

**Genotoxicity**

**1. Studies with Pantoprazole:**

**Microbial Metabolic Activation Test to Assess the Potential Mutagenic Effect of Pantoprazole (GTR-32058).**

**Testing Laboratory:** \_\_\_\_\_

**Date of the Study:** Oct. 13, 1987 to March 1988

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

**Methods:** Pantoprazole (batch no. 4) dissolved in DMSO at concentrations of 312.5-5000  $\mu\text{g}/\text{plate}$  was tested in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 and E. coli WP2 uvrA to detect its mutagenic potential in the presence or absence of metabolic activation. S-9 mix was prepared from rat liver after i.p. injection of \_\_\_\_\_ (induction period: 5 days). The plate were inverted and incubated for 72 hours at 37°C. Following positive controls were used: N-ethyl-NI-nitrosoguanidine (3  $\mu\text{g}/\text{plate}$ ), 9-aminoacridine (80  $\mu\text{g}/\text{plate}$ ), 2-aminoanthracene (0.5-20  $\mu\text{g}/\text{plate}$ ) and 2-nitrofluorene (1-2  $\mu\text{g}/\text{plate}$ ). The criteria for a positive result was a statistically significant dose-related increase in the number of revertants.

**Results:** The data indicate that pantoprazole shows no evidence of mutagenicity in this testing system. Positive controls yielded typical result.

Pantoprazole at concentrations ranging from 312.5 to 5000  $\mu\text{g}/\text{plate}$  in the presence or absence of metabolic activation was negative in the bacterial reverse mutation assay using Salmonella typhimurium tester strains, TA1535, TA1537, TA1538, TA98 and TA100, and Escherichia coli tester strain, WP2 uvrA.

**Metaphase Chromosome Analysis of Human Lymphocytes Cultured In Vitro (GTR-32042).**

**Testing Laboratory:** \_\_\_\_\_

**Date of the Study:** Sept. 28, 1987 to Oct. 3, 1988.

**GLP Requirement:** Testing laboratory stated that the study was carried out following the guidelines of the Japanese Ministry of Health and Welfare and OECD.

**Methods:** The human lymphocytes were treated with pantoprazole (batch no. 4) dissolved in DMSO at concentrations of 28-280  $\mu\text{g}/\text{mL}$  in the presence of metabolic activation and at 38.98-389.8  $\mu\text{g}/\text{mL}$  in the absence of metabolic activation. Due to excessive toxicity study at two highest dose levels, the study was repeated at concentration of 48.73-194.92  $\mu\text{g}/\text{mL}$ . For the investigation with metabolic activation the cells were incubated for 6 hours in the presence of S-9 mix and pantoprazole followed by an additional 18 hours of incubation without S-9 mix before being processed for cytogenetic analysis. For the studies in the absence of S-9 mix, the cells were incubated for 24 or 48 hours before being processed for cytogenetic analysis. The metaphase cells were examined for both chromosomal damage and polyploidy. Ethyl methane sulfonate, mitomycin, cyclophosphamide, carbendazim, and diethylstilbestrol were used as the positive controls. Criteria for a positive result were not provided.

**Results:** In the presence of metabolic activation, pantoprazole failed to produce any significant increase in chromosomal aberrations or polyploidy. In the absence of S-9 mix (24 hour culture) it caused no significant increase in the number of cells with chromosomal aberrations, but a slight increase in the number of cell showing polyploidy at lowest dose level of 48.73  $\mu\text{g}/\text{mL}$ . However, after 48-hr incubation, it caused a statistically significant increase in number of cells with chromosomal damage at dose levels (146.19 and 194.92  $\mu\text{g}/\text{mL}$ ). The results are given below:

Number of Aberrant Cells/200 Cells

Solvent Control	0
48.73 $\mu\text{g}/\text{mL}$	1
97.46	0
146.19	3
194.92	4
Mitomycin C	26

Positive controls exhibited typical positive results.

In conclusion, pantoprazole caused chromosomal aberration in the human lymphocyte in the absence of metabolic activation.

Chromosomal Aberration Assay in Human Whole Blood Lymphocytes With Pantoprazole (GTR-32046 and GTR-32253).

Testing Laboratories: [ ]

Dates Study Started and Completed: February 10, 1995 and April 17, 1996.

Strain Employed: Cultured human lymphocytes cells.

Concentration Employed: 55 – 550  $\mu\text{g}/\text{ml}$ .

**Solvent Control:** Dimethyl Sulfoxide (DMSO)

**Positive Control:** Mitomycin C (0.05  $\mu\text{g/ml}$ ) and cyclophosphamide (20  $\mu\text{g/ml}$ ).

**Source of Metabolic Activation:** Rat liver microsomal enzymes (S - 9 mix).

**Drug Batch No.:** 0494180000

**Methods:** Human lymphocytes cultured cells were treated with pantoprazole in the presence and the absence of S-9 mix. Cells were harvested at about 17 hr after the start of treatment (cells in the presence and absence of S-9 mix were treated only for 4 hr then washed and incubated for additional 13 hours). At the end of experiment, 100 metaphases were examined per treatment group. Criteria of positivity was the same as mentioned above.

**Results:** Irrespective of the presence and absence of metabolic activator (S-9 mix), pantoprazole (incubated for only 4 hours with the cells) was clastogenic in cultured human lymphocytes.

Dose ( $\mu\text{g/ml}$ )	Percent Aberrant Cells Without Gap	Mitotic Index (%)
	+ S-9 Mix	+ S-9 Mix
Control	0.5	5.15
250	0.0	5.10
375	0.0	2.70
500	10.50*	1.70
Positive Control	20.0*	1.05

\* p = <0.001

Dose ( $\mu\text{g/ml}$ )	Percent Aberrant Cells Without Gap	Mitotic Index (%)
	- S-9 Mix	- S-9 Mix
Control	0.0	8.85
400	1.0	7.95
450	6.5*	8.85
500	3.0	6.80
515	15.0*	5.00
530	9.0*	2.6
Positive Control	5.5*	7.55

\* p = <0.001

In GTR-32253, the above experiment was repeated with omeprazole (50-500  $\mu\text{g/ml}$ ). In the absence of S-9 mix, omeprazole induced significant chromosomal aberration (excluding gaps) at 75 and 100  $\mu\text{g/ml}$ , but the percent of aberrant cells were within the range of historical incidence rate (0-5%). In the presence of S-9 mix, data clearly indicated that omeprazole was clastogenic in cultured human lymphocytes.

