Mean plasma pharmacokinetic parameters for pantoprazole from rats that received pantoprazole at doses of 5, 15, and 50 mg/kg/day for 1, 367, and 710 days

Dose, mg/kg	Sex	AUC, mg*hr/L			C_{max}, mg/L			T_{max}, hr			T½, hr		
		D1	D367	D710	D1	D367	D710	D1	D367	D710	D1	D367	D710
5	M	0.96	2.85	3.61	1.43	3.42	3.35	0.25	0.25	0.29	0.36	0.49	0.66
	F	1.37	3.65	2.92	1.85	3.35	2.87	0.25	0.25	0.25	0.41	0.66	0.64
15	M	4.07	8.63	13.30	3.85	9.56	11.90	0.25	0.25	0.25	0.93	0.55	0.67
	F	5.92	11.49	11.34	5.16	9.49	9.54	0.25	0.25	0.25	0.79	0.70	0.70
50	M	20.80	35.17	32.41	12.92	28.14	23.22	0.25	0.25	0.25	1.02	1.03	0.74
	F	28.25	52.62	37.09	17.97	36.05	25.39	0.25	0.25	0.25	0.90	0.96	0.80

Mean plasma pharmacokinetic parameters for the sulfone metabolite of pantoprazole from rats that received pantoprazole at doses of 5, 15, and 50 mg/kg/day for 1, 367, and 710 days

Dose, mg/kg	Sex	AUC, mg*hr/L			C_{max}, mg/L			T_{max}, hr			T½, hr		
		D1	D367	D710	D1	D367	D710	D1	D367	D710	D1	D367	D710
5	M	0.99	1.66	0.59	0.59	1.03	0.34	0.67	0.75	0.92	NA	NA	NA
	F	0.35	0.50	0.47	0.21	0.29	0.27	0.83	0.92	0.80	NA	NA	NA
15	M	4.52	9.23	2.69	1.90	3.80	1.01	1.00	1.00	1.17	2.70	2.83	5.03
	F	1.51	2.40	2.51	0.62	0.94	0.97	1.17	1.17	1.17	3.47	4.55	5.82
50	M	25.51	38.04	7.39	7.42	10.55	1.99	2.00	1.13	1.71	2.32	2.27	3.94
	F	7.98	12.04	9.48	2.15	3.07	2.40	2.00	2.17	2.00	3.09	4.09	4.43

N.A. = Not Ascertainable

Study for Assessing the Tumor Promoting Activity of Pantoprazole in Stomach and Forestomach in Sprague Dawley Rats (GTR-33036).

Testing Laboratory: [redacted]

Dated Started: August 1, 1995

Dated Completed: May 12, 1998

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

Animals: Sprague Dawley rats were used in this study. At the start of treatment, animals were 6 weeks old and body weight ranges were 94-145 g for male rats and 86-131 g for female rats.

Drug Batch: Pantoprazole, batch 0295220000; N-methyl-N-nitroso-guanidine (MNNG), batch 07144 from [redacted]

Methods: The tumor promoting activity of pantoprazole was assessed in the stomach and forestomach of Sprague Dawley rats. This mechanistic study was intended to evaluate the potential tumor promoting activity of pantoprazole in combination with a strong initiating carcinogen, N-methyl-N-nitroso-guanidine (MNNG). The study protocol was based upon the experimental model described by Newberne et al. (Cancer Letters 38: 149-163, 1987). The study design is illustrated in the table below. Each group consisted of 24 rats. Group A served as the control and received no treatment. Group B received MNNG at 75 mg/L in drinking water for 3 months. Additionally, these rats received pantoprazole by oral gavage at 200 mg/kg/day for 52 weeks. Group C received MNNG at 75 mg/L in drinking water for 3 months. Additionally, these rats received the vehicle daily by oral gavage for 52 weeks. Group D received pantoprazole by oral gavage at 200 mg/kg/day for 52 weeks. The vehicle for pantoprazole was double distilled water adjusted to pH 10.4 with 1 M NaOH. Double distilled drinking water was used as the vehicle for MNNG. The rats in groups 3 through 6 received the tumor initiating treatment, MNNG in drinking water during the first 3 months of the study. Simultaneously, rats in groups 3 through 6 received the tumor promoting treatment, pantoprazole or vehicle, by oral gavage. Rats in groups 7 and 8 received only tumor promoting treatment, pantoprazole, by oral gavage for 52 weeks, but no tumor initiating treatment during the first 3 months. The dosing volume for oral gavage was 10 mL/kg. Treatment was initiated on August 1, 1995 for animals in subset 0 and August 8, 1995 for animals in subset 1. Animals were terminated from July 30 to August 9, 1996. Animals were observed once daily for clinical signs of toxicity or mortality. A physical examination of each animal was performed weekly. Body weight was measured weekly. Food consumption was recorded weekly during the first 6 months of the study and thereafter, once per month until study termination. Water consumption was measured weekly throughout the study period. For animals in groups 3 through 6, water consumption was measured daily for the first 3 months during MNNG treatment and weekly, thereafter. Due to poor health and high levels of mortality for animals of groups 3 through 6, the sponsor interrupted treatment beginning at day 94 for 3 weeks. After this 3 week interruption, treatment with the tumor promoter, pantoprazole or vehicle, was restarted. Each animal was subjected to necropsy. Absolute and relative weights for the liver and stomach (empty) were measured. Organs and tissues were collected and fixed as follows: brain, pituitary, tongue, eyes, lacrimal glands, nasal and paranasal cavities, larynx, pharynx, trachea, thyroid, parathyroids, lungs, thymus, heart, aorta, lung-associated lymph nodes, salivary glands, mandibular lymph nodes, liver, pancreas, spleen, kidneys, adrenal glands, esophagus, forestomach, glandular stomach, duodenum, jejunum, ileum, caecum, colon, rectum, mesenteric lymph nodes, urinary bladder, testes, epididymis, prostate, seminal vesicles ovaries, uterus, vagina, mammary glands, skeletal muscle, femur including joints, vertebrae with spinal cord, skin, peripheral nerve, sternum with bone marrow. The sponsor performed a complete histopathological examination for 20 out of 24 animals per group. Selection was based upon two factors: [1] a long survival time, and [2] mortality not caused by technical errors during treatment. The sponsor in amendments dated December 18, 1998 and January 28, 1999 provided gross pathological and histopathological data for the remaining 4 animals per group. For determination of differences in the time dependent tumor incidence, Peto's method was applied. The sponsor stated that the definition of the time intervals by the adaptive method according to Peto was not applicable to this study. This method requires an increasing tumor

incidence in the consecutive intervals as a criteria for the definition of time intervals. In present study, the incidence in the last time interval was low due to the early termination of the study. The definition of the time interval was based upon the first "ad hoc" run. All treatment groups were compared to the control group. Since the purpose of this study was to evaluate the possible tumor promoting activity of pantoprazole, the MNNG + pantoprazole and MNNG + vehicle groups were compared against one another. This comparison was done for both sexes alone and also for male and female rats together.

Study Design

Group	No. of Rats	Sex	Initiatiion[1] (oral)	Promotion[2] (oral)
A01	24	Male	Control (untreated)	-
A02	24	Female	Control (untreated)	-
B03	24	Male	MNNG (0.075%)	Pantoprazole, 200 mg/kg
B04	24	Female	MNNG (0.075%)	Pantoprazole, 200 mg/kg
C05	24	Male	MNNG (0.075%)	Vehicle
C06	24	Female	MNNG (0.075%)	Vehicle
D07	24	Male	-	Pantoprazole, 200 mg/kg
D08	24	Female	-	Pantoprazole, 200 mg/kg

1. MNNG was administered in drinking water at 0.075% for 3 months.
2. The promoter (pantoprazole or vehicle) was administered daily by oral gavage for 52 weeks.

Results:

1. Observed Effects: Severe dyspnea combined with severe breathing sounds were observed for groups that received MNNG + vehicle, MNNG + pantoprazole, and pantoprazole alone. For male and female rats that received MNNG + vehicle, these observed effects started during weeks 2 and 3 of treatment, respectively, and continued throughout treatment. The percentage of male and female rats effected during any given week ranged from 4-47% and 4-33%, respectively. For male and female rats that received MNNG + pantoprazole, these observed effects started during weeks 6 and continued throughout treatment. The percentage of and male and female rats effected during any given week ranged from 4-50% and 4-38%, respectively. For male rats that received pantoprazole alone, these observed effects started during week 23 and continued until week 45. The percentage of male rats effected during any given week ranged from 4-8%. These effects were not observed for female rats that received pantoprazole alone.

