

**Methods:** The acute toxicity of pantoprazole by the oral and intravenous route was examined in NMRI mice, Sprague Dawley rats, and beagle dogs. Mice received drug by the oral or intravenous route in water at pH 10 using a dose volume of 10 mL/kg. Rats received drug by the oral route in water at pH 10 using a dose volume of 10 mL/kg. Rats received drug by the intravenous route in water at pH 10 in a dose volume of 20 mL/kg. Beagle dogs (1/sex/dose) received pantoprazole by the oral route as a dry powder packed in gelatin capsules. Beagle dogs (1/sex/dose) received pantoprazole by the intravenous route dissolved in physiological saline, pH 9.79-10.43. Animals were monitored for clinical signs of toxicity and morbidity/mortality for periods ranging  $\leq$  16 days after treatment. Animals that died as a result of treatment and those that survived to the end of the observation period were subjected to necropsy examination.

**Results:** For NMRI mice that received pantoprazole by the oral route at 250 mg/kg, clinical signs included hypoactivity, ataxia, and hunched sitting. At oral doses  $\geq$  500 mg/kg, additional clinical signs included limb-splay, lateral position, segregation, hunched sitting, and absence of ear reflex. For male and female NMRI mice that received pantoprazole by the intravenous route at 150 mg/kg, clinical signs included short atactic running phase, hypoactivity, and limb-splay (lying). At intravenous doses  $\geq$  300 mg/kg, additional clinical signs for male mice included ataxia, lateral position, segregation, hunched sitting, and absence of ear reflex. Female mice at doses  $\geq$  300 mg/kg were observed with clonic convulsions prior to lateral position. For male and female rats that received oral doses  $\geq$  500 mg/kg in GTR-31639, clinical signs included a decrease in spontaneous movement or prone position, depression of the righting reflex, oligopnea 5-15 min after dosing, and salivation 15-30 min after dosing. In GTR-31635, female rats that received doses  $\geq$  500 mg/kg were observed with slightly decreased activity and hunched sitting. Clinical signs observed following intravenous administration of drug were similar to those observed after oral administration. In GTR-32136, a comparison of the acute intravenous toxicity of old and new batches of pantoprazole in male rats found no significant differences. For old and new batches, doses  $\geq$  100 mg/kg produced prostration, dyspnea, hunched posture, and ataxia. In GTR-31650, a comparison of the acute intravenous toxicity of stressed and unstressed batches of pantoprazole in male rats found no significant differences. For stressed and unstressed batches, doses  $\geq$  100 mg/kg produced prostration and labored breathing. Beagle dogs that received a dose of 1000 mg/kg by the oral route died within 24 hr after administration. The progression of clinical signs was as follows: prone position, shaking of the head, and tremor during the first hour, lethargy, miosis, and lateral position were observed after 2 hours, coma and decreased respiratory rate were observed after 3 hours, and declining body temperature was observed from 5 hr on. Dogs that received 300 mg/kg by the oral route were observed with prone position, shaking of the head, miosis, and tremor by the first hour; however, recovery occurred within 2 days. Beagle dogs that received 300 mg/kg by the intravenous route died 3-4 min after the start of administration. Clinical signs were similar to those observed for the oral route. Beagle dogs that received 150 mg/kg by the intravenous route recovered within a few days after treatment.

## Acute oral and intravenous toxicity of pantoprazole in mice, rats, and beagle dogs.

| Species/<br>Report #   | Route | Dose,<br>mg/kg<br>(Na <sup>+</sup> salt) | Maximum Nonlethal<br>Dose, mg/kg |        | Minimum Lethal<br>Dose, mg/kg |        | LD <sub>50</sub> , mg/kg |        | Time to<br>Death              |
|------------------------|-------|------------------------------------------|----------------------------------|--------|-------------------------------|--------|--------------------------|--------|-------------------------------|
|                        |       |                                          | Male                             | Female | Male                          | Female | Male                     | Female |                               |
| Female mice<br>(31636) | Oral  | 250-<br>1000                             | -                                | 500    | -                             | 750    | -                        | 747    | Within 1<br>day               |
| Male mice<br>(31634)   | Oral  | 250-<br>1000                             | 750                              | -      | 1000                          | -      | >1000                    | -      | Within 1<br>day               |
| Female mice<br>(31637) | IV    | 50-450                                   | -                                | 350    | -                             | 400    | -                        | 395    | Within<br>15 min              |
| Male mice<br>(31633)   | IV    | 150-450                                  | 350                              | -      | 400                           | -      | 399                      | -      | Within<br>15 min <sup>A</sup> |
| M/F<br>rats<br>(31639) | Oral  | 700-<br>2000                             | 700                              | 700    | 900                           | 900    | 1343                     | 1037   | 2 hr-<br>2 days               |
| Female rats<br>(31635) | Oral  | 125-<br>1000                             | -                                | 1000   | -                             | N.D.   | -                        | >1000  | -                             |
| M/F<br>Rats<br>(31640) | IV    | 210-500                                  | 210                              | 260    | 260                           | 320    | 331                      | 343    | Within<br>0-5 min             |
| Female rats<br>(31638) | IV    | 100-400                                  | -                                | 200    | -                             | 250    | -                        | 256    | Within<br>0-15<br>min         |
| Male rats<br>(32136)   | IV    | 100-300<br>#294160<br>= old              | 200                              | -      | 250                           | -      | 200-250                  | -      | Within<br>0-5 min             |
| Male rats<br>(32136)   | IV    | 100-300<br>#513150<br>= new              | 200                              | -      | 250                           | -      | 200-250                  | -      | Within<br>0-5 min             |
| Male rats<br>(31650)   | IV    | 100-300<br>#033927<br>= stressed         | 250                              | -      | 300                           | -      | 250-300                  | -      | Within<br>0-5 min             |
| Male rats<br>(31650)   | IV    | 100-300<br>#349235<br>= cold<br>storage  | 200                              | -      | 250                           | -      | 250-300                  | -      | Within<br>0-5 min             |
| M/F dog<br>(31642)     | Oral  | 100-<br>1000                             | 300                              | 300    | 1000                          | 1000   | -                        | -      | Within<br>24 hr               |
| M/F dog<br>(31643)     | IV    | 37.5-<br>300                             | 150                              | 150    | 300                           | 300    | -                        | -      | During<br>admin.              |

A. At doses 400 and 450 mg/kg, 2/5 and 4/5 male mice, respectively, died within 15 min; however, 1 male at 450 mg/kg died between 6 and 24 hr and 1 male at 400 mg/kg died on day 4.

N.D. = Not Determined.

The acute toxicity of pantoprazole was examined in mice, rats, and dogs. In mice that received pantoprazole by the oral route, the maximum nonlethal doses in males and females mice were 750 and 500 mg/kg, respectively. The minimum lethal doses in male and female mice were 1000 and 750 mg/kg, respectively. Estimated oral LD<sub>50</sub> values in male and female mice were >1000 and 750 mg/kg, respectively. In both male and female mice that received pantoprazole by the intravenous route, the maximum nonlethal and minimal lethal doses were 350 and 400 mg/kg, respectively. Estimated intravenous LD<sub>50</sub> values in male and female mice were 399 and 395 mg/kg, respectively. In rats that

received pantoprazole by the oral route, the maximum nonlethal dose in both male and female rats was 700 mg/kg/day. The minimum lethal dose in both male and female rats was 900 mg/kg/day. Estimated oral LD<sub>50</sub> values in male and female rats were 1343 and 1037 mg/kg/day, respectively. In rats that received pantoprazole by the intravenous route, the maximum nonlethal doses in males and females were 200-250 and 200-260 mg/kg, respectively. The minimum lethal doses in male and female rats were 250-300 and 250-320 mg/kg, respectively. The estimated intravenous LD<sub>50</sub> values in male and female rats were 200-331 and 256-343 mg/kg, respectively. In beagle dogs following oral administration of pantoprazole, the maximum nonlethal and minimum lethal doses were 300 and 1000 mg/kg, respectively. In beagle dogs following intravenous administration of pantoprazole, the maximum nonlethal and approximate minimum lethal doses were 150 and 300 mg/kg, respectively. Clinical signs in mice, rats, and dogs following oral or intravenous administration of pantoprazole were similar and included decreased activity and ataxia.

**Acute Intravenous Toxicity of (+)Enantiomer of the Racemic Pantoprazole (B9010-007) in Mice (GTR-31644).**

**Testing Laboratories:** Byk Gulden  
Konstanz, Germany

**Study Started and Completed:** January 12, 1993 and June 24, 1993.

**Methods:** NMRI mice (3/sex/group) were given a single I.V. dose of 0, 170, 220, 270, 370 and 450 mg/kg of B9010-007 (pH 10). All mice were observed for clinical signs and mortality for 14 days. At the end of observation period all surviving rats were sacrificed and necropsied.

