CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-229

PHARMACOLOGY REVIEW

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: September 13, 2000

FROM: Supervisory Pharamcologist
Division of Gastrointestinal and
Coagulation Drug Products
HFD-180

SUBJECT: NDA 21,229 (Prilosec 1/Omeprazole magnesium) - Summary of Preclinical Findings for Inclusion in the Package for Advisory Committee Meeting on October 20, 2000.

TO: NDA 21,229

The following are the safety issues identified from preclinical testing of omeprazole. These concern the genotoxicity, tumorigenicity and contragestational and developmental toxicity findings in preclinical toxicology studies. These findings raise safety concerns for the intended use of omeprazole as an Over-the-counter drug product.

Genotoxic Effects

Omeprazole was positive in tests for genotoxicity. These tests identify the clastogenic potential of test substances but do not provide safety margins for the intended clinical doses. The positive tests were the in vivo mouse micronucleus test and the in vivo mouse bone marrow cell chromosome aberration test. The positive clastogenic effects of the aforementioned tests were further confirmed by the results of sponsor's most recent in vitro human lymphocyte chromosome aberration tests. The test results clearly demonstrated that omeprazole sodium (racemate) and its S- and R-enantiomers were clastogenic.

Recent published reports also affirm the genotoxic potential of omeprazole. Omeprazole increased the frequency of micronucleated cells indicative of chromosomal aberrations in the in vitro primary human and rat hepatocyte cell cultures (Martelli et al, Toxicology 130:29-41, 1998) and the in vitro human lymphoblastoid $TK^{+/-}$ cell line cultures (Crofton-Sleigh et al, Mutagenesis 8:363-372, 1993).

Tumorigenic Effects

In two 24-month carcinogenicity studies in rats, omeprazole at daily oral doses in a range of 1.7 to 140.8 mg/kg (about 0.7 to 57 times the human dose of 20 mg/day, expressed on a body surface area basis) produced dose-related incidence of gastric ECL cell carcinoid tumors (2 to 40%). A threshold dose could not be identified in these studies since even the lowest dose of omeprazole (about 0.7 times the human dose on a body surface area basis) was tumorigenic. Although this tumorigenic effect was ascribed to the hypergastrinemic effect of the drug, some of the tumorigenic doses (about 0.7 and 1.4 times the human dose on a body surface area basis) in these studies did not produce hypergastrinemia. Treatment of rats with omeprazole for one year was also tumorigenic. In one of the above mentioned carcinogenicity studies, exposure of female rats to omeprazole at a daily dose of 13.8 mg/kg (about 5.6 times the human dose on a body surface area basis) for one year, then followed for an additional year without the drug, produced gastric adenocarcinoma in one female rat (2% incidence). In a recently reported 52-week oral toxicology study in rats, treatment with omeprazole at 0.4, 2 and 16 mg/kg/day (about 0.2, 0.8 and 6.5 times the human dose on a body surface area basis) produced treatment related incidences of astrocytoma in the brains of treated male rats (4.3 to 8.3%) and low incidence of alveolar adenoma of the lungs in both sexes without a dose relationship. Spontaneous incidences of brain astrocytoma and alveolar adenoma of lungs were not reported for 12 month toxicity studies in this strain of rats.

Recently, Martelli et al (Toxicology 130:29-41, 1998) reported that omeprazole, when administered orally on alternate days at 10 mg/kg for 8 weeks to female rats (about 4 times the human dose on a body surface area basis) following treatment with azoxymethane (colon carcinogen), acted as a promoter of putative preneoplastic lesions, i.e. increased number and size of aberrant crypt foci in their colons. The preclinical assessment of the overall carcinogenicity potential of omeprazole remains incomplete due to lack of an adequate two-year mouse carcinogenicity study.

Contragestational and Developmental Toxic Effects

Treatment of male and female rats with omeprazole at 13.8, 43.1 and 138.0 mg/kg/day (about 5.6, 17.5 and 60 times the human dose on a body surface area basis) did not produce any toxicity or apparent adverse effects on the fertility of parental male and

female rats. But, in their offspring, it produced fetal toxicity and postnatal developmental toxicity as evidenced by dose-related increases in postimplantation losses, decreases in the number of viable fetuses, decreases in the number of viable pups born, decreases in survival of pups and retarded body weight gains of pups. Treatment of pregnant rats with omeprazole in a dose range of 3.2 to 320 mg/kg/day (about 1.3 to 130 times the human dose on a body surface area basis) during organogenesis produced no structural teratogenic effects, but affected the postnatal behavioral development of the offspring adversely. This was evidenced by alterations in the responses to open field test and conditional avoidance response test by pups belonging to dams treated by omeprazole at 32 and 320 mg/kg/day (about 13 and 130 times the human dose on a body surface area basis).

In pregnant rabbits, during organogenesis, treatment with omeprazole at doses of 6.9, 27.6 and 69.1 mg/kg/day (about 5.6, 22.4 and 56 times the human dose on a body surface area basis) was disruptive to pregnancy in a dose-related manner. It was also fetotoxic but not teratogenic.

In female rats, treatment during late pregnancy and lactation (perinatal-postnatal period) with omeprazole at 13.8, 43.1 and 138 mg/kg/day (about 5.6, 17.5 and 60 times the human dose on a body surface area basis) produced dose-related developmental toxicity in their offspring as evidenced by their decreased body weights on day 21 postpartum.

9/14/00
Jasti B. Choudary, B.V.Sc., Ph.D.

cc:
NDA
HFD-180
HFD-181/CSO, Ms. Walsh
HFD-180/Dr. Aurecchia
HFD-180/Dr. Choudary
HFD-180/Dr. Robison

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NDA 21,229

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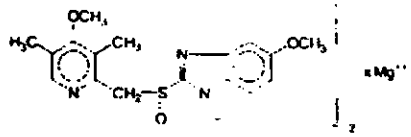
PHARMACOLOGIST'S REVIEW OF NDA 21,229

Sponsor: AstraZeneca LP
Wayne, PA

Reviewer Name: Timothy W. Robison, Ph.D.
Division Name: Gastrointestinal and Coagulation Drug Products
HFD# 180

Review number: #001
NDA number: 21,229
Serial number/date/type of submission: January 27, 2000
Date of HFD-180 Receipt: January 28, 2000
Review Completion Date: September 12, 2000, HFD-180

Drug: Omeprazole magnesium
Code Name:
Generic Name: Omeprazole magnesium
Trade Name: Prilosec 1
Chemical Name: Di-(R,S)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium (salt)
CAS Registry Number:
Molecular Formula/ Molecular Weight: $C_{34}H_{36}N_6O_6S_2Mg$/ 713.1
Structure:



Relevant INDs/NDAs/DMFs:
IND[ ](AstraZeneca LP of Wayne, PA)
NDA 19,810 (AstraZeneca LP of Wayne, PA)
IND[ ](AstraZeneca LP of Wayne, PA)
IND[ ](AstraZeneca LP of Wayne, PA)

Drug Class: Gastric parietal cell H^+,K^+-ATPase inhibitor/Proton Pump Inhibitor

Indication: (1) For **relief** of heartburn, acid indigestion, and sour stomach and (2) for **prevention** of heartburn, acid indigestion, and sour stomach brought on by consuming food and beverages, or associated with events such as stress, hectic lifestyle, lying down, or exercise.

Clinical formulation: The active ingredient is omeprazole magnesium 20.6 mg (equivalent to 20 mg omeprazole). Inactive ingredients include glyceryl monostearate,

hydroxypropyl cellulose, hydroxypropyl methylcellulose, iron oxide, magnesium stearate, methacrylic acid copolymer, microcrystalline cellulose, paraffin, polyethylene glycol 6000, polysorbate 80, polyvinylpyrrolidone, sodium stearyl fumarate, starch, sucrose, talc, titanium dioxide, and triethyl citrate.

Route of administration: Oral

Proposed clinical use: For adults and children 12 years of age and older: (1) for relief of symptoms, swallow 1 tablet with a glass of water, and (2) for prevention of symptoms for 24 hours, swallow 1 tablet with a glass of water anytime during the day, or if preferred, one hour before those events associated with occasional heartburn, such as consuming food and beverages, stress, hectic lifestyle, lying down, or exercise. Not more than one tablet should be taken per day. This product should not be used for more than 10 days in a row unless directed by a doctor. The tablets should not be chewed or crushed.

Previous clinical experience: Omeprazole (Prilosec®) has been marketed as a prescription drug for short-term treatment of active duodenal ulcer, treatment of gastroesophageal reflux disease (GERD), treatment of heartburn and other symptoms associated with GERD, short-term treatment of erosive esophagitis, maintenance of healing of erosive esophagitis, and long-term treatment of pathological hypersecretory conditions (e.g., Zollinger-Ellison syndrome, multiple endocrine adenomas and systemic mastocytosis).