2. Mortality: Mortality for control and treatment groups is shown in the table below. Mortality was highest for male and female rats that received MNNG + pantoprazole at 58.3 and 54.2%, respectively. Gross pathological findings, for animals that died or were sacrificed in a moribund condition during the treatment period, consisted of stomach and intestine severely inflated with air; larynx and/or trachea of firm consistency; and the lungs for 2 animals were totally inflated with air (i.e., suspected emphysema). Histopathological findings, for animals that died or were sacrificed in a moribund condition during the

treatment period, consisted of moderate to severe diffuse squamous cell metaplasia of the larynx combined with a moderate to severe laryngitis. Four animals died due to technical errors that were apparently unrelated to treatment as follows: male #030001 in group 3 (MNNG + pantoprazole) died on day 55; females #040007 and 040008 in group 4 (MNNG + pantoprazole) died on days 67 and 46, respectively; and female # 050008 in group 5 (MNNG + vehicle) died on day 41.

Mortality for control rats and for rats that received treatment with MNNG + pantoprazole, MNNG + vehicle, or pantoprazole alone (n = 24).

Treatment	Control		MNNG + Pantoprazole		MNNG + Vehicle		Pantoprazole	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Dead	1	0	2	4	1	3	0	1
Moribund Sacrifice	0	2	12	9	6	6	1	2
Accidental Deaths	0	0	1	2	1	0	0	0
Total Deaths[1]	1	2	14	13	7	9	1	3
% Death[1]	4.17	8.33	58.3	54.2	29.2	37.5	4.17	12.5

1. Total Deaths = Dead + Moribund Sacrifice

3. Body Weight, Food and Water Consumption: Body weight gain for female rats that received MNNG + pantoprazole was reduced to 88.3% of the control; although, final body at 92.7% of the control was reduced by < 10%. For male and female rats that received treatment with MNNG + pantoprazole, there were no consistent differences in food consumption as compared with respective control groups. For male rats that received MNNG + vehicle, food consumption was generally depressed as compared to the control group; however, food consumption was unaffected for female rats. For male and female rats that received pantoprazole alone, food consumption was elevated from days 91 to 350 and days 77 to 350, respectively, as compared to respective control groups. For male and female rats that received treatment with MNNG with or without pantoprazole, water consumption was decreased from days 1 to 98 as compared to respective controls; however, water consumption was comparable to control groups from days 105 to 364. For male rats that received pantoprazole alone, water consumption was comparable to the control; however, water consumption for female rats was increased throughout most of the treatment period as compared to the control group.

Body weights for control rats and rats that received treatment with MNNG + pantoprazole, MNNG + vehicle, or pantoprazole alone.

Treatment	Control		MNNG + Pantoprazole		MNNG + Vehicle		Pantoprazole	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Day 0	125.5	111.6	124.4	111.5	124.6	112.1	125.5	111.3
Day 364	518.0	290.1	530.7	269.0	490.1	292.7	537.1	296.1
Final BW, % of Control	100	100	102.45	92.7	94.6	100.9	103.7	102.0
BW Gain	392.5	157.5	406.3	157.5	365.5	180.6	411.6	184.8
BW Gain, % of Control	100	100	104.4	88.3	93.8	100.7	104.9	103.8

4. Hematology: No hematological parameters were measured.

5. Blood Biochemisty/Urinalysis: No blood biochemistry or urinalysis parameters were determined.

6. Physical Effects: Animals were examined once per week for physical effects; although, there were no reports for palpable masses.

7. Organ Weights: Absolute and relative liver and stomach weights were determined for animals at necropsy.

Liver: Absolute liver weights for male and female rats that received MNNG + pantoprazole were elevated to 158.1 and 167.8% of controls (14.817 and 7.374 g), respectively. Relative liver weights for male and female rats that received MNNG + pantoprazole were elevated to 156.8 and 180.1% of controls (29.46 and 26.22 g/kg), respectively. Absolute liver weights for male and female rats that received treatment with pantoprazole alone were elevated to 168.5 and 157.6% of controls, respectively. Relative liver weights for male and female rats that received treatment with pantoprazole alone were elevated to 165.3 and 155.7% of the controls, respectively. Absolute liver weight for female rats that received MNNG + vehicle was elevated to 119.3% of the control.

Stomach: Absolute stomach weight for male and female rats that received treatment with MNNG + pantoprazole were elevated to 135.9 and 152.9% of the controls (2.747 and 1.902 g), respectively. Relative stomach weights for male and female rats that received treatment with MNNG + pantoprazole were elevated to 135.3 and 164.7% of the controls (5.478 and 6.769 g/kg)., respectively. Absolute stomach weights for male and female rats that received pantoprazole alone were elevated to 154.5 and 170.3% of the controls, respectively. Relative stomach weights for male and female rats that received pantoprazole alone were elevated to 151.7 and 168.9% of the controls, respectively.

8. Gross Pathology: Review of gross pathological changes was confined to the stomach. The incidence of slimy surface for the glandular stomach was increased for rats that received MNNG + pantoprazole. The incidence of nodules in the glandular stomach was increased for rats that received MNNG with or without pantoprazole.

Gross pathological analysis of organs and tissues from control rats and rats that received treatment with MNNG + pantoprazole, MNNG + vehicle, or pantoprazole (n = 20).

Organ/Tissue	Control		MNNG + Pantoprazole		MNNG + Vehicle		Pantoprazole	
	Male	Female	Male	Female	Male	Female	Male	Female
Glandular Stomach -								
-erosion, red areas	0	2	4	4	2	0	4	0
-slimy surface	2	1	6	5	2	3	0	2
-smooth surface	0	0	2	2	0	1	0	2
-thickened mucosa	0	0	4	2	0	1	0	1
-nodules	0	0	2	5	7	1	1	1

9. Histopathology: Review of histopathological changes was confined to the stomach. Analysis of neoplastic lesions in the glandular stomach suggested that pantoprazole might be a tumor promoter as the incidence of antral adenocarcinoma for female rats that received MNNG + pantoprazole was increased as compared to female rats that received MNNG + vehicle; however, incidences in the corresponding male treatment groups were approximately equal. With regard to non-neoplastic lesions, antral mucosal fibrosis was observed for rats that received MNNG + pantoprazole, but not for rats that received either MNNG or pantoprazole alone.

Neoplastic Lesions: The glandular stomach was study target organ with regard to evaluating the tumor promoting activity of pantoprazole. The incidence of antral adenocarcinoma for female rats that received MNNG + pantoprazole was increased to 35% (7/20) as compared to 5% (1/20) for female rats that received MNNG + vehicle. However, the incidence of antral adenocarcinoma for male rats that received MNNG ± pantoprazole was approximately equivalent [4/20 (20%) vs. 5/20 (25%)]. Adenocarcinomas arose within the mucosa of the antrum and had well preserved glandular structures. A tubular growth pattern predominated. Cystic and papillary patterns were less common. Tumors were at different stages of development.

Non-Neoplastic Lesions: For the forestomach, squamous epithelial hyperplasia and hyperkeratosis were evident for treatment groups that received pantoprazole. For the glandular stomach, hyperplasia of the fundic and antral mucosa were evident for treatment groups that received pantoprazole. The fundic mucosa was increased in height and contained varying levels of eosinophilic chief cell foci consisting of hyperplastic chief cells. Within the hyperplastic fundic glands, secretions appeared to be thickened and dried. The hyperplastic antral mucosa usually had areas of pre-neoplastic focal regenerative hyperplasia and dysplasia. Inflammatory cell infiltration was evident in the fundic and antral mucosa. Antral mucosal fibrosis was evident for rats that received MNNG + pantoprazole.

Tumor incidence by organ for control rats and rats that received treatment with MNNG + pantoprazole, MNNG + vehicle, and pantoprazole (n = 20/group).

Organ/Tissue	Control		MNNG + Pantoprazole		MNNG + Vehicle		Pantoprazole	
	Male	Female	Male	Female	Male	Female	Male	Female
Glandular Stomach								
-antral adenoma (B)	0	0	0	0	1	0	0	0
-antral adenocarcinoma (M)	0	0	4	7	5	1	0	0
-Schwannoma (M)	0	0	0	1	0	0	0	0

Non-neoplastic lesions of organs and tissues from control rats and rats that received treatment with MNNG + pantoprazole, MNNG + vehicle, or pantoprazole (n = 20).