**Results:** Reduced activity, prostration and increased respiration rate were seen in all treated mice. Tremors and convulsions were also seen in some of the treated mice. The highest non-lethal dose was 220 mg/kg for mice of both sexes. The minimum lethal dose was 270 mg/kg (both sexes).

**Addendum:** Intravenous LD<sub>50</sub> values in male and female mice were 302 and 390 mg/kg, respectively.

**Acute I.V. Toxicity of Intravenous (-)Enantiomer of the Racemic Pantoprazole (B9010-026) in Mice (GTR-31645).**

**Testing Laboratories:** Byk Gulden  
Konstanz, Germany

**Study Started and Completed:** October 12, 1993 and April 30, 1993

**Methods:** NMRI mice (5/sex/group) were given a single I.V. dose of 0, 170, 220, 270 and 370 mg/kg/day of B9010-026 (pH 10). All mice were observed for clinical signs and mortality for 14 days. At the end of observation period, all surviving mice were sacrificed and necropsied.

**Results:** Ataxia, loss of muscle tone and prostration were seen in all treated mice. The highest non-lethal I.V. doses were 170 mg/kg for males and 220 mg/kg for females. The minimum lethal I.V. doses were 220 mg/kg in males and 270 mg/kg in females.

**Addendum:** Intravenous LD<sub>50</sub> values in male and female mice were 244 and 220-270 mg/kg, respectively.

**Acute Oral Toxicity of the Thiol Metabolite (B 8401-026) of Pantoprazole in Rats (GTR-32262).**

**Methods:** The thiol metabolite of pantoprazole (B8401-026; 5-difluoromethoxy-1H-benzimidazole-2-thiol) has been associated with pulmonary toxicity in dogs. The acute oral toxicity of the thiol metabolite was assessed in rats. Rats (5/sex/group) received B8401-026 by oral gavage at doses of 160, 240, 360, 540 or 810 mg/kg. Control rats received the vehicle, 1,2- propylene glycol in an aqueous methocel suspension. The dose volume was 20 mL/kg. All animals were observed for clinical signs and mortality daily for 16 days. At the end of observation period, surviving animals were sacrificed and necropsied.

**Results:** The highest non-lethal dose of the thiol metabolite of pantoprazole was 240 mg/kg in male rats and 160 mg/kg in female rats. LD<sub>50</sub> values were 360 mg/kg for male rats and 340 mg/kg for female rats. Clinical signs included ataxia, loss of muscle tone, prostration, hypothermia, reduced activity, ptosis, piloerection and hunchbacked posture.

The highest non-lethal dose of the thiol metabolite of pantoprazole was 240 mg/kg in male rats and 160 mg/kg in female rats. LD<sub>50</sub> values were 360 mg/kg for male rats and 340 mg/kg for female rats.

**Addendum:** The minimum lethal dose of the thiol metabolite of pantoprazole was 360 mg/kg in male rats and 240 mg/kg in female rats. Pulmonary toxicity was evident in rats at doses  $\geq$ 360 mg/kg. Histopathological analysis of the lungs from rats that received doses  $\geq$ 360 mg/kg revealed protein-rich perivascular and focal alveolar edemas.

**Acute Toxicity after Intravenous Administration of \_\_\_\_\_ to Mice and Rats (GTR-31649 and GTR-31648).**

**Testing Laboratory:** Byk Gulden  
Institute of Pathology and Toxicology, FT2  
Friedrich-Ebert-Damm 101  
22047 Hamburg  
Germany

**Study Started:** November 8, 1994 (mice) and October 25, 1994 (rats)

**Study Completed:** April 18, 1995 (mice) and March 27, 1995 (rats)

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

**Animals:** NMRI mice and Sprague Dawley rats were used in this study. On the day of administration, mice were 6-10 weeks of age and body weight ranges were 32-40 g for males and 25-33 g for females. Rats were 6 weeks of age and body weight ranges were 163-211 g for males and 140-172 g for females.

**Drug Batch:** \_\_\_\_\_ Batch number K33/141-1

**Methods:** The compound, \_\_\_\_\_ is an impurity found in the lyophilized formulation of pantoprazole for intravenous injection. The acute intravenous toxicity of \_\_\_\_\_ was examined in mice and rats. Mice (2 to 5/sex/group) received \_\_\_\_\_ by the intravenous route at doses of 50, 65, 85, 110, 143, 200, and 400 mg/kg. Rats (5/sex/group) received \_\_\_\_\_ by the intravenous route of administration at doses of 40, 52, 68, 88, and 114 mg/kg. Mice or rats in the control group received the vehicle composed of ethanol, 1,2-propylene glycol, and trometamol in water for injection at a pH of 5.8 to 7.3. Clinical signs of toxicity and morbidity/mortality were monitored for 14 days after dosing. On day 15, animals were sacrificed and subjected to a general microscopic evaluation.

**Results:** For mice that received doses  $\geq 50$  mg/kg, clinical signs included prostration, loss of muscle tone, and increased respiratory rate. Deaths at doses of 50-400 mg/kg occurred within 8 min and were generally preceded by convulsions or muscle spasms. For rats that received doses  $\geq 40$  mg/kg, clinical signs included prostration, increased respiratory rate/dyspnea, piloerection, reduced activity, hunched position, limp, and lying flat on stomach or side were observed in rats that received doses. Convulsions, tremors, and choking/gasping were observed at doses  $\geq 68$  mg/kg. All deaths occurred within 10 min after dosing. Surviving mice or rats recovered quickly, but some continued to display a hunched posture and/or piloerection for several days after injection.

Acute intravenous toxicity of \_\_\_\_\_ in mice and rats.

| Species | Dose, mg/kg | Maximum Nonlethal Dose, mg/kg |        | Minimum Lethal Dose, mg/kg |        | LD <sub>50</sub> , mg/kg |        | Time to Death |
|---------|-------------|-------------------------------|--------|----------------------------|--------|--------------------------|--------|---------------|
|         |             | Male                          | Female | Male                       | Female | Male                     | Female |               |
| Mice    | 50-400      | N.D.                          | 50     | 50                         | 65     | 119                      | 167    | $\leq 8$ min  |
| Rats    | 40-114      | N.D.                          | 52     | 40                         | 68     | 73                       | 82     | $\leq 10$ min |

The acute intravenous toxicity of \_\_\_\_\_ an impurity found in the lyophilized formulation of pantoprazole for intravenous injection, was examined in mice and rats. The maximum nonlethal dose in female mice was 50 mg/kg; however, it was not determined for male mice. The minimum lethal doses in male and female mice were 50 and 65 mg/kg, respectively. LD<sub>50</sub> values for male and female mice were 119 and 167 mg/kg, respectively. The maximum nonlethal dose in female rats was 52 mg/kg; however, it was not determined for male rats. The minimum lethal doses in male and female rats were 40 and 68 mg/kg, respectively. LD<sub>50</sub> values for male and female rats were 73 and 82 mg/kg, respectively.

**RAT**

**Subacute Toxicology**

**Intravenous Route of Administration**

**The Subacute Toxicity of Pantoprazole after Intravenous Administration in the Rat for 4-Weeks (GTR-31901).**

**Testing Laboratories:** Byk Gulden Pharmaceuticals  
Hamburg, Germany

**Study Started:** January 15, 1987

**Study Completed:** July 27, 1987 (report date)

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

**Animals:** Sprague Dawley rats (7 weeks old, males: 164-243 g and females: 133-190 g).

**Drug Batch No.:** K19/271-3.

**Methods:** Groups of rats (10/sex/group) were given daily I.V. doses of pantoprazole (1, 5 and 30 mg/kg/day, pH 10.0) for 29 days. The volume of administration was 5 mL/kg. The control group animals were given vehicle (physiological saline at pH 9-10) in similar fashion. Two additional groups (8/sex/group) of animals were included in this study, one received the vehicle and another received high dose pantoprazole for a 30-day recovery study. All rats were observed daily for clinical signs and mortality. Body weights and food intakes were recorded twice weekly. Blood samples were collected from retro-orbital venous plexus at pre-test, and at the end of treatment/recovery period for hematology and serum chemistry tests. Urine samples were also collected at the end of treatment/recovery period for urinalysis. Ophthalmoscopic examinations were performed on all rats at pretest and at the end of treatment/recovery period. All animals were sacrificed at the end of treatment/recovery period and necropsied. Only tissues from control and high dose groups were examined microscopically. Additionally, stomach, thyroid and site of injection (tail) of all animals were examined microscopically.

**Results:**

1. **Observed Effects:** Irritation at the injection sites were seen in all treated rats. Additionally, staggering gait was seen in high dose treated males.

2. **Mortality:** One female (# 25) from recovery control group died on day 55 of the study and on female (# 39) from 5 mg/kg dose group died on day 6 of the study. The cause of death was accidental (ether anesthesia).