Pantoprazole (incubated for only 4 hours with the cells) in the presence or absence of metabolic activation was clastogenic in cultured human lymphocytes.

**Chromosome Aberration Assay in Human Lymphocytes In Vitro with Pantoprazole (GTR-32044).**

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

**Date Started:** January 17, 1996

**Date Completed:** December 17, 1996 (Stamp Date of January 6, 1998)

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

**Drug Batch:** Pantoprazole, Batch Number 029522000

**Methods:** The genotoxic potential of pantoprazole was evaluated with human lymphocytes *in vitro*. Phytohemagglutinin-treated human lymphocytes were incubated for 44 hr prior to experiments with pantoprazole. Human lymphocytes in the absence of S-9 metabolic activation were incubated with pantoprazole at concentrations of 0, 500, 1000, 1200, and 1400  $\mu\text{M}$ . Three experiments were performed with human lymphocytes in the presence of S-9 metabolic activation with varying pantoprazole concentrations. In experiment LE0395, lymphocytes were incubated with pantoprazole concentrations of 0, 500, 1000, 1200, and 1400  $\mu\text{M}$ ; however, cell numbers were too low at 1200 and 1400  $\mu\text{M}$  and analysis was only performed with 500 and 1000  $\mu\text{M}$ . In experiment LG0395, human lymphocytes were incubated with pantoprazole concentrations of 0, 500, 1000, 1050, 1175, 1200, 1300, 1350, and 1400  $\mu\text{M}$ ; however, cell numbers were too low and only 1 culture at 1400  $\mu\text{M}$  could be analyzed. In experiment LJ0395, human lymphocytes were incubated with pantoprazole concentrations of 0, 125, 250, 500, 1000, and 1400  $\mu\text{M}$ . For experimental groups, there were 2 parallel cultures, except in LJ0395, where there were 4 cultures for each dose group. Lymphocytes in the presence or absence of S-9 metabolic activation were incubated with pantoprazole for 4 hr. Medium was changed and cells were incubated for an additional 72 hr. At 4 hr prior to harvesting, colcemid was added to cells in culture. Positive controls were methyl methanesulfonate (200  $\mu\text{M}$ ) in absence of metabolic activation and cyclophosphamide (10  $\mu\text{M}$ ) in presence of metabolic activation. At the end of 72-hr incubation period, cells were processed and stained with Giemsa. Only those metaphases, which were well spread and numerically complete ( $n=46$ ), were evaluated. The percentage of mitotic cells were calculated after counting of 1000 transformed cell nuclei per sample. The percentages of aberrant metaphases without achromatic lesions from each treatment group were compared with concurrent solvent controls using Fisher's exact test.

**Results:** Treatment of human lymphocytes with 1400  $\mu\text{M}$  pantoprazole in the presence or absence of metabolic activation led to a significant increase in the incidence of aberrant metaphases.

Human lymphocytes treated with pantoprazole in the absence of metabolic activation (Expt. LD0395).

Treatment	Mitotic Index, %	Break ev. Per cell
Untreated Control	11.10	0.03
	10.80	0.02
Control	7.80	0.01
	7.50	0.02
Positive Control	12.40	0.15
	9.70	0.16
500 $\mu\text{M}$	10.80	0.03
	11.80	0.02
1000 $\mu\text{M}$	8.30	0.04
	10.20	0.00
1200 $\mu\text{M}$	4.80	0.06
	10.00	0.11
1400 $\mu\text{M}$	5.00	0.40
	2.90	0.08

Human lymphocytes treated with pantoprazole in the presence of metabolic activation (Expt. LE0395).

Treatment	Mitotic index, %	Break ev. Per cell
Untreated Control	6.20	0.00
	9.20	0.01
Control	8.60	0.01
	8.20	0.01
Positive Control	7.20	0.17
	8.10	0.16
500 $\mu\text{M}$	3.30	0.03
	11.40	0.06
1000 $\mu\text{M}$	6.60	0.16
	2.00	0.02

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Human lymphocytes treated with pantoprazole in the presence of metabolic activation (Expt. LJ0395).

Treatment	Mitotic Index, %	Break ev. Per cell
Untreated Control	17.10	0.01
	10.80	0.02
Control	6.70	0.00
	7.10	0.04
Positive Control	10.00	0.32
	9.00	0.20
125 $\mu$ M	9.10	0.00
	11.30	0.03
	13.90	0.00
	8.60	0.06
250 $\mu$ M	8.40	0.05
	8.40	0.03
	8.50	0.04
	8.60	0.00
500 $\mu$ M	13.40	0.05
	7.60	0.00
	9.70	0.01
	8.90	0.01
1000 $\mu$ M	7.00	0.02
	6.20	0.05
	10.20	0.08
	9.90	0.04
1400 $\mu$ M	4.00	0.00
	4.10	0.27
	5.90	0.17
	3.10	0.22

Pantoprazole in the presence or absence of metabolic activation displayed clastogenic activity with human lymphocytes in vitro.

**An Assessment of the Mutagenic Potential of Pantoprazole in a Mammalian Cell Mutation Assay Using the Chinese Hamster Ovary/HPRT Locus Assay (GTR-32057).**

**Testing Laboratory:** \_\_\_\_\_

**Date of the Study:** Sept. 28, 1987 to Sept. 1988.

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

**Methods:** The CHO cells were incubated for 4 hours in the presence and absence of metabolic activation with doses of pantoprazole (Batch no. 4) dissolved in 1% DMSO at acid concentrations of 89-890  $\mu$ g/mL. After treatment the cell were cultured for 7 days to allow for expression of mutations. Then the cells were grown in medium containing 6-thioguanine for 7 days and the number of mutant colonies were counted. The criteria used by sponsor for a positive response are: (1) the demonstration of a statistically significant dose-related increase in mutant frequency, (2) the response must be reproducible, and

(3) the mean mutant frequency in treated cultures would be expected to reach a value of twice the highest acceptable mean spontaneous mutant frequency. Based on testing laboratory the spontaneous mutant frequency was 15 mutant/10<sup>5</sup> viable cell. Following positive controls were used: ethylmethane sulfonate (EMS) and 20-methylcholanthrene (20-Mc). The assays were conducted two times in the absence of metabolic activation and three times in the presence of metabolic activation.