Organ/Tissue	Control		MNNG + Pantoprazole		MNNG + Vehicle		Pantoprazole	
	M	F	M	F	M	F	M	F
Forestomach								
-squamous epithelial hyperplasia	2	0	6	5	2	2	8	7
-hyperkeratosis	2	0	8	7	1	1	9	6
-submucosal edema	0	1	2	3	4	1	1	1
-inflammatory cell infiltrations	1	1	5	4	5	3	7	5
Glandular Stomach								
-antral mucosal hyperplasia	0	0	20	19	1	0	20	19
-antral mucosal fibrosis	0	0	11	5	2	0	0	1
-antral mucosal inflam. cell infiltr.	0	0	18	11	3	3	14	15
-fundic mucosal hyperplasia	0	0	20	20	1	0	20	20
-fundic eosinophilic chief-cell foci	0	0	20	20	0	0	19	20
-fundic mucosal fibrosis	0	0	6	1	0	0	4	2
-fundic glandular inspat. secretion	0	0	12	6	0	0	16	9
-fundic mucosal inflam. cell infitr.	1	0	20	20	1	0	20	19
-fundic mucosal erosions	0	0	5	1	0	0	3	0
-fundic submucosal edema	1	0	5	2	0	0	2	0

The tumor promoting activity of pantoprazole was assessed in the stomach and forestomach of Sprague Dawley rats. This mechanistic study was intended to evaluate the potential tumor promoting activity of pantoprazole in combination with a strong initiating carcinogen, N-methyl-N-nitroso-guanidine (MNNG). The incidence of mortality was increased for treatment groups that received MNNG. The sponsor attributed high mortality in these groups to MNNG + oral gavage. Moderate to severe multifocal or diffuse squamous metaplasia of the non-squamous laryngeal epithelium was observed in treatment groups that received MNNG. Partial obstruction of the laryngeal lumen was observed in these animals and appeared to correlate with severe breathing sounds. Focal or multifocal aspiration pneumonia was observed with an increased incidence in treatment groups that received MNNG. It should be noted that esophageal lesions were most prevalent for the treatment group that received pantoprazole alone. Control animals received no treatment, which made it impossible to assess the deleterious effects of oral gavage alone. The glandular stomach was study target organ with regard to evaluating the tumor promoting activity of pantoprazole. The incidence of antral adenocarcinoma in the glandular stomach for female rats that received MNNG + pantoprazole was increased to 35% (7/20) as compared to 5% (1/20) for female rats that received MNNG + vehicle. However, the incidence of antral adenocarcinoma for male rats that received MNNG ± pantoprazole was approximately equivalent [4/20 (20%) vs. 5/20 (25%)]. Significant histopathological findings were evident for the forestomach and glandular stomach. Squamous epithelial hyperplasia and hyperkeratosis in the forestomach were evident for treatment groups that received pantoprazole. Hyperplasia of the fundic and antral mucosa in the glandular stomach were evident for treatment groups that received pantoprazole.

Study for Assessing the Tumor Promoting Activity of Pantoprazole in Liver and Thyroid in Sprague Dawley Rats **(GTR-33037).**

Testing Laboratory: [redacted]

Date Started: July 10, 1995

Date Completed: May 12, 1998

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

Animals: Sprague Dawley rats were used in this study. At the start of treatment, animals were 6 weeks old and body weight ranges were 101-145 g for male rats and 89-128 g for female rats.

Drug Batch: Pantoprazole, batch 0295220000; N-nitroso-N-methylurea, batch 24H08992 from [redacted] and Sodium Phenobarbital, batch 122H0143 from [redacted]

Methods: The tumor promoting activity of pantoprazole was assessed in the liver and thyroid gland of Sprague Dawley rats. This mechanistic study was intended to evaluate the potential tumor promoting activity of pantoprazole in combination with a strong initiating carcinogen, N-nitroso-N-methylurea (NMU). The study protocol was based upon the experimental protocol described by Diwan et al. (Journal of the National Cancer Institute 75: 1099-1105, 1985). The study design is illustrated in the table below. Each group consisted of 24 rats. Treatment was initiated on July 10, 1995 for animals in subset 0, July 17, 1997 for animals in subset 1, July 24, 1995 for animals in subset 2, and July 31, 1995 for animals in subset 3. Animals were terminated from August 12 to September 6, 1996. Group A served as the control and received no treatment. Group B received NMU by the intravenous route at a dose of 0.05 mmole/kg/week for 4 weeks. Two weeks after the last NMU treatment, rats received pantoprazole by oral gavage at a dose of 200 mg/kg/day for 52 weeks. Group C received NMU by the intravenous route at a dose of 0.05 mmole/kg/week for 4 weeks. Two weeks after the last NMU treatment, rats received the vehicle of pantoprazole by oral gavage for 52 weeks. Group D received the vehicle of NMU by the intravenous route once per week for 4 weeks. Two weeks after the last vehicle treatment, rats received pantoprazole by oral gavage at a dose of 200 mg/kg/day for 52 weeks. Group E received NMU by the intravenous route at a dose of 0.05 mmole/kg/week for 4 weeks. Two weeks after the last NMU treatment, rats received phenobarbital in drinking water at a concentration of 0.05% for 52 weeks. Group F received NMU by the intravenous route at a dose of 0.05 mmole/kg/week for 4 weeks. Two weeks after the last NMU treatment, rats received the vehicle of phenobarbital in drinking water for 52 weeks. Group G received the vehicle of NMU by the intravenous route once per week for 4 weeks. Two weeks after the last vehicle treatment, rats received phenobarbital in drinking water at a concentration of 0.05% for 52 weeks. Each group consisted of 24 rats/sex/group. The intravenous dosing volume for NMU or its vehicle was 5 mL/kg. The oral gavage dosing volume for pantoprazole or its vehicle was 10 mL/kg. Two weeks after the last vehicle treatment, rats received phenobarbital in drinking water at a concentration of 0.05% for 52 weeks. The vehicle for pantoprazole was double distilled water, adjusted to pH 10.4 with 1 M NaOH. The vehicle for NMU was — sterilized citrate buffer solution (pH 5) containing 0.15 M NaCl. Rats were not treated by oral gavage in the case of impaired health or a severe defense reaction. Animals were observed for clinical signs of toxicity and mortality at least once daily. A physical examination of each animal was performed weekly. Body weights were measured weekly. Food consumption was measured weekly during the first 6 months of the study and then monthly until the end of the study period. Water consumption was measured weekly throughout the study period. Prior to sacrifice, animals were fasted overnight. The sponsor performed complete gross pathological and histopathological examinations for 20 out of 24 animals per group. Selection was based upon two factors: [1] a long survival time, and [2] mortality not caused by technical errors during treatment. The sponsor in amendments dated December 18, 1998 and January 28, 1998 provided gross pathological and histopathological findings for the remaining 4 animals per groups. Absolute and relative liver and thyroid gland weights were measured. Organs and tissues were collected and fixed as follows: brain, pituitary, tongue, eyes, lacrimal glands, nasal and paranasal cavities, larynx, pharynx, trachea, thyroid, parathyroids, lungs, thymus, heart, aorta, lung-associated lymph nodes, salivary glands, mandibular lymph nodes, liver, pancreas, spleen, kidneys, adrenal glands, esophagus, forestomach, glandular stomach,

duodenum, jejunum, ileum, caecum, colon, rectum, mesenteric lymph nodes, urinary bladder, testes, epididymis, prostate, seminal vesicles ovaries, uterus, vagina, mammary glands, skeletal muscle, femur including joints, vertebrae with spinal cord, skin, peripheral nerve, sternum with bone marrow.

Study Design

Group	No. of Rats	Sex	Initiatiion[1] (i.v.)	Promotion[2] (oral)
A01	24	Male	Control (untreated)	-
A02	24	Female	Control (untreated)	-
B03	24	Male	NMU (0.05 mmol/kg)	Pantoprazole, 200 mg/kg
B04	24	Female	NMU (0.05 mmol/kg)	Pantoprazole, 200 mg/kg
C05	24	Male	NMU (0.05 mmol/kg)	Vehicle of pantoprazole
C06	24	Female	NMU (0.05 mmol/kg)	Vehicle of pantoprazole
D07	24	Male	vehicle of NMU	Pantoprazole, 200 mg/kg
D08	24	Female	vehicle of NMU	Pantoprazole, 200 mg/kg
E09	24	Male	NMU (0.05 mmol/kg)	Phenobarbital, 0.05%
E10	24	Female	NMU (0.05 mmol/kg)	Phenobarbital, 0.05%
F11	24	Male	NMU (0.05 mmol/kg)	Vehicle of phenobarbital
F12	24	Female	NMU (0.05 mmol/kg)	Vehicle of phenobarbital
G13	24	Male	vehicle of NMU	Phenobarbital, 0.05%
G14	24	Female	vehicle of NMU	Phenobarbital, 0.05%

Results:

1. **Observed Effects**: A low incidence of severe breathing sounds were observed primarily for male rats that received NMU + pantoprazole, NMU + vehicle, and pantoprazole alone. For male rats that received NMU + pantoprazole or NMU + vehicle, severe breathing sounds started during week 21 or 22, respectively, and continued to the end of treatment; however, ≤ 3 animals/group were affected for any given week. For male rats that received pantoprazole alone, 1 animal was observed with severe breathing sounds during week 21. From week 33 to the end of treatment, 1 to 3 male rats per week that received pantoprazole alone were observed with severe breathing sounds. For female rats that received pantoprazole alone, a low incidence of severe breathing sounds at 1 animal per week was observed from week 37 to the end of the study.

