swelling of hepatocytes was found to be 2/24, 0/24, 0/24, and 4/24, respectively. Bile duct hyperplasia was found in 8/24 male rats and 1/23 female rats that had received 320 mg/kg/day. For male rats that received 320 mg/kg/day, there was little or no hemosiderin storage. For female rats that received 320 mg/kg/day, there was reduced or no fat staining. Centrolobular swelling of hepatocytes was not found at the end of the recovery period; however, bile duct hyperplasia (male rats), lack of hemosiderin storage (male rats), and reduced or no fat staining (female rats) persisted. A hepatocellular adenoma was seen in 1/24 male rats treated with 320 mg/kg/day.

Thyroid Gland: For female rats that received 16 and 320 mg/kg/day, epithelial cells of the thyroid follicles underwent a change in cell morphology to cuboidal and columnar features. However, normal cell morphology was observed at the end of the recovery period. A C-cell adenoma in thyroid was present in 1/24 females treated with 16 mg/kg/day at the end of the treatment period.

Spleen: For male rats that received 320 mg/kg/day, there was a depletion of iron levels in the spleen at the end of the treatment period. This change persisted through the recovery period.

Histological Findings in 6-Months Oral Toxicity Study in Rats					
Male					
Organs	0 mg/kg	0.8 mg/kg	4 mg/kg	16 mg/kg	320 mg/kg
Stomach/Fundic Region					
# Examined	24	24	23	24	24
Chief Cell Hyperplasia	3	10	19	23	22
Chief Cell Atrophy	1	4	2	8	11
Parietal Cell Degen./Vacuol.	0	3	2	0	14
Parietal Cell Hyperplasia	2	4	12	11	6
Eosinophilic Infiltration	0	1	3	3	5
Mixed Cell Infiltration	0	4	1	3	11
Inspissated Secretory Products	1	2	11	23	23
Forestomach					
# Examined	24	24	23	24	24
Hyperkeratosis	5	4	4	4	9
Liver					
# Examined	24	24	24	24	24
Centrolobular Swelling	1	8	4	5	10
Bile Duct Hyperplasia	0	1	1	0	8
Altered Hepatocytes	0	0	0	0	1
Necrotizing Hepatitis	0	0	0	0	1
Hepatocellular Adenoma	0	0	0	0	1
Thyroid					
# Examined	24	24	19	24	24
C-Cell Hyperplasia	0	0	0	0	1

Histological Findings in 6-Months Oral Toxicity Study in Rats					
Female					
Organs	0 mg/kg	0.8 mg/kg	4 mg/kg	16 mg/kg	320 mg/kg
Stomach/Fundic Region					
# Examined	22	22	24	24	23
Chief Cell Hyperplasia	2	6	18	20	21
Chief Cell Atrophy	0	2	2	2	6
Parietal Cell Degen./Vacuol.	1	2	4	4	15
Parietal Cell Hyperplasia	2	2	8	9	4
Eosinophilic Infiltration	0	0	0	0	5
Mixed Cell Infiltration	0	1	1	0	4
Inspissated Secretory Products	1	2	8	12	23
Forestomach					
# Examined	22	22	24	24	23
Hyperkeratosis	3	1	2	3	7
Liver					
# Examined	23	23	24	24	23
Centrolobular Swelling	0	2	0	0	4
Bile Duct Hyperplasia	2	2	0	1	1
Thyroid					
# Examined	23	20	20	23	23
Activation	0	0	0	3	9
C-Cell Adenoma	0	0	0	1	0

Histopathological changes in rats that received pantoprazole by oral gavage at doses of 0 or 320 mg/kg/day for 6 months followed by an 8-week recovery period.