3. **Body Weight/Food Consumption**: No treatment related effects were seen.
4. **Hematology/Coagulation/Bone Marrow**: No treatment related effects were seen.
5. **Blood Chemistry/Urinalysis**: No treatment related effects were seen.
6. **Vital Signs/Physical Examinations /Ophthalmoscopic Examinations/ECG**: No treatment related effects were seen.
7. **Organ Weights**: In treated males, stomach weights and height of the gastric mucosa were increased by 12-16% and 14-26%, respectively, when compared to control values. The corresponding increases in treated females were 8-14% and 9-18%, respectively. At the end of recovery period, stomach weights and gastric mucosal heights were within normal limits.
8. **Gross Pathology**: No treatment-related effects were seen.
9. **Histopathology**: No treatment related effects were seen.
10. **Plasma Gastrin Levels**: Plasma gastrin levels increased dose-dependently in treated rats (both sexes). At the end of the recovery period, gastrin levels in the treated rats were within normal range.

| Plasma Gastrin Levels (ng/ml) |          |           |          |          |
|-------------------------------|----------|-----------|----------|----------|
|                               | Males    |           | Females  |          |
|                               | Day 5    | Day 27    | Day 5    | Day 27   |
| Control                       | 275 ± 44 | 203 ± 31  | 168 ± 19 | 124 ± 22 |
| Low Dose (1 mg/kg/day)        | 409 ± 42 | >437 ± 83 | 337 ± 44 | 322 ± 53 |
| Mid Dose (5 mg/kg/day)        | 446 ± 44 | 542 ± 80  | 401 ± 56 | 418 ± 48 |
| High Dose (30 mg/kg/day)      | 729 ± 35 | >664 ± 68 | 409 ± 33 | 502 ± 72 |

In this study, the highest tested dose produced staggering gait in some rats, increased stomach weights and height of gastric mucosa. However, no treatment related histopathological findings were seen. The highest tested dose (30 mg/kg/day) can be considered as no effect dose.

#### Addendum:

**Urinalysis**: Calcium excretion on day 29 for female rats that received 1, 5, or 30 mg/kg/day was decreased to 71, 55.3, and 50% of the control (0.076 mOsmol), respectively. Chloride excretion on day 29 for female rats that received 1, 5, or 30 mg/kg/day was decreased to 60.2, 72.5, and 68.5% of the control (2.89 mOsmol), respectively.

**Histopathology**: Grimelius-positive-cell (GPC)-area was increased in male and female pantoprazole treatment groups; although, there was no dose-response relationship. The GPC-area was still increased in the 30 mg/kg/day group following the recovery period as compared to the control group.

Grimelius-positive-cell (GPC)-area in rats that received pantoprazole by the intravenous route at doses of 1, 5, or 30 mg/kg/day for 4 weeks. RA = Reference Area of 168 100 $\mu\text{m}^2$ .

| Dose, mg/kg/day | GPC-Area/RA ( $\mu\text{m}^2$ ) |        | GPC-Area/RA (%) |        |
|-----------------|---------------------------------|--------|-----------------|--------|
|                 | Male                            | Female | Male            | Female |
| 0               | 2028                            | 2376   | 1.2             | 1.4    |
| 1               | 6806                            | 3008   | 4               | 1.8    |
| 5               | 4411                            | 3987   | 2.6             | 2.4    |
| 30              | 4953                            | 3485   | 2.9             | 2.0    |
| 0-Recovery      | 2279                            | 1911   | 1.4             | 1.1    |
| 30-Recovery     | 4418                            | 3753   | 2.6             | 2.2    |

**Local Tolerance Study of Pantoprazole After 4-Week Administration in the Rat by the Intravenous Route (GTR-32004).**

**Testing Laboratories:** Byk Gulden Pharmaceuticals  
Kontanz, Germany

**Study Started:** August 22, 1987

**Study Completed:** July 9, 1992 (report date)

**Drug Batch No.:** K21/120A

**Methods:** Groups of Sprague Dawley rats (2/sex/group) were given daily I.V. doses of 20 and 40 mg/kg/day (5 mL/kg/day) of pantoprazole (pH 10) for 29 days. In this study, no control group was included. Rats were observed for clinical signs and mortality daily (except weekends) Body weights were recorded twice weekly. Twenty-four hours after the last dose, rats were sacrificed. Only the stomach and injection sites were examined microscopically.

**Results:** Irritation at the injection sites were seen in treated rats. Additionally, tachycardia and staggering gait were seen in high dose-treated males. Due to the lack of control data, body weight gains in treated rats could not be interpreted. Hyperkeratosis of the forestomach, increase in glandular dilatation and eosinophilic infiltration were seen in fundus region of treated rats. Bleeding, fibrosis and/or round cell infiltration were seen at the injection sites (tail). This study was not very informative due to the absence of control rats.

**Addendum:** At doses of 20 and 40 mg/kg/day, the borderline between the forestomach and gastric fundus showed hyperkeratosis. In the stomach, spreading of the foveolar zone was observed. An increase of the height of the mucosa in the gastric fundus appeared to exist.

APPEARS THIS WAY  
ON ORIGINAL

**The Toxicity of Pantoprazole Lyophile (Unstressed Batch) After Intravenous Administration in the Rat for 4 Weeks (GTR-32911).**

**Testing Laboratory:** Byk Gulden  
Institute of Pathology and Toxicology, FT2  
Friedrich-Ebert-Damm 101  
22047 Hamburg  
Germany

**Date Started:** June 25, 1997

**Date Completed:** February 4, 1998

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

**Animals:** Wistar rats were used in this study. Animals were 6 weeks of age at the start of treatment and body weight ranges were 134-158 g for male rats and 118-150 g for female rats.

**Drug Batch:** Pantoprazole, batch no. 296310. Each vial contained \_\_\_\_\_ pantoprazole (free acid/vial, within specifications). Impurity \_\_\_\_\_ (within specifications). Total identified impurities = \_\_\_\_\_ Total unidentified impurities = \_\_\_\_\_

**Methods:** The 4 week intravenous toxicity of pantoprazole from an "unstressed" batch, which had been stored at 4°C after manufacture, was evaluated in Wistar rats. In stability tests with the lyophilized form of pantoprazole used for intravenous administration, the formation of several temperature-dependent impurities was detected. In order to extend the specification limits for lyophilized form of pantoprazole used for intravenous administration, the toxicity of batches with low (unstressed) and high (stressed) levels of impurities were compared in two parallel studies. The lyophilized form of pantoprazole used in the present study contained a low level of impurities (unstressed). Rats received the lyophilized form of pantoprazole by the intravenous route at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks. The control group received the vehicle, physiological saline solution. There were 10 rats/sex/group. The dose volume was 5 mL/kg. Drug was administered intravenously in a lateral tail vein. Animals were observed for clinical signs of toxicity and mortality daily, immediately and 0.5, 3, 6, and 24 hr after dosing. Body weight and food and water consumption were measured twice per week. Blood for determination of hematology and clinical chemistry parameters was collected on days -5 and 29. Serum gastrin levels were measured on day 22 at 4 and 24 hr after dosing. Urine specimens for analysis were collected over a 16 hr period on days 28/29 following administration of 15 mL of tap water per animal. Vaginal smears for microscopic evaluation of estrus cycle were collected daily from female rats. Ophthalmic examinations were performed on days -6 and 28. Rats were sacrificed on day 30, 24 hr after the last treatment. All animals were subjected to a gross examination. Absolute organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, spleen,

testes, thymus, thyroids, uterus (with vagina), pituitary, prostate, seminal vesicles, and stomach. Relative organ weights were determined for the liver, kidneys, heart, lungs, spleen, brain, and stomach. Organs were collected and processed as follows: adrenal glands, aorta, brain, esophagus, epididymides, eye, Harder's glands, heart, injection sites, intestines, kidneys, liver, lungs + lung-associated lymph nodes, mammary glands, mesenteric lymph nodes, muscles, nasal turbinates, extraorbital lacrimal glands, ovaries with oviducts, pancreas, pituitary gland, prostate, salivary glands + associated lymph nodes, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, testes, tibia (bone marrow) with knee joint, tongue, trachea, thymus, thyroids (including parathyroids), urinary bladder, uterus, vagina, and any macroscopic abnormalities. For the control and 40 mg/kg/day groups, all organs were microscopically examined with the exception of the nasal turbinates. For any abnormalities identified in organs from the 40 mg/kg/day group, the corresponding organs from 10 and 20 mg/kg/day groups were also microscopically examined. The stomach, thyroid gland, and liver were microscopically examined in the 10 and 20 mg/kg/day groups.

### Results:

1. **Observed Effects:** There were no significant clinical signs of toxicity throughout the study period other than localized tissue irritation at the injection site in the tail. Changes at the injection site (i.e., tail blue, tail red, tail swollen) in the tail were more pronounced for pantoprazole treatment groups and occurred in a dose-related manner.

2. **Mortality:** None.