2. **Mortality**: The incidences of mortality were increased for all female treatment groups as compared with the control, but in particular for those that received NMU. The sponsor did not state causes of death. Evaluation of histopathology data did not reveal any significant trends for animals that died or were sacrificed in a moribund condition for most treatment groups. There were numerous deaths related to technical errors associated with administration of pantoprazole by oral gavage. For the treatment groups that received NMU + pantoprazole, 6 male rats (numbers 030002, 030003, 030004, 030019, 030022, and 030024) and 9 female rats (numbers 040001, 040003, 040004, 040005, 040008, 040012, 040013, 040014, and 040016) died due to technical error. For treatment groups that received vehicle of NMU + pantoprazole, 2 male rats (numbers 070001 and 070015) and 4 female rats (080003, 080004, 080009, 080011) died due to technical error. Apparently, death due to technical error for treatment groups that received NMU + vehicle of pantoprazole was significantly lower or the sponsor did not report deaths due to technical error for these groups. The sponsor reported that male rat #050017 that received NMU + vehicle of pantoprazole apparently died due to technical error and was subsequently replaced by another animal.

Mortality Incidence for male and female treatment groups (n = 24 per group).

Treatment	Control		NMU + Pantoprazole		NMU + vehicle of Pantoprazole		Vehicle of NMU + Pantoprazole		NMU + Phenobarbital		NMU + Vehicle of phenobarbital		Vehicle of NMU + Phenobarbital	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Died	1	0	1	1	1	2	4	1	0	1	0	0	0	0
Moribund Sacrifice	2	1	2	4	1	7	0	2	1	2	1	6	1	2
Death due to technical error	0	0	6	9	0	0	2	4	0	0	0	0	0	0
Treatment-related deaths	3	1	3	5	2	9	4	3	1	3	1	6	1	2
% Treatment-related Deaths	12.5	4.2	12.5	20.8	8.3	37.5	16.7	12.5	4.2	12.5	4.2	25	4.2	8.3

Treatment-related deaths = Died + Moribund Sacrifice

3. **Body Weight, Food and Water Consumption**: Final body weight and body weight gain for male and female rats in treatment groups were not impaired as compared with respective controls. Food consumption for male treatment groups that received pantoprazole was increased from day 105/112 to the end of the study period. Similarly, food consumption for female treatment groups that received pantoprazole was increased from day 84 to the end of the treatment period. Food consumption was decreased for male rats that received NMU + the vehicle for phenobarbital. For treatment groups that received NMU + pantoprazole, water consumption was increased for male rats from days 84 through 273 and female rats from days 56 through 336. Similarly, for female rats that received vehicle + pantoprazole, water consumption was increased from days 91 to 259. For male rats that received NMU + vehicle of pantoprazole, water consumption was decreased from days 28 through 399. For female treatment groups that received phenobarbital, water consumption was decreased from days 49/56 through 399.

Final body weight and body weight gain for male treatment groups.

Treatment	Control	NMU + Pantoprazole	NMU + Vehicle of Pantoprazole	Vehicle of NMU + Pantoprazole	NMU + PB	NMU + Vehicle of PB	Vehicle of NMU + PB
Day 0	119.8	120.1	119.3	120.0	120.6	118.8	119.0
Day 392	534.2	529.4	525.8	537.2	527.9	498.5	529.3
Final Body Weight, % of Control	100	99.1	98.4	100.6	98.8	93.3	99.1
Body Weight Gain	414.4	409.3	406.5	417.2	407.3	379.7	410.3
Body Weight Gain, % of Control	100	98.5	98.5	100.5	97.6	92.4	99.7

Final body weight and body weight gain for female treatment groups.

Treatment	Control	NMU + Pantoprazole	NMU + Vehicle of Pantoprazole	Vehicle of NMU + Pantoprazole	NMU + PB	NMU + Vehicle of PB	Vehicle of NMU + PB
Day 0	108.5	107.6	108.5	107.9	110.4	106.6	108.6
Day 392	291.3	323.3	314.4	308.5	299.9	292.5	292.3
Final Body Weight, % of Control	100	111	107.9	105.9	102.95	100.4	100.3
Body Weight Gain	182.4	215.7	205.9	200.6	189.5	185.9	183.7
Body Weight Gain, % of Control	100	113.3	113.3	111	102.5	104.1	101

4. **Hematology**: Not performed.

5. **Blood Biochemistry/Urinalysis**: Not performed.

6. **Physical Effects**: Incidences of subcutaneous nodules were increased for all female treatment groups as compared to the control (See table below). In contrast, few subcutaneous nodules were found for male treatment groups.

Incidence of subcutaneous nodules for male and female treatment groups.

Treatment	Control		NMU + Pantoprazole		NMU + vehicle of pantoprazole		Vehicle of NMU + Pantoprazole		NMU + Phenobarbital		NMU + Vehicle of phenobarbital		Vehicle of NMU + Phenobarbital	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Subcutaneous nodules	1	5	0	12	1	17	0	4	1	11	0	14	0	13

7. **Organ Weights**: Absolute and relative liver weights were increased for male treatment groups that received pantoprazole or phenobarbital. Absolute and relative liver weights were increased for female treatment groups that received NMU + pantoprazole, NMU + vehicle of pantoprazole, vehicle of NMU + pantoprazole, and NMU + phenobarbital. Absolute and relative thyroid weights were increased for male treatment groups that received pantoprazole or phenobarbital. Absolute and relative thyroid weight were increased for female treatment groups that received pantoprazole.

Absolute and relative liver and thyroid gland weights for male and female treatment groups (n = 20 per group).

Treatment	Liver, % of Control				Thyroid, % of Control			
	Male		Female		Male		Female	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Control	100 (16.165 g)	100 (31.17 g/kg)	100 (8.056 g)	100 (28.85 g)	100 (0.022 g)	100 (0.0422 g/kg)	100 (0.017 g)	100 (0.0603 g/kg)
NMU + Pantoprazole	151.3*	155*	165.9*	146.3*	159.1*	163.5*	135.3*	120.7*
NMU + Vehicle of Pantoprazole	94.7	96.8	130.2*	116.2*	100	103.6	94.1	88.4
Vehicle of NMU + Pantoprazole	150.6*	151.3*	142.5*	137.6*	145.5*	149.8*	135.3*	129.8*
NMU + Phenobarbital	127.7*	129*	117.8*	112.7*	131.8*	135.1*	100	96.1
NMU + Vehicle of phenobarbital	93.1	99.4	108	106.1	95.5	103.1	100	99.3
Vehicle of NMU + phenobarbital	125.4*	125.9*	110.5	109.6	136.4*	138.9*	100	97.8

8. **Gross Pathology**: Review of gross pathology is confined to the liver and thyroid gland. For treatment groups that received pantoprazole or phenobarbital, lobulated livers were observed. For treatment groups that received pantoprazole, enlarged livers were observed.

Gross pathological changes for control rats and rats that received NMU + pantoprazole, NMU + vehicle of pantoprazole, vehicle of NMU + pantoprazole, NMU + phenobarbital, NMU + vehicle of phenobarbital, and vehicle of NMU + phenobarbital (n = 20 per group).

Tissue	Control		NMU + Pantoprazole		NMU + Vehicle of Pantoprazole		Vehicle of NMU + Pantoprazole		NMU + Phenobarbital		NMU + Vehicle of Phenobarbital		Vehicle of NMU + Phenobarbital	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Liver														
-lobulated	0	0	6	5	0	0	5	2	4	3	0	0	6	0
-enlarged	0	0	8	1	0	0	7	0	0	0	0	0	0	0
-nodules	0	0	2	0	0	1	0	0	2	0	0	0	2	0
-colored areas	0	0	0	3	0	1	0	3	0	1	0	1	0	0
-incised margins														
-cyst(s)	0	0	0	0	0	0	0	2	0	1	0	0	0	0
	0	0	0	0	0	0	0	2	0	0	0	0	0	0

9. Histopathology:

Neoplastic Lesions: For the liver, the incidence of hepatocellular carcinoma was 8.3% (2/24) for female rats that received NMU + pantoprazole and 4.2% (1/24) for female rats that received NMU + pentobarbital as compared to 0% for female rats that received NMU + vehicle of pantoprazole. Diwan et al. (JNCI 75: 1099-1105) reported that for male Fischer rats, which received phenobarbital for 52 weeks after NMU treatment, were observed with hepatocellular adenomas at incidence rate of 20% (2/10); however, the primary observation was an increased area of hepatocellular foci of cellular alteration/cm^2 for both male and female rats. For the thyroid gland, the incidence of follicular cell adenoma was 8.3% (2/24) for male rats that received NMU + pantoprazole and 4.2% (1/24) for male rats that received NMU + pentobarbital. Diwan et al. (JNCI 75: 1099-1105, 1985) reported a significantly higher yield of follicular cell adenomas (30-40%) at 52 weeks for both male and female Fischer rats. In the present study, tumor incidences in thyroid gland for the positive control group, NMU + phenobarbital were low and confined to one sex, possibly due to high mortality rates in the study. Thus, the positive control produced a negative response with regard to thyroid gland tumors and the study could be assumed to have no validity with regard to the thyroid gland.

Non-Neoplastic Lesions: The incidence of focus/foci of cellular alteration in the liver was increased for female treatment groups that received pantoprazole or phenobarbital as compared to the control. Foci of cellular alteration consisted of single or multiple clear cell(s) and basophilic or eosinophilic foci. The incidence of bile duct hyperplasia and hepatocellular hypertrophy were increased for treatment groups that received pantoprazole or phenobarbital. The incidence of hepatocellular necrosis was increased for all male treatment groups as compared to the control group; although, the incidence was particularly higher for groups that received phenobarbital. For the thyroid gland, follicular cell hypertrophy was increased for treatment groups that received pantoprazole.