**Results:** No significant increases in mutant frequency were induced by treatment with pantoprazole in two tests in the absence of S-9 mix or in the first test in the presence of S-9 mix. However, significant linear increases were induced in test 2 and test 3 in the presence of S-9 mix (Linear regression analysis is significant in both tests at the 5% level). The results of the studies in the presence of S-9 mix are summarized below:

### Test 1

Concentration ( $\mu\text{g/mL}$ )	Mutants/10 <sup>6</sup> Cells
0	4
178	2
267	4
356	11
20 MC (5 $\mu\text{g/mL}$ )	491

### Test 2

Concentration ( $\mu\text{g/mL}$ )	Mutant/10 <sup>6</sup> Cells
0	2
222.5	9
267	7
311.5	7
356	22
400.5	9
20-MC (5 $\mu\text{g/mL}$ )	649

### Test 3

Concentration ( $\mu\text{g/mL}$ )	Mutant/10 <sup>6</sup> Cells
0	4
267	7
311.5	9
356	7
400.5	14
467.25	13
20-MC (5 $\mu\text{g/mL}$ )	497

Sponsor's third criteria for positive response is not generally accepted.<sup>2</sup> Significant reproducible dose-response relationship is generally used as the criteria for positive response (Mutation Research 189:135-141, 1987).

Since the linear regression analysis shows that it was significant in two tests at concentration up to 400.5 and 467.25  $\mu\text{g/mL}$ , mutagenic potential is indicated.

**Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rat (In Vitro) With Pantoprazole (GTR-32055).**

**Testing Laboratories:** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Date of Study Started:** July 24, 1990

**Date of Study Completed:** December 10, 1990

**Animals:** Male Wistar rats (8-12 weeks old)

**Drug Batch No.:** 109-195

**Solvent Control:** Water/Dimethylsulfoxide (DMSO)

**Positive Control:** 2-Acetylaminofluorene (2-AAF: 2.23  $\mu\text{g/mL}$ )

**Methods:** Primary hepatocyte cultures from the liver of adult male Wistar rats were used for this study. Concentrations of 1, 3, 10, 30 and 100  $\mu\text{g/ml}$  of B 8610-023 (pantoprazole), positive control 2-AAF (2.23  $\mu\text{g/ml}$ ) or negative control (culture medium) were tested. The test was performed in two independent experiments. The hepatocytes were incubated with test article for 18 hr in the presence of [ $^3\text{H}$ ] thymidine. Incorporation was followed by \_\_\_\_\_ of the hepatocytes and grains were counted in 100 nuclei other than S-phase. Net increase in nuclear grains induced by each compound were determined. The test compound is considered positive when there is a linear dose response and the results are statistically significant or a reproducible and statistically significant positive response was seen in at least one of the tested points.

**Results:** Pantoprazole was slightly cytotoxic at dose level of 100  $\mu\text{g/ml}$  (survival rate = 62%). No net increase in nuclear grain counts was observed in hepatocytes exposed to pantoprazole at concentrations ranging from 1-100  $\mu\text{g/ml}$ . The positive controls were genotoxic, while no repair was induced by vehicle (negative control). Thus results suggest that pantoprazole was not genotoxic in this test.

Pantoprazole at concentrations  $\leq 100 \mu\text{g/mL}$  was negative in the unscheduled DNA repair assay with primary rat hepatocytes.

**AS52/GPT Mammalian Cell-Forward Gene Mutation Assay with Pantoprazole (GTR-32048).**

**Testing Laboratories:** \_\_\_\_\_

**Dates Study Started and Completed:** February 10, 1995 and October 12, 1995.

**Cells Employed:** Chinese hamster ovary cells (AS52) cells.

**Concentration Employed:** 401-650  $\mu\text{g/mL}$  in the absence of S-9 Mix and 10-300  $\mu\text{g/mL}$  in the presence of S-9 Mix.

**Solvent Control:** Dimethyl Sulfoxide (DMSO)

**Positive control:** Ethyl methane sulfonate (150 and 300  $\mu\text{g/ml}$ ) in the absence of S-9 mix and N-nitrosodimethylamine (100  $\mu\text{g/ml}$ ) in the presence of S-9 mix.

**Source of Metabolic Activation:** Rat liver microsomal enzymes (S-9 mix).

**Drug Batch No.:** 0494180000

**Criteria of Genotoxic Effect:** Mutation frequency in the exposed culture must be at least twice the mean mutation frequency of the negative control cultures and is increased above the solvent control by at least 10 mutant per million clonable cells.

**Results:** The relative cloning efficiency was about 20% at 650  $\mu\text{g/ml}$  in the absence of S-9 mix and about 30% at 300  $\mu\text{g/mL}$  in the presence of S-9 mix. Mutation frequencies in pantoprazole treated cultures (with and without S-9 Mix) were all within normal limits. Significant increases in the mutation frequencies of the positive test control cultures were observed. Thus, pantoprazole had no mutagenic potential in this test.

Pantoprazole was negative in the AS52/GPT mammalian cell-forward gene mutation assay.

**Cell Mutation Assay at the Thymidine Kinase Locus in Mouse Lymphoma L5178Y Cells with Pantoprazole (GTR-32047).**

**Testing Laboratories:** \_\_\_\_\_

**Date Study Started and Completed:** May 23, 1995 and December 12, 1995.

**Strain Employed:** L5178Y TK+/- mouse lymphoma cells.

**Concentration Employed:** 3-400  $\mu\text{g/mL}$  in the absence of S-9 Mix and 3-300  $\mu\text{g/mL}$  in the presence of S-9 Mix.