3. **Body Weight and Food and Water Consumption:** Body weight gain was impaired for the male 40 mg/kg/day group. Other treatment groups were observed with impairments of body gain that exceeded 10%; however, dose response relationships were not evident. Food consumption for male and female treatment groups were reduced by < 5-10% and appeared to have little biological significance. Water consumption was reduced in male and female treatment groups; although, problems occurred in measurements of water consumption for male and female controls. Body weights for male controls on days 1 and 28 were 175 and 279 g, respectively. Body weight gains for the male 10, 20, and 40 mg/kg/day groups were 88.0, 91.4, and 85.1% of the control, respectively. Body weights for female controls on days 1 and 28 were 137 and 184 g, respectively. Body weight gains for the female 10, 20, and 40 mg/kg/day groups were 102.9, 89.4, and 92.8% of the control, respectively. Food consumption was reduced by <5% in all male treatment groups and the female 10 and 20 mg/kg/day groups. Food consumption for the female 40 mg/kg/day group was reduced to 91.9% of the control (15.85 g/animal/day). Water consumption for the male 10, 20, and 40 mg/kg/day groups were reduced to 84.2, 94.8, and 89.5% of the control (34.7 g/animal/day), respectively. Water consumption for the female 10, 20, and 40 mg/kg/day groups were reduced to 79.6, 83.8, and 73.1% of the control (29.45 g/animal/day), respectively. Errors occurred in measurements of water consumption for male and female control groups.

4. **Hematology and Blood Coagulation:** Reticulocyte counts for the female 10, 20, and 40 mg/kg/day groups were reduced to 80.4, 49, and 58.8% of the control (0.051 per 1000 erythrocytes). Leukocyte counts for the female 40 mg/kg/day group were reduced to 80.8% of the control ( $9.4 \times 10^9/L$ ).

### 5. Blood Biochemistry and Urinalysis:

**Blood Biochemistry:** Serum gastrin levels on day 22/23 were elevated for male and female treatment group at 4 and 24 hr after dosing; although, there was no evidence of a dose response relationship.

Serum gastrin levels (ng/L) on day 22/23 in male and female treatment group at 4 and 24 hr after dosing. Values in parentheses are % of control.

| Dose<br>mg/kg/day | Male Rats   |               | Female Rats   |              |
|-------------------|-------------|---------------|---------------|--------------|
|                   | 4 hr        | 24 hr         | 4 hr          | 24 hr        |
| 0                 | 212         | 219           | 195           | 116          |
| 10                | 1518 (716%) | 840 (383.6%)  | 1296 (664.6%) | 563 (485.3%) |
| 20                | 1374 (648%) | 912 (416.4%)  | 1374 (704.6%) | 712 (613.8%) |
| 40                | 1344 (634%) | 1057 (482.6%) | 1449 (743.1%) | 712 (613.8%) |

**Urinalysis:** Urine osmolality for the male 20 and 40 mg/kg/day groups were increased to 113.5 and 118% of the control (674 mOsmol), respectively. Urinary sodium excretion for the male 40 mg/kg/day group was increased to 172.4% of the control (0.58 mmol/kg BW). Urinary calcium excretion for the male 10, 20, and 40 mg/kg/day groups were decreased to 21.1, 16.8, and 24.8% of the control (0.161 mmol/kg BW), respectively; although, a dose response relationship was not evident. Urinary calcium excretion for the female 10, 20, and 40 mg/kg/day groups were decreased to 57.6, 45.2, and 37.3% of the control (0.177 mmol/kg BW), respectively. Urinary chloride excretion for the female 10, 20, and 40 mg/kg/day groups were decreased to 80.6, 80.6, and 66.8% of the control (3.19 mmol/kg BW), respectively.

**6. Physical Examinations:** There were no treatment-related ophthalmic effects. Microscopic evaluation of daily vaginal smears did not reveal any treatment-related changes of the estrus cycle.

**7. Organ Weights:** Changes in organ weights were evident for the thymus, kidneys, pituitary gland, prostate, ovaries, and stomach; although, histopathological changes were observed only for the stomach.

**Stomach:** Relative stomach weights for the male 10, 20, and 40 mg/kg/day groups were increased to 114.7, 105.9, and 111.8% of the control (0.68%), respectively. Absolute stomach weights for the female 10, 20, and 40 mg/kg/day groups were increased to 111.5, 102.7, and 108.1% of the control (1.48 g), respectively. Relative stomach weights for the female 10, 20, and 40 mg/kg/day groups were increased to 113.6, 107.4, and 114.8% of the control (0.81%), respectively.

**8. Gross Pathology:** There were no treatment-related gross pathological changes.

**9. Histopathology:** The stomach and lungs were the target organs of toxicity. In the fundic part of the glandular stomach, a focal to multifocal eosinophilic discoloration of the cytoplasm of chief cells was found in pantoprazole treatment groups at 10, 20, and 40 mg/kg/day. Histopathological changes in the lungs consisted of foreign body granuloma formation around intravascular hair emboli and interstitial lympho-histiocytic infiltration. The sponsor attributed histopathological changes in the lung to daily intravenous injection of animals; although, the incidence was significantly higher with rats that received pantoprazole at 40 mg/kg/day as compared to the control.

Histopathological changes in rats that received lyophilized pantoprazole (unstressed: low level of impurities) by the intravenous route at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks.

| Organ/Tissue                                                           | 0 mg/kg/day |   | 10 mg/kg/day |    | 20 mg/kg/day |    | 40 mg/kg/day |   |
|------------------------------------------------------------------------|-------------|---|--------------|----|--------------|----|--------------|---|
|                                                                        | M           | F | M            | F  | M            | F  | M            | F |
| <b>Forestomach, gastric fundus</b><br>-eosinophilic chief cells        | 0           | 0 | 1            | 0  | 1            | 0  | 2            | 2 |
| <b>Lung</b><br>-foreign body granuloma                                 | 2           | 2 | NE           | NE | NE           | NE | 9            | 5 |
| <b>Liver</b><br>-centrilobular swelling                                | 1           | 4 | 1            | 1  | 2            | 1  | 4            | 2 |
| <b>Liver, fat stain</b><br>-lipid in single cells                      | 1           | 1 | NE           | NE | NE           | NE | 3            | 2 |
| <b>Salivary and lacrimal glands</b><br>-parotis gland cell hypertrophy | 2           | 2 | NE           | NE | NE           | NE | 5            | 3 |
| <b>Uterus</b><br>-luminal dilatation                                   | -           | 1 | -            | NE | -            | NE | -            | 3 |
| <b>Tail (Injection Site)</b><br>-foreign body granuloma                | 0           | 0 | NE           | NE | NE           | NE | 1            | 0 |
| -hemorrhage                                                            | 0           | 0 |              |    |              |    | 1            | 1 |
| -thrombotic vessel                                                     | 0           | 0 |              |    |              |    | 1            | 1 |
| -perivascular fibrosis                                                 | 0           | 1 |              |    |              |    | 1            | 2 |
| -lympho-histiocytic infiltration                                       | 5           | 1 |              |    |              |    | 5            | 3 |
| -subcutaneous hemorrhage                                               | 0           | 0 |              |    |              |    | 0            | 1 |

NE = Not Examined.

The 4 week intravenous toxicity of "unstressed" pantoprazole (i.e., contained a low-level of temperature-dependent impurities) was evaluated in Wistar rats. Rats received pantoprazole by the intravenous route at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks. The dose of 20 mg/kg/day could be considered a tolerated dose. Body weight gain was impaired for the male 40 mg/kg/day group. Gastrin levels were elevated for all treatment groups on day 22/23 at 4 and 24 hr after dosing. The target organs of toxicity were the stomach and lungs. In the fundic part of the glandular stomach, a focal to multifocal eosinophilic discoloration of the cytoplasm of chief cells was found for 1 male rat in each of the 10 and 20 mg/kg/day groups and for 2 male and 2 female rats in the 40 mg/kg/day group. Histopathological changes in the stomach were most likely a pharmacological response to elevated gastrin levels. For the lung, an increased incidence of foreign body granuloma formation was found for the 40 mg/kg/day group; although, this was not a test article-specific effect. This study was flawed in that the sponsor did not examine corresponding tissues from the low and mid dose groups where histopathological changes were found in the high dose group.

The Toxicity of Pantoprazole Lyophile (Stressed Batch) After Intravenous Administration in the Rat for 4 Weeks (GTR-32910).

Testing Laboratory: Byk Gulden  
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Germany

Date Started: June 18, 1997

Date Completed: April 28, 1998

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

Animals: Wistar rats were used in this study. Animals were 6 weeks of age at the start of treatment and body weight ranges were 152-204 g for male rats and 119-146 g for female rats.