Organ	0 mg/kg/day		320 mg/kg/day	
	Male	Female	Male	Female
Stomach/fundic region				
-number examined	**8**	**6**	**7**	**8**
-chief cell hyperplasia	3	2	2	3
-degeneration/vacuolation	0	0	0	0
-parietal cell hyperplasia	1	3	7	4
-eosinophilic infiltration	0	0	2	1
-inspissated secretory products	0	1	7	8
-chief cell atrophy	0	0	3	2
Forestomach				
-number examined	**8**	**6**	**7**	**8**
-hyperkeratosis	1	1	3	0
Liver				
-number examined	**8**	**7**	**7**	**8**
-centrolobular swelling	0	0	0	0
-bile duct hyperplasia	0	0	2	0
Hemosiderin/Liver				
-number examined	**4**	**4**	4	**4**
-hemosiderin storage	2	2	1	1
-no storage	2	2	3	2
Fat Stain/Liver				
-number examined	-	**5**	-	**5**
-no fat staining		1		1
-reduced fat staining		0		2
Thyroid gland 1				
-number examined	-	8	-	8
-activation (i.e., change of cell morphology)		0		0
Thyroid gland 2				
-number examined	-	8	-	8
-activation		0		0

10. Plasma Drug Levels: The sponsor has monitored levels of drug at only two time point, therefore, no pharmacokinetic parameters can be calculated. Furthermore, data for the sulfone metabolite is not valid due to interfering peak in chromatograph. Therefore, one can only comment on the levels of pantoprazole at 0.5 and 2 hr after drug administration. Pantoprazole levels at 2 hr after drug administration were always lower than that seen at 0.5 hr after the dose. Levels of pantoprazole increased with increasing dose and levels at pre-dose were always below detection limits, suggesting no accumulation of the drug.

In a 6-month oral toxicity study, Sprague Dawley rats received pantoprazole at doses of 0, 0.8, 4, 16, and 320 mg/kg/day. Additional animals were assigned to the control and 320 mg/kg/day groups for an 8-week recovery period. The dose of 4 mg/kg/day could be considered a tolerated dose given that stomach changes, described below, were most likely a result of the pharmacological action of the drug. Final body weight was impaired by >10% for only female rats at 320 mg/kg/day. During the treatment period at all sampling times, gastrin levels were elevated at doses ≥4 mg/kg/day. During the recovery period, gastrin levels were elevated in the 320 mg/kg/day group at week 1, but not at week 8. The

stomach, liver, thyroid gland, and spleen were the target organs of toxicity. For the fundic region of the stomach, increased incidences of chief cell hyperplasia and inspissated secretory products occurred at doses ≥ 0.8 mg/kg/day. An increased incidence of chief cell atrophy was found at doses ≥ 0.8 mg/kg/day. An increased incidence of parietal cell degeneration/vacuolation and cell infiltrates was found primarily for rats that received 320 mg/kg/day. The incidence of parietal cell hyperplasia was increased for rats that received 4 and 16 mg/kg/day, but decreased at 320 mg/kg/day. Parietal cell hyperplasia and inspissated secretory products persisted through the recovery period. Other changes were not found at the end of the recovery period. For the forestomach, there was an increased incidence of hyperkeratosis for rats that received 320 mg/kg/day; however, this change was not observed at the end of the recovery period. For the liver at the end of the treatment period, centrolobular swelling of hepatocytes was observed for male rats at doses ≥0.8 mg/kg/day and female rats at 320 mg/kg/day. Bile duct hyperplasia was observed for male rats at 320 mg/kg/day. For male rats that received 320 mg/kg/day, there was little or no hemosiderin storage. For female rats that received 320 mg/kg/day, there was reduced or no fat staining. A hepatocellular adenoma was seen in 1/24 male rats treated with 320 mg/kg/day. Centrolobular swelling of hepatocytes was not found at the end of the recovery period; however, bile duct hyperplasia (male rats), lack of hemosiderin storage (male rats), and reduced or no fat staining (female rats) persisted. For thyroid gland, epithelial cells of the thyroid follicles underwent a change in cell morphology to cuboidal and columnar features for female rats at 16 and 320 mg/kg/day. Normal cell morphology was observed at the end of the recovery period. A C-cell adenoma in thyroid was present in 1/24 females treated with 16 mg/kg/day at the end of the treatment period. For the spleen, there was a depletion of iron levels at the end of the treatment period for male rats at 320 mg/kg/day. This change persisted through the recovery period.

12-Month Oral Toxicity Study of Pantoprazole in the Sprague Dawley Rat (GTR-31377, GTR-31999, and GTR-32041).