Combining Hepatocellular Neoplasms and Foci of Cellular Alterations: The sponsor combined incidences of hepatocellular neoplasms and foci of cellular alterations for female rats to reveal rates of 40% (8/20) for NMU + pantoprazole; 10% (2/20) for NMU + vehicle of pantoprazole; 25% (5/20) for vehicle of NMU + pantoprazole; 40% (8/20) for NMU + phenobarbital; 5% (1/20) for NMU + vehicle of phenobarbital; and 40% (8/20) for vehicle of NMU + phenobarbital. The combined incidences of hepatocellular neoplasms and foci of cellular alterations for NMU + pantoprazole as compared to NMU + vehicle of pantoprazole suggests that pantoprazole could be acting as a promoter. However, for male treatment groups, the combined incidences of hepatocellular neoplasms and foci of cellular alterations were not different from the control group. Diwan et al. (JNCI 75: 1099-1105) reported that for both male and female Fischer rats, which received phenobarbital for 52 weeks after NMU treatment, were observed with an increased area of hepatocellular foci of cellular alteration/cm^2. The sponsor reported foci of cellular alteration on an incidence basis, while Diwan et al. reported this parameter on an area basis. It should be noted that Diwan et al. did not combine hepatocellular adenomas/carcinomas with foci of cellular alteration. The lack of change in male treatment groups suggests that observations in female treatment groups occurred possibly by chance and were influenced by high mortality in the study.

Tumor incidence by organ for control rats and rats that received NMU + pantoprazole, NMU + vehicle of pantoprazole, vehicle of NMU + pantoprazole, NMU + phenobarbital, NMU + vehicle of phenobarbital, and vehicle of NMU + phenobarbital (n = 24 per group).

Tissue	Control		NMU + Pantoprazole		NMU + Vehicle of Pantoprazole		Vehicle of NMU + Pantoprazole		NMU + Phenobarbital		NMU + Vehicle of Phenobarbital		Vehicle of NMU + Phenobarbital	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Liver														
-hepatocellular carcinoma (M)	0	0	0	0	0	0	0	0	0	1	0	0	0	0
-hepatocellular adenoma (B)	0	0	0	2	0	0	0	0	0	0	0	1	0	0
-cholangioma (B)	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Thyroid gland														
-C-cell adenoma (B)	0	1	1	1	1	1	1	1	2	0	0	0	0	0
-follicular cell adenoma	0	0	2	0	0	0	0	0	1	0	0	0	1	0

Histopathological changes for control rats and rats that received NMU + pantoprazole, NMU + vehicle of pantoprazole, vehicle of NMU + pantoprazole, NMU + phenobarbital, NMU + vehicle of phenobarbital, and vehicle of NMU + phenobarbital (n = 20 per group).

Tissue	Control		NMU + Pantoprazole		NMU + Vehicle of Pantoprazole		Vehicle of NMU + Pantoprazole		NMU + Phenobarbital		NMU + Vehicle of Phenobarbital		Vehicle of NMU + Phenobarbital	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Liver														
-focus (foci) of cellular alteration	1	0	1	7	3	2	1	5	2	7	3	0	1	8
-bile duct hyperplasia	6	1	14	3	7	1	10	6	17	12	8	7	10	5
-hepatocellular hypertrophy	0	0	20	15	0	4	20	19	20	20	0	2	20	20
-hepatocellular necrosis	2	0	8	0	5	0	4	0	12	0	4	0	11	0
-fatty vacuol. of hepatocytes	0	0	1	0	1	0	0	0	17	0	0	0	11	0
-mononuclear/ inflam. Cell infiltr.	6	0	12	0	7	0	8	0	13	0	5	0	12	0
Thyroid gland														
-follicular cell hyperplasia	0	0	3	0	2	0	0	0	3	0	1	0	0	0
-follicular cell hypertrophy	0	0	18	14	0	0	20	19	2	0	0	0	0	0

Combined incidence of hepatocellular neoplasms and foci of cellular alterations (Sponsor's Table 14)[A]. (n = 20 per group)

Lesion	Control		NMU + Pantoprazole		NMU + Vehicle of Pantoprazole		Vehicle of NMU + Pantoprazole		NMU + Phenobarbital		NMU + Vehicle of Phenobarbital		Vehicle of NMU + Phenobarbital	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Liver -Hepatocellular neoplasms/focus (foci) of cellular alteration	1	0	1	8	3	2	1	5	2	8	3	1	3	8
Liver -Focus (foci) of cellular alteration	1	0	1	7	3	2	1	5	2	7	3	0	1	8
Liver Tumors														
-Hepatocellular adenoma (M)	0	0	0	0	0	0	0	0	0	1	0	0	0	0
–Hepatocellular adenoma (B)	0	0	0	2	0	0	0	0	0	0	0	1	0	0
–Cholangioma (B)	0	0	0	0	0	0	0	1	0	0	0	0	0	0

The tumor promoting activity of pantoprazole was assessed in the liver and thyroid gland of Sprague Dawley rats. This mechanistic study was intended to evaluate the potential tumor promoting activity of pantoprazole in combination with a strong initiating carcinogen, N-nitroso-N-methylurea (NMU). The study protocol was based upon the experimental protocol described by Diwan et al. (Journal of the National Cancer Institute 75: 1099-1105, 1985. In the study conducted by the sponsor to assess the tumor promotional activity of pantoprazole, there was a high incidence of mortality related to technical errors associated with administration of pantoprazole by oral gavage; although, this was not observed with the vehicle of pantoprazole. Tumor incidences in thyroid gland were low and confined to one sex in the positive control group, NMU + phenobarbital, possibly due to high mortality rates in the study. Diwan et al. (JNCI 75: 1099-1105, 1985) reported a significantly higher yield of follicular cell adenomas at 52 weeks for both male

and female Fischer rats. In the present study conducted by the sponsor, the positive control produced a negative response with regard to thyroid gland tumors and thus, the study could be assumed to have no validity with regard to the thyroid gland. The sponsor combined incidences of hepatocellular neoplasms and foci of cellular alterations for female rats to suggest that pantoprazole could be acting as a promoter. However, similar results were not obtained with corresponding male treatment groups. Diwan et al. (JNCI 75: 1099-1105) reported that for both male and female Fischer rats, which received phenobarbital for 52 weeks after NMU treatment, were observed with an increased area of hepatocellular foci of cellular alteration/cm^2. It should be noted that Diwan et al. (JNCI 75: 1099-1105) did not combine hepatocellular adenomas/carcinomas with foci of cellular alteration. Further, Diwan et al. (JNCI 75: 1099-1105) and Harada et al. (Toxicol Pathol. 17:579-593, 1989) have reported that there is no constant proportion between foci of cellular alteration and subsequent development of hepatic neoplasms. The increase of combined incidence of hepatocellular neoplasms and foci of cellular alterations for female rats that received NMU + pantoprazole possibly suggests that pantoprazole possesses tumor promoting activity; however, incidences for corresponding male treatment groups were approximately equivalent suggesting these observations in female rats occurred by chance and were influenced by high mortality in the study.

Reproductive Toxicity

Rat

Oral Segment I Male Fertility Study in Rats (GTR-32063).

Testing Laboratory: [redacted]

Date of the Study: January 30, 1989 to March 1990.

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

Animals: Sprague-Dawley rats 50 days (males) or 40 days (females) of age were used.

Methods: Four groups of animals each consisting of 25 males rats were given pantoprazole (lot no. 58905-88PD477) dissolved in water by gavage at constant volume of 10 mL/kg and at dose levels of 0, 5, 50 and 500 mg/kg/day for 70 days prior to mating with untreated female groups each consisting of 25 rats. Fertility incidences in the first trial were less than the historical control, therefore, after 112 days of treatment, the same males were placed in a second mating trial with untreated females. Dose selection was based on a 12-day dose range finding study in male rats with doses of 0, 200, 400, 600, and 800 mg/kg/day (GTR-32036). Retardation of body weight gain was noted at doses ≥ 600 mg/kg/day (22-23%) and increased liver weights (17-39%) were observed at doses ≥ 200 mg/kg/day. All females were killed on day 21 of pregnancy and were necropsied. Mating rates and fertility rates were determined. Fetuses were subjected to external examination. Males were killed on days 113-127 of study. Testicular and epididymal spermatid counts were performed and the testis and epididymides were evaluated histologically.

Results:

Clinical Signs: Salivation and urine stained genital-abdominal area was noted dose-dependently. Prostration, ataxia, decreased motor activity, abdominal gait and/or abnormal reflex were detected in the 500 mg/kg/day group.

Body Weight: A slight retardation of body weight gain (12%) was observed in the 500 mg/kg/day group. However, this difference was not statistically significant.

Mortality: None.

Food Consumption: Normal.