Drug Batch: Pantoprazole, batch no. 195220: Each vial contained \_\_\_\_\_ pantoprazole (free acid/vial), Impurity \_\_\_\_\_ Total identified impurities = \_\_\_\_\_ and Total unidentified impurities = \_\_\_\_\_ Batch number 195220 was manufactured in March 1995 and stored at 15-20°C until February 1996. Thereafter, the batch was stored at 40°C until shipment in April 1997. Vials were subsequently stored at room temperature until use.  
Pantoprazole, batch no. 195220-A: Each vial contained \_\_\_\_\_ pantoprazole (free acid/vial), Impurity \_\_\_\_\_ Total identified impurities = \_\_\_\_\_ and Total unidentified impurities = \_\_\_\_\_ Batch 195220-A was stored at 40°C from February 1996 to August 1997. Vials were subsequently stored at room temperature until use.

Methods: The 4-week intravenous toxicity of pantoprazole, from a "heat-stressed" batch, was evaluated in Wistar rats. In stability tests with the lyophilized form of pantoprazole used for intravenous administration, the formation of several temperature-dependent impurities was detected. In order to extend the specification limits for lyophilized form of pantoprazole used for intravenous administration, the toxicity of batches with low (unstressed) and high (stressed) levels of impurities were compared in two parallel studies. The lyophilized form of pantoprazole used in the present study contained a high level of impurities (stressed). Rats received the lyophilized form of pantoprazole by the intravenous route at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks. The control group received the vehicle, physiological saline solution. There were 10 rats/sex/group. The dose volume was 5 mL/kg. Drug was administered intravenously in a lateral tail vein. Animals were observed for clinical signs of toxicity and mortality daily, immediately and 0.5, 3, 6, and 24 hr after dosing. Body weight and food and water consumption were measured twice per week. Blood for determination of hematology and clinical chemistry parameters was collected on days -5 and 29. Serum gastrin levels were measured on day 22/23 at 4 and 24 hr after dosing. Urine specimens for analysis were collected over a 16-hr period on days 28/29 following administration of 15 mL of tap water per animal. Vaginal smears for microscopic

evaluation of estrus cycle were collected daily from female rats. Ophthalmic examinations were performed on days -6 and 28. Rats were sacrificed on day 30, 24 hr after the last treatment. All animals were subjected to a gross examination. Absolute organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus, thyroids, uterus (with vagina), pituitary, prostate, seminal vesicles, and stomach. Relative organ weights were determined for the liver, kidneys, heart, lungs, spleen, brain, and stomach. Organs were collected and processed as follows: adrenal glands, aorta, brain, esophagus, epididymides, eye, Harder's glands, heart, injection sites, intestines, kidneys, liver, lungs + lung-associated lymph nodes, mammary glands, mesenteric lymph nodes, muscles, nasal turbinates, extraorbital lacrimal glands, ovaries with oviducts, pancreas, pituitary gland, prostate, salivary glands + associated lymph nodes, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, testes, tibia (bone marrow) with knee joint, tongue, trachea, thymus, thyroids (including parathyroids), urinary bladder, uterus, vagina, and any macroscopic abnormalities. For the control and 40 mg/kg/day groups, all organs were microscopically examined with the exception of the nasal turbinates. For any abnormalities identified in organs from the 40 mg/kg/day group, the corresponding organs from 10 and 20 mg/kg/day groups were also microscopically examined. The stomach, thyroid gland, and liver were microscopically examined in the 10 and 20 mg/kg/day groups.

### Results:

**1. Observed Effects:** Localized tissue irritation at the injection site in the tail was increased in severity for pantoprazole-treatment groups. Slight tissue irritation at the injection site was observed for some female control rats.

Observed effects for rats that received pantoprazole by the intravenous route of administration at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks (n = 10 rats/group).

| Observed Effects | 0 mg/kg/day |   | 10 mg/kg/day |   | 20 mg/kg/day |   | 40 mg/kg/day |   |
|------------------|-------------|---|--------------|---|--------------|---|--------------|---|
|                  | M           | F | M            | F | M            | F | M            | F |
| Tail dark blue   | 0           | 2 | 0            | 1 | 0            | 5 | 0            | 5 |
| Tail red         | 0           | 0 | 0            | 0 | 0            | 3 | 4            | 4 |
| Tail swollen     | 0           | 2 | 3            | 2 | 1            | 4 | 7            | 5 |

**2. Mortality:** One female rat (#42) that received pantoprazole at 40 mg/kg/day died on day 12 of treatment. Histopathological evaluations of organs and tissues from this rat did not reveal a cause of death.

**3. Body Weight and Food and Water Consumption:** Body weight gain was impaired by >10% for female rats that received pantoprazole at 40 mg/kg/day. Food and water consumption were not affected in a dose-related manner. Body weights for male control rats on days 1 and 28 were 174 and 267 g, respectively. Body weight gains for male rats that received pantoprazole at 10, 20, or 40 mg/kg/day were 96.3, 95.1, and 94.1% of the control, respectively. Body weights for female control rats on days 1 and 28 were 132 and 181 g, respectively. Body weight gains for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were 91.8, 97.2, and 88.5% of the control, respectively.

4. **Hematology and Blood Coagulation:** There were no treatment-related changes of hematological or blood coagulation parameters.

5. **Blood Biochemistry and Urinalysis:**

**Blood Biochemistry:** Serum gastrin levels were elevated in pantoprazole treatment groups; however, there was no evidence of a dose response relationship (see table below). Gastrin levels at 4 hr after dosing were higher than at 24 hr after dosing. Gastrin levels were comparable between male and female rats.

Serum gastrin levels (ng/L) on day 22/23 at 4 and 24 hr after dosing for rats that received pantoprazole by the intravenous route at doses of 0, 10, 20, or 40 mg/kg/day. Values in parentheses represent % of control.

| Dose, mg/kg/day | 4 hr After Dosing |               | 24 hr After Dosing |              |
|-----------------|-------------------|---------------|--------------------|--------------|
|                 | Male              | Female        | Male               | Female       |
| 0               | 418               | 217           | 488                | 192          |
| 10              | 1317 (315.1%)     | 1451 (668.7%) | 966 (198%)         | 630 (328%)   |
| 20              | 1364 (326.3%)     | 1425 (656.7%) | 954 (195.5%)       | 742 (386.5%) |
| 40              | 1284 (307.2%)     | 1925 (887.1%) | 980 (200.8%)       | 876 (456.3%) |

**Urinalysis:** There were a number of statistically significant changes in urinalysis parameters for pantoprazole treatment groups; however, changes were relatively flat and lacked dose response relationships. Further, there were no treatment-related histopathological findings for the kidney. Urinary pH values for male rats that received pantoprazole at 10, 20, or 40 mg/kg/day were decreased to 91.4, 92.6, and 88.9% of the control (pH 8.1), respectively. Urinary Ca<sup>2+</sup> excretion for male rats that received pantoprazole at 10, 20, or 40 mg/kg/day were decreased to 19.4, 14.1, and 20% of the control (0.170 mmol/kg), respectively. Urinary Cl<sup>-</sup> excretion for male rats that received pantoprazole at 10, 20, or 40 mg/kg/day were decreased to 72.8, 75.9, and 78.9% of the control (2.28 mmol/kg), respectively. Urine osmolality values for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were increased to 105.5, 108.7, and 125.3% of the control (689 mOsmol), respectively. Urine volume for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were decreased to 78.5, 89.4, and 79.0% of the control (42.4 mL/kg), respectively. Urinary Na<sup>+</sup> excretion for female rats that received pantoprazole at 20 or 40 mg/kg/day were increased to 127.5 and 137.7% of the control (0.69 mmol/kg), respectively. Urinary Ca<sup>2+</sup> excretion for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were decreased to 20.9, 29.1, and 29.1% of the control (0.220 mmol/kg), respectively. Urinary Cl<sup>-</sup> excretion for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were decreased to 47.3, 77.9, and 77.2% of the control (2.81 mmol/kg), respectively.

6. **Physical Examinations:** There were no treatment-related findings with ophthalmic examinations. There were no treatment-related effects on estrus cycling in female rats.

7. **Organ Weights:** Organ weight changes were observed for the stomach, lungs, and spleen; however, treatment-related histopathological findings were only observed for the stomach.

**Stomach:** Absolute stomach weights for male rats that received pantoprazole at 10, 20, or 40 mg/kg/day were increased to 106.4, 107.5, and 110.4% of the control (1.73 g), respectively. Relative stomach weights for male rats that received pantoprazole at 10, 20, or 40 mg/kg/day were increased to 109, 110.4, and 112% of the control (0.67%), respectively. Absolute stomach weights for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were increased to 107.5, 106.8, and 114.3% of the control (1.47 g), respectively. Relative stomach weights for female rats that received pantoprazole at 10, 20, or 40 mg/kg/day were increased to 110.7, 108.3, and 116.7% of the control (0.84%), respectively.

