Table 1. Number of adducts/10^8 nucleotides detected in the Omeprazole and Pantoprazole spots and background values for the equivalent areas in controls

		Exp. 1	Exp. 2	Exp. 3	Mean	SD	Average	SD	Significance
Omeprazole									
200mg/kg	1				2.28	1.5			
	2				2.11	0.6	2.30	0.2	NS
	3				2.5	0.9			
600mg/kg	1				2.82	0.6			
	2				2.81	0.5	2.52	0.5	NS
	3				1.93	0.8			
Control	1				2.13	1.8			
(Background)	2				2.18	2.3	1.90	0.5	
	3				1.29	0.9			
Pantoprazole									
200mg/kg	1				8.04	5.8			
	2				11.74	6.0	10.26	1.96	NS
	3				11	3.7			
Control	1				7.22	4.2			
(Background)	2				9.11	5.8	7.53	1.5	
	3				6.27	3.2			

* No visible spot
NS, no significant difference between the dosed and the corresponding control (background) samples.

Table 2. Number of DNA adducts/10^8 nucleotides in Control and Pantoprazole treated samples in ——— systems 1, 3, and 4 following nuclease P1 enhancement.

Solvent System 1			
Experiment Number	Spot	Control	Pantoprazole
5	Common	14.8	8.5
	Extra		11
6	Common	48	10
	Extra-1		12.6
	Extra-2		6.3
8	Common	14	12
	Extra-1		19.9
	Total	**42.2**	**73.6**
Solvent System 3			
Experiment Number	**Spot**	**Control**	**Pantoprazole**
5	Common	9.6	9.6
	Extra		9.1
	Total	**85.8**	**84.1**
8	Common	9.3	9.3
	Extra		17.3
	Total	**57.3**	**113.9**
Solvent System 4			
Experiment Number	**Spot**	**Control**	**Pantoprazole**
5	Common	15.5	15.5
	Extra		22.3
	Total	**37.8**	**87.8**
8	Common	11.3	11.3
	Extra		19.5
	Total	**38.8**	**89.4**

Extra spot (1) = Unique adduct.

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7. Toxicokinetics: Systemic exposure to pantoprazole or omeprazole in rats was relatively similar; however, exposure was significantly lower for lansoprazole assuming linear extrapolation to 1200 mg/kg/day.

Pharmacokinetic parameters for pantoprazole, omeprazole, and lansoprazole after oral administration for 7 days:

Treatment, mg/kg/day	C_{max}, µg/mL	T_{max}, hr	AUC0-8hr, µg*hr/mL	T½, hr
Pantoprazole, 200	45.5	0.5	81.3	5.0
Omeprazole, 600	33.8	2.0	121	Not available
Lansoprazole, 600[A]	5.96	2.0	28.2	Not available

A. Due to solubility problems, lansoprazole was administered at 600 mg/kg/day in this satellite study.

The sponsor assessed the nature of hyperplastic and hypertrophic changes in the liver and the potential for DNA damage in rats following treatment with pantoprazole and compared it with omeprazole and lansoprazole. Female rats received the vehicle, pantoprazole at 200 mg/kg/day, omeprazole at 200 or 600 mg/kg/day, or lansoprazole at 200 or 1200 mg/kg/day by the oral route for 4 weeks. Doses of omeprazole at 200 and 600 mg/kg/day and lansoprazole at 200 and 1200 mg/kg/day were unusually high and exceeded the highest doses used in rat carcinogenicity studies with these respective compounds. Absolute and relative liver weights were increased in all treatment groups. Centrilobular enlargement of hepatocytes (i.e., hypertrophy) was evident in all treatment groups and greatest for lansoprazole at 1200 mg/kg/day. Hepatocyte proliferation (i.e., hyperplasia) was greatest for rats that received pantoprazole at 200 mg/kg/day or omeprazole at 600 mg/kg/day. Hypertrophy and hyperplasia were reversible in all treatment groups following a 4-week recovery period. DNA damage assessed by ^{32}P-postlabeling suggested that pantoprazole treatment led to the formation of a unique DNA adduct not observed in control samples. An "extra spot" was observed with pantoprazole samples in three different chromatography systems that was not found in control samples. These results suggests that pantoprazole or one of its metabolites directly interacts with DNA to form an adduct. It is unclear how DNA adducts were quantified; however, the chromatography pattern clearly indicates that pantoprazole treatment lead to the formation of a unique adduct. This data clearly opens the concern that pantoprazole or one of its metabolites directly interacts with DNA.

2. Studies with the Thiol Metabolite of Pantoprazole (B8401-026):

Ames Test with B8401-026 (GTR-32256).

Testing Laboratories: Byk Gulden Pharmaceuticals
Dept. of Microbiology
Kontanz, Germany

Dates Studies Started and Completed: September 19, 1991 and August 20, 1992

Strain Used: Salmonella Typhimurium Strains TA98, TA100, TA1535, TA1537 and TA1538.

Concentration Levels: 125, 250, 500 and 1000 μg/plate.

Solvent Control: DMSO

Positive Controls: 9-aminoacridine (30-60 μg/plate), 2-aminoanthracene (0.5-2 μg/plate), 2-nitrofluorene (1-5 μg/plate), N-Methyl-N-nitro-N-nitrosoguanidine (2-4 μg/plate) and N-(3-[5-nitrofuryl-(2)-propenylidene])-O-toluene hydrazide (1.56-6.25 μg/plate).

Source of Metabolic Activation: Mouse liver microsomal enzymes.

Drug Batch No.: 200085

Criteria of Positivity: Two fold increase in the number of revertant colonies above the solvent control value are considered positive provided if the effect is also dose related.

Results: The drug inhibited the growth of most of the strains at 1000 µg/plate. Drug was not mutagenic in any of the tester strains, irrespective of the ~~treatment with~~ metabolic activation system ~~(S-9 Mix)~~. Increase ~~in mutant~~ colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

The thiol metabolite of pantoprazole (B8401-026) was negative in the bacterial reverse mutation assay with tester strains, TA98, TA100, TA1535, TA1537 and TA1538.

Addendum: Tests with strain TA98 may indicate weak mutagenic activity.

TA98 revertant colony count with Pantoprazole concentrations of 0, 125, 250, 500, and 1000 µg/plate.