Disclaimer: The sponsor's material has been incorporated in parts of this review.

Introduction and drug history: Prilosec® has been marketed as a prescription drug product for over 10 years. Prilosec 1 is under development to be marketed as an over-the-counter drug product for relief or prevention of symptomatic heartburn.

Studies reviewed within this submission:

Study Title	Study No.	Testing Laboratory	Drug Batch	Page No.
PHARMACOLOGY:				3-19
TOXICOLOGY:				
Acute Toxicity in Mice and Rats	T2244 T2243		300/87 300/68	19-21
Subacute/Subchronic/Chronic Toxicity				
Rats				
52-week oral toxicology study in rats (Submitted to IND [redacted] Amendment #376 dated February 18, 2000).				21-26
Dogs				
Comparative 3-month oral toxicity study of omeprazole magnesium salt with omeprazole in dogs.	T2237	Astra Hassle AB Sodertalje, Sweden	300/87	26-32
H199/18 Magnesium: 3 month oral (gavage)	97103	Safety Assessment	602/97	32-37

toxicity study in the dog – a comparison with omeprazole magnesium.		Leics, England Safety Assessment Sodertalje, Sweden		
Immunotoxicology				
Lymphocyte Transformation Test of H168/68	8604\1	Astra Safety Assessment		37-39
Genetic Toxicology				
Human lymphocyte chromosomal aberration assay with omeprazole, and its R- and S-enantiomers. (Submitted to NDA 19,810 as correspondence dated March 29, 2000).	524/12	Covance Laboratories Limited England	H199/18, #600/93; H199/19, #600/93; omeprazole, #13	40-44

The sponsor has cross-referenced preclinical pharmacology and toxicology studies submitted to NDA 19,810. This review has incorporated portions of previous pharmacology reviews of NDA 19,810 (Official Review Dates, May 25, 1989 and August 9, 2000), IND [redacted] (Official Review Dates, August 11, 1994 and August 22, 2000) IND [redacted] (Official Review Date, April 2, 1998), and IND [redacted] (Official Review Date, February 9, 1999).

PHARMACOLOGY:

Mechanism of Action:

The Pharmacology of the Gastric Acid Pump: The H^+,K^+-ATPase (Annual Review of Pharmacology 35: 277-305, 1995).

Gastric H^+,K^+-ATPase is the molecular target for proton-pump inhibitors, such as omeprazole. The H^+,K^+-ATPase is responsible for the elaboration of HCl, when it is present in the canalicular membrane of the parietal cell, where it is associated with a K^+ and Cl^- conductance. KCl effluxes from the cytoplasm of the cell, and external K^+ is exchanged for cytoplasmic H^+ at the expense of ATP breakdown. H^+,K^+-ATPase is a heterodimer composed of a 1034 amino acid catalytic α subunit and a glycosylated 291 amino acid β subunit. The α subunit contains approximately 10 membrane-spanning sequences, while the β subunit contains a single transmembrane segment. Proton-pump inhibitors have a pK_a of approximately 4.0 leading to accumulation in the acidic secretory canaliculus of the stimulated parietal cell. It should be noted that proton-pump inhibitors, such as omeprazole, are acid labile and undergo rapid degradation in the acid environment of the stomach. Thus, omeprazole is administered as an enteric-coated granule formulation. Following intestinal absorption, the drug reaches the gastric mucosa by way of the blood circulation. In the acidic environment of the secretory canaliculus of the stimulated parietal cell, proton pump inhibitors undergo conversion to a cationic sulfenamide. Acid activation is believed to involve protonation of the benzimidazole N in acidic solutions, which increases reactivity of C2, and thereby facilitates attack by the unprotonated pyridine N. This N has to deprotonate at relatively acidic pH values in order to account for the transformation to the sulfenamide. To facilitate this deprotonation, there is an intramolecular transfer of the proton from the pyridine N to the benzimidazole N. This sulfenamide reacts with cysteine residues on the extracytoplasmic face of the α subunit. The loop between transmembrane

segments, M5 and M6, contains cysteine$_{813}$, which may be relevant for inhibition by the 2-pyridyl methylsulfinyl benzimidazoles, such as omeprazole. Other cysteine residues to be considered in this context are cysteine$_{321}$ in the M3-M4 region and cysteine$_{892}$ in the M7-M8 loop. Omeprazole interacts in a covalent manner with cysteine$_{813}$(or $_{822}$) and cysteine$_{892}$. The half-life of H^+,K^+-ATPase in rat (and inhibition of acid secretion in humans) is 50 hr. Thus, with a once-a-day dosing regimen, a significant quantity of newly synthesized H^+,K^+-ATPase, that has not been exposed to omeprazole, will be present 24 hr after dosing. Given a half-life of 50 hr, approximately 25% of available H^+,K^+-ATPase is synthesized over a 24-hr period. This is most likely the predominant mechanism for recovery of acid secretion following covalent inhibition of the pump by benzimidazoles. At maximum, 75% of H^+,K^+-ATPase pumps, old or new, are active during exposure to omeprazole. Thus, steady-state inhibition of acid secretory capacity (i.e., approximately 30%), as reflected by the number of active pumps, should be achieved at approximately 48 hr after initiation of treatment. To improve inhibition of acid secretion, it more judicious to increase the frequency of dosing rather than the quantity of drug administered. With maximal inhibition of active H^+,K^+-ATPase pumps, newly synthesized or newly active pumps will not be inhibited 3 hr after dosing, given negligible blood levels of omeprazole due to the drug's short half-life. A second dose will be required along with meal stimulation to inhibit these pumps. With two doses per day, inhibition is more rapid and greater, and maximal secretory capacity is reduced to about 10%.

Turnover of the Gastric H^+,K^+-Adenosine Triphosphatase α Subunit and Its Effect on Inhibition of Rat Gastric Acid Secretion (Gastroenterology 109: 1134-1141, 1995).

The half-life of the α-subunit of H^+,K^+-ATPase in the stomach mucosa was examined in female Sprague-Dawley rats following treatment with omeprazole at oral doses of 0 or 28 mg/kg/day for 7 days. The half-life of the α-subunit of H^+,K^+-ATPase was compared with the recovery of H^+,K^+-ATPase activity. The half-life of the α-subunit of H^+,K^+-ATPase was determined by evaluating the turnover of ^{35}S-methionine-labeled enzyme in intact rats using a pulse chase method. Omeprazole treatment had no effect on levels of the α-subunit of H^+,K^+-ATPase. The half-lives of the α-subunit for control and omeprazole-treated groups were relatively similar at 54 and 49 hr, respectively. This result suggests that covalent binding of sulfenamide to the two relevant cysteine residues of the catalytic subunit does not affect enzyme turnover. Following discontinuation of omeprazole treatment, H^+,K^+-ATPase activity recovered with a half-life of 15 hr. Thus, activity recovered more rapidly than predicted from turnover of the α-subunit. This finding suggests that recovery of H^+,K^+-ATPase activity after covalent inhibition by omeprazole does not depend solely on the de novo synthesis of pump protein. It appears that there may be some loss of covalent bound drug in vivo to account for difference in recovery of enzyme activity and turnover of the α-subunit. In vitro studies have demonstrated that the disulfide bound formed between omeprazole and H^+,K^+-ATPase is not inherently unstable. It has been suggested that glutathione may reduce these disulfide bonds in vivo leading to some restoration of activity.

Reducing agents, such as β-mercaptoethanol, have been shown to reverse inhibition of H^+,K^+-ATPase activity by omeprazole in vivo.

Drug Activity Related to Proposed Indication:

Inhibition of Gastric Acid Secretion by Omeprazole in the Dog and Rat (Gastroenterology 85: 900-907, 1983).