Mating and Fertility: Fertility incidences were 54-80% for mating trial I (at 70 days of treatment) and 83-96% for trial II (day 112 of treatment). Mating incidences of 92%-100% in both mating trial were observed. No treatment-related differences on mating or fertility were evident.

Dams: There were no effects on maternal body weight, numbers of corpora lutea, implantations, resorptions, live fetuses and fetal weights.

Organ Weight of Male Rats: An increase in liver weight (13-53%) was observed in the 50 and 500 mg/kg/day groups. There were no effects on reproductive organ weights. Seminal vesicle weight was increased in the 500 mg/kg/day male rats. Testis and epididymis counts and histological change in testis or epididymides were normal.

In conclusion, treatment with pantoprazole at doses up to 500 mg/kg/day for 112 days prior to mating had no effect on fertility, mating and gonadal function in male rats.

Oral Segment I Fertility Study in Female Rats (GTR-32062).

Testing Laboratory: [redacted]

Date of the Study: January 16, 1989 to March 1990.

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

Animals: Sprague-Dawley rats 65-70 days of age were used.

Methods: Six groups of animals each consisting of 24 female rats were given pantoprazole (Code 589085 - 38PD477) dissolved in water at acid dose levels of 0, 50, 150 and 450 mg/kg/day 14 days prior to mating, continued throughout gestation and lactation. Dose selection was based on a dose-range finding study (GTR-32037) that toxicity (increased liver weight and low pup body weight) was evident in dams administered 450 mg/kg/day pantoprazole. Twelve females from each group were killed on day 21 of gestation. Fetuses were examined externally. The remaining dams in each group were allowed to deliver their young naturally and nursed them for 21 days.

Results:

Clinical Signs: Dose-related salivation was observed.

Mortality: One, one and three dams in the control, 150 and 450 mg/kg/day were found dead or killed in extremis on day 24 of gestation or day 3 of lactation. Macroscopic examination at necropsy did not reveal lesions related to the drug.

Estrous Cycle and Mating: There were no treatment-related effects on estrous cycle. Lower mating rate were observed in the 150 and 450 mg/kg (86 and 82%). However, these rates are within the range of normal values for this laboratory according to sponsor.

Body Weight and Food Consumption: Retardation of body weight gain (18%) was found in the 450 mg/kg/day groups during gestation period. However, this group gained more weight than the control groups during lactation period. Reduction in food intake (9-17.8%) was noted in the 450 mg/kg/day during mating and gestation period. Reduction in food intake was also seen in the 150 mg/kg/day group during lactation period (6%).

Cesarean Delivery Data: It had no effects on pre-implantation loses, live birth index or fetal sex ratio. Increase in post-implantation loss (18 vs 6%) was noted in the 450 mg/kg/day group. Lower fetal body weights (10-13%) in the 150 and 450 mg/kg/day groups were found. Reduced live fetuses (13%) and lower uterine weight (16%) were observed in the 450 mg/kg/day group.

Fetal Examination: A dose-related increase in the number of pups with reduced sternal center was observed and a reduced number of metacarpals occurred in the 450 mg/kg/day group.

Natural Delivery Data: There were no effects on the length of pregnancies, incidence of still-births, live birth index, pup sex ratio or number of uterine implantation sites. Delayed parturition was apparent in the 450 mg/kg/day group (12.9 vs 8.5 minutes for control). Litter size was reduced (20.7%) in the 450 mg/kg/day than that of the controls. Pup survival to day 21 of lactation was smaller in the 450 mg/kg/day group (71 vs 96%) than that of the controls.

Pup Growth: At day 21 of gestation, pup body weight were lower in drug-treated groups (14-20%).

Dam Liver Weight: Liver weight in female rats receiving 450 mg/kg/day was higher than controls (23.7% and 17.3%) in cesarean and natural-delivered dams.

In conclusion, pantoprazole at oral dose of 450 mg/kg/day given 14 day prior to mating produced maternal toxicity, increased prenatal death, reduced fetal weight, incomplete or delayed parturition, increased incidence of postnatal death and reduced growth of pups during the lactation period. At lower doses, decreased fetal weight (150 mg/kg/day) and reduced pup growth (15 and 150 mg/kg/day) were observed. No interim sacrifice of dams on day 13 of gestation was performed to detect any effects on early event of pregnancy. It had no effect on fertility and general reproductive performance in female rats.

Addendum: In the dose range finding study (GTR-32037), rats received pantoprazole by oral administration at doses of 0, 50, 150, 300, 450, and 600 mg/kg/day for 14 days prior to mating, throughout the mating period, and until day 7 of lactation. During the 14 day period prior to mating and during the gestational period, body weight gain for dams that received 600 mg/kg/day was impaired by >10%. The average delivery time per pup was increased in dose-related manner. Delivery time/pup was 12.5, 19.2, and 19.9 min in the 300, 450, and 600 mg/kg/day groups, respectively, as compared to a control value of 8.5 min. One dam at 600 mg/kg/day was euthanized on day 24 of gestation due to severe dystocia. Live pups per litter in the 600 mg/kg/day group were reduced to 9.4 (40.1% decrease) as compared to a control value of 15.7 pups/litter. Post-implantation loss at 600 mg/kg/day was increased to 41.3% as compared to a control value of 7.7%. Postnatal mortality for the 600 mg/kg/day group was 24 deaths as compared to 4 for the control group. Average pup body weight on postnatal day 7 was reduced by 15.7% as compared to the control value. Pup viability on day was 48.2% at 600 mg/kg/day as compared to 95.7% for the control group. For the main Segment I study with female rats, 450 mg/kg/day was selected as the high dose.

In the Segment I study (GTR-32062) with female rats, overall skeletal ossification was delayed in fetuses from dams that received doses of 150 and 450 mg/kg/day (i.e., variation) as noted above.

I.V. Segment II Teratology Study in Rats (GTR-32031).

Testing Laboratories: Byk Gulden Pharmaceuticals
Hamburg, Germany

Study Started: October 18, 1987

Study Completed: April 20, 1988 (report date)

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

Animals: Pregnant Crl:CD (SD) BK Sprague-Dawley rats.

No. of Animals: 25-26 pregnant rats/group.

Drug Batch No.: K23/161.

Methods: Pregnant rats were given I.V. (into tail vein) doses of 0 (vehicle: 0.9% NaCl, pH 10.2), 1, 4 and 20 mg/kg/day of pantoprazole from days 6 to 15 of gestation. The volume of administration was fixed at 5 mL/kg. All rats were observed for clinical signs and mortality daily. On day 20 of gestation, all dams were sacrificed and were examined for the number of corpora lutea, the number of implants, pre- and post-implantation loss and number of live fetuses. Live fetuses were weighed and sexed. About one-half of the fetuses in each litter were eviscerated and examined for skeletal major/minor abnormalities, and the remaining fetuses were examined for visceral abnormalities and variations.

Results: No treatment-related effects were seen. Treatment had no significant effect on fertility rate. The number of corpora lutea, number of implants, early and late resorption and mean fetal weight did not show any significant difference between the treated and control groups. No treatment related abnormalities were seen on external, skeletal and visceral examinations in any group, except delays in fetal ossification was evident in the high dose treated group.

Effect of Pantoprazole on Maternal and Fetal Parameters in Rats				
Parameters	Control	Low Dose	Mid Dose	High Dose
Total Mated	25	25	26	26
# of Pregnant	25	25	26	26
% Pregnant	100	100	100	100
# of Corpora Lutea/dam	16.1 ± 0.4	16.5 ± 0.5	16.5 ± 0.5	16.1 ± 0.4
# of Implants/dam	14.1 ± 0.6	14.6 ± 0.4	15.5 ± 0.3	13.8 ± 0.7
Pre-Implantation Loss (%)	12.4	11.2	6.3	13.9
Post Implantation Loss (%):				
Early	3.4	3.8	3.2	4.2
Late	0.8	1.4	0.2	1.4
Mean Live Fetuses/dam	13.6 ± 0.6	13.9 ± 0.4	15.0 ± 0.4	13.1 ± 0.7
Mean Fetal Wt. (g)	3.21 ± 0.07	3.25 ± 0.05	3.25 ± 0.03	3.32 ± 0.04
Sex Ratio (F/M)	1.09	1.09	1.05	0.99
Fetal Malformation/Variations				
Visceral	0/164	0/159	0/190	0/165
Skeletal	0/176	0/178	0/191	0/159

In this study, no teratogenic effects were observed in rats at intravenous doses ≤20 mg/kg/day.

Oral Segment II Teratology Study in Rats (GTR-32034).

Testing Laboratory: Byk Gulden Pharmaceuticals.

Date of the Study: January 2 1988 to Oct. 2, 1989.

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

Animals: Female Crl:CD rats 12 weeks of age were used.

Methods: Four groups of animals each consisting of 24-26 pregnant rats were given pantoprazole (Batch no. 579-015) dissolved in distilled water at acid dose levels of 0, 50, 150 and 450 mg/kg/day during days 6-15 of gestation period. Dams were sacrificed on day 20 of gestation. Fetuses were subjected to external examination and half of the fetuses were examined for visceral abnormalities and the remaining fetuses underwent skeletal examination.