Pantoprazole, µg/plate	Trial 1				Trial 2			
	-S9		+S9		-S9		+S9	
	Revertants	MAR	Revertants	MAR	Revertants	MAR	Revertants	MAR
0	24	1.00	19	1.00	22	1.00	17	1.00
125	25	1.04	17	0.91	25	1.13	17	1.00
250	24	1.01	19	1.00	29	1.31	15	0.90
500	28	1.17	19	1.02	27	1.22	19	1.12
1000	32	1.32	23	1.21	31	1.40	21	1.22

MAR = Mutagenic activity ratio.

Mouse Micronucleus Test with Thiol Metabolite (B8401-026) (GTR-32252).

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

Dates Study Started and Completed: September 3, 1991 and June 11, 1992.

No. of Animals: 5 mice/sex/group

Route of Administration: Orally (gavage).

Dose Selection: Dose selection was based on range finding experiment in which mortality was seen at 300 mg/kg and higher dose levels.

Dose Levels: 50, 125 and 250 mg/kg (10 mL/kg).

Solvent Control: Methocel and propylene glycol.

Drug Batch No.: 200085

Positive Control: Cyclophosphamide (25 mg/kg)

Methods: Animals were given a single dose of B8401-026 or positive control at 24 hours prior to sacrifice and preparation of the bone marrow. Additional groups of animals were also included which received 250 mg/kg of the drug at 48 and 72 hr prior to sacrifice and preparation of bone marrow slide. On the Giemsa-stained slides, 2000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. The compound is considered positive if the number of micronucleated polychromatic erythrocytes at one or more doses are significantly greater than the negative control value.

Results:

Micronucleus Test With B8401-026

Dose (mg/kg)	Sampling Time (hr)	Mean Micronucleated PCE	p-value
0	24	2.7	
50	24	3.3	NS
125	24	2.6	NS
250	24	3.1	NS
250	48	6.1	0.011
250	72	1.3	NS
Positive control	24	45.6	<0.001

NS = not significant according to non-parametric Fisher-Pitman permutation test (one-side)

The increase in the number of micronucleated polychromatic erythrocytes at the 48 hr. sampling time in high dose group was statistically significant. Thus, the metabolite of pantoprazole (B8401-026) was positive in this test. The positive control gave the expected findings.

The thiol metabolite of pantoprazole (B8401-026) was positive in the mouse micronucleus assay.

Addendum: COBS® hybrid mouse B6C3F1/CrlBr ([redacted] was used in this study.

Repeat Mouse Micronucleus Test with B8401-026 (GTR-32251).

In the earlier reported study (GTR-32252) thiol metabolite of pantoprazole was positive in mouse micronucleus test. It significantly increased the number of micronucleated polychromatic erythrocytes at 48 hr sampling point in 250 mg/kg treated mice. Lower dose levels were not tested at 48 hr sampling time. To confirm this finding sponsor repeated the test at the same testing laboratory using same age, sex and strain (B6C3F1) of mice and same dose levels. Samples from 36 and 48 hr sampling point were analyzed for the presence of micronuclei.

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

Dates Study Started and Completed: September 18, 1992 and January 21, 1993.

No. of Animals: 5 mice/sex/group.

Route of Administration: Orally (gavage).

Dose Levels: 50, 125 and 250 mg/kg

Solvent Control: Methocel and propylene glycol.

Drug Batch No.: 292109

Positive Control: Cyclophosphamide (25 mg/kg).

Methods: Animals were given a single dose of B8401-026 at 36 and 48 hours prior to sacrifice and preparation of the bone marrow (positive control animals were sacrificed at 24 hr after cyclophosphamide administration). On the Giemsa-stained slides, 2000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. The compound is considered positive if the number of micronucleated polychromatic erythrocytes at one or more doses are significantly greater than the negative control value.

Results: B8401-026 did not induce a significant increase of micronucleated polychromatic erythrocytes in mice bone morrow. In contrast, the positive control gave the expected results. These findings suggest that B8401-026 is not mutagenic in this repeat test.

The thiol metabolite of pantoprazole (B8401-026) was negative in a repeat mice micronucleus test.

Action of Thiol Metabolite of Pantoprazole on Malignant Transformation in C3H-M2 Mouse Fibroblasts In Vitro (GTR-32254).

Testing Laboratory: [redacted]

Date Started: September 30, 1992

Date Completed: February 23, 1993

GLP Compliance: None.

Methods: The ability of thiol metabolite of pantoprazole (B8401-026) to induce cell transformation in vitro with the M2 clone of C3H mouse fibroblasts (originally obtained from C3H mouse prostate) was evaluated in the absence of a metabolic activation system. Cells were treated with B8401-026 concentrations of 10, 25, 50,and 100 μg/mL for 24 hr. After, the 24-hr treatment period, medium containing the metabolite was removed and replaced with new medium. For assessment of cytotoxicity, 2 culture dishes/dose with 100 cells/dish were incubated for 2 weeks. For assessment of transformation, 18 culture dishes/dose with 1000 cells/dish were incubated for 8 weeks. The negative control was DMSO. The positive controls were N-methyl-N-nitro-N-nitroguanidine (MNNG, 0.5 μg/mL) and methylcholanthrene (10 μg/mL). For determination of cytotoxicity, the number of colonies/dish was determined and the plating efficiency (control = 100%) was calculated. For determination of malignant transformation, cultures are fixed and stained with Giemsa. Transformed foci of type III are counted. The study can only be evaluated when the rate of transformation induced by MNNG is approximately one focus/dish.

Results: Plating efficiency at B8401-026 concentrations of 10, 25, 50, and 100 μg/mL were 40, 36, 29, and 22%, respectively. There was no evidence of transformed foci following treatment with B8401-026. Positive controls produced expected responses. Experiments assessing the effects of metabolic activation were omitted from the present study.

The thiol metabolite of pantoprazole (B8401-026) was negative for induction of cell transformation in vitro with the M2 clone of C3H mouse fibroblasts in the absence of a metabolic activation system.

Cell Transformation Assay Using Syrian Hamster Embryo (SHE) Cells with B8401-026 (GTR-32255).