Inhibition of gastric acid secretion by omeprazole was examined in dogs and rats. Picoprazole (another benzimidazole) and cimetidine (histamine H_2-receptor antagonist) were used as a reference compounds. Most studies with dogs and rats were performed with animals that had a gastric fistula for sampling of gastric juice. Intraduodenal administration of omeprazole inhibited histamine-stimulated gastric acid secretion in dogs in a dose-dependent manner with an ED_{50} of 0.09 mg/kg (i.e., 0.26 µmole/kg). Intraduodenal ED_{50} values for picoprazole and cimetidine were 1.9 and 1.8 µmole/kg, respectively. Intravenous administration of omeprazole inhibited histamine-stimulated gastric acid secretion in dogs in a dose-dependent manner with an ED_{50} of 0.12 mg/kg. Oral administration of omeprazole to the Heidenhain pouch dog inhibited histamine-stimulated gastric acid secretion in a dose-dependent manner with an ED_{50} of 0.4 mg/kg (i.e., 1.2 µmole/kg). Oral ED_{50} values for picoprazole and cimetidine were 4.2 and 2.5 µmole/kg, respectively. Inhibition of histamine-stimulated gastric acid secretion in dogs at 22-24 hr after oral administration of omeprazole at doses of 0.17, 0.43, 0.86, and 1.7 mg/kg was 37, 56, 81, and 92%, respectively. There was no direct correlation between plasma concentrations of omeprazole and inhibitory effects on gastric acid secretion. Bioavailability values for omeprazole in the dog following intraduodenal or oral administration were 70 and 15%, respectively. Intraduodenal, oral, or intravenous administration of omeprazole to the vagotomized acute fistula rat inhibited pentagastrin-stimulated gastric acid secretion in a dose-dependent manner. Omeprazole administered by the oral route ($ED_{50} \approx 5.2$ mg/kg) was less potent than found with either the intraduodenal or intravenous routes ($ED_{50} = 0.52$ mg/kg). Following intraduodenal administration to the vagotomized acute or chronic fistula rat, omeprazole was found to be significantly more potent than cimetidine with regard to inhibition of pentagastrin-stimulated gastric acid production. Following intravenous administration of omeprazole to chronic fistula rats at doses of 1.7 and 6.9 mg/kg, antisecretory effects (i.e., inhibition of pentagastrin-stimulated gastric acid production) returned to basal levels at 10 and 13 hr after dosing, respectively.

Effect of Omeprazole – A Gastric Proton Pump Inhibitor – On Pentagastrin Stimulated Acid Secretion in Man (Gut 24: 270-276, 1983).

Omeprazole administered to healthy male volunteers at single oral doses of 20, 40, 60, or 80 mg produced a dose-dependent maximal inhibition of pentagastrin-stimulated gastric acid secretion at 36, 65, 90, and 99%, respectively. Maximal plasma concentrations of omeprazole were obtained at 30-40 min after dosing and then declined with a half-life of approximately 50-min. With repeated administration of 15 mg per day, 47% inhibition of the maximal response to pentagastrin-stimulated gastric acid secretion was obtained at 2 hr after the first dose. Inhibition at 2 hr after the third and fifth doses was 75 and 80%, respectively. This data suggested a steady-state inhibition

of gastric acid secretion was obtained after approximately 3 days. Omeprazole produced a profound inhibition of gastric acid secretion; however, this effect was achieved after the third dose with a one dose per day regimen.

Effects of Omeprazole, a Substituted Benzimidazole, on Gastrointestinal Secretions, Serum Gastrin, and Gastric Mucosal Blood Flow in Dogs (Gastroenterology 86: 71-77, 1984).

The effects of omeprazole on gastric, duodenal, and pancreatic secretions, gastric microcirculation, and the release of gastrin in conscious dogs were examined. Eight dogs were fitted with esophageal (EF), gastric (GF), and pancreatic fistula (PF). Four of these animals were provided with vagally denervated pouches of the oxyntic gland area [i.e., Heidenhain pouch (HP)]. Four separate animals were prepared with GF and denervated antral pouches and four others were prepared with GF and two duodenal pouches fashioned from the upper and distal portions of the duodenum. Omeprazole administered by intravenous infusion produced dose-dependent inhibition of histamine-stimulated acid output for gastric fistula and Heidenhain pouch dogs with ID_{50} values of 0.37 and 0.22 mg/kg/hr, respectively. Omeprazole treatment also reduced pepsin output in gastric fistula and Heidenhain pouch dogs due to a decrease in the volume of gastric juice rather than in the concentration of pepsin. Omeprazole, administered by intravenous infusion, in a dose-dependent manner, inhibited maximal histamine, pentagastrin, or urecholine-stimulated acid output in gastric fistula and Heidenhain pouch dogs. Omeprazole, administered by topical application to the mucosa of the Heidenhain dog, produced a dose-dependent inhibition of histamine-stimulated acid output. Omeprazole at an intravenous dose of 0.7 mg/kg inhibited histamine-stimulated acid output in gastric fistula and Heidenhain pouch dogs for at least 24 hr; however, acid output was completely restored by 36 hr after dosing. Omeprazole administered by the intraduodenal route at a dose of 0.35 mg/kg inhibited sham feeding and peptone meal-induced gastric acid output. Omeprazole at an intravenous dose of 0.7 mg/kg had no effect on alkaline secretion in oxyntic, antral, and duodenal pouches. Omeprazole administered by the intraduodenal route reduced pancreatic bicarbonate secretion in response to ordinary feeding without affecting pancreatic protein secretion. However, pancreatic bicarbonate and protein secretion in response to secretin and duodenal acidification were unaffected by intraduodenal treatment with omeprazole. Omeprazole was shown to inhibit gastric acid secretion induced by a variety of secretagogues.

Gastric Acid Antisecretory Effect of Two Different Dosage Forms of Omeprazole During Prolonged Oral Treatment in the Gastric Fistula Dog (Scandinavian Journal of Gastroenterology 23: 1013-1019, 1988).

Two studies were performed in gastric fistula dogs to investigate inhibitory effects of oral treatment with omeprazole on gastric acid secretion. In the first study, dogs received omeprazole at an oral dose of 0.7 mg/kg/day administered as a 0.5% methylcellulose suspension for 8 weeks. Histamine-stimulated gastric acid secretion was measured at 1-3 hr or 22-24 hr after dosing at selected intervals. In the second study, dogs received omeprazole at an oral dose of 0.17 mg/kg/day administered as enteric-coated granules for 3 weeks. Histamine-stimulated gastric acid secretion was

measured at 2-4 hr or 22-24 hr after dosing at selected intervals. In the first study when omeprazole was administered at 0.7 mg/kg/day in suspension, inhibition of acid secretion was 54% after the first dose. Steady-state inhibition of gastric acid secretion was achieved within 1 week after the start of dosing. During steady-state conditions, the mean maximal antisecretory effect observed at 3 hr after dosing was 82% with a range of 50-98% inhibition. The mean minimal antisecretory effect observed at 24 hr was 35% with a range of 13-52% inhibition. Following the discontinuation of treatment, acid secretion returned to the control level within 4 days. In the second study when omeprazole was administered at 0.17 mg/kg/day in enteric-coated granules, inhibition of acid secretion was 20% after the first dose. Steady-state was achieved after 5 days. During steady-state conditions, the maximal inhibitory effect observed at 4 hr after dosing was 60% and the minimal inhibitory effect observed at 24 hr after dosing was 40%. Following the discontinuation of treatment, acid secretion returned to the control level within 8 days. Both studies demonstrated that antisecretory effects of omeprazole cumulated within the first week of treatment and effects were maintained over the remaining period of treatment. With discontinuation of treatment, no rebound effect (i.e., increased gastric acid production) was observed; however, other studies have demonstrated a rebound effect (See Report #222-0054 and American Journal of Physiology 254: G33-G39, 1988 below).

Duration of Gastric Antisecretory Effect After High Oral Doses of Omeprazole Administered to the Gastric Fistula Dog (Report #222-0015).

Omeprazole, 16 or 400 µmole/kg (5.4 or 138 mg/kg, respectively), was given orally to 4 harrier dogs once a day for 7 days. Gastric acid secretion tests were performed 24, 48, 72 and 120 hr after the last administration. The results showed that the duration of the inhibitory effect on acid secretion was the same at both doses and little or no effect was observed 72 hr after the last treatment.

Studies on the Morphology and Function of the Gastric Mucosa During and After 12 Months of Treatment with Omeprazole in the Dog (Report #222-0054).

Dates of Study Started and Completed: 8/16/82 and 8/15/83

Animals: Male beagle dogs (aged 13-21 months and weighing 14-18.2 kg) were used.

Methods: Groups of 2 dogs each were treated orally with 0 (1 mL/kg 0.25% Methocel), 0.68 or 27.6 mg/kg omeprazole (batch # 313/81, micronized before suspended in Methocel) once daily for one year. The gastric acid and pepsin secretory response to histamine and the morphology of the gastric mucosa were determined once a month during the treatment period and frequently after the end of treatment. Meal-stimulated plasma gastrin levels and plasma somatostatin levels were analyzed once every 3 months during the treatment and frequently after the treatment. Gastroscopy and biopsy analysis were performed monthly during the treatment.

Results:

1. Clinical Examination: No treatment-related clinical signs were observed. Food and water intake were not affected by omeprazole.

2. Morphology Findings: Gastroscopy of the high-dose dogs showed an increase in mucosal folding which reached a maximum at the end of treatment, but recovery was evident at 4 months after the cessation of treatment.

3. Gastric Acid Secretion: There was a dose-dependent inhibition of histamine-induced gastric acid secretion (averaged -36% and -66% during treatment period for the 0.68 and 27.6 mg/kg dose groups, respectively). The acid secretory response to histamine returned to normal 3 days after cessation of omeprazole treatment.