**8. Gross Pathology:** There were no treatment-related gross pathological findings.

**9. Histopathology:** In the fundic part of the glandular stomach, a minimal focal to multifocal eosinophilic discoloration of the cytoplasm of chief cells was found in pantoprazole-treated animals at doses of 20 and 40 mg/kg/day. A dose-related increased incidence of centrilobular swelling of the liver was observed in pantoprazole treatment groups. Changes for the lungs that occurred with an increased incidence for pantoprazole-treated rats at 40 mg/kg/day included infiltration with eosinophilic granulocytes, the formation of foreign body granulomas surrounding intravascular hair emboli, and interstitial lympho-histiocytic infiltrations.

Histopathological findings for rats that received pantoprazole (stressed batch) by the intravenous route at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks.

| Organ/Tissue                                | 0 mg/kg/day |        | 10 mg/kg/day |       | 20 mg/kg/day |       | 40 mg/kg/day |       |
|---------------------------------------------|-------------|--------|--------------|-------|--------------|-------|--------------|-------|
|                                             | M           | F      | M            | F     | M            | F     | M            | F     |
| <b>Forestomach, gastric fundus (n = 10)</b> |             |        |              |       |              |       |              |       |
| -eosinophilic chief cells                   | 0           | 0      | 0            | 0     | 4            | 2     | 5            | 3     |
| -apoptosis of glandular cells               | 0           | 0      | 0            | 0     | 0            | 0     | 1            | 0     |
| <b>Liver (n = 10)</b>                       |             |        |              |       |              |       |              |       |
| -centrilobular swelling                     | 1           | 2      | 2            | 3     | 2            | 3     | 5            | 6     |
| <b>Eyes, Harderian glands</b>               | (n=10)      | (n=10) | (n=0)        | (n=0) | (n=0)        | (n=0) | (n=10)       | (n=9) |
| -Harderian gland inflammation               | 1           | 1      |              |       |              |       | 4            | 2     |
| <b>Uterus</b>                               |             | (n=10) |              | (n=0) |              | (n=0) |              | (n=9) |
| -luminal dilatation                         |             | 0      |              |       |              |       |              | 3     |
| <b>Lungs</b>                                | (n=10)      | (n=10) | (n=0)        | (n=0) | (n=1)        | (n=1) | (n=10)       | (n=9) |
| -eosinophilic infiltration                  | 1           | 4      |              |       | 0            | 0     | 4            | 4     |
| -foreign body granuloma                     | 2           | 2      |              |       | 0            | 0     | 6            | 4     |
| -interstitial lymphocytic infiltration      | 2           | 0      |              |       | 0            | 0     | 6            | 0     |
| <b>Submandibular and parotid gland</b>      | (n=10)      | (n=10) | (n=0)        | (n=0) | (n=0)        | (n=0) | (n=9)        | (n=9) |
| -submandibular gland cell hypertrophy       | 0           | 1      |              |       |              |       | 2            | 2     |

The 4-week intravenous toxicity of "heat-stressed" pantoprazole (i.e., containing high levels of temperature-dependent impurities) was evaluated in Wistar rats. Rats received pantoprazole by the intravenous route at doses of 0, 10, 20, and 40 mg/kg/day for 4 weeks. The dose of 20 mg/kg/day could be considered a tolerated dose. Mortality occurred for 1 female rat at a dose of 40 mg/kg/day. Body weight gain was impaired by >10% for female rats that received pantoprazole at 40 mg/kg/day. Gastrin levels were elevated for all treatment groups on day 22/23 at 4 and 24 hr after dosing; although, there was no evidence of a dose response relationship. The stomach and liver were the target organs of toxicity. In the fundic part of the glandular stomach, a minimal focal to multifocal eosinophilic discoloration of the cytoplasm of chief cells was found in pantoprazole-treated animals at doses of 20 and 40 mg/kg/day. A dose-related increased incidence of centrilobular swelling of the liver was observed in pantoprazole treatment groups. This study was flawed in that the sponsor did not examine corresponding tissues from the low and mid dose groups where histopathological changes were found in the high dose group. Toxicological findings observed with the "unstressed" and "stressed" batches were similar, although, mortality was observed for 1 female (10%) rat that received "stressed" pantoprazole at 40 mg/kg/day.

The Toxicity of \_\_\_\_\_ in Comparison to Pantoprazole After Intravenous Administration in the Rat for 4 Weeks (GTR-32006).

Testing Laboratory: Byk Gulden  
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Date Started: July 3, 1996

Date Completed: January 16, 1997 (Document stamp date of January 5, 1998)

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

Animals: CRL:(WI)WU BR (SPF strain) Wistar rats were used in this study. At the start of administration, animals were 6 weeks old and body weight ranges were 159-210 g for male rats and 114-162 g for female rats.

Drug Batch: \_\_\_\_\_ batch no. \_\_\_\_\_, Pantoprazole, batch no. 296300.

Methods: The intravenous toxicity of \_\_\_\_\_ a temperature-dependent impurity identified in stability tests with pantoprazole, was examined in Wistar rats. The chemical name for \_\_\_\_\_

\_\_\_\_\_ and it has a molecular weight of \_\_\_\_\_ g/mole. Rats received \_\_\_\_\_ by the intravenous route at doses of 0, 5, or 25 mg/kg/day for 4 weeks. For comparison, rats received treatment with

pantoprazole by the intravenous route at doses of 0 or 25 mg/kg/day for 4 weeks. The vehicle group for \_\_\_\_\_ received aqueous 30% polyethylene glycol solution. The vehicle group for pantoprazole-Na-sesquihydrate lyophilizate received saline. There were 10 rats/sex/group. The dosing volume was 5 mL/kg and solutions were administered by intravenous bolus over a period of 10-15 seconds. Animals were observed for clinical signs of toxicity and morbidity/mortality immediately after drug treatment and at 0.5, 3, 6, and 24 hr after administration each day of the study period. Body weight and food and water consumption were measured twice per week. Blood for determination of hematology and clinical chemistry parameters was collected on days -5 and 29 (24 hr after the previous administration). Urine specimens for analysis were collected over a 16-hr period on days 28/29 following administration of water at 15 mL/rat. Vaginal smears were collected daily from Monday through Friday during the study period for microscopic determination of estrus cycle. Ophthalmic examinations were performed on days -6 and 28. On day 30, 24 hr after the last treatment, animals were sacrificed and subjected to a gross examination. Absolute and relative organ weights were determined for liver, kidneys, heart, lungs, spleen, and brain. Absolute organ weights were determined for the adrenal glands, thyroid gland, thymus, pituitary gland, testes, seminal vesicles, prostate, uterus, and ovaries. Organs were collected, preserved, and subjected to histopathological examination as follows: liver, kidney, urinary bladder, stomach, intestines, pancreas, salivary glands, lungs, trachea, heart, aorta, brain, thymus, spleen, mesenteric lymph node, tibia (bone marrow), femur and knee joint, muscles, testes, epididymides, seminal vesicles, prostate, ovaries, uterus, thyroid gland (including parathyroid gland), adrenal glands, pituitary gland, eye (with optic nerve and chiasm), skin, tongue, sciatic nerve, mammary gland, spinal cord, esophagus, sternum, vagina, Harder's glands, nasal turbinates, injection site (tail), and any abnormalities.

### Results:

- 1. Observed Effects:** Male and female rats that received \_\_\_\_\_ at 25 mg/kg/day were observed with muscle spasms and twitches or short atactic movements immediately following intravenous administration throughout the study period. These observed effects generally had a duration  $\leq 1$  min.
- 2. Mortality:** None.
- 3. Body Weight and Food and Water Consumption:** Body weight gain for male and female rats that received pantoprazole at 25 mg/kg/day was impaired by  $>10\%$  during the treatment period; however weight gain was unaffected for rats received that received \_\_\_\_\_ at 5 and 25 mg/kg/day. Body weights for male rats that received the vehicle of \_\_\_\_\_ on days 1 and 28 were 186 and 286 g, respectively. Body weight gains for male rats that received \_\_\_\_\_ at 5 and 25 mg/kg/day were 100.6 and 106.7% of the control, respectively. Body weights for female rats that received the vehicle of \_\_\_\_\_ on days 1 and 28 were 137 and 185 g, respectively. Body weight gains for female rats that received \_\_\_\_\_ at 5 and 25 mg/kg/day were 113.75 and 94.45% of the control, respectively. Body weights for male rats that received the vehicle of pantoprazole on days 1 and 28 were 179 and 291 g, respectively.

The body weight gain for male rats that received pantoprazole at 25 mg/kg/day was 77.75% of the control. Body weights for female rats that received the vehicle of pantoprazole on days 1 and 28 were 139 and 196 g, respectively. The body weight gain for female rats that received pantoprazole at 25 mg/kg/day was 82.46% of the control. Food consumption for treatment groups that received \_\_\_\_\_ was unaffected. Food consumption for male and female rats that received pantoprazole at 25 mg/kg/day was reduced to 95.7 and 95.35% of the control (21.15 and 15.6 grams/animal/day), respectively. Water consumption for male rats that received \_\_\_\_\_ at 25 mg/kg/day was increased to 114.4% of the control (31.925 grams/animal/day). Water consumption for female rats that received either \_\_\_\_\_ or pantoprazole at 25 mg/kg/day was reduced to 93.1 and 92.9% of the control (27.35 and 26.2 g/animal/day), respectively.