**Solvent Control:** Dimethylsulfoxide (DMSO).

**Positive Control:** Methyl methane sulfonate (13  $\mu\text{g/mL}$ ) in the absence of S-9 mix and 3-methylcholanthrene (3  $\mu\text{g/mL}$ ) in the presence of S-9 mix.

**Source of Metabolic Activation:** Rat liver microsomal enzymes (S-9 mix).

**Drug Batch No.:** 0494180000

**Methods:** Sponsor has conducted two identical independent experiments. Cells were incubated for 4 hours with vehicle, pantoprazole or positive control, then cells were washed, resuspended in media and viability rates and mutation frequency were determined at appropriate time. At the highest tested dose, the relative survival rates in experiment 1 were 19.5% and 12.5% in the absence and presence of S-9 mix respectively, and the corresponding values in experiment 2 were 36.8% and 10.7% respectively. The test substance was considered to be mutagenic if the mutation frequency at 1 or more doses were significantly greater than that of the negative control (or 2 time higher than the mean spontaneous mutation frequency) and it was dose-related. The size distributions of the mutant colonies were also compared with that seen in solvent control and positive control.

**Results:** Irrespective of the treatment with metabolic activation system (S-9 mix), the drug was not mutagenic at the tk locus of L5178Y mouse lymphoma cells.

Pantoprazole was not mutagenic at the tk locus of L5178Y mouse lymphoma cells.

**Action of Pantoprazole on Malignant Transformation in C3H-M2 Mouse Fibroblasts In Vitro (GTR-32053).**

**Testing Laboratories:** \_\_\_\_\_  
\_\_\_\_\_  
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**Study Started:** May 26, 1992

**Study Completed:** November 13, 1992

**Cell Cultures:** M2-clone of C3H-mouse fibroblasts obtained from C3H mouse prostate.

**Concentrations Used:** 25, 50, 100, 200 and 300  $\mu\text{g/mL}$  in the absence of S-9 mix and 100, 200, 300, 400, 500 and 1000  $\mu\text{g/mL}$  in the presence of S-9 mix.

**So'vent Control:** Dimethylsulfoxide (DMSO)

**Source of Metabolic Activation:** S-9 mix

**Positive Control:** N-methyl-N-nitro-N-nitrosoguanidine (MNNG: 0.5  $\mu\text{g/ml}$ ), methyl cholanthrene (MCA: 10  $\mu\text{g/ml}$ ) and 2acetylaminofluorene (AAF: 10  $\mu\text{g/ml}$ ).

**Drug Batch No.:** 500205

**Methods:** The malignant transformation of M2 clone of C3H mouse fibroblasts by pantoprazole was assessed by the method of Marquardt et al. (Cancer Research 36: 2065-2069, 1976). Briefly, cell were incubated for 24 hr with 25-300  $\mu\text{g}/\text{mL}$  of pantoprazole in the absence of S-9 mix or incubated for 2 hour with 100-1000  $\mu\text{g}/\text{mL}$  of pantoprazole in the presence of S-9 mix. Three independent experiments were performed. Cells were washed after the exposure with drug, then 8 weeks after the initial plating, the cultures were stained (Giemsa) and the transformed foci of the type III were counted according to Reznikoff et al. (Cancer Research 33: 3239-3249, 1973). The rate of transformation was expressed as transformed foci in treated dishes (1000 cells/dish were plated). There were 18 dishes per point.

**Results:** The plating efficiencies decreased in a dose-dependent manner in treated culture (plating efficiencies usually ranged from 20-40% depending on the batch of serum used). Irrespective of presence or absence of S-9 mix, no malignant transformed foci were evident in treated culture. The positive controls gave expected results.

Pantoprazole at concentrations  $\leq 300 \mu\text{g}/\text{mL}$  in the absence of metabolic activation or  $\leq 1000 \mu\text{g}/\text{mL}$  in the presence of metabolic activation did not cause formation of malignant transformed foci with the M2-clone of C3H mouse fibroblasts obtained from C3H mouse prostate.

**In Vitro Cell Transformation Assay Using Syrian Hamster Embryo (SHE) Cells (GTR-32051).**

**Testing Laboratories:**

**Study Started:** November 2, 1992

**Study Completed:** December 11, 1992

**Cell Cultures:** SHE cell cultures

**Concentrations Used:** 5, 20, 30 and 50  $\mu\text{g}/\text{ml}$  in the absence of S-9 mix and 10, 25, 50 and 100  $\mu\text{g}/\text{mL}$  in the presence of S-9 mix.

**Solvent Control:** Dimethylsulfoxide (DMSO)

**Source of Metabolic Activation:** S-9 microsomal fraction of Aroclor-treated rats.

**Positive Control:** N-methyl-N-nitrosoguanidine (MNNG: 0.25  $\mu\text{g}/\text{mL}$ ), benzo(a)pyrene (BaP: 2.5  $\mu\text{g}/\text{mL}$ ).

**Drug Batch No.:** 500205

**Methods:** This study was conducted to assess the potential of pantoprazole to induce morphological transformation (phenotypic changes) in Syrian hamster embryo cells (*in vitro*). It has been reported that when transformed cells are injected into suitable hosts it produces cancer. Therefore, this test has been used for screening potential carcinogens (Piental *et al.*; *Int. J. Cancer*, 19: 642-655, 1977). Two independent experiments were conducted. Cells (500 cells/flask, 15 flask/test point) were incubated for 48 hr with 5-50  $\mu\text{g}/\text{ml}$  of pantoprazole in the absence of S-9 mix or incubated for 4 hr with 10-100  $\mu\text{g}/\text{mL}$  of pantoprazole in the presence of S-9 mix. Cells were washed after exposure with drug/vehicle/positive control, then 10 days after the initiation of cell culture, the cells were fixed, stained and a maximum of 100 colonies/flask were examined for morphological transformation. The test compound is considered positive when it induces dose-related response and the results are statistically significant or a reproducible and statistically significant positive response was seen in at least one of the tested points.