Results:

Clinical Signs: Prostration, eating bedding material, and piloerection were seen in the 450 mg/kg/day group.

Body Weight: Retardation of body weight gain (12%) was observed in the 450 mg/kg/day group.

Fertility Rate: Normal.

Implantation Rate, Pre- and Post-Implantation Loss: Increase in pre-implantation loss was seen in the 450 mg/kg/day group (14.9% vs 10% for controls).

Number of Live Fetuses: There were no effects.

Fetus Ratio and Fetal Body Weight: There were no effect on sex ratio. Slight increase (5%) in fetal body weight was seen in the 150 mg/kg/day groups.

Fetal Examinations:

Visceral Examination: Two fetus in the 50 mg/kg/day group showed a hydrocephalus internus or a microphthalmos. One litter (five fetuses) in the 150 mg/kg/day group exhibited malformation of eyes. Increases in enlarged renal pelvis were observed in the drug treated groups (2/179, 13/171, 7/174 and 7/158 for 0, 50, 150 and 450 mg/kg/day groups, respectively).

Skeletal Examination: Fused sternebrae were found in 5 fetuses of one litter in the 50 mg/kg/day group and four fetuses of one litter in the 150 mg/kg/day group. Dose-dependent increased incidence of delayed ossification of the cranial bone was observed (68-82% vs 34% for controls).

In conclusion, pantoprazole at oral acid doses ≤ 450 mg/kg/day during days 6-15 of the gestation period did not induce any teratogenic effect, but produced delayed ossification.

Oral Segment II Teratology Study in Rats (GTR-32059).

Testing Laboratory: Byk Gulden
Institute of Pathology and Toxicology, FT3
Friedrich-Ebert-Damm 101
22047 Hamburg
Germany

Date Started: May 24, 1994

Date Completed: April 13, 1995

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Pregnant female Sprague Dawley were used in the present study. At the start of treatment, rats were 12 weeks old.

Drug Batch: Pantoprazole, Batch number 500205.

Methods: In a Segment II teratology study, pregnant female rats received pantoprazole by the oral route at doses of 0, 5, 15, and 50 mg/kg/day from days 6 to 15 of gestation. In the first Segment II teratology study with rats (GTR-32034) using doses of 50, 150, and 450 mg/kg/day, a dose-dependent increase in incomplete ossification of skull bones was detected. It should be noted that this effect is a variation, which has no effect on the survival of the animal. The sponsor conducted the present study in order to identify a no effect dose with regard to incomplete ossification of skull bones. Control animals received the vehicle, distilled water adjusted to pH 10 with NaOH. The vehicle or drug solution was administered using a stomach tube. The dose volume was 10 mL/kg. There were 24-28 pregnant female rats per group. Clinical signs of toxicity and morbundity/mortality were monitored daily. Body weight was measured on day 0, daily from days 6 to 15, and day 20. Food consumption was measured daily. Dams were sacrificed on day 20 of gestation and the gravid uterus was weighted. Numbers of corpora lutea, implantation sites, and live fetuses were determined. Fetuses were sexed, weighted, and examined for external malformations and variations. Approximately half of the fetuses of each litter/test group were fixed in Bouin's solution and examined for visceral malformations and variations. Remaining fetuses were examined for skeletal malformations and variations following fixation in alcohol after clearing in 1-2% KOH and staining of the skeleton with alizarin red.

Results: Treatment of dams with pantoprazole by the oral route at doses of 5, 15, and 50 mg/kg/day from days 6 to 15 of gestation had no effects on the following: body weight gain of dams, numbers of corpora lutea/dam, numbers of implantations/dam, pre-implantation loss, numbers of live fetuses/dam, post-implantation loss, fetal body weight, or fetal sex. Examination of fetuses revealed no treatment-related external or visceral malformations or variations. In addition, there were no treatment-related skeletal malformations; however, a number of variations were observed as listed below.

Incidence of skeletal variations in fetuses from pregnant female rats that received pantoprazole by the oral route at doses of 0, 5, 15, and 50 mg/kg/day from days 6 to 15 of gestation. Values in parentheses represent the percentage effected.

Parameter	**0**	**5**	**15**	**50**
# Fetuses/Litters examined	210/27	168/24	201/24	203/28
Rib Defects:				
11th rib-wavy	0/0	1 (0.6)/1(4.2)	5(2.5)/4(16.7)	1(0.5)/1(3.6)
6th-12th rib-wavy	0/0	2(1.2)/2(8.3)	4(2.0)/4(16.7)	0/0
8th-12th rib-wavy	0/0	1(0.6)/1(4.2)	3(1.5)/2(8.3)	0/0
Skull Bone Defects:				
Interparietal bone- incomplete ossified	42(20.0)/ 20(74.1)	42(25)/ 18(75)	44(21.9)/ 18(75)	61*(30)/ 24(85)
Interparietal and supraocciptal bones-incomplete ossified	33 (15.7)/ 17(63.0)	21(12.5)/ 9(37.5)	52*(25.9)/ 19(79.2)	47(23.2)/ 19(67.9)
Interpariparietal supraocciptial squamous bones-incomplete ossified	7 (3.3)/ 4 (14.8)	5(3.0)/ 4(16.7)	25*(12.4)/ 8(33.3)	17*(84)/ 10(35.7)
Jugal bone- incomplete ossified	11(5.2)/ 7(25.9)	12(7.1)/ 8(33.3)	34*(16.9)/ 12(50.0)	18(8.9)/ 10(35.7)
Os maxillare-incomplete ossified	0/0	2(1.2)/ 2(8.3)	2(1.0)/ 2(8.3)	5(2.5)/ 5(17.9)
Parietal bones-incomplete ossified	4(1.9)/ 2(7.4)	5(3.0)/ 4(16.7)	9(4.5)/ 7*(29.2)	13*(6.4)/ 9*(32.1)
Sternebral Defects:				
4th sternal vertebrae body and xiphisternum-incomplete ossified	85(40.5)/ 24(88.9)	91*(54.2)/ 21(87.5)	77(38.3)/ 21(87.5)	105*(51.7)/ 25(89.3)
Vertebrae Defects:				
First caudal vertebral body-incomplete ossified	115(54.8)/ 25(92.6)	76(45.2)/ 22(91.7)	133*(66.2)/ 24(100)	91(44.8)/ 27(96.4)
2nd-4th sacral vertebral bodies-incomplete ossified	0/0	1(0.6)/1(4.2)	8*(4.0)/5*(20.8)	1(0.5)/1(3.6)

*$p \leq 0.05$

In a Segment II teratology study, pregnant female rats received pantoprazole by the oral route at doses of 0, 5, 15, and 50 mg/kg/day from days 6 to 15 of gestation. Pantoprazole at doses ≤ 50 mg/kg/day produced no evidence of teratogenic effects. There was no evidence of maternal toxicity (i.e., decreased body weight or food consumption, clinical signs of toxicity) at doses ≤ 50 mg/kg/day. Skeletal examination of fetuses revealed evidence of incomplete ossification for bones in the skull at doses of 15 and 50 mg/kg/day; however, these effects were variations, which have no effect on survival.

Rabbits

I.V. Segment II Teratology Study in Rabbits (GTR-32033).

Testing Laboratories: Byk Gulden Pharmaceuticals
Hamburg, Germany

Study Started: April 5, 1988

Study Completed: August 24, 1988

GLP Requirements: A Statement of Compliance with GLP regulations and quality assurance unit was included.

Animals: "Little Russians" Himalaya rabbits (6-months old, 2 kg).

No. of Animals: 13 - 15 pregnant rabbits.

Route of Administration: I.V.

Dose Levels: 0, 1.5, 5 and 15 mg/kg/day.

Drug Batch No.: 579015.

Methods: In this study, dose selection was based on preliminary study (# 87-095) in which 15 mg/kg was maternal toxic (reduced food intake and body weight). In the main study pregnant rabbits were given I.V. (into ear vein) doses of 0 (vehicle: demineralized water pH 10), 1.5, 5 and 15 mg/kg/day of pantoprazole from days 6 to 18 of gestation. The volume of administration was fixed at 2 mL/kg. All rabbits were observed daily for mortality and clinical signs. Body weights were recorded on day 0 and 6 through 29 of gestation. Food intakes were recorded daily. All surviving dams were sacrificed on day 29 of gestation and were examined for number of implants, pre- and post- implantation loss, live fetuses were weighed, sexed and examined for external, skeletal and visceral abnormalities.

Results: No treatment-related clinical signs and mortality were seen during study period. The number of corpora lutea, the number of implants, pre- and post-implantation loss, mean fetal weights and sex ratio did not show any significant difference between the treated and control groups. No treatment-related abnormalities were seen in external, skeletal and visceral examinations in any group, except delayed dental growth was seen in mid and high dose treated groups.

