

Pantoprazole, rat 1 year toxicity study

dose mg/kg	sex	n	ECL-cell hyperplasia: focal		
			diffuse*	groups/chains*	micronodules
0	M	18	0.4	0.06	
	F	20	0.5	0	
5	M	17	1.8	0.3	
	F	17	2.6	1.2	
50	M	19	1.7	0.4	
	F	20	2.4	2.0	
300	M	18	0.9	0.5	1 x
	F	17	1.7	2.2	4 x

(G89025)

* score index.

BEST POSSIBLE COPY

10. Reversibility Study (GTR-31999): Animals assigned for reversibility study (control and low dose groups) were treated for 12 months and then maintained drug free for 9 months. The purpose of this study was to assess the reversibility of gastric effects of the drug. The lowest dose was used in this part of the study because 5 mg/kg/day was associated with gastric mucosal hyperplasia. Only clinical signs, mortality, body weight, food intakes and ophthalmoscopic examination were monitored during in-life phase as mentioned above (see methods). At the end of 9-months, all surviving rats were sacrificed and subjected to complete necropsy. Only stomach and macroscopic lesions were examined microscopically.

- a. **Observed Effects:** No treatment-related effects were seen.
- b. **Mortality:** During the treatment and recovery period, there were 35 (18/40 from control group and 17/40 from 5 mg/kg/day) deaths. Causes of deaths were not considered to be treatment-related.
- c. **Body Weight/Food Consumption:** No treatment related effects were seen.
- d. **Ophthalmoscopic Examinations:** Normal.
- e. **Organ Weights:** At the end of recovery period absolute weights of the stomach were increased by 15-16% in rats which were treated with 5 mg/kg/day (both sexes), when compared to the control values.
- f. **Gross Pathology:** Sponsor did not provide a summary table, however, the text indicated that there were no treatment-related effects seen.

g. Histopathology: At the end of 9 months of recovery period 11/13 treated males and 9/11 treated females had minimal fundic gland ectasia, and minimal eosinophilic chief cells in the fundic mucosa was seen in 1/13 treated males. Additionally, a malignant neuroendocrine cell tumor (fundus) was seen in 1/11 treated females. Although, no evidence of increase fundic mucosal height or increased incidence of hyperplasia of chromogranin-positive cells were evident at the end of 9-month of recovery period (these findings were evident at the end of 12-months of treatment, see above), the presence of a gastric carcinoid at the end of recovery period in the 5 mg/kg/day dose group indicates that cellular changes initiated during the treatment phase must have persisted during the 9-month recovery period. Hence, the gastric effects of the drug observed at the lowest tested dose were not reversible.

In a 12-month oral toxicity study, Sprague Dawley rats received pantoprazole at doses of 0, 5, 50, and 300 mg/kg/day. Additional rats were included in the 0 and 5 mg/kg/day groups for a 9-month recovery period following treatment. The dose of 5 mg/kg/day could be considered a tolerated dose as stomach changes, described below, were more than likely due to the pharmacological action of the drug. Final body weight was impaired by >10% in female rats that received 300 mg/kg/day. Serum gastrin, cholesterol, and triglyceride levels were elevated at doses \geq 5 mg/kg/day during the treatment period. The stomach, liver, thyroid gland, spleen, and kidney were the target organs of toxicity at the end of the treatment period. For the stomach at doses \geq 5 mg/kg/day, findings were increased height of fundic mucosa, fundic gland ectasia, eosinophilic chief cells (fundus), mixed inflammatory cell infiltrate (fundus), mild fibrosis of the lamina propria (fundus), and hyperplasia of chromogranin-positive cells (fundus). 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. For the stomach at doses \geq 50 mg/kg/day in male rats, focal squamous cell hyperplasia (non-glandular stomach) was observed. For the liver at doses \geq 5 mg/kg/day, centrilobular hepatocellular hypertrophy was observed. Hepatocellular necrosis was observed at doses \geq 50 mg/kg/day. Additionally, one low dose treated male had a hepatocellular adenoma and another low dose male had a hepatocellular carcinoma. For the thyroid gland at doses \geq 50 mg/kg/day, follicular cell hypertrophy was observed. For the spleen at doses \geq 50 mg/kg/day, there was reduced hemosiderin. For the kidney, the incidence of mild to severe nephropathy was increased at doses \geq 50 mg/kg/day. The incidence of urothelial hyperplasia was increased at a dose of 300 mg/kg/day. Changes the kidney correlated with increased incidences of proteinuria at 50 and 300 mg/kg/day. At the end of 9-month recovery period, minimal fundic gland ectasia and minimal eosinophilic chief cells in the fundic mucosa were observed in rats that had received pantoprazole at 5 mg/kg/day. Additionally, a malignant neuroendocrine cell tumor (fundus) was observed for 1 of 11 female rats at 5 mg/kg/day. For rats that had received 5 mg/kg/day, there was no evidence of increased fundic mucosal height and hyperplasia of chromogranin-positive cells at the end of the recovery period. The presence of a gastric carcinoid at the end of recovery period for a female rat at 5 mg/kg/day suggests that cellular changes initiated during the treatment phase persisted through the recovery period. Gastric effects induced by pantoprazole at 5 mg/kg/day were not reversible.

DOG

Subacute Toxicology

Intravenous Route of Administration

Toxicity of Pantoprazole in Beagle Dogs Following Oral or Intravenous Administration for 2 Weeks with Special Emphasis on Toxic Effects on the Eye and Ear (GTR-32001).

Testing Laboratory: Byk Gulden
Institute of Pathology and Toxicology

Date Started: October 2, 1995

Date Completed: March 25, 1996

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

Animals: Beagle dogs were used in this study. Male and female dogs had average ages of 8.7 and 9.3 months, respectively. Body weight ranges were 8.6-11.2 kg for male dogs and 9.4-12.4 kg for female dogs.

Drug Batch: Pantoprazole for Oral Administration, Batch No. BY1023-20-1-1 and Lyophilized Pantoprazole for Intravenous Administration, Batch No. Ch.B.: 513150.

Methods: Compounds of the proton pump inhibitor class are suspected to cause visual and auditory disturbances. The subacute toxicity of pantoprazole administered by the intravenous and oral routes to beagle dogs was examined, with special emphasis on possible visual and auditory disturbances. Dogs received pantoprazole by the intravenous route at doses of 0 and 60 mg/animal/day or the oral route at doses of 40 or 160 mg/animal/day for 2 weeks (15-18 days). Approximate oral doses of pantoprazole were 3.8-4.0 and 15.1-16.0 mg/kg/day, respectively. The approximate intravenous dose of pantoprazole was 5.7-6.0 mg/kg/day. Dogs in the control groups received the vehicle, 0.9% NaCl, by the intravenous route. There were 2 dogs/sex/group. For the intravenous route, the dosing volume was 15 mL/kg. Hard gelatinous coated 40-mg tablets were used for oral administration. The plasma C_{max} for pantoprazole was found to occur within 3 hr after oral administration of these tablets. Animals were monitored for clinical signs of toxicity and mortality daily at 1, 2, 3, 4, and 24 hr after dosing. Body weight was measured on days -18, -4, 1, 4, 8, 11, and 15. Food consumption was measured daily. Physical examinations