Testing Laboratory: [redacted]

Date Started: July 14, 1992

Date Completed: December 3, 1992

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

Drug Batch: B8401-026, Batch number 292109

Methods: The ability of the thiol metabolite of pantoprazole (B8401-026) to induce in vitro cell transformation of Syrian hamster embryo (SHE) cells was evaluated in the absence of a metabolic activation system. Initially, the plating efficiency of SHE cells with B8401-025 was evaluated at concentrations of 0.5, 1, 5, 10, 25, 50, 75, 100, and 128.4 μg/mL for 48 hr in the absence of metabolic activation. Concentrations >128.4 μg/mL exceeded the limit of solubility (i.e., precipitation). For the cell transformation assay, B8401-026 concentrations of 6, 15, 30, and 60 μg/mL were selected for the cell transformation assay.

Cells were treated with B8401-026 for 48 hr in the absence of metabolic activation. Within 10 days of the initiation of cell culture, cells were fixed, stained, and 100 cells per flask were examined for morphological transformation. The negative control was DMSO. The positive control was N-methyl-N'-nitro-nitrosoguanidine (0.25 μg/mL). A test was considered positive if it induced a significant dose-related increase in the transformation frequency or a significant and reproducible response for at least one of the test concentrations.

Results: Cytotoxicity studies with B8401-026 found that plating efficiency at a concentration of 50 μg/mL was 47.3%. There was no cell survival at concentrations of 100 and 128.4 μg/mL. No cell transformation was evident with B8401-026 concentrations of 6, 15, 30, and 60 μg/mL in the absence of metabolic activation. The positive control produced expected responses. Experiments assessing the effects of metabolic activation were omitted from the present study.

The thiol metabolite of pantoprazole (B8401-026) in the absence of metabolic activation was negative in the cell transformation assay using Syrian hamster embryo cells.

Addendum: The sponsor conducted the SHE cell transformation assay at pH 7.2. Recent studies (Kerckaert et al. Mutation Research 356: 65-84, 1996 and LeBoeuf et al. 356: 85-127, 1996) have demonstrated significant advantages of conducting this assay at pH 6.70 as compared to higher pH values (pH 7.10-7.35). These advantages include reduction of the influence of SHE cell isolates and fetal bovine serum lot variability on the assay, an increase in the frequency of chemically-induced morphological transformation (MT) compared to controls, and an increased ease in scoring the MT phenotype. Conducting the assay at pH 6.70 can greatly increase reproducibility and the predictive value of the assay. The results obtained by the sponsor with the thiol metabolite (B8401-026) in the SHE cell assay must be considered highly questionable. Thus, the assay cannot be considered valid.

3. Studies with [redacted] an Impurity Found in the Lyophilized Formulation of Pantoprazole for Intravenous Injection:

Reverse Mutation Assay Using Bacteria (Salmonella Typhimurium and Escherichia Coli) with [redacted] (GTR-32045).

Testing Laboratory: [redacted]

Byk Gulden
Konstanz, Germany

Date Started: June 14, 1996

Date Completed: May 20, 1998

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

Drug Batch: ——— batch number Zi20/107A

Methods: The genotoxic potential of ——— an impurity found in the lyophilized formulation of pantoprazole for intravenous injection, was assessed in bacterial reverse mutation assays using Salmonella typhimurium strains, TA1535, TA1537, TA98, and TA98, and Escherichia coli strains, WP2 and WP2uvrA. Both plate incorporation and pre-incubation techniques were used in these studies. The S9 liver microsomal fraction was obtained from ——— Positive controls in the absence of metabolic activation were NaN3 for TA1535 and TA100, 4-nitro-O-phenylene-diamine for TA1537 and TA98, and methyl methane sulfonate for WP2 and WP2uvrA. The positive in the presence of metabolic activation was 2-aminoanthracene for all strains. A dose ranging toxicity assay used ——— levels ranging from 0 to 5000 μg/plate with strains TA98 and TA100. For the plate incorporation and pre-incubation mutation assays, ——— levels were 33.3, 100, 333.3, 1000, 2500, and 5000 μg/plate. For the pre-incubation assay, ——— was pre-incubated with the tester strain for 60 min at 37°C in the presence or absence of metabolic activation and subsequently poured onto the surface of a minimal agar plate. For the plate incorporation assay, ——— and the tester strain in the presence or absence of metabolic activation were mixed and immediately poured onto the surface of a minimal agar plate. Plates were incubated at 37°C for 48 hr. Colonies were counted following the 48 hr incubation period. A test article was considered positive if a dose-related and reproducible increase in the number of revertants occurred or a substantial and reproducible increase for at least one test concentration occurred. A substantial increase was defined as follows: if in strain TA100, the number of revertants was at least twice the spontaneous reversion rate; and if in strains TA1535, TA1537, TA98, WP2, and WP2uvrA, the number of revertants was at least three times the spontaneous reversion rate.

Results: In a dose range finding assay, ——— was not toxic with tester strains, TA98 and TA100, at levels ≤5000 μg/plate. Using both plate incorporation and pre-incubation techniques, ——— at levels ≤5000 μg/plate did not produce an increase in the number of revertant colonies for any tester strain in the presence or absence of metabolic activation. ——— was negative in bacterial reverse mutation assays using Salmonella typhimurium strains, TA1535, TA1537, TA98, and TA98, and Escherichia coli strains, WP2 and WP2uvrA, in the presence or absence of metabolic activation. Positive controls produced expected responses with each tester strain.

——— possessed no genotoxic potential at levels ≤ 5000 μg/plate in bacterial reverse mutation assays using Salmonella typhimurium strains, TA1535, TA1537, TA98, and TA98, and Escherichia coli strains, WP2 and WP2uvrA, in the presence or absence of metabolic activation. Both plate incorporation and pre-incubation techniques were used.

Special Toxicity Studies

Pulmonary Studies

Rat

The Effect of Pantoprazole, Omeprazole, and B8410-26 (Thiol Metabolite) on the Lung After Intravenous Administration to Rats (GTR-31983 and GTR-31982).

Testing Laboratory: Byk Gulden Pharmaceuticals

Date of the Study: April 7, 1988 to May 1989.

GLP Requirement: A statement of compliance with GLP was not included.

Animals: Sprague-Dawley rats weighing 190-280 g were used.

Methods: Four groups of animals, each consisting of 8 rats/sex, received one of the following treatments for 4 days by the intravenous route: vehicle, 30 mg/kg/day of pantoprazole, 30 mg/kg/day of omeprazole, or 3 mg/kg/day of B-8410-26 (thiol metabolite).