4. Pepsinogen Content in Gastric Mucosa and in Gastric Juice: No significant changes were found.

5. Plasma Concentration of Omeprazole:

Group	Omeprazole at 1 hr (mg/L)	AUC (0-6 hr) (mg x h/L)	T½ (min)
0.6 mg/kg	0.31	0.41	50
27.6 mg/kg	5.78	19.5	50

6. Plasma Levels of Gastrin: The peak gastric response to feeding was elevated in one of the low-dose dogs after 6 and 9 months of treatment. Both dogs in the high-dose group showed more than a 10-fold increase in gastrin levels and 10-15 fold increases in the gastrin response to feeding throughout the treatment period; however, gastrin levels returned to pretreatment values 15 days after discontinuation of treatment.

7. Plasma Levels of Somatostatin, Secretin and CCK-8: No consistent changes of these parameters were found.

8. Conclusion: Omeprazole treatment at both 0.68 and 27.6 mg/kg/day for one year inhibited acid secretory response to histamine for a prolonged period. The high dose treatment caused a reversible hypergastrinemia and hypertrophy of gastric mucosa (increased mucosal folding).

Addendum:

Gastric Acid Secretion: From 1 to 3 months after the cessation of treatment for the high dose omeprazole group, histamine-stimulated gastric acid secretion was increased to 150-160% of the control, although, basal gastric acid secretion was comparable to the control. Comparison of the dose response curves for histamine-stimulated gastric acid secretion prior to the start of treatment and during the recovery period found that

ED_{50} values for histamine were similar. This result suggested that the capacity to produce acid had increased and the sensitivity to histamine was unaffected.

Microscopic Examination of the Stomach: Biopsies of the oxyntic and antral mucosa were collected prior to treatment, during the treatment period, and the cessation of treatment. Comparison of the control group to the omeprazole group found no changes in mucosal thickness, volume densities of endocrine cells, parietal cells, or lamina propria. There were no changes in the size of parietal or zymogen cells.

General Toxicity of Omeprazole (H168/68) Given Orally to Dogs for 7 Years (Project #: 826-01, Study #: 84001).

Testing Laboratory: Astra Hassle AB, Sweden

Dates Started and Completed: March 15, 1984 and June 23, 1992

Compliance with GLP and QAU Requirements: Sponsor included a statement of compliance with GLP regulations and a QAU statement.

Animals: Males (1 1.0-15.5 kg, 12.5-13.5 months old)
Females (9.0-15.0 kg, 13.0-13.5 months old)
Beagle Dogs

Methods: The objective of this study was to investigate if omeprazole at 0.17 mg/kg/day given orally for 7 years would have any toxicological and morphological gastric effects in dogs. Omeprazole at 0.17 mg/kg/day was given by capsule to five male and five female beagle dogs for seven years. In control group, control vehicle was administrated to three male and three female beagle dogs for seven years. These dogs were observed daily for clinical signs. The food consumption was recorded daily. The body weight and rectal temperature were recorded weekly. ECGs were recorded from all dogs immediately before, 1, 5 and 24 hours after dosing started on the first day of the study and every six months thereafter. Ophthalmoscopy was performed on all dogs before the study started and once a year after the study started. Gastroscopic examinations including general survey of the entire gastric lumen and biopsies from gastric mucosa were performed before the study started and once a year thereafter. The urine and blood samples were collected from all dogs between 8:00 to 10:00 a.m. before the study started and every six months thereafter. All animals were fasted during the night before the blood samples were withdrawn and food- and water-fasted during the night before the urine samples were collected. Plasma concentrations of omeprazole were measured using liquid chromatography every six months during the study. The basal and food-stimulated plasma concentrations of gastrin were determined using radioimmunoassay before the study started, at 2, 4, 6 and 10 months after the study started and every six months between the second year and the end of the study. For determination of the basal gastrin level, the blood samples were taken after the animals were food-fasted for 12 hours and three samples were taken at 30 minutes intervals. For. determination of food-stimulated gastrin level, the blood samples were

taken 30, 60 and 90 minutes after a test food was given. Gastric acid secretion test was performed at ~ five years after dosing started in both control and test groups. At the termination, gross pathology, histopathology, and morphometry were performed and organ weights were determined in both control and test groups.

Results:

1. **Clinical Signs:** There were no treatment-related clinical signs observed during the study. Major clinical signs including vomiting, soft stool, diarrhea and body tremor were observed in both control and test groups.

2. **Mortality:** None

3. **Body Weight:** All animals gained weight during the study. The mean value of the relative body weight gain was slightly lower in the test group (43%) than in the control group (47%) during the dosing period of seven years. Sponsor did not indicate whether this difference is statistically significant.

4. **Food Consumption:** The food consumption was not markedly different between the control and test groups.

5. **Rectal Temperature:** All dogs in both control and test groups had a normal rectal temperature during the study.

6. **ECG:** There were no treatment related abnormal ECGs observed during the study.

7. **Ophthalmoscopy:** There were no treatment-related abnormalities observed during the study.

8. **Gastroscopic Examination:** There were no treatment-related alterations observed during the study.

9. **Hematology:** There were no treatment-related alterations.

10. **Blood Chemistry/Urinalysis:** There were no treatment-related alterations.

11. **Plasma Level of the Drug:** The mean maximal blood concentration of omeprazole (C_{max}, 159-550 nmol/L) was usually obtained between 1 to 2 hours after dosing. The mean total area under the curve, (AUC) was ~ 990 nmol/L,h. These values were close to those obtained in man following repeated dose of 20 mg (0.4 mg/kg, 50 kg body weight assumed) of omeprazole daily (Drug Invest. 3: 45-52, 1991). In this study, C_{max} and AUC after the first dose were 520 nmol/L and 890 nmol/L,h respectively. After the fifth close, C_{max} and AUC were 790 nmol/L and 1510 nmol/L,h, respectively.

12. Plasma Level of Gastrin: Both basal and food-stimulated gastrin levels were not significantly different between the control and test groups during the entire period of the study.

13. Gastric Acid Secretion Test: The gastric acid section was stimulated by a continuous intravenous infusion of histamine for three hours. In the control group, the acid outputs during second and third hours of the histamine stimulation were 30.2±3.3 and 24.9±1.3 mmol H^+/h (mean±SEM), respectively. In contrast, these values in the test group were 16.0±2.1 and 14.1 ± 1.6 mmol H^+/h, suggesting that the acid secretion was inhibited by omeprazole ~ 50% compared to the control. However, no data indicated whether this test was also performed before dosing started or at other times after dosing started in this study to make a comparison. Sponsor stated that in a different study in dogs omeprazole (0.5 µmol/kg), reduced histamine-stimulated acid secretion about 50% (Scand. J. Gastroenterol, 1988; 23:1013-1019). In this study, the maximal inhibition was ~60% at 4th hour and ~ 40% after 24 hours.

14. Gross Pathology: There were no treatment-related alterations observed during the study. Figures 1 and 2 are not readable.

15. Organ Weight: There were no treatment-related alterations observed during the study except that the ratio of stomach weights to the body weights was higher in the test male group (0.89×10^{-2}) than in the control group (0.74×10^{-2}). Sponsor did not indicate whether this difference was statistically significant.

16. Microscopic pathology: There were no treatment-related abnormalities observed during the study. No significant difference in the argyrophil cell counts between the control and test groups were reported but the number of the argyrophil cells at 4 and 7 years after dosing started was slightly higher in the test group (166 and 145 cells per linear mm oxyntic mucosa) than in the control group (150 and 130 cells per linear mm oxyntic mucosa) (data from table 3:2 on page 54(453)). The argyrophil cells were also increased at terminal autopsy (97, 106 cells/mm in control, and 113, 114 cells/mm in test group). Sponsor did not indicate whether this difference was statistically significant.

In summary, to study the chronic toxicity, omeprazole was given orally at 0.17 mg/kg/day for 7 years in dogs. No treatment-related signs of toxicity were observed during the study. There were no treatment-related changes in the gross pathological and histopathological examinations. The mean maximal blood level of omeprazole was usually reached within 1 to 2 hours after dosing. The mean total area under the curve was ~ 990 nmol/L,hr. The gastric acid secretion was inhibited ~50% at ~ five years after the treatment.

Addendum:

Plasma Level of the Drug: The mean maximal blood concentration of omeprazole (C_{max}, 55-190 µg/L) was usually obtained between 1 to 2 hours after dosing. The mean total area under the curve, (AUC) was ~ 342 µg/L,h. These values were close to those

obtained in man following repeated dose of 20 mg (0.4 mg/kg, 50 kg body weight assumed) of omeprazole daily (Drug Invest. 3: 45-52, 1991). In this study, C_{max} and AUC after the first dose were 180 µg/L and 307 µg/L,h respectively. After the fifth close, C_{max} and AUC were 273 µg/L and 522 µg/L,h, respectively.