consisting of inspection and palpation, pulse rate, body temperature, senses (i.e., muscle tone, sensitivity, reflexes, vision, hearing, CNS, autonomic nervous system), percussion (i.e., lungs), auscultation (i.e., lungs, heart, heart rate, abdomen) were performed at weeks -1 and 2. Ophthalmic examinations were performed as follows: pupillometry at days -14, 4, and 11; gonioscopy at day -18; tonometry at days -18, 5, and 12; slit light examination on days -18, 5, and 12, and funduscopy at days -18, 5, and 12. Electroretinograms, visual evoked cortical potentials, and auditory evoked potential were performed on days -3, 5, and 12 at approximately 3 hr after drug administration to coincide with the plasma C_{max} for pantoprazole. Blood for determination of hematology and clinical chemistry parameters was collected on days -20, -5, 3, and 11. Blood for measurement of plasma pantoprazole was collected on days 1 and 10 at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 24 hr after oral administration or at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 24 hr after intravenous administration. Cerebrospinal fluid and vitreous fluid from one eye were collected for measurement of pantoprazole levels following sacrifice. Dogs were sacrificed 3 hr after the last dose between days 15 and 18 due to collection of cerebrospinal fluid and vitreous fluid. Animals were subjected to a gross examination and organ weights were determined for the heart, liver, kidneys, brain, thyroid gland, adrenal glands, pancreas, testes, ovaries, uterus, spleen, lungs, pituitary gland, and prostate. Bone marrow from the femur (i.e., proximal and distal samples from the metaphysis and the diaphysis) was microscopically evaluated. Organs were collected, fixed, and microscopically evaluated as follows: heart, liver, kidneys, brain, thyroid gland, adrenal glands, pancreas, testes, ovaries, uterus, spleen, lungs, pituitary gland, prostate, skin, muscle (longissimus dorsi), various lymph nodes, aorta, tongue, esophagus, thymus, trachea, salivary glands, stomach, sections of the intestine, gall bladder, urinary bladder, eyes, spinal cord, sciatic nerve, optic nerve, cochlea, epididymides, mammary gland, and bone marrow (sternum).

Results:

1. Observed Effects: None.

2. Mortality: None.

3. Body Weight and Food Consumption: Body weight and food consumption were unaffected by either oral or intravenous treatment with pantoprazole. Body weights for male controls on days 1 and 15 were 9.65 and 9.80 kg, respectively, yielding a net change of 0.15 kg or a 1.55% increase of initial body weight. Body weights on day 15 for the male 40 mg/day-oral and 60 mg/day-IV groups were increased by 2.0 and 2.8% of initial body weights, respectively. Body weight for the male 160 mg/day-oral group was unchanged. Body weights for female controls on days 1 and 15 were 9.80 and 9.75 kg, respectively, yielding a net change of -0.05 kg or a 0.5% decrease of initial body weight. Body weights on day 15 for the female 40 mg/day-oral, 160 mg/day-oral, and 60 mg/day-IV groups were increased by 5.4, 4.8, and 1.3% of initial body weights, respectively. Food consumption for the male 160 mg/day-oral and 60 mg/day-IV groups were both increased to 108.2% of the control (305 g/animal/day), while consumption for the male 40 mg/day-oral group was unchanged. Food consumption for the female 40 mg/day-oral, 160 mg/day-oral, and 60 mg/day-IV groups were increased to 104, 105.6, and 106.4% of the control (312.5 g/animal/day), respectively.

4. Hematology, Blood Coagulation, and Bone Marrow: There were no treatment-related changes of hematological or blood coagulation parameters on days 3 and 11. The sponsor provided individual line listing for each animal. Values for male and female dogs from identical treatments were averaged together to allow an evaluation of the effects of pantoprazole. For microscopic evaluation of bone marrow, the sponsor evaluated the quantity of hematopoietic marrow and fat in the metaphysis (proximal and distal) and diaphysis (proximal and distal). There were no treatment-related changes in the quantity of hematopoietic marrow and fat in proximal and distal portions of the metaphysis and diaphysis.

5. Blood Biochemistry: ASAT activity for the 60 mg/day-IV group on day 11 was elevated to 263.6% of the control (13.75 U/L); although, this increase was due to 1 female dog with an activity at — U/L. There were no changes of serum electrolyte levels. The sponsor provided individual line listing for each animal. Values for male and female dogs from identical treatments were averaged together to allow an evaluation of the effects of pantoprazole.

6. Physical Examinations:

Body Temperature, Pulse, and Respiratory Rate: There were no treatment-related changes of body temperature, pulse, or respiratory rate on day 9. The sponsor provided individual line listing for each animal. Values for male and female dogs from identical treatments were averaged together to allow an evaluation of the effects of pantoprazole.

Ophthalmic and Auditory Examinations: Electroretinograms, visual evoked cortical potentials, and intraocular pressures for the right and left eyes on days 5 and 12 were unaffected by treatment. Auditory evoked potentials for the right and left ears on days 5 and 12 were unaffected by treatment. The sponsor provided individual line listing for each animal. Values for male and female dogs from identical treatments were averaged together to allow an evaluation of the effects of pantoprazole.

7. Organ Weights: Changes were observed for absolute heart, absolute right kidney weight, and relative brain weight in the 60 mg/day-IV group; although, there were no corresponding histopathological changes.

8. Gross Pathology: For one male and one female dog of the 60 mg/day-IV group, emphysema was observed in the right and left cranial lobes of the lung.

9. Histopathology: The target organs of toxicity were the stomach and the lungs. Parietal cell vacuolation and eosinophilic parietal cells were observed in the stomach for 1 male dog in 60 mg/day-IV group. Increased activity of lymph follicles in the gastric antrum was observed for 1 male dog in the 160 mg/day-oral group. Alveolar histiocytosis was observed in all three pantoprazole treatment groups. Alveolar emphysema was observed in the 40 mg/day-oral and 60 mg/day-IV groups.

Histopathological changes for beagle dogs that received pantoprazole at 0 mg/day-IV, 40 mg/day-oral, 160 mg/day-oral, or 60 mg/day-IV for 2 weeks. There were 2 dogs/sex/group.

Tissue/Organ	0 mg/day-IV		40 mg/day-Oral		160 mg/day-Oral		60 mg/day-IV	
	Male	Female	Male	Female	Male	Female	Male	Female
Gastric fundus								
-parietal cell vacuolation	0	0	0	0	0	0	1	0
-eosinophilic parietal cells	0	0	0	0	0	0	1	0
Gastric Antrum								
-activity of lymph follicles	0	0	0	0	1	0	0	0
Liver								
-lymphocytic infiltration	0	0	0	0	0	1	0	0
Lung								
-alveolar emphysema	0	0	0	1	0	0	1	1
-alveolar histiocytosis	0	0	0	1	1	0	0	1
-foamy alv. macrophage accumulation	0	0	0	0	0	0	1	0
-foreign body granuloma	0	0	0	0	1	0	0	0
-purulent bronchopneumonia	0	0	0	0	1	1	0	0
-interstitial pigment deposits	0	0	0	0	1	0	0	0
-subpleural lymphatic dilatation	0	0	0	0	0	0	0	0
-lympho-histiocytic infiltration	0	0	0	0	0	0	0	1
Thyroid, C-cells, 1								
-hyperplasia, diffuse	1	0	0	0	1	1	0	0
Thyroid, C-cells, 2								
-hyperplasia, diffuse	1	0	1	0	1	1	0	0
Parathyroid 1								
-cyst	0	0	0	0	0	0	0	1

10. Drug Levels: Data for drug levels in the plasma, cerebrospinal fluid, and vitreous fluid of one eye was not presented.

Compounds of the proton pump inhibitor class are suspected to cause visual and auditory disturbances. The subacute toxicity of pantoprazole administered by the intravenous and oral routes to beagle dogs was examined, with special emphasis on possible visual and auditory disturbances. Dogs received pantoprazole by the intravenous route at doses of 0 and 60 mg/animal/day or the oral route at doses of 40 or 160 mg/animal/day for 2 weeks (15-18 days). The study was flawed in that there were no corresponding controls for groups that received pantoprazole by the oral route. Electroretinograms, visual evoked cortical potentials, and intraocular pressures for the right and left eyes on days 5 and 12 were unaffected by treatment. Auditory evoked potentials for the right and left ears on days 5 and 12 were unaffected by treatment. The target organs of toxicity were the stomach and the lungs. Parietal cell vacuolation and eosinophilic parietal cells were observed in the stomach for 1 male dog in the 60 mg/day-IV group. Increased activity of lymph follicles in the gastric antrum was observed for 1 male dog in the 160 mg/day-oral group. Alveolar histiocytosis was observed in all three pantoprazole treatment groups. Alveolar emphysema was observed in the 40 mg/day-oral and 60 mg/day-IV groups.

NDA 20,987

Page 139

30-Day Intravenous Toxicity Study of Pantoprazole in the Dog (GTR-31904 and GTR-31193).