**Results:** In the preliminary experiment, concentrations of pantoprazole (0.33-500  $\mu\text{g}/\text{ml}$ ) were tested for its cytotoxic effects in SHE cells. The relative survival was about 41% at 33.3  $\mu\text{g}/\text{ml}$  in the absence of S-9 mix and about 36% at 100  $\mu\text{g}/\text{ml}$  in the presence of S-9 mix. Higher tested concentrations of pantoprazole were highly cytotoxic. Irrespective of presence or absence of S-9 mix, no increase in morphologically transformed colonies were seen in treated cultures. Positive controls gave expected results.

Pantoprazole at concentrations  $\leq 50 \mu\text{g}/\text{mL}$  in the absence of metabolic activation or  $\leq 100 \mu\text{g}/\text{mL}$  in the presence of metabolic activation was negative in the SHE cell assay.

**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 pantoprazole in the SHE cell assay must be considered highly questionable. Thus, the assay cannot be considered valid.

#### Mouse Micronucleus Test with Pantoprazole (GTR-32056).

##### Testing Laboratory: \_\_\_\_\_

Date of the Study: Oct. 6, 1987 to Sept. 20, 1988.

**Methods:** Four groups of Swiss mice each consisting of 15 males and 15 females were given pantoprazole (batch no, 4) dissolved in 4% Methocel E45 by gavage at dose levels of 0 (vehicle), 177.5, 355 and 710 mg/kg. Dose selection was based on a preliminary study that  $\text{LD}_{10}$  was 710 mg/kg. Bone marrow cells were obtained 24, 48 and 72 hours after dosing. Presence of micronuclei in 1000 polychromatic erythrocytes and ratio of polychromatic to normochromatic erythrocytes were measured. Mitomycin C (12 mg/kg) was used as the positive control. Criteria for a positive result was not provided.

**Results:** At the 24 hour sampling time, statistically significant increases in the incidence of micronucleated polychromatic erythrocytes were obtained at the 177.5 and 710 mg/kg dose levels, but not in a dose-related manner. At the 24 hour sampling time there was a significant dose-related decrease in the p/n ratio but only significant at 355 mg/kg dose level as compared with the vehicle control. The results are summarized below:

Dosage (mg/kg)	Incidence of Micronucleated Polychromatic Erythrocyte
Vehicle Control	0.2
177.5	0.9
355	0.5
710	1.0
Mitomycin C (12 mg/kg)	61.6

Sponsor stated that the values obtained were comparable with the historical control and concluded that the increases were not a result of treatment. However, an adequate historical control database is used to assess the consistency of concurrent control data with historical controls. The usual criteria of positive response are a dose-response trend or a statistically significant elevation of individual values above the control values. Although, there was no statistically significant dose-related trend in the study, it produced statistically significant increases in micronucleated polychromatic erythrocytes at two dose levels.

#### Mouse Micronucleus Test with Pantoprazole (GTR-32054).

Sponsor earlier submitted the result of mouse micronucleus test (GTR-32056). The earlier study was conducted at \_\_\_\_\_  
In this study CD-1 outbred Swiss mice of both sexes (40 day old, 18-25 g) were used, and doses of 177.5, 355 and 710 mg/kg of pantoprazole were used. At three time points (24, 48 and 72 hr after drug administration) 1000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. At 24 hr sampling point, the incidence of micronucleated polychromatic erythrocytes was significantly higher than the concurrent control group at two dose levels (177.5 and 710 mg/kg). To confirm this previous positive finding, sponsor repeated the test at the same laboratory, using same strain and age of mice with minor modifications. There were two modifications in the experiment: (1) only 24 hr and 30 hr sampling time were evaluated and (2) an additional low dose level of 88.75 mg/kg was included in the protocol.

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Testing Laboratories: \_\_\_\_\_

Study Started: July 22, 1991

Study Completed: June 22, 1992

No. of Animals: 5 mice/sex/group

Route of Administration: Oral (gavage)

Dose Levels: 88.75, 177.5, 355, and 710 mg/kg (pH 10.5, 20 mL/kg)

Drug Batch No.: 109195

Positive Control: Mitomycin C (12 mg/kg)

Methods: Animals were given a single dose of pantoprazole or positive control at 24 or 30 hours prior to sacrifice and preparation of the bone marrow. 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 and it is dose-related. Sponsor also stated that at least two animals must have more than six micronucleated cells per 2000 polychromatic erythrocytes in any individual compound treated group (this is based on — historical data May 1986 - May 1991).

Results: Pantoprazole did not induce an increase of micronucleated polychromatic and normochromatic erythrocytes in mice bone marrow. In contrast, the % of micronucleated polychromatic erythrocytes in mitomycin treated group was markedly higher than the negative control. These findings suggest that pantoprazole is not mutagenic in this repeat test.

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TABLE I

Summary of results - group totals/mean for the entire experiment and results of statistical analysis

Sex	Treatment	Dose <sup>a</sup> (mg/kg)	Ratio p/n		Incidence sup	Incidence sup
			mean	mean	P	total
24 Hour	Vehicle control	-	1.198	1.9	>0.01	1.2
	96022	88.75	1.108	1.3		0.3
		177.5	1.110	2.2		1.2
		355	1.081	1.5		1.2
		710	0.962	1.3	1.1	
Mitomycin C	12	0.753	88.7	<0.01	2.0	
30 Hour	Vehicle control	-	0.913	1.4	>0.01	0
	96022	88.75	0.979	1.1		0.4
		177.5	1.121	2.1		0.4
		355	1.023	1.4		0
		710	1.009	0.7	0.4	
Mitomycin C	12	0.610	127.5	<0.01	1.7	

p/n Ratio of polychromatic to normochromic erythrocytes  
 sup Number of micronucleated polychromatic erythrocytes observed per 2000 cells  
 n Number of micronucleated normochromic erythrocytes observed per 2000 cells  
 P Results of statistical analysis using Dunnett's test for inter-group comparisons and Linear Regression Analysis (1-tailed probabilities)  
 - Dose levels of 96022-Z are expressed in terms of the free acid 96022

Pantoprazole at doses  $\leq 710$  mg/kg at 24 or 30 hr after treatment was negative in the mouse micronucleus assay.