Results:

Clinical Signs: Three male rats of the omeprazole group showed signs of ataxia and weakness (reduced movement) on day 4.

Mortality: None.

Body Weight: Normal.

Serum Gastrin: Elevation of gastrin level was seen in both omeprazole and pantoprazole groups.

Organ Weight: Normal.

Gross Pathology: One male in the omeprazole group showed a small grainy nodule on the liver.

Histopathology: Height of the gastric mucosa was similar for all test groups. No differences in the morphology of the thyroid or stomach were seen between the test groups. Four rats in the omeprazole group and 3 in the 841-26 group showed signs of a interstitial pneumonia, while 8 rats in the pantoprazole group were affected. However, this interstitial pneumonia is not uncommon in this strain of rat. In another 4-day intravenous toxicity study in rats (GTR-31982) conducted by the [redacted] pantoprazole at a dose of 30 mg/kg did not produce any evidence of pulmonary toxicity.

Addendum: Treatment of Sprague Dawley rats with pantoprazole at 30 mg/kg/day, omeprazole at 30 mg/kg/day, or B8410-26 at 3 mg/kg/day by the intravenous route for 4 days produced no evidence of pulmonary edema or changes in cellular content of the lung. The incidence of mild interstitial pneumonia was increased in all treatment groups as compared to the control; however, this is a common histopathological finding for Sprague Dawley rats. In a second 4-day intravenous toxicity study in rats (GTR-31982), pantoprazole at a dose of 30 mg/kg did not produce any evidence of pulmonary toxicity.

Pulmonary Toxicity of [redacted] 97165 (Thiol metabolite of pantoprazole) in Male Rats (GTR-32260).

Methods: This study was conducted to evaluate pulmonary toxicity of thiol metabolite of pantoprazole ([redacted] 97165). Male Sprague Dawley rats were given an intravenous dose of 90 mg/kg/day (given over 30 min) of [redacted] 97165 for 1 to 3/4 days. The control group rats were given the vehicle (0.9% saline, pH 11) in a similar fashion. At 6 and 24 hr after the first dose and 6 hr after the last dose, 5-7 rats from treated and control groups were sacrificed and lungs were examined microscopically.

Results: One rat that received [redacted] 97165 died on second day of the study. The cause of death could not be established (no microscopic abnormalities were seen in the lungs). Decreased motor activity and/or prostration were seen in treated rats. These clinical signs were not seen at 24 hr after drug administration. Single dose of [redacted] 97165 had no significant effect on lungs weight and did not produce any histological changes in lungs. However, after 3/4 days of daily treatment with [redacted] 97165, lungs relative weights were increased by 23% compared to control values. For this group, only 1 out of 7 rats had histological changes in the lungs that consisted of diffuse alveolar histiocytosis with limited alveolar proteinaceous exudate (focal, peripheral). This histological finding for 1 of 7 rats could be considered an incidental finding. Data indicated that [redacted] 97165 had little or no potential of pulmonary toxicity in male rats.

Addendum: [redacted] 97165 is 2-mercapto-5-difluoromethoxy benzimidazole. The increase in relative lung weight for one group treated with [redacted] 97165 was considered incidental due to lack of corresponding histopathological changes, no change in absolute weight, and no change in the wet weight to dry weight ratio.

Dog

Comparison of Toxicological Responses and Pharmacokinetic Behavior of [redacted] and [redacted] Dogs to Intravenous and Oral Treatment with Pantoprazole (GTR-32137).

Testing Laboratory: [redacted]

Date of the Study: Nov. 11, 1987 to March 29, 1988.

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

Animals: Female —— (13 months and 9.8-11.2 kg) and ——— 5-6 months of age and 7.2-8.8 kg) Beagle dogs were used.

Methods: Four groups of dogs (2 female dogs/group) were given pantoprazole for 5 days either by the oral route in enteric coated tablets (batches 4 and 5) at a dose of 100 mg/kg/day or by the intravenous route (batch no. 4) at a dose level of 50 mg/kg/day. The purpose of the study was to evaluate and compare the responses of — and ——— dogs when given doses of pantoprazole orally or intravenously with particular emphasis on lung changes or pharmacokinetics.

Results:

Clinical Signs: Salivation and emesis were noted in all dogs. Ataxia and subdued behavior were observed in both —— and ——— dogs with more prolonged effects being observed in the —— dogs. Respiratory effects (i.e., coughing) were noted in most — dogs and only one ——— dog (50 mg/kg, I.V.).

Mortality: One —— dogs (100 mg/kg, p.o.) was killed in extremis.

Body Weight and Food Consumption: Reduced body weights (0.8 kg) and food intake were seen in the —— dogs.

Hematology: There were no drug-treated effects.

Blood Chemistry: Reduction in ALT activity was seen in all dogs.

Gross Pathology: Mottled red or cream/red discoloration in the lung and white froth in the trachea were observed in —— dogs. Red areas in few lobes of lungs were detected in ——dogs.

Histopathology: Alveolar hemorrhage, foamy alveolar macrophages, eosinophilic material in alveoli (i.e., protein) and peribronchial and perivascular edema in lung tissues were observed. The —— dogs showed greater degree of foamy alveolar macrophages compare to the ——— dogs. The ——— dogs showed greater degree of alveolar hemorrhage compare to the —— dogs. Thymus involution was noted in all the —— dogs but not in the ——— dogs.

Plasma Drug/Metabolites Levels: The major metabolites were the same after I.V. and oral dosing (sulfone and thiol derivatives). The AUC of parent compound and metabolites were up to 2-3 times greater in ——dogs than in ——— dogs.

In conclusion, the data showed evidence of fluid effusion into the lung irrespective of supplier or route of administration. The —— dogs showed a greater degree of lung changes and clinical signs of toxicity than the —— dogs. The greater response by —— dog was associated with quantitative difference in pharmacokinetics of parent compound and its metabolites. However, these differences between —— and —— could be attributed to differences in age of dogs (13 months vs 5-6 months) rather than in suppliers as stated by sponsor. In addition, the number of animals (2 per group) used in the study is too small to reach a conclusion.

Addendum:

Mortality: For the ——dog that died on day 3 after treatment with pantoprazole at a dose of 100 mg/kg/day, gross findings included clear fluid discharge from the nose and a gelatinous thymus and mediastinal adipose tissue, which both dripped fluid when cut. These findings were considered secondary to pulmonary edema, which was regarded as the cause of death.