Testing Laboratories: []

Study Started: February 23, 1987

Study Completed: March 25 1988 (report date)

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

Animals: Beagle dogs (males: 6 months old, 9.6-12.6 g and females: 8 months old, 9.0-11.4 kg).

Drug Batch No.: 3 and 4.

Methods: Dose selection in this study was based on dose ranging studies (GTR-31903 and GTR-31905). In report GTR-31903, dogs were given escalating I.V. (30 min infusion) doses of 10, 20, 40, and 60 mg/kg/day of pantoprazole. Doses were escalated on alternate days. The dose of 60 mg/kg/day was given for 4 days followed by 80 mg/kg/day for one day. Female dog became agitated and hyperreactive after a second dose of 20 mg/kg. A dose of 40 mg/kg/day caused both male and female dogs to be lethargic and slightly unsteady. The dose of 60 mg/kg produced symptoms of loss of motor coordination and unsteady gait (both sexes) and vomiting in the female. Clinical signs seen after 80 mg/kg dose were similar to that seen at 60 mg/kg, but more severe in nature (female dog collapsed). Histopathological examinations revealed parietal cell vacuolation/degeneration in the stomach of each dog. Since 1 out of 2 dogs collapsed at the 80 mg/kg dose level, this dose was considered to have exceeded the maximum tolerated dose (MTD). In report GTR-31905, dogs (1/sex) were given I.V. dose of 40 mg/kg/day (infused over 30 min) for 10 days. This dose level produced clinical signs (slight ataxia, staggering gait on one or two occasions), subcutaneous hemorrhages at the injection sites (tail) and parietal cell vacuolation/degeneration in the stomach. Sponsor also administered a single dose of 60 mg/kg/day (I.V. infusion given over 30 min) to one male and one female dog. These dogs showed the above mentioned clinical signs and marked ataxia and collapsed to their hind limbs during infusion period. Based on the results of the above two dose ranging studies, 40 mg/kg/day was considered to be a maximum tolerated dose.

In the present study, groups of dogs (5/sex/group) were given I.V. (30-min infusion, 4 mL/kg/day) doses of 10, 20 and 40 mg/kg/day of pantoprazole for 30 days. Control group dogs received the vehicle (not identified) in similar fashion. A positive control group was also included which received — 95448-Z (40 mg/kg/day; it produces necrotizing vasculitis). All animals were observed daily for clinical signs and mortality. Body weight and food intakes were recorded daily. Ophthalmoscopic examinations were performed on all dogs at pre-test and during the last week of study period. ECG recordings were recorded during pre-test, prior to drug administration on days 15 and 30 of the study. Venous blood

samples were collected from all dogs at pre-test and prior to dosing on day 2, 15 and 30 of the study for hematology and clinical chemistry tests. Blood samples were also collected at pre-test and at 4 and 24 hr post-dosing on days 8, 16 and 28 of the study for measuring plasma gastrin. Additionally, blood samples were also collected at pre-dose and 30, 45, 90 min, 2.5, 4 and 7 hr after start of I.V. infusion on days 1 and 30 of the study to measure plasma drug levels. At the end of study period all dogs were sacrificed and subjected to complete histopathological examinations.

Results:

1. **Observed Effects:** Three out of 5 high dose treated males had unsteady gaits on 1-3 occasions. This unsteady gait lasted for about 30 min. Necrotizing arteritis was seen in one positive control treated dog and this finding was not seen in pantoprazole-treated dogs.
2. **Mortality:** None. One of the dogs from positive control group was killed on day 16 of the study due to treatment-related necrotizing vasculitis.
3. **Body Weight/Food Consumption/Water Consumption:** Treatment had no significant effect of body weights in males. Most of the females (including control) lost 0-5.7% of their weights during study period. Food intakes were not affected by the treatment.
4. **Hematology/Coagulation/Bone Marrow:** No treatment-related effects were seen. Significant increases in white blood cell counts were seen in dogs treated with positive control.
5. **Blood Chemistry/Urinalysis:** No treatment-related effects were seen.
6. **Vital Signs/Physical Examination/Ophthalmic Examinations/ECG:** No treatment-related effects were seen.
7. **Organ Weights:** In high dose-treated dogs, liver weights in males and females were increased by 17% and 27% of control values, respectively.
8. **Gross Pathology:** No tabulated summary was provided.
9. **Histopathology:** Parietal cell vacuolation in the stomach was seen in treated dogs (males: control = 0/5, low dose = 2/5, mid dose = 5/5 and high dose = 5/5; females: control = 0/5, low dose = 5/5, mid dose = 5/5 and high dose = 5/5). The degree of parietal vacuolation was minimal to slight. Positive control also produced similar degree of parietal cell vacuolation in the stomach.
10. **Plasma Gastrin Levels:** Both at 4 hr and 24 hr after drug administration on days 8, 16 and 28 of the study, plasma gastrin levels were increased significantly in treated dogs when compared to control values, however, the increase was not dose-related (increase in plasma gastrin levels were also seen in positive control group).

Plasma Gastrin Levels (pg/ml) on Day 28				
Treatment	Males		Females	
	4 hr	24 hr	4 hr	24 hr
Control	34.6 ± 19.0	46.4 ± 16.2	27.2 ± 7.9	35.8 ± 13.2
Low Dose (10 mg/kg)	514.6 ± 381.0	295.8 ± 198.9	379.8 ± 285.8	431.4 ± 384.0
Mid Dose (20 mg/kg)	733.8 ± 533	765.4 ± 909.8	273.8 ± 165.4	339.4 ± 89.7
High Dose (40 mg/kg)	1052.0 ± 1049.6	596.8 ± 328.1	485.2 ± 425.8	536.0 ± 291.3

11. Toxicokinetics: Plasma C_{max} and AUC values for male dogs on days 1 and 30 were approximately proportional to dose. However, plasma C_{max} and AUC values for female dogs were not proportional to dose due to unexpectedly high values with a dose of 10 mg/kg/day. Plasma C_{max} and AUC values for male dogs at a dose of 10 mg/kg/day on days 1 and 30 were approximately one-half of values observed for female dogs at the same dose; however, these observations were not found with doses > 10 mg/kg/day. These differences in C_{max} and AUC values for male and female dogs at 10 mg/kg/day do not appear to be related to differences in the terminal rate constant. The terminal rate constants for male dogs at 20 and 40 mg/kg/day on day 1 were similarly elevated as compared to values for female dogs; although, plasma pharmacokinetic parameters were similar.

Plasma pharmacokinetic parameters for — 96022 on days 1 and 30 for dogs that received — 96022 by the intravenous route at doses of 10, 20, or 40 mg/kg/day.

Day	Dose, mg/kg/day	C_{max} , $\mu\text{mole/L}$		T_{max} , hr		$AUC_{0-\infty}$, $\mu\text{mol}\cdot\text{hr/L}$		Terminal Rate Constant, hr^{-1}	
		Male	Female	Male	Female	Male	Female	Male	Female
1	10	54.02	93.14	0.5	0.5	38.46	81.7	2.17	1.63
	20	114.9	111.6	0.55	0.5	93.1	112.1	1.84	1.37
	40	192.6	201.4	0.5	0.5	221.4	267	1.16	0.92
30	10	50.9	92.36	0.5	0.5	36.3	76.46	2.40	2.06
	20	106.24	97.62	0.5	0.5	100.22	86.04	1.59	1.79
	40	182.6	192.2	0.5	0.5	209.6	209	1.20	1.44

12. Hepatic Cytochrome P450 Levels (GTR-31207): Cytochrome P450 content was only measured in control and high dose treated dogs. Pantoprazole had no significant effects on hepatic ethylmorphine N-demethylase activity (control: males = 26.0 I.U., females = 20.9 I.U.; 40 mg/kg/day: males = 31.5 I.U. and females = 22.3 I.U.) nor on cytochrome P450 contents (control = — nmoles/g liver and 40 mg/kg/day = — nmoles/g liver).