Bone Marrow Chromosomal Aberration Assay in Sprague Dawley Rats with Pantoprazole (GTR-32049).

Testing Laboratories: \_\_\_\_\_

Dates Study Started and Completed: February 10, 1995 and June 30, 1995.

Animals: Sprague Dawley rats (7 weeks old, males = 172-244 g and females = 187-209 g).

Solvent Control: Distilled water

Positive Control: Cyclophosphamide (20 mg/kg)

Drug Batch No.: 0494180000

**Methods:** Groups of rats (5/sex/group) were given single oral (gavage) dose of vehicle (distilled water), 300, 600 and 1200 mg/kg (10 mL/kg) of pantoprazole. A control group containing 5 males were given single i.p. dose of cyclophosphamide (20 mg/kg). Bone marrow cells were sampled (2 hr prior to sample collection, each rat was given colchicine [2 mg/kg, i.p.] to arrest dividing cell at metaphase) at 17 hr and 41 hr after the drug administration. 100 metaphases from each rat were examined for chromosomal damage. Criteria for positive response were a significant one-tailed trend test for the frequency of metaphase cells containing one chromosome aberration (excluding gaps) and a significant increase in at least one dose group using Fisher's exact test.

**Results :** No significant increase in chromosomal aberration (excluding gaps) was evident in treated females at 17 and 41 hours and in treated males at 41 hours. A statistical significant ( $p = 0.062$  [one-tailed Fisher's exact test]) increase in chromosomal aberration (excluding gaps) was evident in high dose treated male rats at 17 hr sampling time (control = 0%, 300 mg/kg = 0%, 600 mg/kg = 0.2% and 1200 mg/kg = 0.8%; positive control = 24.8%). This small increase in chromosomal aberration i.e. 0.8% at 1200 mg/kg/day dose level was statistically significant, but was not considered to be biologically significant. Positive control gave expected results. Thus in vivo pantoprazole had no clastogenic activity in rats.

Pantoprazole was not clastogenic in vivo in the bone marrow chromosomal aberration test.

**Potential for DNA Binding of Pantoprazole (GTR-32052).**

**Testing Laboratories:** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Study Started:** August 1992

**Date Study Completed:** November 2, 1992 (Stamp Date of May 20, 1998)

**Animals:** Female Sprague Dawley rats (2/group)

**Dose Used:** 200 mg/kg/day (10 mL/kg)

**Drug Batch No.:** 500205

**Solvent Used:** Water

**Positive Control:** Not included

**Methods:** This study was conducted to find out whether pantoprazole (or its reactive metabolites) interact covalently with liver DNA isolated from female rats. Lutz (Mutation Res. 65: 289-3561, 1979) has suggested to quantify that covalent DNA binding data by calculating covalent binding index (CBI = micromol chemical bound per mol nucleotide/millimole chemical applied per kg body wt.). Groups of rats (n=2/group) were given orally (gavage) vehicle (water) or 200 mg/kg/day of pantoprazole for 2 weeks. Twenty-four hours after the last dose each rat was given a single oral dose of  $^{14}\text{C}$ -pantoprazole (200 mg/kg, 21.8  $\mu\text{Ci}/\text{ng}$ ). Additionally, 2 rats were also included in this study as cage control. Radioactivity excretion in urine and expired air were monitored for the first 24 hr after the administration of radiolabelled pantoprazole, then all the animals were killed. The livers were excised and radioactivity was measured in purified liver DNA and chromatin proteins. The binding data were expressed as CBI.

**Results:** About 35-37% of the administered radioactivity was excreted in the urine during the first 24 hr after drug administration. Pretreatment with unlabelled pantoprazole (14 days) had no effect on urinary excretion. Less than 0.1% of administered radioactivity was excreted in the expired air during this period. The covalent bindings to purified liver DNA were 0.70 and 1.6 CBI units in rats not pretreated with the drug and in rats pretreated with the drug respectively. The data indicated two findings: (1) pantoprazole radioactivity binds with purified liver DNA and (2) prolonged treatment with drug increased the percentage of binding to liver DNA (i.e., binding to liver DNA is increased about 2.3 fold upon pretreatment of the rats with pantoprazole for 14 days). This increase in binding upon pretreatment may be related to its property as a marginal metabolizing enzyme inducer. Sponsor tried to identify DNA-adduct by \_\_\_\_\_ method but failed to do so. Due to low specific radioactivity of the DNA isolated from  $^{14}\text{C}$ -pantoprazole treated rats (# 3 & 4) no analysis of DNA constituents was possible. However, when DNA isolated from rats (# 1 & 2) which were pretreated with pantoprazole for 14 days and then given a single dose of  $^{14}\text{C}$ -pantoprazole was pooled and then analyzed for DNA-adducts, about 20% of the DNA radioactivity was bound to nucleotide fraction. Hence, the data clearly indicated that pantoprazole or its metabolite(s) bind to rat liver DNA.

This report clearly indicated that pantoprazole binds with liver DNA in rats (covalent binding index (CBI) = 1.6 units, when rats were treated with pantoprazole 200 mg/kg/day for 14 days). In the present report, sponsor referred to a published report (Lutz, W.K., J. Cancer Res. Clin. Oncol. 112, 85-91, 1986) which indicated that CBI's in the order of  $10^3$ - $10^4$  are strong hepatocarcinogens, values around  $10^2$  are moderate hepatocarcinogens and values around 10 are weak hepatocarcinogens. Sponsor further stated that the low CBI for pantoprazole cannot explain the mechanism of tumor formation in the female SD rat liver.

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ON ORIGINAL

Studies on the Hepatic Effects of Pantoprazole, Lansoprazole, and Omeprazole in Rats Including <sup>32</sup>P-Postlabeling Experiments (GTR-32977).