#### 4. Hematology and Blood Coagulation:

Reticulocyte counts for male rats that received \_\_\_\_\_ at 25 mg/kg/day were increased to 175% of the control (0.008 per 1000 erythrocytes). Leukocyte counts for female rats that received \_\_\_\_\_ at 5 or 25 mg/kg/day were increased to 116 and 126.7% of the control ( $7.5 \times 10^9/L$ ). There were no changes in blood coagulation parameters.

#### 5. Blood Biochemistry and Urinalysis:

**Blood Biochemistry:** Aspartate aminotransferase activities for female rats that received \_\_\_\_\_ at 5 or 25 mg/kg/day were increased to 114.6 and 124.4% of the control (41 U/L), respectively. Alkaline phosphatase activities for female rats that received either \_\_\_\_\_ or pantoprazole at 25 mg/kg/day were increased to 111.2 and 116.1% of the control (116 and 112 U/L), respectively. Urinary  $Na^+$  excretion for male rats that received pantoprazole at 25 mg/kg/day was increased to 176.4 of the control (0.89 mmol/kg BW). Urinary volume,  $Ca^{2+}$  excretion, and  $Cl^-$  excretion for female rats that received pantoprazole at 25 mg/kg/day were decreased to 84.1, 67.9, and 69.1% of control values (44 mL/kg BW, 0.056 mmole/kg BW, and 1.94 mmole/kg BW), respectively.

6. Ophthalmic Examination: There were no treatment-related ophthalmic changes for rats that received either \_\_\_\_\_ or pantoprazole; although, no data was provided for independent analysis.

7. Organ Weights: Increases of absolute and relative liver weight for male rats that received \_\_\_\_\_ at 25 mg/kg/day appeared to correlate with liver centrilobular hypertrophy. Increased relative lungs, kidney, spleen, and brain weights for male rats that received pantoprazole at 25 mg/kg/day had no histopathological correlations. Further, increased relative lungs for female rats that received pantoprazole at 25 mg/kg/day had no histopathological correlations. Absolute and relative liver weight for male rats that received \_\_\_\_\_ at 25 mg/kg/day were increased to 106.6 and 109.8% of the control (13.4 g and 4.78%), respectively.

8. **Gross Pathology:** There were no treatment-related gross pathological changes for rats that received either \_\_\_\_\_ or pantoprazole; although, no data was provided for independent analysis.

9. **Histopathology:** The target organs of toxicity for rats that received \_\_\_\_\_ were the liver and stomach corpus. The target organ of toxicity for rats that received pantoprazole was the stomach corpus. Centrilobular hypertrophy of the liver was observed for rats that received \_\_\_\_\_ at 25 mg/kg/day. Increased mucosal thickness of the stomach corpus was observed for rats that received either \_\_\_\_\_ or pantoprazole at 25 mg/kg/day.

| Tissue                                      | Polyethylene glycol |   | _____       |   | _____        |   | Saline |   | Pantoprazole 25 mg/kg/day |   |
|---------------------------------------------|---------------------|---|-------------|---|--------------|---|--------|---|---------------------------|---|
|                                             |                     |   | 5 mg/kg/day |   | 25 mg/kg/day |   |        |   |                           |   |
|                                             | M                   | F | M           | F | M            | F | M      | F | M                         | F |
| Liver<br>-centrilobular hypertrophy         | 0                   | 0 | 0           | 0 | 5            | 4 | 0      | 0 | 0                         | 0 |
| Stomach corpus<br>-eosinophilic chief cells | 0                   | 0 | 0           | 0 | 0            | 0 | 0      | 0 | 0                         | 1 |
| -increased mucosal thickness                | 1                   | 0 | 0           | 0 | 4            | 1 | 3      | 0 | 7                         | 3 |
| Urinary bladder<br>-proteinous plug         | 2                   | 0 | 0           | 0 | 4            | 0 | 0      | 0 | 6                         | 0 |
| Esophagus<br>-inflammation                  | 0                   | 0 | 0           | 0 | 0            | 1 | 0      | 0 | 0                         | 3 |
| Injection site<br>-fibrin                   | 3                   | 3 | 0           | 0 | 5            | 1 | 0      | 0 | 5                         | 7 |
| -arterial wall disruption                   | 3                   | 2 | 0           | 0 | 3            | 4 | 0      | 0 | 4                         | 6 |

The 4-week intravenous toxicity of \_\_\_\_\_, a temperature-dependent impurity identified in stability tests with pantoprazole for injection, was examined in Wistar rats. Rats received \_\_\_\_\_ by the intravenous route at doses of 0, 5, or 25 mg/kg/day for 4 weeks. For comparison, rats received treatment with pantoprazole by the intravenous route at doses of 0 or 25 mg/kg/day for 4 weeks. The no effect dose for \_\_\_\_\_ was 5 mg/kg/day. The target organs of toxicity for rats that received \_\_\_\_\_ were the liver and stomach corpus. The target organ of toxicity for rats that received pantoprazole was the stomach corpus. Centrilobular hypertrophy of the liver was observed for rats that received \_\_\_\_\_ at 25 mg/kg/day. Increased mucosal thickness of the stomach corpus was observed for rats that received either \_\_\_\_\_ or pantoprazole at 25 mg/kg/day.

Oral Route of Administration

4-Week Oral Toxicity Study in Rats (GTR-31902).

Testing Laboratory: Byk Gulden Pharmaceuticals.

Date of the Study: Sept. 1 to Dec. 2, 1987.

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

Animals: Sprague-Dawley rats weighing 155-205 g were used.

Methods: Five groups of animals each consisting of 10 males and 10 females were given pantoprazole (batch no. K 23/144) suspended in water at constant volume of 10 mL/kg and at acid dose levels of 0, 1, 5, 20 and 500 mg/kg/day for 4 weeks. An additional 8 males and 8 females each were assigned to the control and 500 mg/kg/day groups for a recovery period of 8 weeks. The ECL-cells in a section of the fundus region of the stomach were stained according to GRIMELIUS. Examinations of all organs of the control, the 500 mg/kg/day and the recovery groups were conducted. In the case of changes in the high dose group, the respective organs of the middle and low dose groups were also examined. All animals dying before termination as well as abnormalities in all groups were also evaluated.

Results:

Clinical Signs (daily): Piloerection was noted more frequently in the 500 mg/kg/day group.

Mortality: One in the 20 mg/kg/day and two in the 500 mg/kg/day groups died after the blood sampling. There were no treatment-related deaths.

Body Weight (twice weekly): Significant retardation of body weight gain (22-36%) was observed only in the 500 mg/kg/day group during days 1-7 of dosing. Overall decrease was 5% as compared to the control.

Food Consumption: Reduction in food intake (8%) was observed in the 500 mg/kg/day group. It returned to normal at end of recovery period.

Hematology (days 0, 29 and 85): Decreases in hemoglobin (9-15%) and hematocrit (9%) were observed in the 500 mg/kg/day group. They returned to normal at end of recovery period except that in the 500 mg/kg/day female group, lower hemoglobin (4%) was still present. Increase in leukocyte count (46%) was found in the 500 mg/kg/day female group. They returned to normal at end of recovery period.

Blood Chemistry (days 0, 29 and 85): Elevation of protein (8%) and reduction of urea (25%) were noted in female 500 mg/kg/day group. They were normal at end of recovery period.

**Gastrin Level (days 3/4, 31/32, & 86):** Increase in (211-654%) gastrin level was seen in the 5 mg/kg/day and above groups. It returned to normal during recovery period.

**Urinalysis (days 28/29 and 84/85):** There were no treatment-related changes.

**Ophthalmoscopy (days 0, 25 and 85):** No treatment-related abnormalities were detected.

**Organ Weight:** Increases in liver (22-29%), lung (9%), thyroid (60%) and stomach (11-16%) and decreases in thymus (26%) and seminal vesicle (17%) were observed in the 500 mg/kg/day group. They were normal at end of recovery period.

**Gross Pathology:** One animal in the 500 mg/kg/day group exhibited ulceration in stomach.

**Histopathology:** Parietal cell hyperplasia and epithelial cell degeneration in the cardiac and pyloric region were found in the 5 mg/kg/day group. In the 20 mg/kg/day and above groups, additional parietal cell degeneration/vacuolation were found. Parietal hyperplasia and degeneration/vacuolation were still present in the 500 mg/kg/day group at end of recovery period. No increase in ECL cell were noted. Spleens of the 20 and 500 mg/kg/day groups showed no hemosiderin storage or iron.

In conclusion, pantoprazole given orally for 4 weeks produced clinical signs of piloerection, slight retardation of body weight gain, slight hematological changes, elevation of protein, increased liver, thyroid, lung and stomach weights, decreases in thymus and seminal vesicle weights and histopathological changes in stomach. A no effect dose of 1 mg/kg/day was established. However, animals tolerated well at 5 mg with exception of morphological changes for stomach being observed.