In this study, the target organ of toxicity was stomach, which can be considered as exaggerated pharmacological effect of proton pump inhibitor (pantoprazole). Therefore, if we disregard this effect on stomach, then 40 mg/kg/day can be considered as no effect dose.

Four Week Toxicity of Pantoprazole in the Unrestrained Dog By 24 Hour Continuous Intravenous Infusion (GTR-32002).

Testing Laboratory:



Byk Gulden
Konstanz, Germany

Date Started: July 9, 1996 for female dogs
August 13, 1996 for male dogs

Date Completed: February 24, 1997

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

Animals: Beagle dogs were used in this study. At the start of treatment, male and female dogs were 7 to 9.5 months of age and had a body weight range of 6.2-10.2 kg.

Drug Batch: Pantoprazole, batch number 513150.

Methods: Beagle dogs received pantoprazole by continuous intravenous infusion at doses of 0, 6, 18, and 36 mg/kg/day for 4-weeks. The control and 36 mg/kg/day groups had 5 dogs/sex/group. The 6 and 18 mg/kg/day groups had 3 dogs/sex/group. Two dogs/sex from the control and 36 mg/kg/day groups entered an 8-week recovery period following the 4-week treatment period. Pantoprazole was administered as a 0.4 mg/mL solution in 0.9% NaCl. Control animals received the vehicle, 0.9% NaCl. The dose volume for the control and 36 mg/kg/day groups was 90 mL/kg/day. The dose volume for the 6 and 18 mg/kg/day groups was 15 and 45 mL/kg/day, respectively. Eleven or 21 days prior to the start of treatment, an intravenous polyurethane catheter fitted with a silicon, resealable access port was surgically implanted into the jugular vein of each dog. Following recovery from surgery, but at least 7 days prior to the start of treatment, dogs were fitted with a jacket designed to hold a _____ infusion pump. During the course of the study, it was necessary to implant new catheters into a number of dogs due to accidental removal of catheters, defects in catheters, or occlusion of the vein. Dogs were observed for clinical signs of toxicity and morbidity/mortality at least twice per day. Food consumption was measured daily. Body weight was measured weekly. Ophthalmic examinations were performed prior to start of treatment, during week 4 of the treatment period, and during week 8 of the recovery period. Electrocardiograms were obtained from each dog using _____ and _____ leads prior to the start of treatment, during weeks 2 and 4 of treatment, and during week 8 of the recovery period. Heart rate, P wave duration and amplitude, and P-Q, QRS, and Q-T intervals were measured using a

representative section of the electrocardiogram from lead II. Blood for determination of hematological and clinical chemistry parameters was collected prior to start of treatment, during weeks 1 and 4 of treatment, and during week 8 of recovery. Thyroid stimulating hormone (TSH) was measured by _____ Urinalysis was performed prior to start of treatment, during weeks 1 and 4 of treatment, and during week 8 of recovery. Blood for determination of serum gastrin levels was collected prior to the start of treatment, during weeks 1 and 4 of the treatment, and during week 8 of recovery at 07⁰⁰, 10⁴⁵ (shortly before feeding), and 15⁰⁰. Gastrin was measured by _____. After the 4-week treatment period, 3 dogs/sex/group were sacrificed. Following an 8-week recovery period, 2 dogs/sex from the control and 36 mg/kg/day groups were sacrificed. Animals were subjected to a gross pathological examination and any macroscopic abnormalities were recorded. Organ weights (absolute, relative to body weight, and relative to brain weight) were determined for the adrenal glands, brain (including brainstem), heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes with epididymides, and thyroid gland with parathyroid. Organs and tissues were collected, processed, and examined microscopically as follows (organs in parentheses were only examined if indicated by signs of toxicity or target organ involvement): adrenal glands, aorta, bone-femur including articular surface, bone marrow-sternum, brain-including sections of medulla/pons, cerebral and cerebellar cortex, optic chiasma, facial nerve, contralateral jugular vein, epididymides, esophagus, eyes with optic nerve, female mammary gland area, (male mammary gland area), gallbladder, heart, infusion site, kidneys, large intestine-cecum, colon, and rectum, larynx, liver, lung-infused with formalin, lymph nodes-retropharyngeal and mesenteric, ovaries, pancreas, pituitary gland, prostate gland, salivary glands-mandibular, parotid, and (sublingual), sciatic nerve, skeletal muscle, skin, small intestine-duodenum, jejunum, and ileum, spinal cord-cervical, midthoracic, and lumbar segments, spleen, stomach, testes, thymus, thyroid gland including parathyroid gland, tongue, trachea, urinary bladder, uterus (with vagina), and all gross lesions.

Results:

1. Observed Effects: There were no treatment-related observed effects.

2. Mortality: There was no treatment-related mortality. One control male died on day 28. The sponsor attributed death to circulatory failure following complications with intravenous administration through the jugular vein. This male control dog as well as two other control animals (1 male and 1-female) were not infused with saline during the last 5 to 8 days of treatment due to occlusion of the jugular vein.

3. Body Weight and Food Consumption: There were no treatment-related effects on body weight gain or food consumption during the treatment or recovery periods. Body weights for male controls on days 1 and 29 of the treatment period were 8.2 and 8.4 kg, respectively, yielding a net change of 0.2 kg or a 2.4% increase of initial body weight. Body weights for the male 6 and 18 mg/kg/day groups were by 1.27 and 2.60% of initial body weight, respectively. Body weight for the male 36 mg/kg/day group was unchanged. Body weights for male controls on days 8 and 57 of recovery were 9.1 and 10.0 kg, respectively, yielding a net change of 0.9 kg or a 9.9% increase of body weight on day 8 of recovery. Body weight for the male 36 mg/kg/day group was increased by 12.8% during the recovery period. Body weights for female controls on days 1 and 29 were 8.0 and 7.8 kg, respectively, yielding a net change of -0.2 kg or a 2.5% decrease of initial body weight.

Body weight for the female 6 mg/kg/day was unchanged. Body weight for the female 18 mg/kg/day group was increased by 1.45% of initial body weight, while it was decreased by -2.78% for the female 36 mg/kg/day group. Body weights for the female controls on days 8 and 57 of recovery were 8.0 and 8.8 kg, respectively, yielding a net change of 0.8 kg or a 10% of body weight on day 8 of recovery. Body weight for the female 36 mg/kg/day group was increased 7.8% during the recovery period.

4. Hematology: A number of changes in hematological parameters were observed for treatment groups; although, dose response relationships were weak and the relation of changes to treatment was not clear.

Male Dogs at Week 1: Platelet counts for the male 6, 18, and 36 mg/kg/day groups were increased to 192, 223.1, and 222.2% of the control ($117 \times 10^9/L$), respectively. The relative lymphocyte percent for the male 36 mg/kg/day group was increased to 153.3% of the control (0.15). The absolute lymphocyte count for the male 18 and 36 mg/kg/day groups were increased to 125 and 137.5% of the control ($2.4 \times 10^9/L$), respectively.

Male Dogs at Week 4: White blood cell counts for the male 18 and 36 mg/kg/day groups were decreased to 86.1 and 75.9% of the control ($13.7 \times 10^9/L$), respectively. The absolute segmented neutrophil counts for the male 18 and 36 mg/kg/day groups were decreased to 73.2 and 63.4% of the control ($11.2 \times 10^9/L$), respectively. The relative lymphocyte percent for the male 18 and 36 mg/kg/day groups were increased to 153.3 and 193.3% of the control (0.15), respectively. The absolute lymphocyte counts for the male 6, 18, and 36 mg/kg/day groups were increased to 120, 130, and 145% of the control ($2.0 \times 10^9/L$), respectively.

Male Dogs at Week 8 of Recovery: Red blood cell counts, hemoglobin levels, and hematocrit for the male 36 mg/kg/day group during week 8 of recovery were increased to 117.1, 115.5, and 116.2% of the control ($5.49 \times 10^{12}/L$, 7.7 mmole/L, and 37%), respectively. The relative lymphocyte percent for the male 36 mg/kg/day group was increased to 128.6% of the control. The absolute segmented neutrophil count for the male 36 mg/kg/day group was decreased to 82.6% of the control ($13.2 \times 10^9/L$).