Testing Laboratory: Main study was conducted at the \_\_\_\_\_  
\_\_\_\_\_ <sup>32</sup>P-Postlabeling Studies were  
performed by the \_\_\_\_\_  
\_\_\_\_\_ (Electron microscopy of liver)  
Byk Gulden (Histopathology of Liver)  
Byk Gulden (Measurements of hepatocyte proliferation)  
Wyeth-Ayerst Research (Pharmacokinetics)

Date Started: January 22, 1996

Date Completed: March 20, 1998

GLP Compliance: The main studies and satellite studies dealing with electron microscopy were in compliance with GLP Regulations and the Quality Assurance Unit. The satellite studies covering histopathology, hepatocyte proliferation, and pharmacokinetics were conducted in laboratories that were GLP compliant, but were not audited by a Quality Assurance Unit.

Animals: Female Sprague Dawley rats.

Drug Batch: Not Indicated.

Methods: 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, omeprazole, or lansoprazole. Liver cell hyperplasia and hypertrophy were assessed by light microscope morphometry, electron microscope examination, bromodeoxyuridine (BrdU) incorporation into hepatic nuclei and identification of proliferating cell nuclear antigen (PCNA). The potential for DNA damage was assessed using the <sup>32</sup>P-postlabeling technique. Drug exposure levels were measured in a toxicokinetic study. 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. There were 15 rats/group. Following the 4-week treatment period, 6 rats/group were allowed a 4-week recovery period. Pantoprazole at 200 mg/kg/day was identical to the high dose used in the carcinogenicity study with Sprague Dawley rats. Omeprazole and lansoprazole at 200 mg/kg/day were identical to the pantoprazole dose. Omeprazole at 600 mg/kg/day and lansoprazole at 1200 mg/kg/day were selected to provide plasma drug levels that were similar to those observed with pantoprazole at 200 mg/kg/day. Omeprazole and lansoprazole were administered as suspensions in 0.5% carbonate-buffered pH 9.0 hydroxypropylmethylcellulose. Pantoprazole was administered as an aqueous solution (20 mg/mL) adjusted to pH 10.5 with 0.1 M NaOH. Control rats received 0.5% aqueous hydroxypropylmethylcellulose. One week prior to sacrifice at the end of the 4-week treatment period or the 4-week recovery period, 6 rats/group received BrdU in the drinking water at 80 mg/mL. At the time of sacrifice, body and liver weights were measured. Samples of liver were collected and processed as follows: from rats treated with BrdU, samples of liver were fixed in Carnoy's solution; from 3 rats/group not treated with BrdU,

samples of liver were frozen and stored at -80°C until DNA extraction and subsequent <sup>32</sup>P-postlabeling; from all rats in control and treatment groups, samples of liver were fixed for subsequent electron microscopic examination; and from all rats in control and treatment groups, samples of liver were fixed for light microscopic examination and determination of hepatocyte proliferation. For estimation of the number of proliferating hepatocytes, the immunohistological detection of PCNA was performed. Additionally, the immunohistological detection of BrdU antigen was performed from animals, which were treated with BrdU. From each animal, 1000 hepatocytes were counted and number of PCNA (or BrdU) positive stained hepatocytes were determined. The numbers of PCNA or BrdU positive cells per 1000 hepatocytes were calculated (i.e., fewer cells per field indicated increased individual cell size). From the 4-week treatment groups, liver sections for electron microscopy were taken from 9 animals each of the 600 mg/kg/day omeprazole, pantoprazole, and 1200 mg/kg/day lansoprazole groups and 8 control animals. From recovery groups, liver sections for electron microscopy were taken from 6 animals each of the control, 600 mg/kg/day omeprazole, and pantoprazole groups and 5 animals from the 1200 mg/kg/day lansoprazole group. For <sup>32</sup>P-postlabeling of DNA isolated from rat liver, DNA was digested with deoxyribonucleotide 3'-monophosphates and radiolabeled with [<sup>32</sup>P]-ATP. Radiolabeled adduct were applied to — plates, resolved in two directions using 5 different solvent systems, and visualized after exposure of — plates to film. Radioactivity was quantified and the number of adducts per 10<sup>9</sup> unadducted nucleotides was calculated. For comparison, liver DNA from a rat treated with tamoxifen at 40 mg/kg for 6 months was also analyzed. Blood for determination of plasma levels of omeprazole, pantoprazole, and lansoprazole was collected on day 7 at 0, 0.5, 1, 2, 4, and 8 hr after dosing. Plasma drug levels were quantified using a — method.

**Results:**

1. **Mortality:** There was no treatment-related mortality.

2. **Liver Weight:** Absolute and relative liver weights were increased in all treatment groups. This change was attributed to hepatocyte hypertrophy (i.e., SER proliferation) and hyperplasia. Increased absolute and relative weights were reversible following a 4-week recovery week.

Treatment, mg/kg/day	4-Week Treatment Period		4-Week Recovery Period	
	Abs. Liver wt., g	Rel. Liver wt., % <sup>A</sup>	Abs. Liver wt., g	Rel. Liver wt., %
Control, 0	8.778	3.3544	10.133	3.6900
Omeprazole, 200	10.970	4.5429	10.573	3.7129
Omeprazole, 600	11.836	4.5884	11.232	3.7812
Pantoprazole, 200	10.620	4.4011	10.142	3.6150
Lansoprazole, 200	11.512	4.3870	10.413	3.8013
Lansoprazole, 1200	11.687	4.7589	9.558	3.6556

A. Relative liver weights provided by sponsor appeared to be in error. Values were calculated from raw data.

**3. Measurement of Hepatocyte Proliferation by Determination of BrdU Incorporation into Hepatic Nuclei and Identification of Proliferating Cell Nuclear Antigen:** For estimation of the number of proliferating hepatocytes, the immunohistological detection of PCNA was performed. Additionally, the immunohistological detection of BrdU antigen was performed from animals, which were treated with BrdU during the week prior to sacrifice. Hepatocyte proliferation (i.e., hyperplasia) as assessed by BrdU labeling was found for rats that receive omeprazole at 600 mg/kg/day, pantoprazole at 200 mg/kg/day, or lansoprazole at 200 mg/kg/day. Hepatocyte proliferation (i.e., proliferation) as assessed by a positive reaction with PCNA was found for rats that received omeprazole at 200 or 600 mg/kg/day and pantoprazole at 200 mg/kg/day. Hyperplasia was not evident for rats that lansoprazole at 1200 mg/kg/day, which may be related to problems in methodology. Hepatocyte proliferation (i.e., hyperplasia) was reversible following a 4-week recovery period.