Ancillary Pharmacology Studies:

Parietal Cell Function After Prolonged Inhibition of Gastric Acid Secretion (American Journal of Physiology 254: G33-G39, 1988).

Female Sprague-Dawley rats received omeprazole at oral doses of 13.8 and 138 mg/kg/day for 3 months. The effects of omeprazole treatment were evaluated on secretory function of parietal cells and oxyntic mucosal cells. Four weeks prior to the end of treatment, rats undergoing secretory studies were fitted with chronic gastric fistulas. Basal and carbachol + pentagastrin-stimulated gastric acid secretion rates at 2 hr after the last dose for both omeprazole treatment groups were completely inhibited. Twenty-four hr after the last dose, basal gastric acid secretion for both omeprazole treatment groups had returned to control levels (~125 µmole H^+/hr). However, on day 3, basal gastric secretion for rats at 138 mg/kg omeprazole was increased to ~250% of the control. Three days after the end of treatment, carbachol + pentagastrin-stimulated gastric acid secretion rates for both omeprazole groups were increased to 160% of the control (~350 µmole H^+/hr). The pentagastrin + carbachol-stimulated gastric acid secretion rates for both omeprazole groups declined toward control levels and by day 70, the secretion rate for the 13.8 mg/kg group was comparable to the control. However, the rate for the 138 mg/kg group was still slightly elevated. Pepsinogen secretion paralleled acid output. Gastrin levels were dose-dependently increased within 2 hr after the first omeprazole treatment and continued to increase for the first 3 days of treatment. However, elevated gastrin levels were relatively stable from day 3 to the end of treatment at 8 and 15-20 times the control level (~120 pg/mL) with doses of 13.8 and 138 mg/kg/day, respectively. By 9 days after the discontinuation of treatment, gastrin levels were comparable to control in both omeprazole treatment groups. At 24 hr after the last dose, H^+,K^+-ATPase activities in the corpus mucosal tissue and microsomal fraction at 13.8 and 138 mg/kg were inhibited by 42-47% and 63-69%, respectively, relative to control activities (microsomal fraction, 10 µmole P_i/mg protein/hr). At 7 days after the last dose, H^+,K^+-ATPase activities in the corpus mucosal tissue and microsomal fraction of the two omeprazole treatment groups had returned to control levels. At the end of treatment, the corpus mucosal mass was increased by 45 and 33% relative to the control (0.4 g) for the 13.8 and 138 mg/kg omeprazole groups, respectively. Based upon an elevated histamine content, the increased mass was attributed to an increase of ECL cell density. No change in parietal cell density was observed.

Effects of Omeprazole on Gastric and Pancreatic Secretion in the Dog (Hassle Report No. 222-0055).

Omeprazole (0.17 or 3.4 mg/kg, i.v.) has neither direct effect on pancreatic secretion nor any effect on the pancreatic amylase secretion induced by pentagastrin or by secretin.

Addendum:

Pentagastrin stimulation induced both gastric acid secretion and pancreatic secretion (i.e., volume flow and bicarbonate output). Treatment of dogs with omeprazole inhibited pentagastrin-stimulated gastric acid secretion, but had had no significant effect on pancreatic volume flow, bicarbonate flow, or amylase output. Omeprazole treatment had no effect on secretin stimulation of pancreatic volume flow or bicarbonate flow. These results suggest that omeprazole had no direct effect on pancreatic secretion.

Exocrine Pancreatic Function After Prolonged Oral Administration of the Proton Pump Inhibitor Omeprazole in Rats (Bulletin of the Osaka Medical College 38: 49-53, 1992).

Treatment of male Wistar rats with omeprazole at oral doses of 5 and 50 mg/kg/day for 6 weeks had no significant effects on pancreatic function or histological appearance.

Hypergastrinemia Produces Trophic Effects in Stomach But Not in Pancreas and Intestines (Regulatory Peptides 13: 225-233, 1986).

Hypergastrinemia produced in female Sprague-Dawley rats by treatment with omeprazole at oral doses of 13.8 or 138 mg/kg/day for 20 weeks or in male Sprague-Dawley rats by treatment with omeprazole at an oral dose of 13.8 mg/kg/day for 16 weeks appeared to have a direct correlation to increased stomach oxyntic mucosal thickness and ECL cell density; however, there were no effects on the stomach antrum, pancreas, or small and large intestines.

Hypergastrinemia Evoked by Omeprazole Stimulates Growth of Gastric Mucosa But Not of Pancreas or Intestines in Hamster, Guinea Pig and Chicken (Regulatory Peptides 23: 105-115, 1988).

Administration of omeprazole to chickens at an intramuscular dose of 138 mg/kg/day for 10 weeks and to male hamsters and guinea pigs at an oral dose of 138 mg/kg/day for 10 weeks inhibited gastric acid secretion, elevated plasma gastrin levels and increased the weight and thickness of acid-producing mucosa. For all three species, the pancreas and other parts of the gastrointestinal tract appeared to be unaffected.

Effect on Intestinal Motility in the Rat.

Omeprazole administered to female Sprague-Dawley rats at oral doses of 6.8, 13.8, and 68 mg/kg did not affect intestinal motility as assessed by the transit distance of a charcoal meal. Under the same experimental conditions, cimetidine at an oral dose of 10 mg/kg also did not affect intestinal motility.

Effect of Omeprazole on Gastric Emptying in Rats.

Omeprazole administered to female rats at an oral dose of 138 mg/kg produced a delay in gastric emptying. However, omeprazole administered at an intravenous dose of 27.8 mg/kg, which produced similar plasma omeprazole concentrations, had no

effects on the rate of gastric emptying. These results suggested that the delay in gastric emptying observed following oral administration of omeprazole might be due to a localized effect rather than a circulating compound.

Summary of Pharmacology: Omeprazole is a substituted benzimidazole that suppresses gastric acid secretion by specific inhibition of the H^+,K^+-ATPase enzyme system at the secretory surface of the gastric parietal cell. Given that omeprazole is acid labile and undergoes rapid degradation in the acid environment of the stomach, the drug is administered as an enteric-coated granule formulation. Following intestinal absorption, the drug reaches the gastric mucosa by way of blood circulation. Omeprazole has a pK_a of approximately 4.0 leading to accumulation in the acidic secretory canaliculus of the stimulated parietal cell. In this acidic environment, omeprazole undergoes conversion to a cationic sulfenamide. The sulfenamide interacts in a covalent manner with cysteine residues at critical sites in the extracellular (lumenal) domain of the membrane-spanning H^+,K^+-ATPase. Full inhibition occurs with two molecules of inhibitor bound per molecule of enzyme. Since this enzyme system is regarded as the acid (proton) pump within the gastric mucosa, omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. In studies with both animals and humans, this effect has been found to be dose-related and leads to inhibition of both basal and agonist-stimulated acid secretion. Administration of omeprazole results in permanent inhibition of enzyme activity in vivo. Given the short plasma half-life of omeprazole (i.e., less than 1 hr), inhibition of acid secretion persists for significant periods after the drug has been eliminated from the plasma. With an enzyme half-life of 50 hr, approximately 25% of available H^+,K^+-ATPase is synthesized over a 24 hr period. This is the predominant mechanism for recovery of acid secretion following covalent inhibition of the pump by benzimidazoles. In once-a-day dosing regimens with animals or humans, a significant quantity of newly synthesized H^+,K^+-ATPase, that has not been exposed to omeprazole, will be present 24 hr after dosing. At maximum, 75% of H^+,K^+-ATPase pumps, old or new, are active during exposure to omeprazole. With once-a-day dosing regimens, steady-state inhibition of acid secretory capacity at greater than or equal to 70% was found to be achieved at 2-3 days after initiation of treatment with rats or humans and 5-7 days with dogs. With maximal inhibition of active H^+,K^+-ATPase pumps, newly synthesized or newly active pumps were not inhibited 3 hr after dosing due rapid elimination of the drug. Treatment of rats and dogs with omeprazole for 3 and 12 months, respectively, produced profound inhibitory effects on both basal and stimulated gastric acid secretion; however, within a few days after the cessation of treatment, a rebound phenomenon of increased acid secretion was observed in both species. Basal and/or agonist-stimulated acid secretion were found to be increased in rats and dogs that had received omeprazole, as compared to corresponding untreated controls, for periods up to 3 months after completion of treatment. This effect was attributed to an increased acid secretion capacity as no changes in agonist dose response relationships were found.

SAFETY PHARMACOLOGY:

Effects on Exploratory and Basal Locomotor Activity in The Rat.

Omeprazole administered to male Sprague-Dawley rats at oral doses of 6.8, 68 or 138 mg/kg did not affect exploratory activity. Whether omeprazole possessed sedative or neuroleptic effects was not conclusive, since it significantly reduced basal motor activity at the two lower doses (-46% and -49%, respectively), but exerted no significant effect at the high dose.