Histopathology: Electron microscopy from selected dogs revealed evidence of necrosis of a small proportion of alveolar capillary endothelial cells. Histopathological changes were considered indicative of vascular leakage with fluid effusion into the alveoli.

Plasma Drug/Metabolites Levels: Differences in pantoprazole and metabolite AUC values between —— and —— dogs were greatest after intravenous administration of pantoprazole.

Plasma $AUC_{0-\infty}$ values (μmol*hr/L) for pantoprazole and its sulfone (—— 97167) and thiol (—— 97165) metabolites on days 1, 3, and 5 in —— and —— beagle dogs that received pantoprazole by the intravenous route at a dose of 50 mg/kg/day (n = 2).

Supplier	Day 1			Day 3			Day 5		
	Pantoprazole	—— 97167	—— 97165	Pantoprazole	—— 97167	—— 97165	Pantoprazole	—— 97167	—— 97165
——	509	677.5	303	580	-	-	517	-	-
—— ——	362.5	375	208	375.5	-	-	339	-	-

Plasma $AUC_{0-\infty}$ values (μmol*hr/L) for pantoprazole and its metabolites on days 1, 3, and 5 in Fisons and Interfauna beagle dogs that received pantoprazole by the oral route at a dose of 100 mg/kg/day (n = 2).

Supplier	Day 1			Day 3			Day 5		
	Pantoprazole	—— 97167	—— 97165	Pantoprazole	—— 97167	—— 97165	Pantoprazole	—— 97167	—— 97165
——	683.5	754	549.5	1211	-	-	632.5	-	-
—— ——	672	681	309.5	689.5	-	-	655	-	-

5-Day Intravenous toxicity Study to Establish Dose/Response Relationships for Effusion of Fluid into Pulmonary Alveoli Following Daily Administration of Pantoprazole to Beagle Dogs (GTR-31987).

Testing Laboratory: ———————— and Byk Gulden

Date Started: December 2, 1987

Date Completed: December 16, 1997

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

Animals: Beagle dogs supplied by ———————— were used in this study. At the start of treatment, the body weight range was 9 to15 kg.

Drug Batch: Pantoprazole, Batch 4

Methods: This study was intended to establish a dose response relationship and no effect level for pulmonary changes in beagle dogs following daily intravenous treatment with pantoprazole for 5 days. In phase I of the study, two dogs/sex received pantoprazole at an intravenous dose of 50 mg/kg/day. In phase II of the study, 2 dogs/sex/group received pantoprazole at doses of 0, 7.5, 15, 30, and 50 mg/kg/day. Body weights and food consumption were measured daily. Clinical signs of toxicity were observed frequently during the first 12 hr after dosing each day. Lung sounds were assessed by auscultation. Dogs were necropsied following death, morbund sacrifice, or terminal sacrifice on day 6. The right apical lung lobe was removed and lavaged with a balanced salt solution. Lavage fluid was examined for total and differential cell count and total protein. Major organs and tissues were collected and preserved. Only samples of the lungs, tracheo-bronchial lymph node, and macroscopically abnormal tissue were processed and subjected to histopathological examination. Cell preparations from the pulmonary lavage fluid were examined degree of macrophage vacuolation and number of neutrophils present.

Results: In phase I of the study, 3 of the dogs that received pantoprazole at 50 mg/kg/day were observed with respiratory problems (i.e., lung sounds), which led to the sacrifice of 2 animals on days 3 and 4, respectively. In phase II of the study, 1 dog treated with 50 mg/kg/day died on day 3 due to fluid effusion into the pulmonary alveoli. The other 3 animals that received 50 mg/kg/day showed clinical evidence of respiratory problems. Gross necropsy examination of the lungs in dogs that received 30 and 50 mg/kg/day revealed some degree of red mottled discoloration. Only 1 dog at 15 mg/kg/day was observed with red mottled discoloration. White froth or frothy material was observed in the trachea for 2 dogs at 30 mg/kg/day and all but 1 animal at 50 mg/kg/day. For the 2 dogs sacrificed in Phase I and for the 1 dog that died in Phase II, there was evidence of edema in the thyrnus and other mediastinal structures. Slight to moderate vacuolation of macrophages was evident at doses ≥15 mg/kg/day. Elevated levels of protein in lavage fluid were observed

at doses ≥30 mg/kg/day. Histopathological analysis of the lung revealed areas of alveolar hemorrhage, eosinophilic material in alveoli (i.e., protein), and neutrophils in alveoli for dogs at 50 mg/kg/day. Areas of foamy alveolar macrophages were evident at doses of 15, 30, and 50 mg/kg/day. Moderate erythrophagocytosis was evident in the tracheobronchial lymph node for animals that received 30 and 50 mg/kg/day.

Histopathological changes for the lungs in dogs that received pantoprazole by the intravenous route at doses of 0, 7.5, 15, 30, and 50 mg/kg/day for periods up to 5 days (n = 2/group).

Lungs	Phase II-0	Phase II-7.5	Phase II-15	Phase II-30	Phase II-50	Phase I-50
Areas of alveolar hemorrhage						
-absent	2	2	2	2	1	1
-minimal	0	0	0	0	1	0
-slight	0	0	0	0	0	1
Eosinophilic material in alveoli						
-absent	2	2	2	2	1	0
-minimal	0	0	0	0	1	0
-moderate	0	0	0	0	0	1
-marked	0	0	0	0	0	1
Areas of foamy alveolar macrophages						
-absent	1	1	1	0	0	0
-minimal	1	1	0	0	0	0
-moderate	0	0	1	1	0	0
-marked	0	0	0	1	2	2
Areas with neutrophils in alveoli						
-absent	1	1	1	1	0	1
-minimal	1	1	0	1	0	0
-moderate	0	0	0	0	2	1
-marked	0	0	1	0	0	0