Female Dogs at Week 1: Reticulocyte counts for the female 6, 18, and 36 mg/kg/day groups were increased to 112.6, 149, and 149% of the control ($0.0484 \times 10^{12}/L$), respectively. White blood cell counts for the female 6, 18, and 36 mg/kg/day groups were decreased to 56.6, 51.6, and 67.9% of the control ($15.9 \times 10^9/L$), respectively. The absolute segmented neutrophil counts for the female 6, 18, and 36 mg/kg/day groups were decreased to 45.1, 41.8, and 55.7% of the control ($12.2 \times 10^9/L$), respectively. The relative lymphocyte percentages for the female 6, 18, and 36 mg/kg/day groups were increased to 178.95, 168.4, and 173.7% of the control (0.19), respectively.

Female Dogs at Week 4: None.

Female Dogs at Week 8 of Recovery: Red blood cell counts, hemoglobin levels, and hematocrit for the female 36 mg/kg/day group were decreased to 87.6, 87.6, and 87.8% the control ($7.24 \times 10^{12}/L$, 10.5 mmole/L, and 49%), respectively. The relative and absolute reticulocyte counts for the female 36 mg/kg/day group were decreased to 69.5 and 60.7% of the control (0.82% and $0.0595 \times 10^{12}/L$), respectively.

5. Blood Biochemistry and Urinalysis: A number of changes in clinical chemistry and urinalysis parameters were observed for treatment groups; although, dose response relationships were generally flat or weak and the relation of changes to treatment was not clear.

Male Dogs at Week 1: Urea levels for the male 6, 18, and 36 mg/kg/day groups were increased to 181.6, 135.4, and 141.1% of the control (3.53 mmole/L), respectively. Bilirubin levels for the male 18 and 36 mg/kg/day groups were increased to 133.5 and 123.4% of the control ($3.55 \mu\text{mole}/L$), respectively. For urinalysis, osmolality values for the male 6, 18, and 36 mg/kg/day were increased by $> 200\%$; however, there was no dose response relationship.

Male Dogs at Week 4: Urea levels for the male 6, 18, and 36 mg/kg/day groups were increased to 226.7, 142.6, and 155.7% of the control (3.52 mmole/L), respectively. Total lipids for the male 36 mg/kg/day group was increased to 140.6% of the control (3.2 g/L). Cholesterol levels for the male 18 and 36 mg/kg/day groups were increased to 122.4 and 157.2% of the control (6.80 mmol/L), respectively. Triglyceride levels for the male 18 and 36 mg/kg/day groups were increased to 135.2 and 185.2% of the control (0.54 mmol/L), respectively. Absolute albumin levels for the male 36 mg/kg/day group were increased to 126.8% of the control (17.9 g/L). For urinalysis, osmolality values for the male 6, 18, and 36 mg/kg/day were increased by $> 150\%$; however, there was no dose response relationship.

Male Dogs at Week 8 of Recovery: γ -Glutamyl transpeptidase activity for the male 36 mg/kg/day group was increased to 154.7% of the control (38.11 nKat/L). Serum iron levels were decreased to 74.7% of the control ($36.96 \mu\text{mole}/L$). For urinalysis, the osmolality value was increased to 156% of the control (623 mmole/kg).

Female Dogs at Week 1: Urea level for the female 6, 18, and 36 mg/kg/day groups were increased to 195.8, 214.8, and 243% of the control (2.63 mmole/L), respectively. Creatinine levels for the female 18 and 36 mg/kg/day groups were increased to 125.3 and 122% of the control ($45.1 \mu\text{mole}/L$), respectively. Total bilirubin levels for the female 36 mg/kg/day group were increased to 127.2% of the control ($4.08 \mu\text{mole}/L$). ALAT activities for the female 6, 18, and 36 mg/kg/day groups were increased to 127.5, 132.5, and 135% of the control ($0.40 \mu\text{Kat}/L$), respectively. γ -Glutamyl transpeptidase activities for the female 6, 18, and 36 mg/kg/day groups were increased to 141-147.6% of the control (25.04 nKat/L). Serum iron levels for the female 6, 18, and 36 mg/kg/day groups were increased to 131.4, 180.4, and 190.7% of the control ($17.65 \mu\text{mole}/L$), respectively. Magnesium levels

for the female 6, 16, and 36 mg/kg/day groups were increased to 109.2-118.4% of the control (0.76 mmol/L). Slight alterations ($\pm 20\%$ of control) in triiodothyronine (T_3) and thyroxine (T_4) levels observed for females at 6, 18, and 36 mg/kg/day appeared to have no biological significance. Total protein levels for the female 18 and 36 mg/kg/day groups were decreased to 87.7 and 86% of the control (61.6 g/L), respectively.

Female Dogs at Week 4: Urea level for the female 6, 18, and 36 mg/kg/day groups were increased to 171.1, 174, and 157.9% of the control (3.11 mmole/L), respectively. Total bilirubin levels for the female 36 mg/kg/day group were increased to 121% of the control (4.20 μ mole/L). Total lipid levels for the female 18 and 36 mg/kg/day groups were increased to 139.4 and 166.7% of the control (3.3 g/L), respectively. Total cholesterol levels for the female 6, 18, and 36 mg/kg/day groups were increased to 131.4, 166.9, and 182% of the control (4.90 mmole/L), respectively. Triglyceride levels for the female 18 and 36 mg/kg/day groups were increased to 121.3 and 167.2% of the control (0.61 mmole/L), respectively. Phospholipid levels for the female 36 mg/kg/day group were increased to 142.5% of the control (4.35 mmol/L), respectively. γ -Glutamyl transpeptidase activities for the female 6, 18, and 36 mg/kg/day groups were increased to 164.5, 137.6, and 175.4% of the control (24.44 nKat/L), respectively. Iron levels for the female 6 and 36 mg/kg/day groups were increased to 146.9 and 156.3% of the control (30.85 μ mole/L), respectively; although, no change was evident for the 18 mg/kg/day group. Magnesium levels for the female 6, 18, and 36 mg/kg/day groups were increased to 115.4-120.5% of the control (0.78 mmole/L). Slight alterations ($\pm 20\%$ of control) in T_4 levels for females at 6, 18, and 36 mg/kg/day appeared to have no biological significance.

Female Dogs at Week 8 of Recovery: Urea and creatinine levels for the female 36 mg/kg/day group were increased to 129 and 122.3% of the control (4.38 and 5.65 mmole/L), respectively. Total lipid and triglyceride levels for the female 36 mg/kg/day group were increased to 140 and 184.4% of the control (2.5 g/L and 0.32 mmol/L), respectively. ASAT, ALAT, LDH, and CK activities for the 36 mg/kg/day group were increased to 173.8, 144.7, 132.9, and 161.8% of the control (0.47, 0.47, 1.43, and 2.33 μ Kat/L), respectively. Slight alterations ($\pm 20\%$ of control) in TSH and T_4 levels for females at 36 mg/kg/day appeared to have no biological significance. Relative and absolute γ -globulin levels for the female 36 mg/kg/day group was decreased to 60.5 and 53.7% of the control (0.086 and 5.4 g/L), respectively. No treatment-related changes in urinalysis were observed.

Serum Gastrin Levels: Serum gastrin levels were increased at weeks 1 and 4 for pantoprazole treatment groups; however, there was not a dose response relationship.

Serum gastrin levels (ng/L) at weeks 1 and 4 for dogs (male + female) that received pantoprazole by continuous intravenous infusion at doses of 0, 6, 18, and 36 mg/kg/day.

Dose mg/kg/day	Week 1			Week 4		
	07 ⁰⁰	10 ⁴⁵	15 ⁰⁰	07 ⁰⁰	10 ⁴⁵	15 ⁰⁰
0	< 29.8	< 27.0	< 56.0	< 27.0	< 27.0	< 31.4
6	< 197.6	< 110.7	421.8	< 220.1	< 96.9	325.6
18	360.9	244.3	453.4	393.9	192.2	507.5
36	< 100.6	71.1	303.2	346	117.6	643

6. Physical Examinations: Ophthalmic and electrocardiogram examinations were performed prior to the start of treatment, and during the treatment and recovery periods.