Bromodeoxyuridine labeling of liver cell nuclei.

Treatment, mg/kg/day	4-Week Treatment Period	4-Week Recovery Period
Control, 0	92.2	60.33
Omeprazole, 200	102.5	31.50
Omeprazole, 600	287.3 (199.4 <sup>A</sup> )	21.00
Pantoprazole, 200	329.33	24.50
Lansoprazole, 200	170.40	60.83
Lansoprazole, 1200	53.17	59.00

A. Mean without outlying value.

PCNA-positive hepatocytes after 4 weeks of treatment.

Treatment, mg/kg/day	4-Week Treatment Period	4-Week Recovery Period
Control, 0	3.2	2.7
Omeprazole, 200	5.3	2.4
Omeprazole, 600	18.6	2.1
Pantoprazole, 200	7.8	1.7
Lansoprazole, 200	3.6	4.9
Lansoprazole, 1200	3.9	2.4

**4. Light and Electron Microscopy Analysis for Hepatocyte Hypertrophy:** Light microscopy indicated centrilobular enlargement of hepatocytes in all treatment groups as shown in the table below. Electron microscopy of liver sections was confined to the 600 mg/kg/day omeprazole, pantoprazole, and 1200 mg/kg/day lansoprazole groups. A minimal increase in the amount of smooth endoplasmic reticulum in centrilobular hepatocytes was found for 1/9 (11.1%) animals treated with pantoprazole at 200 mg/kg/day and 2/9 (22.2%) animals treated with 1200 mg/kg/day lansoprazole. Liver hypertrophy was most prominent for rats that received lansoprazole at 1200 mg/kg/day. There were no findings of hypertrophy at the end of the recovery period.

Table 3. Comparison of hepatic centrilobular hypertrophy in PPI treated groups.

Finding/ Group	Omeprazole 200 mg/kg		Omeprazole 600 mg/kg		Pantoprazole 200 mg/kg		Lansoprazole 200 mg/kg		Lansoprazole 1200 mg/kg		Control	
	m	r	m	r	m	r	m	r	m	r	m	r
Centrilobular hypertrophy												
- minimal								1				
- slight	1		4		2	1	5		3	1		1
- moderate			1						6			
Total	1/7	0/6	5/9	0/6	2/9	1/6	5/8	1/6	9/9	1/5	0/8	1/5

**5. Histopathology:** Histopathological analysis was confined to the liver and stomach. For the liver, slight to moderate centrilobular hypertrophy in all treatment groups was evident. At the end of the recovery period, no treatment-related centrilobular hypertrophy was evident. For the non-glandular stomach, hyperkeratinization was evident for rats that received omeprazole at 600 mg/kg/day, pantoprazole at 200 mg/kg/day, or lansoprazole at 1200 mg/kg/day. For the fundic portion of the stomach, parietal cell vacuolation was evident for rats that received omeprazole at 600 mg/kg/day, pantoprazole at 200 mg/kg/day, or lansoprazole at 1200 mg/kg/day. Foveolar hyperplasia and eosinophilic chief cells were evident for rats that received pantoprazole at 200 mg/kg/day or lansoprazole at 1200 mg/kg/day. Inspissated secretion, subepithelial cysts, and mucosal atrophy were only evident for rats that received lansoprazole at 1200 mg/kg/day. At the end of the recovery period, the only treatment-related change still evident was an increased incidence of eosinophilic chief cells for rats that received lansoprazole at 1200 mg/kg/day.

Histopathological changes for rats that received vehicle, omeprazole at 200 or 600 mg/kg/day, pantoprazole at 200 mg/kg/day, or lansoprazole at 200 or 1200 mg/kg/day by the oral route for 4 weeks.

Organ/Tissue	Control	Omeprazole		Pantoprazole, 200	Lansoprazole	
		200	600		200	1200
n =	8	9	9	9	8	9
<b>Liver, H&amp; E stain</b>						
-centrilobular hypertrophy	0	1	5	2	5	9
-hepatocellular vacuolation	0	1	4	0	1	5
<b>Stomach, non-glandular</b>						
-hyperkeratinization	0	0	5	5	0	7
<b>Stomach, fundic</b>						
-parietal cell vacuolation	0	0	9	1	0	8
-foveolar hyperplasia	0	0	0	5	0	6
-inspissated secretion	0	0	0	0	0	2
-eosinophilic chief cells	0	0	0	4	0	8
-subepithelial cysts	0	0	0	0	0	2
-mucosal atrophy	0	0	0	0	0	4

**6. Detection of DNA Damage with <sup>32</sup>P-Postlabeling:** Pantoprazole treatment led to the formation of a unique DNA adduct not observed in control samples (see below). Pantoprazole samples were tested in 4 different solvent systems following nuclease P1 enhancement of DNA adducts or extraction of adducts into butanol. Following nuclease P1 enhancement, it appeared that there was an extra spot visible in treated samples following separation in solvent systems 1, 3, and 4. This extra spot was located to the lower left of the main adduct spot that was seen in both control and treated samples (see figure below): This "extra spot" suggests that pantoprazole or one of its metabolites directly interacts with DNA. Quantitation of DNA adducts provided by the sponsor is unclear. Quantitation of adducts by the sponsor is shown in table 1; however, table 2 was prepared from line listings from pantoprazole provided by the sponsor following nuclease P1 enhancement. Numbers in tables 1 and 2 do not correlate with one another. In four experiments, the number of DNA adducts following pantoprazole treatment were elevated above control levels. Quantitation of DNA adducts by the sponsor was unreliable; however, chromatography patterns clearly indicate that pantoprazole treatment leads to the formation of a unique adduct.