This study was intended to establish a dose response relationship and no effect level for pulmonary changes in beagle dogs following daily intravenous treatment with pantoprazole for 5 days. Dogs received pantoprazole at doses of 0, 7.5, 15, 30, and 50 mg/kg/day. At a dose of 50 mg/kg/day, there were clinical signs of respiratory problems (i.e., lung sounds), which resulted in the death of 1 animal due to fluid effusion into the pulmonary alveoli and morbund sacrifice of 2 other dogs. Gross necropsy examination of the lungs in dogs that received 30 and 50 mg/kg/day revealed some degree of red mottled discoloration. Only 1 dog at 15 mg/kg/day was observed with red mottled discoloration. White froth or frothy material was observed in the trachea for 2 dogs at 30 mg/kg/day and all but 1 animal at 50 mg/kg/day. For dogs that died or were morbund sacrificed at 50 mg/kg/day, there was evidence of edema in the thymus and other mediastinal structures. Slight to moderate vacuolation of macrophages was evident at doses ≥15 mg/kg/day. Elevated levels of protein in lavage fluid were observed at doses ≥30 mg/kg/day. Histopathological analysis of the lung revealed areas of alveolar hemorrhage, eosinophilic material in alveoli (i.e., protein), and neutrophils in alveoli for dogs at 50 mg/kg/day. Areas of foamy alveolar macrophages were evident at doses of 15, 30, and 50 mg/kg/day. Moderate erythrophagocytosis was evident in the tracheobronchial lymph node for animals that received 30 and 50 mg/kg/day. The no effect dose for pantoprazole-induced pulmonary toxicity appeared to be 7.5 mg/kg/day.

5-Day Intravenous Pulmonary Toxicity Study of Pantoprazole and —— 97165 in Dogs (GTR-31985).

Testing Laboratory: ————————————

Date of the Study: Sept. 8, 1989 to March 1990.

Animals: Beagle dogs weighing 7-12 kg and 9-11 month of age were used.

Methods: Five groups of animals each consisting of 2 males and 2 female were given pantoprazole (lot no. 589085-88PD477) intravenously for 30 minutes at acid dose levels of 0, 15 and 50 mg/kg/day or —— 97165 (thiol metabolite) at dose levels of 2.5, 5 and 15 mg/kg/day for 5 days. The dosage for the 50 mg/kg/day group was reduced to 40 mg/kg/day on day 2 because of their aggressive behavior. The objective of the study was to investigate the toxicokinetic relationship between pulmonary effects and systemic exposure to —— 97165 either following direct administration of —— 97165 or following administration of pantoprazole.

Results:

Clinical Signs: The animal became hyperexcitable and aggressive following first dose of 50 mg/kg. Uncoordinated gait and loss of coordination was seen in the dogs treated with pantoprazole. Moist rales were detected in the 50/40 mg/kg pantoprazole and 15 mg/kg —— 97165 groups. Inappetance was noted in 5 and 15 mg/kg —— 97165 groups.

Mortality: None.

Body Weight: One dogs of 15 mg/kg —— 97165 group lost body weight.

Drug Metabolism: The AUC values for —— 97165 following I.V. administration of 15 and 50 mg/kg of pantoprazole were equivalent to AUC values of —— 97165 following I.V. injection of 2.5 and 5 mg/kg of —— 97165, respectively.

Pulmonary Lavage: Increases in red blood cell were observed in the 50/40 mg/kg pantoprazole and 5 and 15 mg/kg 97165 groups. Increases in white blood cells were noted in the 50/40 mg/kg pantoprazole and 5 mg/kg 97165 groups. Protein was elevated in all drug-treated groups. Moderate vacuolation of macrophage cytoplasm was observed in the 50/40 pantoprazole and 5 and 15 mg/kg —— 97165 groups.

Lung Wet Weight/Dry Weight Data: Increases in lung water content was noted in the 50/40 mg/kg pantoprazole and 5 and 15 mg/kg —— 97165 groups.

Gross Pathology: Discoloration of the lung was noted in the 50/40 mg/kg pantoprazole and 5 and 15 mg/kg —— 97165 groups.

Histopathology: Foamy alveolar macrophages were found in the 50/40 mg/kg pantoprazole and 5 and 15 mg/kg —— 97165 groups.

In conclusion, intravenous administration of pantoprazole and —— 97165 can induce pulmonary alveolar effusion. The syndrome was characterized by increased lung sound (moist rales), lung discoloration, increased red cells and protein in the pulmonary alveolar lavage fluid, increased water content of lung tissue and histological findings of increased foamy alveolar macrophages and alveolar hemorrhage. Parameters studied indicated that the same process was occurring following administration and that a dose-responsive relationship existed for both compounds. The finding may support the hypothesis that the pulmonary changes induced by pantoprazole are a consequence of its metabolite —— 97165 (thiol metabolite) in dogs.

Addendum: Dogs were supplied by ———— Systemic exposure (AUC) to the thiol metabolite was >700 μg*min/mL in all dogs that had evidence of alveolar effusion, which supports the concept that pantoprazole-induced pulmonary toxicity might be due to its thiourea containing metabolite (—— 97165).

Effects on Thyroid Gland

Effects of Pantoprazole on the Thyroid Hormone Metabolism in Rats – UDPGT Induction By Consecutive Administration of Pantoprazole (GTR-31307).

Testing Laboratories: ————

Study Started and Completed: January 25, 1994 and July 17, 1995.

Drug Batch Number: 500205

Methods: In this experiment groups of female Sprague-Dawley rats (n=3/group) were given orally vehicle (0.5% CMC), pantoprazole, omeprazole or lansoprazole (5, 50 or 300 mg/kg/day) for 1 week. There were two positive control groups, one received MC (3-methylcholanthrene: 30 mg/kg/day) in corn oil (i.p.) for 3 days and the other group received PB (sodium phenobarbital: 80 mg/kg/ day) in physiological saline (i.p.) for 1 week. Twenty-four hours after the last dose, animals were sacrificed, blood and livers were collected to determine hepatic UDP-glucuronyltransferase (UDPGT) activities and plasma levels of thyroid hormones. Some of the animals from 300 mg/kg/day (pantoprazole, omeprazole and lansoprazole) dose group along with animals from positive control groups underwent bile ducts cannulation to assess biliary excretion of thyroid hormones. Levels of thyroid hormone and its glucuronide in bile were measured by ————

Results: All three proton pump inhibitors, significantly induced UDPGT activities (as measured by O-aminophenol UDPGT activities), however, the induction of UDPGT by pantoprazole was smaller than that seen with omeprazole or lansoprazole (induction at 300 mg/kg/day: pantoprazole = 2.4-fold, omeprazole = 4.5-fold and lansoprazole = 3.1-fold).