Effects on Autonomic Control of Cardiovascular Function in the Dog.

Omeprazole at doses of 2.7, 13.8 and 69 mg/kg (which is 500 times the ED_{50} for inhibition of gastric secretory activity) administered by the intraduodenal route to male and female dogs did not cause any change in basal mean arterial blood pressure or heart rate, indicating no interference with autonomic cardiovascular control mechanisms in dogs.

Effects of Intravenous Injections of Polyethylene Glycol 400 and Omeprazole on Electrocardiographic and Cardiovascular Findings.

Neither the vehicle (polyethylene glycol 400) nor omeprazole produced any alteration in the P-Q interval in conscious dogs. Systemic blood pressure was slightly decrease by omeprazole treatment.

Acute Effects on Circulation and Acid-Base Balance in the Dog at Rest and During Exercise (Pilot Study).

Omeprazole administered to dogs at an oral dose of 34 mg/kg completely inhibited gastric acid secretion, but produced no acute circulatory or respiratory effects. In addition, the behavior of the dogs was not influenced by the treatment.

TOXICOLOGY:

Acute Toxicity

Mice and Rats

The Acute Toxicity of Single Oral Omeprazole Magnesium Salt, and Omeprazole in Mice and Rats: (Study # 89128 and 89140 resp, report # T2244 and T2243 resp).

Methods: The aim here was to compare the acute oral toxicity of omeprazole magnesium, with that of omeprazole in mice and rats.

Mice: Three groups of mice (BALB/cj, n=5/dose) were given single oral doses of omeprazole magnesium (batch # 300/87) as follows: group 1 (males only) received 710 mg/kg (2000 µmole/kg), and group 2 and 3 (male and female mice) received 1400 and 1900 mg/kg (3800 and 5200 µmole/kg), respectively, by a gavage in a dose volume of 10 mL/kg. Three additional groups of mice (groups 4-6, n=5/dose/sex) similarly received

equal doses of omeprazole (H168/68, at 690, 1300, and 1800 mg/kg, or 2000, 3800, and 5100 µmole/kg, respectively, batch #165). Both compounds were dissolved in 0.5% Methocel solution, at concentrations of 200, 380 and 510-520 µmole/mL, respectively. These were the highest doses that could be given due to viscosity of the test formulations.

Rats: Male and female Sprague Dawley rats (5/dose) received oral 460, 930, and 1800 mg/kg (or 1300, 2600, and 5100 µmole/kg, respectively, batch # 300/68) of omeprazole magnesium, and 900 and 2500 mg/kg (or 2600 and 7300 µmole/kg, respectively H168/68, batch # 165) of omeprazole. Both compounds were dissolved in 0.5% Methocel solution. All animals were observed for toxic signs and mortality, daily for 14 days. Body weights were determined at baseline and on days 1, 2, 3, 4, 5, 7, and 14 during the treatment. At the end of observation period, all animals were necropsied and subjected to standard examinations.

Results:

Mice: Mortalities were noted in male mice, at 5200 µmole/kg (1900 mg/kg) of omeprazole magnesium (1 of 5 males died). Whereas, with omeprazole, mortalities were noted in male mice at 3800 and 5100 µmole/kg (1300 and 1800 mg/kg, 2 males and 1 male died at these doses, respectively). All died within 24 hrs of receiving the drug. For surviving animals, no effects on body weights were noted during the 14 day observation period. However, both oral drugs (at all doses) caused similar clinical signs (reduced motor activity, reduced reaction to stimuli, prostration and piloerection from 0.5 hrs after dosing). The clinical signs increased in intensity and duration with the increase in dose. No gross lesions were observed in any animal. The surviving animals were free of these signs within 2 days of dosing.

Rats: After oral administration of omeprazole magnesium, mortalities were noted in rats at 2600 µmole/kg (930 mg/kg, 1 of 5 females died) and 5100 µmole/kg (1800 mg/kg, 1 male and 2 females died). Whereas, with omeprazole, no mortalities were noted at the highest dose used (7300 µmole/kg, or 2500 mg/kg). For rats receiving omeprazole magnesium at 930 and 1800 mg/kg, all died within 48 hrs of receiving the drug. For surviving animals, no effects on body weights were noted during the 14-day observation period. However, both oral drugs (at all doses) caused similar clinical signs as noted in mice (reduced motor activity, reduced reaction to stimuli, prostration and piloerection from 0.5 hrs after dosing). The clinical signs increased in intensity and duration with the increase in dose. No gross lesions were observed in any animal. The surviving animals were free of these signs within 1 day of dosing. The minimum lethal doses of both compounds, given orally are shown in Table 7.

Table 7. Highest tolerated doses, and minimum lethal doses in acute toxicity studies in mice and rats, after oral doses of omeprazole magnesium and omeprazole (H168/68).

Species (strain)	No/Sex/Group	Doses (mg/kg/day)	Highest Tolerated Doses (mg/kg/day)	Minimum Lethal Dose (mg/kg/day)
BALB/cj Mice				
Omeprazole Magnesium				
Oral				
Males	5	710,1400 & 1900	1400	1900
Females	5	1400 & 1900	1900	----
Omeprazole				
Oral				
Males	5	690, 1300 & 1800	690	1300
Females	5	690, 1300 & 1800	1800	----
Sprague-Dawley Rats				
Omeprazole Magnesium				
Oral				
Males	5	460, 930 & 1800	930	1800
Females	4-5	460, 930 & 1800	460	930
Omeprazole				
Oral				
Males	5	900 & 2500	2500	----
Females	5	900 & 2500	2500	----

In conclusion, in acute toxicity studies, the minimum lethal oral doses (died within 24 hrs after treatment) of omeprazole magnesium and omeprazole in male mice were 5200 and 3800 µmole/kg (or 1900 and 1300 mg/kg), respectively. The highest administered (1800-1900 mg/kg) doses in female mice were not lethal. The minimum lethal oral doses (died within 48 hrs after treatment) of omeprazole magnesium in male and female rats were 5100 and 2600 µmole/kg (or 1800 and 930 mg/kg), respectively. The highest administered (2500 mg/kg or 7300 µmole/kg) doses of omeprazole in male and female rats were not lethal. Clinical signs (reduced motor activity, reduced reaction to stimuli, prostration and piloerection), which increased in intensity and duration with the increase in dose, in both mice and rats, with both compounds (omeprazole magnesium and omeprazole in both sexes) were similar. All surviving animals recovered from these effects within 1-2 days after treatment. These studies indicate that the acute toxicities with both the drugs (omeprazole magnesium and omeprazole) in mice and rats were in general comparable.

Subacute/Subchronic/Chronic Toxicity

Rats

52-Week Chronic Oral Toxicity Trial of Omeprazole in Rats (Report #63-067).

Testing Laboratory: [redacted]

Date Started: September 16, 1986

Date Completed: July 15, 1988 (Translated from to English July 8, 1999).

GLP Compliance: A statement of compliance with the Quality Assurance Unit was included. There did not appear to be any statement regarding GLP compliance.

Animals: Sprague-Dawley rats (Crj: CD) were obtained from Japan Charles River Co. At the start of treatment, animals were 5 weeks old with body weight ranges of 82.1-94.6 g for male rats and 75.2-87.2 g for female rats.

Drug Batch: Omeprazole, Lot No. 124

Methods: In a 52-week oral toxicology study, Sprague-Dawley rats received omeprazole at doses of 0, 0.4, 2, and 16 mg/kg/day. Control animals received the vehicle, 0.5% methyl cellulose containing 2 mg/mL $NaHCO_3$ adjusted to pH 9 with 1 N NaOH. Dose selection was based upon results obtained from an earlier 26-week chronic toxicity study with omeprazole in rats conducted by Asutora Co. (spelling problem with translation?) in which doses ≥13.8 mg/kg/day produced increased eosinophilicity of the secretory granules in chief cells and an increased incidence of chronic nephropathy. There were 31 rats/sex/group of which 7 rats/sex/group were designated to satellite groups. Animals in satellite groups were sacrificed 2 hr after the last dose for measurement of serum gastrin levels and histopathological examination of the stomach. The remaining 24 rats/sex/group composed the main toxicology study. Rats were monitored for clinical signs of toxicity and mortality on a daily basis before and after dosing. Body weights were measured weekly. Food consumption was measured once per week through week 12 and every two weeks thereafter. Water consumption for 10 rats/sex/group was measured in metabolic cages during week 24 and 52 when urine was collected for urinalysis. Ophthalmic examinations were conducted during weeks 24 and 52 for 5 rats/sex/group. Blood for determination of hematological and blood biochemistry parameters was collected during week 52. Myelograms were prepared by obtaining bone marrow cells from femurs at the time of autopsy; however, they were not examined due to a lack of significant changes in hematological parameters. Animals in the main toxicology study were sacrificed at 18-24 hr after the last dose. Absolute and relative organ weights were determined as follows: brain, thymus, heart, lung, liver, spleen, kidneys, testicles, prostate, ovaries, uterus, submandibular gland (including sublingual glands), pituitary gland, thyroid gland (including parathyroid), adrenal glands, and stomach (from the cardiac orifice to the pylorus). Organs and tissues were collected and preserved as follows: brain, spinal cord (cervical chord), heart, aorta, trachea, bronchi, lungs, liver, spleen, kidneys, urinary bladder, tongue, esophagus, stomach, duodenum, ileum, cecum, colon, rectum, pancreas, testes, seminal vesicles, epididymides, prostate, ovaries, uterus, vagina, mammary glands, submandibular glands (including sublingual glands), pituitary glands, thyroid gland, parathyroid, adrenal glands, femoral marrow, thymus, mesenteric lymph