Ophthalmic Examination: There were no treatment-related ophthalmic effects found during the treatment or recovery periods.

Electrocardiogram Examination: Minor electrocardiogram variations were observed between the control and treatment groups during the treatment and recovery periods that appeared to have little biological significance. P wave amplitude for the male 6, 18, and 36 mg/kg/day groups during week 4 were increased to 131.6, 147.4, and 131.6% of the control (0.19 mV), respectively; although, there was no evidence of a dose response relationship. P wave amplitude for the female 6, 18, and 36 mg/kg/day groups during week 4 were all decreased to 79.3% of the control (0.29 mV). P wave amplitude for the female 36 mg/kg/day group at the end of the recovery period was decreased to 60.6% of the control (0.33 mV).

7. Organ Weights: There were no treatment-related changes in absolute or relative organ weights for the male pantoprazole groups. Absolute and relative liver weight for female 36 mg/kg/day group were increased to 124.7 and 130.3% of the control (253.5 g and 3.3%), respectively.

8. Gross Pathology: There were no treatment-related gross pathological findings at the end of the treatment or recovery periods.

9. Histopathology: The stomach and lungs were the target organs of toxicity. For the stomach, an increased incidence of apoptosis of parietal cells was found for pantoprazole treatment groups. This histopathological change was attributed to a pharmacological alteration in the cell cycle of the parietal cell, the acid-producing cell of the gastric mucosa. For the lungs, subacute inflammation was observed for all pantoprazole treatment groups; however, there was no dose response relationship. Changes in stomach parietal cells were not evident following an 8 week recovery period. For the lung, subacute inflammation was still present following the recovery period.

APPEARS THIS WAY
ON ORIGINAL

Histopathological changes following a 4 week treatment period in which dogs received pantoprazole by continuous intravenous infusion at doses of 0, 6, 18, and 36 mg/kg/day (n = 3 dogs per group).

Organs/Tissues	0 mg/kg/day		6 mg/kg/day		18 mg/kg/day		36 mg/kg/day	
	Male	Female	Male	Female	Male	Female	Male	Female
Stomach -apoptosis, parietal cells	0	1	2	1	2	2	3	3
Lungs -subacute inflammation	0	1	3	2	2	3	3	2
Bone marrow-sternum -fatty replacement -erythroid hyperplasia	0 0	1 0	3 0	3 0	3 0	3 0	2 0	3 2
Mandibular gland -microsialoliths	0	0	0	2	0	4	4	2
Parotid gland -lymphoid cell foci	0	1	2	0	1	0	2	2
Kidneys -inflammation, interstitium -tubular vacuolation	0 0	1 0	0 0	0 0	4 0	0 0	0 0	4 2
Eyes, n = 6 -corneal dystrophy	0	0	0	0	0	0	1	0
Infusion port site -inflammation, chronic	0	2	4	6	6	6	4	6

Histopathological changes following an 8 week recovery period for dogs that received pantoprazole by continuous intravenous infusion at doses of 0 or 36 mg/kg/day for 4 weeks.

Organ/Tissues	0 mg/kg/day		36 mg/kg/day	
	Male	Female	Male	Female
Lungs -subacute inflammation	1	1	2	2
Kidneys -hyaline casts -inflammation, pelvic -inflammation, interstitium -fibrosis, interstitial -tubular basophilia	0 1 0 0 0	0 0 0 0 0	2 2 1 2 0	0 2 0 0 1
Mandibular gland -microsialoliths	0	0	2	2
Thyroid gland, n = 4 -cyst/cystic follicle -mineralization -lymphoid cell foci	0 1 0	2 0 0	0 1 1	0 3 0
Brain-optic chiasm -inflammatory cells	0	0	0	2

Beagle dogs received pantoprazole by continuous intravenous infusion at doses of 0, 6, 18, and 36 mg/kg/day for 4-weeks. The control and 36 mg/kg/day groups had 5 dogs/sex/group. The 6 and 18 mg/kg/day groups had 3 dogs/sex/group. Two dogs/sex from the control and 36 mg/kg/day groups entered an 8-week recovery period following the 4-week treatment period. The dose of 36 mg/kg/day appeared to be well tolerated. The stomach and lungs were the target organs of toxicity. For the stomach, an increased incidence of apoptosis of parietal cells was found for pantoprazole treatment groups. This histopathological change was attributed to a pharmacological alteration in the cell cycle of the parietal cell, the acid-producing cell of the gastric mucosa. For the lungs, subacute inflammation was observed for all pantoprazole treatment groups; however, there was no dose response relationship. Changes in stomach parietal cells were not evident following an 8 week recovery period. For the lung, subacute inflammation was still present following the recovery period.

Oral Route of Administration

10-Day Oral Toxicity Study in Dogs (GTR-32003).

Testing Laboratory:

Date of the Study: Oct 12, 1987 to Dec. 2, 1988.

GLP Requirement: A statement of compliance with GLP regulations was included.

Animals: Beagle dogs were obtained from _____ At the start of treatment, dogs were 9-11 months of age and the body weight range was 10.8-15.4 kg.

Drug Batch: Enteric and uncoated tablets were manufactured from pantoprazole batches 3 and 4.

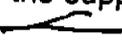
Methods: The toxicity of enteric coated tablets of pantoprazole (75 and 100 mg/kg/day) and uncoated tablets (50, 75, 100, and 150 mg/kg) were compared in beagle dogs over a 10-day treatment period. The treatment schedule is shown in the table below. There was 1 dog/sex/group. Tablets were administered in hard gelatin capsules. Animals were monitored several times per day for clinical signs of toxicity and morbidity/mortality. Body weight and food consumption were measured daily. Electrocardiograms were measured in all dogs prior to the start of treatment and daily for surviving animals in Groups 1-4 from days 4 to 10 and Groups 5 and 6 from days 1-10. Blood for determination of hematology and clinical chemistry parameters was collected prior to the start of treatment and on days 2 and 10. Urine for determination of urinalysis parameters was collected prior to the start of treatment and on days 2 and 10. Animals that died or were sacrificed during the 10-day treatment period and animals sacrificed after the treatment period were subjected to a gross necropsy. Organs/Tissues were examined by light microscopy as follows: liver, heart, lungs, thymus, adrenals, kidneys, stomach, bone marrow (sternum), and macroscopic abnormalities.

10-Day Treatment Schedule

Group	Dose, mg/kg/day	Tablet Formulation
1	100 ^A	Enteric-Coated
2	75	Enteric-Coated
3	150	Uncoated
4	100	Uncoated
5	75 ^B	Uncoated
6	50 ^B	Uncoated

A. Dose for male dog was reduced to 75 mg/kg/day on day 5.

B. These groups were added due to toxicity observed in Groups 1-4.

Results: Reversible ataxia sometimes accompanied by vomiting was observed in all treatment groups, with the male dog in Group 3 observed with most severe changes. Approximately 11 hr after the third dose, female dogs in Groups 1 and 2 were found dead. Animals in Group 3 were sacrificed in moribund condition on day 4. Groups 5 and 6 were added due to toxicity observed in Groups 1-4. The female dog in Group 6 developed audible respiratory problems on the second day of treatment, and was sacrificed on day 5. Body weight and food consumption were unaffected. There were no treatment-related changes found in the electrocardiogram. White blood cell counts were increased for all dogs on day 2 of treatment. Serum alkaline phosphatase activity was increased on day 10 for all surviving dogs. Urinalysis for female dogs on day 2 suggested increases of osmolality, protein, and glucose levels. Necropsy examinations of all dogs that were found dead or sacrificed in a moribund condition during the treatment period were found to have evidence of pulmonary edema. Histopathological analysis of the lungs from these dogs revealed effusion of fluid into the pulmonary alveoli. Findings included foamy alveolar macrophages and eosinophilic material in alveoli suggestive of proteinaceous fluid. In dogs that received uncoated tablets at doses of 75 and 100 mg/kg/day, there were also findings of peribronchiolar/perivascular edema and edema of the mediastinal structures (i.e., thymus, heart/pericardium, and esophagus). Dogs sacrificed after 10 days of treatment showed no evidence of pulmonary edema. Minimal to moderate parietal cell vacuolation was found in the stomach for most of the dogs. The sponsor speculated that pulmonary toxicity induced by pantoprazole was most severe on days 3 to 5; however, if the effects were not severe enough to be fatal, resolution occurred with continued dosing by day 10. Further, the sponsor speculated that pulmonary toxicity may be related to the supplier, with dogs supplied by  being more sensitive than those supplied by .