Effect of Pantoprazole on Maternal and Fetal Parameters in Rabbits				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
Total Mated	13	14	13	15
# of Pregnant	13	14	13	15
% Pregnant	100	100	100	100
# of Corpora Lutea/dam	8.5 ± 0.4	8.2 ± 0.4	8.5 ± 0.3	7.7 ± 0.3
# of Implants/dam	6.9 ± 0.5	6.6 ± 0.5	7.2 ± 0.5	7.1 ± 0.4
Pre-Implantation Loss (%)	18.2	20.0	14.5	7.8
Post-Implantation Loss (%):				
Early	5.6	5.4	6.4	6.6
Late	2.2	4.3	1.1	1.9
Mean Live Fetuses/dam	6.4 ± 0.4	5.9 ± 0.6	6.7 ± 0.7	6.5 ± 0.4
Mean Fetal WT. (g)	40.5 ± 0.8	40.5 ± 1.3	37.2 ± 1/4	38.9 ± 0.9
Sex Ratio (F/M)	1.08	1.02	1.29	1.26
Fetal Malformation/Variations:				
Visceral	0/83	0/83	0/87	0/87
Skeletal	0/83	0/83	0/87	0/87
Delayed Dental Growth	1/83	1/83	3/87	3/87

In this study, no teratogenic effects were found at intravenous doses ≤ 15 mg/kg/day in rabbits.

Addendum: In the summary preceding the report, a reference to compound "B8510-023 (a metabolite of pantoprazole)" was made under the Conclusion on page IV. This appears to be an error. This study examined the effects of pantoprazole (B8610-023).

Oral Segment II Teratology Study in Rabbits (GTR-32035).

Testing Laboratory: Byk Gulden Pharmaceuticals.

Date of the Study: July 18, 1988 to December 20, 1988

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

Animals: Himalaya rabbits weighing 2.4 kg and 6 months of age were used.

Methods: Four groups of animals each consisting of 14-16 pregnant rabbits were given pantoprazole (batch no. 579 015) by gavage at acid dose levels of 0, 2.5, 10 and 40 mg/kg/day during days 6-18 of gestation period. Dose selection was based on a drug-range study (GTR-32061) that signs of maternal toxicity (reduced food intake) was seen at 40 mg/kg/day. Dams were sacrificed on day 29 of gestation. All fetuses were subjected to visceral and skeletal examinations.

Results:

Clinical Signs: Normal.

Body Weight Change: Normal.

Food Consumption: According to sponsor, food intake was reduced in the 40 mg/kg/day group from days 7-25 of gestation. However, the figure was not legible. Sponsor should be asked to provide the basis of the dose selection by providing the report of the dose-range study (GTR-32061) and the comprehensive data of food consumption of this study for our review.

Fertility: Normal.

Implantation Rate, Pre- and Post-Implantation Loss: Normal.

Living Fetuses: There were no drug-related effects.

Fetal Body Weight: Normal.

Wet Weight of Lens: Normal.

Fetal Examinations: No external changes were found.

Visceral Examination: One fetus of the 40 mg/kg/day showed a myocardial hemorrhage and one fetus of the same litter showed urethrophraxis and hydronephrosis.

Skeletal Examination: Delayed dental growth was observed in the 40 mg/kg/day group.

In conclusion, pantoprazole at oral acid doses up to 40 mg/kg/day did not produce any teratogenic effect except delayed dental growth was seen at 40 mg/kg/day. It produced only retardation of food consumption, but no readable data was submitted for review. It had no effect on maternal body weight gain. We could not determine whether maximal tolerated dose was employed in the study at this time. Sponsor should be asked to provide the report of the dose-range finding study and the comprehensive data of the food consumption in this study for our review.

Addendum: Using line listing in the main study (GTR-32035), food consumption from days 6 to 18 of gestation for rabbits that received pantoprazole at 2.5, 10, and 40 mg/kg/day was 100.4, 97.35, and 90.75% of the control (89.7 g/animal/day), respectively. Food consumption was reduced by almost 10% at the high dose of 40 mg/kg/day. Dose selection in the Segment II study with rabbits appears to be adequate. In the oral dose range finding Segment II study with rabbits (GTR-32061), the sponsor only used one dose of 40 mg/kg/day with 2 rabbits. There was no concurrent control group. The sponsor claimed reduced food consumption for dams at 40 mg/kg/day; however, there was no control group for comparison.

Rats

Oral Segment III, Perinatal and Postnatal Study in Rats (GTR-32081).

Testing Laboratories: [redacted]

Study Started: August 20, 1990

Study Completed: March 9, 1992

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

Test Species: Pregnant Sprague Dawley Rats (Crl: CD-VAF)

No. of Animals: 22 Pregnant Rats/Group

Drug Batch No.: 589085 - 88PD480

Dose Levels: 0, 1, 3 and 30 mg/kg/day (10 mL/kg body weight)

Methods: In this study, the dose selection was based on preliminary Segment III perinatal and postnatal studies in rats. Three experiments were conducted. In the first experiment, pregnant rats were given oral (gavage) doses of 0, 10, 30, 100, 300 or 600 mg/kg of [redacted] 96022-Z (pH 11.0-11.3, 10 mL/kg) from day 15 of gestation to day 21 after parturition. In the second experiment, doses of 0.1, 0.3, 1.0, 3.0 and 10 mg/kg/day were used. In the third experiment, doses of 0 and 10 mg/kg/day were used. In the first experiment, body weight gains during day 15-21 of gestation were reduced by 12%, 12%, 27%, 28% and 48% in dams treated with 10, 30, 100, 300 and 600 mg/kg/day, respectively, when compared to control values. In the second experiment, drug (0.1-10 mg/kg/day) had no effect on dams body weight gains. Body weight data was not reported from the third experiment. Food intake in dams treated with 600 mg/kg/day was also significantly reduced (24%) compared to control dams during gestation days 15-21. No abnormality was seen in this study, except body weight gains in the pups (day 2-21) were decreased by 14-15% (in experiment 2; 19%), 21%, 18-19%, 21-23% and 29-30% in 10, 30, 100, 300 and 600 mg/kg/day treated groups, respectively, when compared to the control values. [redacted] 96022-Z at 0.1-3 mg/kg/day had no effect on dams or their pups. Based on these findings, sponsor indicated in the preliminary report (# 26E/91) that "doses of ≤3 mg/kg/day should be used for the main segment III perinatal and postnatal study in rats". However, sponsor selected dose levels of 1, 3 and 30 mg/kg/day for the main study. The dose selection is appropriate and 30 mg/kg/day is the maximum tolerated dose, it reduced body weight gains in dams by 12% and pups by 21%.

In the main study, pregnant rats (22/group) were given oral (gavage) doses of 0 (vehicle, purified water pH 11.0-11.3), 1, 3 and 30 mg/kg/day of ——— 96022-Z from day 15 of gestation to day 21 after parturition. All dams were observed for clinical signs daily, body weights were recorded on days 0, 7, and 15 of postcoitum and daily thereafter until the termination of the study. Food consumptions were recorded between days 15-18 and 18-21 of gestation and during postnatal days 2-7, 7-14 and 14-21. All dams were allowed to litter normally and raise their pups to weaning. The number of live/dead pups were recorded and the live pups were weighed and sexed. On day 7 after birth, culling was carried out to make 8 offspring (4 males and 4 females). The offspring were reared by the dam until weaning. On day 21 of post partum, all dams were sacrificed and necropsied and examined externally and internally for abnormalities. During nursing period the growth and development of the pups were observed. Development parameters that were assessed included pinna detachment, incisor eruption, eye opening, negative geotaxis reflex, pupillary reflex, startle reflex, passive avoidance test, swimming maze test, and reproductive performance test at sexual maturity. F_1 males were killed after mating and F_1 females were killed 10 days after mating and numbers of corpora lutea and implantation sites were counted.

Results: Body weight gains in dams during gestation and lactation period were not affected by the treatment. Food consumptions during lactation period (days 7-14 and 14-21) were significantly reduced (7%) in high dose treated dams compared to controls. Length of gestation was comparable in all groups. No abnormalities were observed at autopsy of F_0 dams, which could be attributed to treatment. No drug related effects were seen in the F_1 pups during postnatal period except body weight gains of pups from high dose group was significantly reduced (19-22%) during the lactation period (day 1-21) compared to control values. This retardation of body weight gain was still evident in male offspring until 12 weeks of age. At the end of 12 weeks, weights of male pups from the high dose group were about 8% lower than that seen in pups of the control group. Development and reproductive performance were comparable in all groups. At 30 mg/kg/day, drug suppressed body weight gains in the pups. However, no adverse effects were seen in rats following oral administration of up to 30 mg/kg/day of ——— 96022-Z during perinatal and postnatal period.

In a Segment III perinatal and postnatal development study, pregnant rats received pantoprazole by oral gavage at doses of 0, 1, 3 and 30 mg/kg/day from day 15 of gestation to day 21 after parturition. Body weight gain in pups, from dams that received a dose of 30 mg/kg/day, was suppressed. Pantoprazole at doses ≤30 mg/kg/day had no significant effects on perinatal and postnatal development in rats.