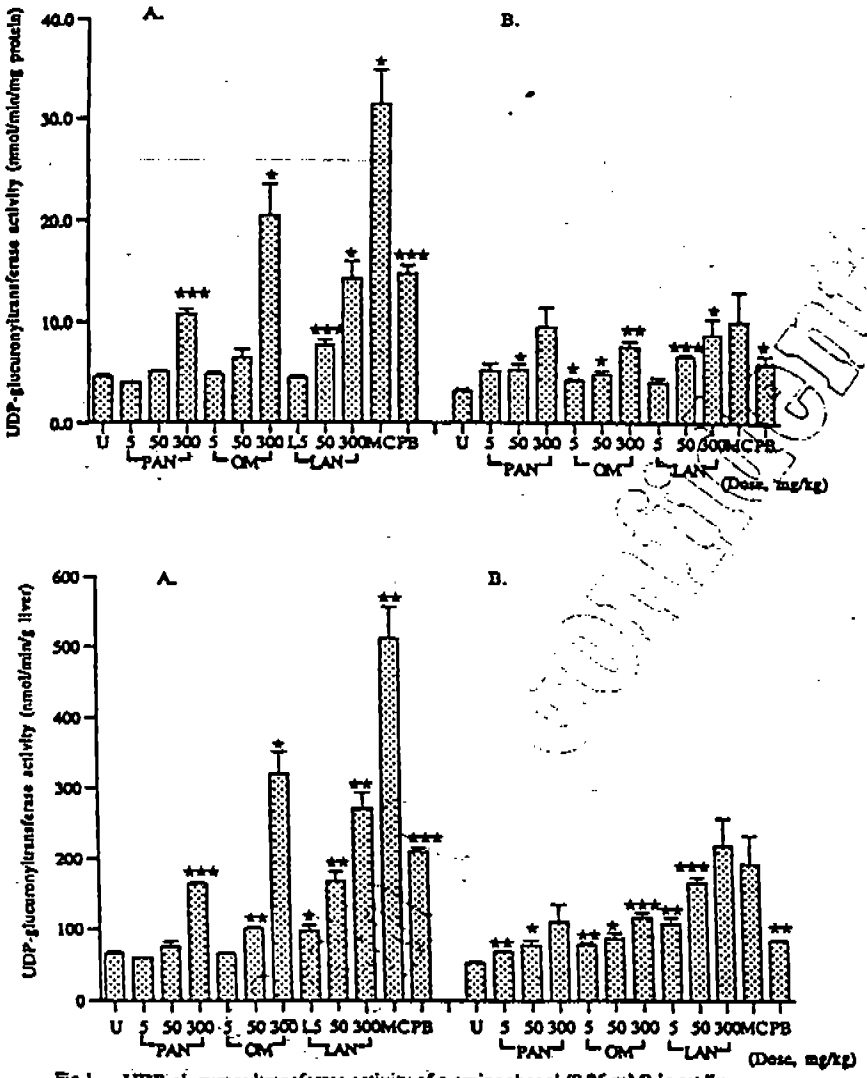

Fig.1. UDP-glucuronyltransferase activity of o-aminophenol (0.25 mM) in rat liver microsomes after consecutive administration of pantoprazole, omeprazole and lansoprazole.
A, 24 hr after the last dose; B, 1 week after the last dose.
U, untreated; PAN, pantoprazole; OM, omeprazole; LAN, lansoprazole; MC, 3-methylcholanthrene; PB, phenobarbital. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Plasma levels of T_3 and T_4 (which are metabolized by UDGT) decreased in rats treated with 300 mg/kg/day of pantoprazole, omeprazole, or lansoprazole; however, decreases were not statistically significant.

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Table 2. Effect of consecutive administration of pantoprazole, omeprazole and lansoprazole on plasma triiodothyronine (T3) and thyroxine (T4)

Treatment	Total T3 (ng/ml)	Free T3 (pg/ml)	Total T4 (μg/dl)	Free T4 (ng/dl)
U	0.620 ± 0.116	3.43 ± 0.05	3.68 ± 0.49	1.51 ± 0.20
PAN	0.711 ± 0.035	3.21 ± 0.16	2.85 ± 0.50	1.21 ± 0.06
OM	0.508 ± 0.027	2.97 ± 0.10[a]	2.93 ± 0.02	1.24 ± 0.03
LAN	0.541 ± 0.040	2.95 ± 0.32	2.01 ± 0.23[a]	0.81 ± 0.06[b]
MC	0.350 ± 0.058	2.86 ± 0.13	0.96 ± 0.23[b]	0.47 ± 0.09[b]
PB	0.452 ± 0.037	2.65 ± 0.13[a]	1.78 ± 0.12[a]	0.78 ± 0.03[a]

Rats were exposed to pantoprazole (300 mg/kg/day for 7 days), omeprazole (300 mg/kg/day for 7 days), lansoprazole (300 mg/kg/day for 7 days), 3-methylcholanthrene (30 mg/kg/day for 3 days), and phenobarbital (80 mg/kg/day for 7 days). 24 hours after the last dose, plasma levels of total T3, total T4, free T3 and free T4 were determined by ———

Each value represents mean ± S.E. for 3 rats. a, p<0.05, b, p<0.01, c, p<0.001
U, untreated; PAN, pantoprazole; OM, omeprazole; LAN, lansoprazole; MC, 3-methylcholanthrene; PB, phenobarbital.

Table 3. The effect of pantoprazole, omeprazole and lansoprazole on the biliary excretion of thyroid hormone in rats

Treatment	Bile flow (mg/min)	Bile excretion (cpm/min)	Bile concentration (cpm/mg)
U	11.5 ± 0.6	3352 ± 227	293 ± 21
PAN	21.2 ± 0.8[c]	4567 ± 160[b]	216 ± 5[c]
OM	17.9 ± 1.1[c]	9160 ± 562[c]	515 ± 29[c]
LAN	24.1 ± 0.8[c]	7514 ± 421[c]	311 ± 9
MC	13.9 ± 1.0	13938 ± 1069[c]	1008 ± 27[c]
PB	18.4 ± 1.7[b]	7645 ± 361[c]	427 ± 27[b]

Each value represents mean ±S.E. for 3 rats. [a], p<0.05, [b], p<0.01, [c], p<0.001
U, untreated; PAN, pantoprazole; OM, omeprazole; LAN, lansoprazole; MC, 3-methylcholanthrene; PB, phenobarbital.