Testing Laboratories: [ ]

Study Started: October 4, 1989

Study Completed: February 25, 1992

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

Animals: 8-week old albino Sprague-Dawley rats (male: 268-354 g and females: 162-228 g).

Drug Batch No.: 299155

Methods: In this study dose selection was based on 6-months oral toxicity study in rats in which the highest tested dose of 320 mg/kg/day produced significant toxicities (decreased body weight gain, decreased food consumption, microscopic changes in stomach, liver and thyroid etc). In the present study, groups of rats (20/sex/group) were given orally (gavage) 96022-Z at daily doses of 5, 50 and 300 mg/kg/day for 12 months. The control group

animals received the vehicle (purified water) in similar fashion. The volume of administration was 10 mL/kg and the drug solution pH was adjusted to 10.5 (± 0.1) before administration. Additionally, two groups (20/sex/group) were also included in this study, one received the vehicle and the other received low dose of the drug and were used for a 9-month recovery study. Only reversibility of gastric mucosal hyperplasia will be evaluated in the recovery study. All animals were observed daily for clinical signs and mortality. Body weights and food consumption were recorded at pretest weekly for the first 13 weeks of treatment and then monthly thereafter. Ophthalmoscopic examinations were performed at pretest, at week 26 of the study, and at the end of the study. Blood samples were collected from tail vein at 3, 6, 9 and 12 months of the study for hematological and serum chemistry tests. Overnight urine samples were also collected for urinalysis. Electron microscopic scanning of red blood cells to identify any ultrastructural pathology were performed at the end of 6 months of treatment. All surviving rats were sacrificed at the end of treatment/recovery period and subjected to complete necropsy. Only control and high dose group animals were examined histologically. The stomach, liver, kidneys, thyroid and spleen were also examined microscopically from low and mid dose groups. Additionally, sections of stomach from all rats were put on poly-L-lysine treated slides and were stained immunohistochemically to identify chromogranin –antigen using [redacted] chromogranin –

Results:

1. Observed Effects: Salivation was seen in rats that received doses ≥50 mg/kg/day.

2. Mortality: During the treatment period, 11 rats (2 males from control group, 3 males and 2 females from low dose group, 1 male from mid dose group and 1 male and 2 females from high dose group) died. The cause of deaths were not considered to be treatment-related.

3. Body Weight/Food Consumption/Water Consumption: Final body weight was impaired by >10% in female rats that received 300 mg/kg/day. Final body weights for male rats that received 5, 50, and 300 mg/kg/day were 106.1, 101.1, and 94.8% of the control (822 g), respectively. Final body weights for female rats that received 5, 50, and 300 mg/kg/day were 93, 104, and 88.4% of the control (474 g), respectively.

4. Hematology/Coagulation/Bone Marrow: No biological significant effects were seen except increased incidence of poikilocytosis in high dose treated males.

5. Blood Chemistry/Urinalysis: Alterations in cholesterol and triglyceride levels were observed on days 274 and 363. At the end of the treatment period, dose-related increases in serum cholesterol (males: low dose 25%, mid dose 31% and high dose 59% and females: low dose 16%, mid dose 55% and high dose 136%) and triglyceride (males: low dose 33%, mid dose 51% and high dose 97% and females: low dose 2%, mid dose 60% and high dose 41%) levels were evident in treated rats. Total protein levels for male and female rats that received 300 mg/kg/day were elevated to 107-120% and 108.7-113.5%

of control values (6.18-6.78 and 7.17-7.56 g/dL), respectively, on days 92, 183, 274, and 363. Dose related increases in serum gastrin levels were also seen (males: low dose 164%, mid dose 343% and high dose 1016% and females: low dose 269%. mid dose 466% and high dose 1158%) in treated rats (blood collection was done just before the drug administration. Increased incidence of proteinuria were seen in mid and high dose treated rats.