Urinalysis for female treatment groups at pretest and on day 2.

Female Group #	Osmolality, mOsm		Protein g/L		Glucose, mmol/L	
	Pretest	Day 2	Pretest	Day 2	Pretest	Day 2
1	147	1360	0.05	0.43	0.03	0.55
2	269	1779	0.07	0.49	ND	0.45
3	942	994	0.15	0.35	0.14	0.28
4	783	1357	0.19	0.43	0.19	0.50
5	2196	2595	0.61	0.66	0.07	0.52
6	469	2892	0.56	0.53	0.05	0.35

Note: Dogs in Groups 1, 2, 3, and 6 died or were sacrificed prior to urinalysis on day 10.

The toxicity of enteric coated tablets of pantoprazole (75 and 100 mg/kg/day) and uncoated tablets (50, 75, 100, and 150 mg/kg) were compared in beagle dogs over a 10-day treatment period. The following dogs died or were sacrificed in moribund condition during the treatment period: females that received the enteric-coated formulation at 75 or 100 mg/kg/day, the male and female that received the uncoated formulation at 100 mg/kg/day, and the female that received the uncoated formulation at 50 mg/kg/day. Necropsy examinations of all dogs that were found dead or sacrificed in a moribund condition during the treatment period were found to have evidence of pulmonary edema. Histopathological analysis of the lungs from these dogs revealed effusion of fluid into the pulmonary alveoli. Findings included foamy alveolar macrophages and eosinophilic material in alveoli suggestive of proteinaceous fluid. In dogs that received uncoated tablets at doses of 75 and 100 mg/kg/day, there were also findings of peribronchiolar/perivascular edema and edema of the mediastinal structures (i.e., thymus, heart/pericardium, and esophagus). Dogs sacrificed after 10 days of treatment showed no evidence of pulmonary edema. Minimal to moderate parietal cell vacuolation was found in the stomach for most of the dogs.

30-Day Oral Toxicity Study in Dogs (GTR-32082 and GTR-31200).

Testing Laboratory: _____

Date of the Study: Jan. 8, 1988 to Dec. 15, 1988.

GLP Requirement: A statement of compliance with GLP Regulations was included.

Animals: Beagle dogs weighing 7.4-13.2 kg and 7-8 months of age were used. Dogs were obtained from _____

Methods: Five groups of animals each consisting of five males and five females were given pantoprazole (batch no. 5) in capsules at acid dose levels of 0, 7.5, 15, 30 and 100 mg/kg/day. Two animals per sex from each groups were sacrificed after 5 days and examined specifically for pulmonary changes. The remaining animals were treated for 30 days. The right apical lung lobe of each dogs was lavaged on days 6 or 30 with a balanced salt solution and the resulting lavage fluid was examined for protein and cell content. Plasma toxicokinetic parameters were determined for pantoprazole and its sulfone metabolite on days 1, 5, and 30 of the 30-day oral toxicity study in dogs. Beagle dogs received pantoprazole by the oral route at doses of 7.5, 15, 30, and 100 mg/kg/day. Pantoprazole and its sulfone metabolite were measured _____

Results:

1. Clinical Signs (daily): Ataxia was observed in the 100 mg/kg/day group. Loose/liquid feces and vomiting were seen in all dose groups.

2. Mortality: None.

3. Body Weight and Food Consumption (daily): There were no treatment-related changes.

4. Ophthalmoscopic Examination (day 30): Normal.

5. EKG (days 0, 3 and 30): Normal.

6. Hematology (days 0, 2 and 30): Platelet counts on days 19 and 30 for male dogs that received 30 mg/kg/day were increased to 112.9 and 127.7% of control values ($387 \times 10^9/L$ and $347 \times 10^9/L$), respectively. Platelet counts on days 2, 19, and 30 for male dogs that received 100 mg/kg/day were increased to 130.2, 125.8, and 140.35% of control values ($338 \times 10^9/L$, $387 \times 10^9/L$, and $347 \times 10^9/L$), respectively. The reticulocyte percentage on day 2 for dogs that received 30 and 100 mg/kg/day were increased to 175 and 275% of the control (0.4%), respectively. There were increases in white cell counts at all dose levels on day 2 and 19 (13 - 37%) but not on day 30. However, none of the values were outside the normal range for the laboratory. There were no effects on prothrombin time and activated partial thromboplastin time.

7. Blood Chemistry (days 0, 2 and 30): Decreases in AST (20 - 35%) and ALT (26 - 74%) were seen in male dogs on day 30 at doses ≥ 7.5 mg/kg/day. For the female dogs on day 30, there was a dose-related fall (10 - 50%) in ALT activity at doses ≥ 15 mg/kg/day. There were no treatment-related changes of fibrinogen levels on days 2, 4, and 7. BUN levels on day 30 for female treatment groups were elevated to 129.8-144.7% of the control (4.7 mmol/L).

8. Urinalysis (days 0, 2 and 30): Glucose levels on days 2 and 30 for female treatment groups were elevated to 550-850% and 185.7-285.7% of control values (0.02 and 0.07 mmol), respectively; although, dose response relationships were not evident. Protein levels on days 2 and 30 for female treatment groups were elevated to 118.8-175% and 132.3-209.7% of control values (0.32 and 0.31 g/L), respectively; although, dose response relationships were not evident. Urinary pH values on day 30 for male rats that received doses ≥ 15 mg/kg/day were elevated to 122.8-133.5% of the control (pH 5.7); although, dose response relationships were not evident. Protein levels on day 30 for male treatment groups were elevated to 240.7-629.6% of the control (0.27 g/L); although, dose response relationships were not evident.

9. Organ Weight: Higher liver weights (8-34%) were reported in the male of all dose groups on days 5 and 30. For females, higher liver weights were seen in the 15 mg/kg/day and above groups on day 5 (11-20%) and in the 100 mg/kg/day group on day 30 (15%). Increase in stomach weights were (5-11%) reported in the 15 mg/kg/day and above groups on days 5 and 30. Decrease in thyroid weight (11-22%) were observed in female groups at 15 mg/kg/day and above dose levels whereas increases in thyroid weight were observed in male groups (15-29%) at 30 and 100 mg/kg/day dose levels. Higher protein concentration (360%) were observed in the lung lavage fluid in the 100 mg/kg/day male groups treated for 5 days but no such changes were detected after 30 days of dosing.

10. Gross Pathology: Normal.

11. Histopathology: Alveolar foamy macrophage were detected in the 100 mg/kg/day group on day 6 but no such changes were noted on day 30. One dogs receiving 30 mg/kg/day was killed on day 6 had alveolar hemorrhage. However, similar change was noted in the control female on day 30. Parietal cell vacuolation in the stomach was detected at doses ≥ 15 mg/kg on day 30. There were no histopathological changes in the kidney that correlated with elevations of BUN levels and urinalysis changes. Based upon lung lavage content of protein and foamy alveolar macrophages, lung injury appears to be most severe 3 to 5 days after the start of treatment, with resolution (i.e., adaptation) of changes occurring during continued treatment. The sponsor stated that beagle dogs obtained from different commercial suppliers demonstrated different sensitivities to pantoprazole-induced lung injury.