nodes, submandibular lymph nodes, eyes, lacrimal glands (including Harder's glands), skin, and any gross abnormalities. Tissues (except eyes, tongue, and bronchi) from all animals were stained with hematoxylin and eosin and submitted to microscopic examination. Grimelius-stained specimens were prepared from the stomachs of all animals. For 3 rats/sex/group, pieces of the stomach were prepared for electron microscopic examination.

Results:

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

2. **Mortality:** There appeared to be no treatment-related deaths as no mortality occurred at 16 mg/kg/day. Two control male rats (#128 and #142), 1 male rat (#160) and 1 female rat (#35) at 0.4 mg/kg/day, and three male rats (#195, #211, and #212) at 2 mg/kg/day died. The cause of deaths for the two control male rats and 1 male rat (#211) at 2 mg/kg/day were considered to be gavage errors. The female rat (#35) at 0.4 mg/kg/day that died on day 38 was found with a pituitary gland tumor. For both the male rat at 0.4 mg/kg/day (#160; died on day 49) and the male rat at 2 mg/kg/day (#212; died on day 31), astrocytomas spreading into the brain stem region were observed. One male rat (#195) at 2 mg/kg/day died immediately prior to scheduled sacrifice; however, a cause of death could not be determined.

3. **Body Weight and Food and Water Consumption:** There were no treatment-related effects on body weight gain and food consumption. Initial and final body weights for male controls were 136.2 and 721.5 g, respectively. Final body weights for male rats at 0.4, 2, and 16 mg/kg/day were 95.8, 101.65, and 103.2% of the control, respectively. Body weight gains for male rats at 0.4, 2, and 16 mg/kg/day were 95.9, 103.4, and 103.6% of the control, respectively. Initial and final body weights for female controls were 118.5 and 432.5 g, respectively. Final body weights of female rats at 0.4, 2, and 16 mg/kg/day were 101.8, 97.2, and 100.4, respectively. Body weight gains for female rats at 0.4, 2, and 16 mg/kg/day were 102.2, 95.7, and 100.3% of the control, respectively. There were no dose-related changes in water consumption.

4. **Hematology and Blood Coagulation:**

Hematology: The reticulocyte percentages for female rats at 0.4, 2, and 16 mg/kg/day were increased to 136.8, 136.8, and 200% of the control (0.19%), respectively. The reticulocyte percentage for male rats at 16 mg/kg/day was increased to 869% of the control (0.16%).

Blood Coagulation: There was no treatment-related change of prothrombin time.

5. **Blood Biochemistry and Urinalysis:** Observed changes in blood biochemistry parameters appeared to have no correlations to histopathological changes.

Biochemistry: Gastrin levels for male and female rats at 16 mg/kg/day were increased to 258 and 415% of control values (366.71 and 408.29 pg/mL), respectively. Creatine phosphokinase activities for female treatment groups were increased to 164-171% of the control (133.5 IU/L). Inorganic phosphorus levels for female rats at 0.4, 2, and 16 mg/kg/day were increased to 116.8, 129.6, and 145.4% of the control (3.28 mg/dL), respectively. GOT activities for female treatment groups were increased to 119-152% of the control (87.6 mU/mL). Blood urea nitrogen levels for female rats at 2 and 16 mg/kg/day were increased to 110.6 and 111.8% of the control (16.1 mg/dL), respectively. Potassium levels for female treatment groups were increased to 109-117% of the control (3.452 mEq/L). Potassium levels for male treatment groups were decreased to 91.6-94.2% of the control (3.916 mEq/L). α_3-Globulin levels for female rats at 16 mg/kg/day was increased to 122.7% of the control (2.2%). Albumin levels for male rats at 16 mg/kg/day were decreased to 92.5% of the control (48.0%). α_2-Globulin levels for male rats at 2 and 16 mg/kg/day were increased to 116 and 139% of the control (3.1%).

Urinalysis: There were no treatment-related changes of urinalysis parameters at weeks 24 or 52.

6. Ophthalmic Examination: There were apparently no treatment-related ophthalmic changes; although, no data was provided for independent verification.

7. Organ Weights: Changes in stomach and adrenal gland weights appeared to correlate with observed histopathological changes.

Stomach: The absolute and relative stomach weights for female rats at 16 mg/kg/day were increased to 131 and 133.5% of the control (1767 mg and 4.33 mg/g), respectively. The absolute and relative stomach weights for male rats at 16 mg/kg/day were increased to 121.4 and 126.7% of the control (2402 mg and 3.52 mg/g), respectively.

Adrenal glands: Absolute adrenal glands weights for female rats at 16 mg/kg/day were decreased to 86.8% of the control (74 mg).

8. Gross Pathology: Results of gross pathological examinations were not reported.

9. Histopathology: The stomach was the target organ of toxicity. Observed histopathological changes in the stomach could be attributed to the exaggerated pharmacological activity of omeprazole (i.e., inhibition of gastric acid secretion).

Non-neoplastic lesions: The target organ of toxicity was the stomach. Hyperplasia/hypertrophy of ECL (Grimelius positive) cells was observed in all male and female treatment groups. Thickening of the gastric mucosa was observed for male and female rats at doses ≥2 mg/kg/day. For rats treated with 16 mg/kg/day, an increase in the ECL cells was observed in the cardiac glands and a thickening of the stomach mucosa, accompanied by branching and tortuosity of the cardiac gland ducts, was

observed. For rats treated with 0.4 or 2 mg/kg/day, increases in ECL cells were observed; however, no branching or tortuosity of the ducts was observed. An increase of eosinophilic granules in chief cells (focal) was observed for all male and female treatment groups. For these cells, both large and small zymogen granules and markedly developed rough-surfaced endoplasmic reticulum were observed. For the kidneys, an increased incidence of chronic nephropathy was observed for male and female rats at 16 mg/kg/day. For the adrenal glands, an increase of pale cells (i.e., poorly stainable ground-glass-like or blister-like cytoplasm) of zona glomerulosa was observed for male and female rats at 16 mg/kg/day. For the liver, an increased incidence of altered cell foci was observed for male treatment groups; however, increased incidences were not observed for female treatment groups. Altered cell foci in the liver are not generally considered as neoplastic lesions (Charles River Laboratories, February 1992) as the sponsor has reported them.

Neoplastic Lesions: For the brain, astrocytomas were observed in male rats at 0.4, 2, and 16 mg/kg/day with incidences of 4.3, 4.3, and 8.3%, respectively. There was no reported spontaneous incidence rate for brain astrocytoma in 12-13 month studies (Charles River Laboratories, February 1992). Bronchiolar/alveolar adenomas were observed for 1 female rat at 16 mg/kg/day and 1 male rat at 2 mg/kg/day. There was no reported spontaneous incidence rate for bronchiolar/alveolar adenoma in 12-13 month studies (Charles River Laboratories, February 1992).

Non-neoplastic lesions for rats that received omeprazole at oral doses of 0, 0.4, 2, and 16 mg/kg/day for periods up to 52 weeks .

Tissue/Organs	Female rats				Male rats			
	0	0.4	2	16	0	0.4	2	16
n =	24	23	24	24	22	23	23	24
Stomach								
-erosions or ulcer in glandular stomach	0	1	0	2	2	1	1	3
-thickening of gastric mucosa	0	0	11	21	0	0	3	21
-increase of eosinophilic granules in chief cells (focal)	0	2	6	24	6	3	10	23
-dilatation of gastric glands	9	12	12	11	13	9	16	17
-hyperplasia/hypertrophy of ECL (Grimelius positive) cells	0	3	11	24	0	1	2	23
Kidney								
-chronic nephropathy	6	6	6	11	15	17	18	19
Adrenal glands								
-increase of pale cells of zona glomerulosa	4	1	2	9	9	6	8	15
Liver								
-altered cell foci	4	4	1	3	1	2	3	4

Neoplastic lesions for rats that received omeprazole at oral doses of 0, 0.4, 2, and 16 mg/kg/day for periods up to 52 weeks (Animals that died during the treatment period are denoted with an asterisk).