Biliary excretion of [^{125}I]thyroxine (T4) was significantly increased by the treatment of rats with proton pump inhibitors (pantoprazole, omeprazole or lansoprazole). Increase in biliary excretion of radiolabeled T4 by pantoprazole was much smaller than that seen with omeprazole or lansoprazole.

Thus, overall, pantoprazole had minimal effect on thyroid metabolism in rats. ———) discussed the effect of pantoprazole on thyroid function in rat and compared with omeprazole. TSH-driven thyroid follicular hypertrophy and hyperplasia can result from either direct inhibition of thyroid peroxidase (which incorporate iodine into T_4 and T_3) or indirectly enhancing thyroid plasma protein binding, metabolism and/or excretion and receptor - mediated effects at hypothalamic and pituitary levels. Pantoprazole (200 mg/kg x 14 days) and omeprazole (500 mg/kg/day x 7 days) had no effect on thyroid peroxide activity. Pantoprazole (300 mg/kg x 28 days) also increases hepatic clearance of T_4 by 106% in rats. In another study in SD rats, pantoprazole (500 mg/kg/day for 28 days) resulted in decrease of T_3 levels (30 - 40%) and concomitant increase in plasma TSH level (93-234%). Taking all these findings together, it is evident that pantoprazole at high dose indirectly affects thyroid metabolism and increases TSH levels in rats. However, 200 mg/kg/day (the highest dose used in SD rat carcinogenicity study) had no significant effect on thyroid metabolism. Therefore, increased incidence of thyroid tumors (adenomas + carcinomas) seen in SD rat carcinogenicity study is not due to imbalance of thyroid metabolism (i.e. TSH-driven).

Effects on Thyroid Function After 4 Weeks of Treatment in Female SD Rats (GTR-31720).

Testing Laboratories: [redacted]

Dates Study Started and Completed: March 1995 and May 17, 1995

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

Drug Batch Number: Pantoprazole, batch number 510235.

Methods and Results: In bile duct-cannulated Sprague Dawley female rats, pantoprazole (300 mg/kg/ day, pH 10.5, for 4 weeks) produced significant increase in biliary clearance of total ^{125}I-T_4 as result of an increased excretion of conjugated ^{125}I-T_4 (excretion of free ^{125}I-T_4 in the bile decreased following pantoprazole treatment). In this experiment phenobarbital (75 mg/kg/day for 4 weeks) had similar effects.

Treatment	CL_b (ml/min)	T_4 Excreted (ng/24 hr)		
		Total	Free	Conjugated
Control	26.9 ± 7.8	29.5	10.0	19.5
Pantoprazole (300 mg/kg/day for 4 weeks)	55.4 ± 20.0	50.9	5.2	45.7
Phenobarbital (75 mg/kg/day for 4 weeks)	52.6 ± 11.0	48.7	6.3	42.4

CL_b = biliary clearance of thyroxine (T_4)

Effects on Thyroid Function After 4-Weeks of Treatment in Female Rats (GTR-31306)

Testing Laboratories: [redacted]

Dates Study Started and Completed: December 12, 1995 and April 19, 1996.

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

Drug Batch Numbers: 0295250000 and 122H0143

Methods: Groups of SD female rats (8/group) were given daily oral (gavage) doses of vehicle (de-ionized distilled water, pH 10.5), 50, 200 and 500 mg/kg/day of pantoprazole for 28 days. One additional group was also included in the study which received 75 mg/kg/ day of phenobarbital (solvent for phenobarbital was distilled water) for 28 days. Subsequently, all animals were maintained drug free for 4-week of recovery period. All rats were observed daily for clinical signs and mortality. Body weights and food intakes were recorded weekly. on day 4 and 28 of the treatment phase and on day 14 and 28 of the recovery phase blood samples were collected at 6 and 24 hours after drug administration to monitor T3, T4 and TSH levels. At the end of recovery period at least 6 rats/group (if possible) were sacrificed, livers were collected, weighed and then stored for future use.

Results:

1. **Observed Effects:** Low and mid dose rats, showed clinical signs such as salivation and paddling of the limbs. In addition to above signs, hunched posture, abnormal gait and piloerection were seen in high dose-treated rats. Rats treated with phenobarbital showed clinical signs such as unsteadiness, collapse, lethargy, piloerection, and hunched posture. No clinical signs were seen in control rats and in rats during 4-week recovery phase.

2. **Mortality:** One phenobarbital treated female was killed due to poor condition an day 8 of treatment phase. The cause of death was dosing error.

3. **Body Weight/Food Consumption:** At the end of treatment period body weight gains were reduced by 14.1%, 13.3% and 4.1% at low, mid and high dose levels respectively (body weight gains were reduced by 25.6% in phenobarbital treated rats). During recovery, body weights did not recover completely (final body weights were still 7-9% lower in all treated rats than the control body weights). Treatment had no significant effect on food consumptions, however, during recovery period, food intakes were decreased by 9% and 13% in rats previously treated with high dose of pantoprazole and phenobarbital respectively.

4. **T_3, T_4 and TSH Levels:** Only at high dose (500 mg/kg/day of pantoprazole) T_3 levels were decreased significantly (29-40%) and TSH levels were increased significantly 2 to 3-fold. At 24 hr after the last dose of phenobarbital, TSH level was increased by 66% compared to control values.

Effect on Thyroid Function After 4 Weeks of Treatment in Female Rats

Parameters	Control				Pantoprazole (500 mg/kg/day)			
	Day 4		Day 28		Day 4		Day 28	
	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr
T_3 (ng/dl)	52	54	58	63	31*	33*	51	45*
T_4 (µg/dl)	2.5	2.6	2.8	2.8	2.5	2.4	2.9	2.4
TSH (ng/ml)	7.7	10.5	10.1	13.6	25.7	23.7	31.1	26.2*

* $p = <0.01$

5. **Organ (liver) Weights:** Not affected by the treatment.

In female rats, high dose of pantoprazole (500 mg/kg/day for 28 days) was associated with reversible decrease in T_3 and increase in TSH levels in plasma. However, at 200 mg/kg/day no effect on thyroid metabolism was evident.

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