12. Plasma Drug Levels: Plasma C_{max} and AUC values for pantoprazole on days 1, 5, and 30 in male and female dogs generally increased in a manner proportional to increasing dose (i.e., linear toxicokinetics). Half-life values at doses of 7.5 to 30 mg/kg/day ranged from _____ hr. However, at a dose of 100 mg/kg/day, half-life values ranged from _____ hr. Slower absorption of pantoprazole at the high dose may have contributed to the longer rate of elimination. Bioavailability of pantoprazole at oral doses of 7.5 and 15 mg/kg/day for male dogs ranged from 70.6 to 97.3%, while for female dogs, values ranged from 41.6 to 58.8%. Bioavailability of pantoprazole at oral doses of 30 and 100 mg/kg/day for male dogs ranged from 126 to 188%, while for female dogs, values ranged 71.8 to 78.9%. Despite the apparent higher bioavailabilities in male dogs, there were no gender-related differences in observed C_{max} and AUC values. Bioavailability values were probably only valid at the lower doses of 7.5 and 15 mg/kg/day as bioavailability values in male dogs at the higher doses of 30 and 100 mg/kg/day exceeded 100%.

Plasma C_{max} values ($\mu\text{mole/L}$) and $\text{AUC}_{0-24\text{hr}}$ values ($\mu\text{mole}\cdot\text{hr/L}$) for pantoprazole on days 1, 5, and 30 for beagle dogs that received pantoprazole by the oral route at doses of 7.5, 15, 30, and 100 mg/kg/day.

Dose, mg/kg/day	Day 1				Day 5				Day 30			
	C_{max} ($\mu\text{mole/L}$)		$\text{AUC}_{0-24\text{hr}}$ ($\mu\text{mole}\cdot\text{hr/L}$)		C_{max} ($\mu\text{mole/L}$)		$\text{AUC}_{0-24\text{hr}}$ ($\mu\text{mole}\cdot\text{hr/L}$)		C_{max} ($\mu\text{mole/L}$)		$\text{AUC}_{0-24\text{hr}}$ ($\mu\text{mole}\cdot\text{hr/L}$)	
	M	F	M	F	M	F	M	F	M	F	M	F
7.5	5.77	7.56	33.0	43.0	4.51	5.86	25.5	30.7	6.72	6.23	38.2	33.5
15	12.0	11.6	71.8	76.3	5.69	9.47	31.1	56.4	11.2	10.3	59.2	58.5
30	29.5	29.4	187	183	29.2	24.7	174	141	43.0	40.0	171	185
100	83.8	70.6	659	489	88.0	79.9	564	470	85.4	92.7	530	539

In a 30-day oral toxicity study, beagle dogs received pantoprazole at doses of 0, 7.5, 15, 30, and 100 mg/kg/day. There were 5 dogs/sex/group. Two dogs/sex/group were sacrificed after 5 days and examined specifically for pulmonary changes. Remaining dogs were sacrificed after the 30-day treatment period. It appears that the dose of 15 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. Higher protein concentrations were observed in the lung lavage fluid from male dogs at 100 mg/kg/day for 5 days, but no such changes were detected after 30 days of dosing. Alveolar foamy macrophage were detected in the 100 mg/kg/day group on day 5, but no such changes were noted on day 30. One dog at 30 mg/kg/day for 5 days had alveolar hemorrhage; however, a similar

change was observed for a control female on day 30. Following treatment for 30 days, parietal cell vacuolation in the stomach was detected at doses ≥ 15 mg/kg/day. Based upon lung lavage content of protein and foamy alveolar macrophages, lung injury appears to be most severe 3 to 5 days after the start of treatment, with resolution (i.e., adaptation) of changes occurring with continued treatment.

Chronic Toxicity

Oral Route of Administration

6-Month Oral Toxicity Study in Dogs (GTR-31376, GTR-31276, and GTR-31319).

Testing Laboratory: Byk Gulden Pharmaceuticals.

Date of the Study: Sept. 19, 1988 to April 12, 1989.

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

Animals: Beagle dogs weighing 8.1-16 kg and 11-16 months of age were used.

Methods: Five groups of animals each consisting of four males and four females were given pantoprazole (batch no. DRe 1418, 1413, 1415, 1419 and 1446) in capsules at acid dose levels of 0, 5, 15, 45 and 90 mg/kg/day for 6 months. An additional 2 males and 2 females were assigned to the 90 mg/kg/day group for a 4-week recovery periods. In order to minimize lung toxicity at doses of 45 and 90 mg/kg/day that had been observed in earlier studies (GTR-32082), doses were elevated in an incremental fashion. Animals on day 1 received 15 mg/kg/day and doses were increased in 15 mg/kg steps over a 3 to 7 day period. When the final dose was reached, the 6-month treatment period was started. Due to clinical signs of toxicity observed at 45 and 90 mg/kg/day, dosages were reduced to 30 and 60 mg/kg/day, respectively. For the 90 mg/kg/day group, treatment was stopped and then resumed at 60 mg/kg/day following a 6-day withdrawal period. For the 45 mg/kg/day group, the dose was lowered to 30 mg/kg/day; however, there was no interruption of treatment. The total period of treatment remained at 6 months. Blood samples for determination of serum levels of pantoprazole and its sulfone metabolite were collected at predose, 0.5, 1, 1.5, 2, 3, 5, 8 and 24 hours after drug administration of days -7, -3, 1, weeks 14 and 26 of the study. Serum concentrations of pantoprazole and its sulfone metabolite were measured by _____ (limit of detection _____)

Results:

1. Clinical Signs: 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. Diarrhea was observed in all dose groups. Vomiting was seen in the 90/60 mg/kg/day group.

2. Mortality: None.

3. Body Weight and Food Consumption: Final body weights were suppressed by >10% for male and female dogs that received 90/60 mg/kg/day. Final body weights on day 183 for male dogs that received 5, 15, 45/30, and 90/60 mg/kg/day were 119.3, 117.5, 107.9, and 87.7% of the control (11.4 kg), respectively. Final body weights on day 183 for female dogs that received 5, 15, 45/30, and 90/60 mg/kg/day were 117.9, 110.4, 99.1, and 86.8% of the control (10.6 kg), respectively.

4. Hematology (weeks 0, 13, 26 and 30): Decreases in hemoglobin (8-20%) and hematocrit (7-19%) were reported in the 90/60 mg/kg/day group. They returned to normal at end of recovery period.

5. Blood Chemistry (weeks 0, 13, 26 and 30) and Urinalysis (weeks 0, 12, and 24): Triglyceride levels at week 13 were elevated for all male treatment groups to 111.4-140% of the control (0.35 mmol/L); although, there was not a dose response relationship. Triglyceride levels for all male and female treatment groups at week 26 were elevated to 110.7-214.3% and 142.9-192.9% of control values (both 0.28 mmol/L), respectively; although, there were no dose response relationships. Triglyceride levels at week 30 for the male and female 90/60 mg/kg/day groups were elevated to 160.3 and 141.8% of control values (0.58 and 0.67 mmol/L), respectively. Cholesterol levels were elevated by 79-100% for all male treatment groups at weeks 13, 26, and 30, the female high dose group at week 13, and all female treatment groups at weeks 26 and 30. Urea levels at week 26 were elevated for all male treatment groups to 144.7-173.7% of the control (3.8 mmol/L). Serum alkaline phosphatase activity at week 26 was elevated by 32 to 114% for the male 45/30 and 90/60 mg/kg/day groups and all female treatment groups. Lactate dehydrogenase at week 26 was elevated by 14 to 68% for all male and female treatment groups. 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.

6. Physical Examinations: There were no treatment-related changes found in electrocardiograms (i.e., cardiac conduction) during weeks 12 and 25. Heart rates for female treatment groups at week 12 were increased to 155-165 beats/minute as compared to a control value of 100 beats/minute; although, there was no dose response relationship. Heart rates for female treatment groups at week 25 were increased to 128-168 beats/minute as compared to a control value of 115 beats/minute; although, there was no dose response relationship. There were no treatment-related ophthalmic effects.

7. Organ Weight: Increases in stomach weight was seen in all drug treated groups (52 - 79%) and they were still present in the 90/60 mg/kg/day at end of recovery period. Liver weights were increased in the 45/30 and 90/60 mg/kg/day groups (18 - 30%). Increase in thyroid (8 - 53%) was seen in all drug-treated groups but not in dose-related manner.

8. Gross Pathology: None.