**Addendum:**

**Serum Gastrin Levels:** Serum gastrin levels on day 3/4 were elevated in male and female treatment groups that received pantoprazole at doses  $\geq$  5 mg/kg/day. Serum gastrin levels on days 31/32 and 86 were not determined due to damage of samples (i.e., deterioration) during shipment.

Serum gastrin levels ( $\mu$ g/L) on treatment day 3/4 at 4 and 24 hr after dosing.

| Dose, mg/kg/day | Serum Gastrin Levels ( $\mu$ g/L) |       |             |       |
|-----------------|-----------------------------------|-------|-------------|-------|
|                 | Male Rats                         |       | Female Rats |       |
|                 | 4 hr                              | 24 hr | 4 hr        | 24 hr |
| 0               | 349                               | 220   | 131         | 114   |
| 0-Recovery      | 218                               | 183   | 194         | 128   |
| 1               | 291                               | 283   | 187         | 118   |
| 5               | 967.7                             | 516   | 768         | 229   |
| 20              | 705                               | 794   | 610         | 339   |
| 500             | 795                               | 884   | 644         | 763   |
| 500-Recovery    | 779                               | 841   | 601         | 660   |

**Urinalysis:** Calcium excretion on day 29 in female rats that received pantoprazole at doses of 5, 20, and 500 mg/kg/day were decreased to 58.6, 46, and 50.6% of the control (0.87 mmol/kg), respectively. Chloride excretion on day 29 in female rats that received pantoprazole at doses of 5, 20, and 500 mg/kg/day were decreased to 66.0, 59.5, and 64.9% of the control (2.62 mmol/kg). No difference in calcium or chloride excretion were found between the control and 500 mg/kg/day groups at the end of the recovery period.

**Histopathology:** The stomach, thyroid gland, and spleen were the target organs of toxicity. In fundic region of the stomach, changes were observed as follows: an increased incidence of chief cell hyperplasia at doses  $\geq 5$  mg/kg/day, an increased incidence of parietal cell hyperplasia at doses  $\geq 5$  mg/kg/day, and an increased incidence of parietal cell degeneration/vacuolation at doses  $\geq 20$  mg/kg/day. Parietal cell hyperplasia and degeneration/vacuolation were still evident for the male 500 mg/kg/day group at the end of the recovery period. The incidence of glandular epithelial cell degeneration was increased in the cardiac region of the stomach at a dose of 500 mg/kg/day and in the pyloric region of the stomach at doses  $\geq 1$  mg/kg/day. Glandular epithelial cell degeneration was not evident in these regions at the end of the recovery period. For the thyroid gland in female rats that received 500 mg/kg/day, epithelial cells in the follicle underwent a change in cell morphology from mainly flat follicle cells to mainly cuboidal follicle cells. This change of cell morphology was not evident at the end of the recovery period. For the spleen at doses  $\geq 20$  mg/kg/day, there was evidence of iron depletion. There were no findings of iron depletion at the end of the recovery period.

Histopathological changes after a 4-week treatment period for rats that received pantoprazole by oral gavage at doses of 0, 1, 5, 20, and 500 mg/kg/day and after a 8-week recovery period for rats in the 0 and 500 mg/kg/day groups.

| Organ/Tissue                     | Treatment Period |    |    |    |    |    |    |    |     |   | Recovery Period |   |     |   |
|----------------------------------|------------------|----|----|----|----|----|----|----|-----|---|-----------------|---|-----|---|
|                                  | 0                |    | 1  |    | 5  |    | 20 |    | 500 |   | 0               |   | 500 |   |
|                                  | M                | F  | M  | F  | M  | F  | M  | F  | M   | F | M               | F | M   | F |
| n =                              | 10               | 10 | 10 | 10 | 10 | 10 | 9  | 10 | 9   | 9 | 8               | 8 | 8   | 8 |
| <b>Stomach/Fundic Region</b>     |                  |    |    |    |    |    |    |    |     |   |                 |   |     |   |
| -chief cell hyperplasia          | 3                | 3  | 3  | 2  | 5  | 3  | 5  | 6  | 9   | 7 | 1               | 2 | 4   | 3 |
| -parietal cell hyperplasia       | 0                | 0  | 0  | 0  | 1  | 1  | 7  | 6  | 7   | 5 | 0               | 0 | 3   | 0 |
| -parietal cell degen/vacuolation | 0                | 0  | 0  | 0  | 0  | 0  | 3  | 3  | 8   | 9 | 0               | 0 | 2   | 0 |
| <b>Stomach/Cardiac Region</b>    |                  |    |    |    |    |    |    |    |     |   |                 |   |     |   |
| -epithelial cell degeneration    | 1                | 1  | 0  | 0  | 0  | 1  | 1  | 0  | 2   | 2 | 0               | 1 | 1   | 1 |
| <b>Stomach/Pyloric Region</b>    |                  |    |    |    |    |    |    |    |     |   |                 |   |     |   |
| -epithelial cell degeneration    | 1                | 0  | 3  | 0  | 7  | 4  | 4  | 5  | 8   | 3 | 4               | 0 | 1   | 4 |
| <b>Thyroid gland</b>             |                  |    |    |    |    |    |    |    |     |   |                 |   |     |   |
| -mainly flat follicle cells      | 2                | 8  | 1  | 7  | 1  | 4  | 1  | 3  | 0   | 0 | 1               | 7 | 2   | 8 |
| -mainly cuboidal follicle cells  | 6                | 2  | 8  | 3  | 8  | 4  | 8  | 6  | 7   | 9 | 7               | 1 | 6   | 0 |
| <b>Spleen</b>                    |                  |    |    |    |    |    |    |    |     |   |                 |   |     |   |
| -hemosiderin storage             | 8                | 10 | 8  | 10 | 7  | 10 | 4  | 4  | 0   | 0 | 8               | 8 | 8   | 8 |
| -no hemosiderin                  | 2                | 0  | 2  | 0  | 3  | 0  | 5  | 6  | 9   | 9 | 0               | 0 | 0   | 0 |

In a 4-week oral toxicity study, rats received pantoprazole at doses of 0, 1, 5, 20, and 500 mg/kg/day. Following the treatment period, rats in the control and 500 mg/kg/day groups entered an 8-week recovery period. Serum gastrin levels on day 3/4 were elevated in male and female treatment groups that received pantoprazole at doses  $\geq$  5 mg/kg/day. The dose of 5 mg/kg/day could be considered a tolerated dose given that changes in the stomach were most likely due to the pharmacological actions of pantoprazole. The stomach, thyroid gland, and spleen were the target organs of toxicity. In fundic region of the stomach, changes were observed as follows: an increased incidence of chief cell hyperplasia at doses  $\geq$  5mg/kg/day, an increased incidence of parietal cell hyperplasia at doses  $\geq$  5 mg/kg/day, and an increased incidence of parietal cell degeneration/vacuolation at doses  $\geq$  20 mg/kg/day. Parietal cell hyperplasia and degeneration/vacuolation were still evident for the male 500 mg/kg/day group at the end of the recovery period. The incidence of glandular epithelial cell degeneration was increased in the cardiac region of the stomach at a dose of 500 mg/kg/day and in the pyloric region of the stomach at doses  $\geq$  1 mg/kg/day. Glandular epithelial cell degeneration was not evident in these regions at the end of the recovery period. For the thyroid gland in female rats that received 500 mg/kg/day, epithelial cells in the follicle underwent a change in cell morphology from mainly flat follicle cells to mainly cuboidal follicle cells. This change of cell morphology was not evident at the end of the recovery period. For the spleen at doses  $\geq$  20 mg/kg/day, there was evidence of iron depletion. There were no findings of iron depletion at the end of the recovery period.

**Serum Gastrin Levels in Rats After Oral Administration of Pantoprazole for 4 Weeks (GTR-31278).**

**Testing Laboratory:** Byk Gulden Pharmaceuticals  
Konstanz, Germany

**Study Started:** May 24, 1988

**Study Completed:** May 19, 1989 (Stamp Date of December 22, 1997)

**GLP Requirements:** There were no statements of compliance with GLP regulations or the Quality Assurance Unit.

**Animals:** Sprague Dawley rats were used in this study. Mean body weights at the start of treatment were 200 g for male rats and 165 g for female rats.

**Drug Batch:** Pantoprazole, Batch 189-025

**Methods:** The sponsor submitted a 4-week oral toxicity study in rats (GTR-31902) in which doses of 1, 5, 20 and 500 mg/kg/day of pantoprazole were used. In that study, the sponsor could not measure serum gastrin levels due to technical mistakes. In the present study, the sponsor repeated the study with fewer animals per group and doses of 1, 5 and 500 mg/kg/day (dose of 20 mg/kg/day was omitted).