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

7. Organ Weights: Absolute stomach weights were increased by 41%, 57% and 59% in treated males at low, mid and high dose groups when compared to the control values, respectively. The corresponding increases in treated females at low, mid and high dose groups were 45%, 78% and 65% of the control, respectively. Absolute liver weights were increased by 10%, 28% and 63% in low, mid, and high dose groups, respectively. In females, liver weights were increased by 26% and 44% in mid and high dose, respectively, when compared to their respective control values. Absolute kidney weights were increased by 17%, 15% and 31% for male rats in the low, mid and high dose groups, respectively. In females, kidney weights were increased by 18% in mid dose group and 23% in high dose group, when compared to the control values. Relative heart weights were increased by 13-15% in high dose treated rats (both sexes).

8. Gross Pathology: Sponsor has not provided a summary table for macroscopic findings. According to the text, enlarged liver was seen in treated males (low dose = 1/20, mid dose = 1/20 and high dose = 3/20) and enlarged thyroid was also seen in high dose treated rats (males = 1/20 and females = 1/20). Additionally, thickened fundic mucosa of stomach was in all treated males (low dose: 3/20, mid dose: 9/20 and high dose: 4/20) and in females at the mid dose (4/20) and high dose (4/20). Findings in the control group were not indicated.

9. Histopathology: Increased incidence of hyperplasia of the fundic mucosa of the stomach along with increased numbers of eosinophilic chief cells and hyperplasia of ECL cells (chromogranin-positive cells) were seen in treated rats (both sexes). There was a dose-related increase in focal ECL cell hyperplasia in fundus including micronodules in pantoprazole-treated rats. Diffuse and focal ECL cell hyperplasia were evaluated separately and correlated to histomorphometric measurements of fundic mucosal height. The diffuse ECL cell index was increased at a dose of 5 mg/kg/day; however, the index decreased with increasing dose. In contrast, focal hyperplasia was more prominent at 300 mg/kg/day than at 5 mg/kg/day. Centrilobular hypertrophy of the liver, hepatocellular necrosis, hypertrophy of the thyroid follicular epithelium and chronic progressive nephropathy were seen in treated rats of both sexes. Additionally, one low dose treated male had hepatocellular adenoma and another low dose male had hepatocellular carcinoma. The incidence for the above abnormalities were as follows:

Histopathological Findings in 1-Year Oral Toxicity Study in Rats					
Organs	Sex	Control	5 mg/kg	50 mg/kg	300 mg/kg
# Examined	(M/F)	20	20	20	20
Stomach					
Increased height of fundic mucosa[1]	M	0	12	15	16
	F	0	3	13	15
Fundic gland ectasia[1]	M	0	17	20	20
	F	0	20	20	20
Eosinophilic chief cells (fundus)[1]	M	0	17	20	20
	F	0	18	20	20
Inflammatory cell infiltrate, mixed (fundus)[1]	M	1	2	8	15
	F	1	0	2	5
Mild fibrosis, lamina propria (fundus)	M	0	2	12	19
	F	0	0	8	13
Hyperplasia, chromogranin-positive cells (fundus)[2]	M	0	10	13	15
	F	0	13	14	12
Focal squamous cell hyperplasia (non-glandular stomach)	M	0	0	1	1
	F	0	0	0	0
Liver					
Hepatocellular necrosis	M	0	0	1	3
	F	0	0	1	1
Centrilobular hepatocellular hypertrophy[1]	M	0	1	19	20
	F	0	1	9	19
Hepatocellular adenoma	M	0	1	0	0
	F	0	0	0	0
Hepatocellular carcinoma	M	0	1	0	0
	F	0	0	0	0
Kidney					
Nephropathy (mild-severe)[1]	M	10	9	15	18
	F	3	3	7	15
Urothelial hyperplasia (mild-moderate)[1]	M	0	1	1	7
	F	4	2	1	4
Thyroid					
Follicular cell hypertrophy	M	0	0	4	17
	F	0	0	2	13
Spleen					
Hemosiderin (reduced)	M	0	0	1	4
	F	0	0	0	1

1 = Severity of the changes increased with dose
2 = Hyperplasia was categorized as nodular, focal/multifocal or diffuse. Nodular hyperplasia was observed in only 3/20 high dose treated females. Multifocal ECL-cell hyperplasia was seen of high dose group. Focal ECL-cell hyperplasia was seen in 1/20 high dose treated males and 2/20 and 2/20 of females treated with mid and high dose group respectively.