Tissue/Organs	Female rats					Male rats				
	0	0.4	2	16	Bkgd.	0	0.4	2	16	Bkgd.
n =	24	23	24	24	-	22	23	23	24	-
Brain -astrocytoma	0	0	0	0	NA	0	1*	1*	2	NA
Lung -bronchiolar/alveolar adenoma	0	0	0	1	NA	0	0	1	0	NA

Bkgd. = Spontaneous neoplastic lesions and selected non-neoplastic lesions in the CRL:CD®BR rats (Charles River Laboratories, February 1992). For 12-13 month studies, there were no reported incidences for brain astrocytoma and bronchiolar/alveolar adenoma.
NA = Not Applicable.

In a 52-week oral toxicology study, Sprague-Dawley rats received omeprazole at doses of 0, 0.4, 2, and 16 mg/kg/day. A no effect dose was not established. There was no treatment-related mortality. The target organs of toxicity were the stomach, kidneys, adrenal glands, and liver. Hyperplasia/hypertrophy of ECL (Grimelius positive) cells was observed in all male and female treatment groups. Thickening of the gastric mucosa was observed for male and female rats at doses ≥2 mg/kg/day. An increase of eosinophilic granules in chief cells (focal) was observed for all male and female treatment groups. For the kidneys, an increased incidence of chronic nephropathy was observed for male and female rats at 16 mg/kg/day. For the adrenal glands, an increase of pale cells of zona glomerulosa was observed for male and female rats at 16 mg/kg/day. For the liver, an increased incidence of altered cell foci was observed for male treatment groups; however, increased incidences were not observed for female treatment groups. For the brain, astrocytomas were observed for male rats at 0.4, 2, and 16 mg/kg/day with an incidence of 1 of 23 (4.3%), 1 of 23 (4.3%), and 2 of 24 (8.3%), respectively. There was no reported spontaneous incidence rate for brain astrocytoma in 12-13 month studies (Charles River Laboratories, February 1992). Bronchiolar/alveolar adenomas were observed for 1 of 24 (4.2%) female rats at 16 mg/kg/day and 1 of 23 (4.3%) male rats at 2 mg/kg/day. There was no reported spontaneous incidence rate for bronchiolar/alveolar adenoma in 12-13 month studies (Charles River Laboratories, February 1992).

Dogs

A Comparative 3-Month Oral Toxicity Study of Omeprazole Magnesium Salt With Omeprazole, in Dogs: (Study # 89037, report # T2237).

Testing Laboratories: Astra Hassle AB,
Sodertalje, Sweden.

Study Started: May 8, 1989

Study Completed: August 17, 1989

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

Animals: Beagle dogs (males 11-12 months old, females 10-12 months old), males 10-21 kg, females 7-13 kg.

Drug Batch No.: Omeprazole magnesium salt batch # 300/87,
Omeprazole (H168/68) batch # 136

Methods: The aim of this study was to compare the 3-month oral toxicity of omeprazole magnesium salt (batch # 300/87), with that of omeprazole (neutral) in dogs, using the equimolar conc of the 2 compounds. Omeprazole magnesium is a 2:1 salt, i.e. one mole of the magnesium salt dissociates to give two moles of omeprazole, whereas omeprazole (neutral) is in the acid form. In this study, the µmole dose and conc of magnesium salt was expressed as the equivalent µmole dose of omeprazole. Four groups of 3 male and 3 female dogs were given oral omeprazole magnesium (by gavage), at 0, 0.71, 5.7, and 29 mg/kg/day (or 0, 2, 16 and 80 µmole/kg, respectively, groups 1-4) for 3 months, at volumes of 2 mL/kg. An additional group of 3 male and 3 female dogs received the highest dose (28 mg/kg/day or 80 µmole/kg) of omeprazole, for 3 months (group 5). Both, omeprazole magnesium salt and omeprazole were formulated as a suspension in 0.5% carbonate buffered hydroxypropyl methyl cellulose solution (0.5% Methocel). Control group received the vehicle only (2 mL/kg of 0.5% Methocel). The dosing solutions were analyzed for conc, homogeneity, and stability, from the first and the last days of the study. The conc of the test substances varied between 92-111%, homogeneity between 93-99%, and all formulations were found to be stable throughout the study. Mortality and clinical signs were observed once daily. Body weights and rectal temperatures were noted once weekly, and food consumptions daily. Electrocardiography (ECGs) were recorded on the first day, and after 1 and 3 months, before dosing, and at 1, 2, 4, and 24 hrs after dosing. In all dogs, the amplitudes of P, Q, R, S waves, ST-j, ST segments, T-waves and the PR, QRS and QT intervals were measured in all the leads. Heart rates were measured by counting the number of R-waves in a time interval of 15 seconds. Ophthalmological examinations were carried out on all dogs, both before and at the end of the 3 month study. Eyes were examined after application of 0.5% solution of Mydriacyl, using indirect ophthalmoscope. Hematology, clinical chemistry tests, and urinalysis (following deprivation of food and water) were carried out at baseline, and after 3 and 11 weeks of dosing. For plasma conc of the drug, blood was collected from the jugular vein of dogs, on day 0 and after 1 and 3 months of dosing, at 0, 0.5, 1, 2, 4, and 24 hrs after treatment. The drug conc (of both, omeprazole magnesium and omeprazole) in plasma was measured by liquid chromatography methods, with quantification limit of 0.02 µmol/L of plasma. Basal and food stimulated gastrin levels in plasma were measured, at the end of 3 months study, by an established method using liquid phase radioimmunoassay. Blood (5 mL) for gastrin levels was drawn from dogs, prior to

feeding (basal), animals were offered ~300 g of food, (for 15 min), blood was drawn again at 0.5, 1, 1.5 and 3 hrs, from the time food was offered. All animals were sacrificed at the end of 3-months. Gross pathology, and complete histopathological examinations were carried out on all animals. The specimens of the stomach were additionally stained with Alcian Blue and Periodic Acid Schiff (AB/PAS) stain.

Results:

1. **Observed Effects:** No treatment-related effects were observed.

2. **Mortality:** None.

3. **Body Weight/Food Consumption:** No treatment-related effects were observed.

4. **Rectal Temperatures:** No treatment-related effects were observed.

5. **Electrocardiography:** No treatment-related effects were observed.

6. **Ophthalmoscopy:** One of 3 male dogs, at 0.71 mg/kg/day had mild unilateral keratitis in the left eye (vs none found in the controls) at the end of 3 months, sponsor indicates that this finding is of traumatic origin, and is not an unusual finding in dogs, and is not drug-related. Prolapse of the nictitating gland in either left or right eye was noted in 1 of 3 female dogs at 0.71, and 29 mg/kg/day of omeprazole magnesium, and in 1 of 3 female dogs at 29 mg/kg/day of omeprazole, along with lens luxation and microphthalmia in this dog (vs none in controls).

7. **Hematology:** No treatment-related effects were observed.

8. **Blood Chemistry/Urinalysis:** A slight increase in serum cholesterol levels were observed with 29 mg/kg/day (80 µmole/kg) of omeprazole magnesium, or 28 mg/kg/day (80 µmole/kg) of omeprazole (males 4.1-4.7 and 4.2-6.8 resp vs 3.7-4.0 mmol/L in controls, females 5.9-7.8 and 3.4-7.4 resp vs 3.6-6.3 mmol/L in controls). No treatment related changes in urinalysis were observed.

9. **Organ Weights:** A dose-related increase in the relative stomach weight was observed in all treated dogs with omeprazole magnesium at 0.7, 5.7 and 29 mg/kg/day (males 1.11, 1.16, and 1.45% vs 0.9% in controls, females 1.11, 1.32, and 1.45% vs 0.9% in controls). Similar increase was also noted with 28 mg/kg/day (80 µmole/kg) of omeprazole (males 1.51, females 1.54% vs both controls 0.9%). An increase in the relative weight of liver was observed in the dogs treated with omeprazole magnesium (males 2.74, 2.74, and 3.09 vs 2.63% in controls, females 2.97, 3.22, and 3.50 vs 2.82% in controls), and omeprazole (males 3.06, females 3.24%). These increases in liver weights were not associated with any histopathological changes.

10. Gross Pathology: At 29 mg/kg/day (80 µmole/kg) of omeprazole magnesium, or 28 mg/kg/day (80 µmole/kg) of omeprazole, the stomach mucosa of dogs had a thickened appearance with increased folding.

11. Histopathology: At 29 mg/kg/day (80 µmole/kg) of omeprazole magnesium, or 28 mg/kg/day (80 µmole/kg) of omeprazole, there was a slight increase in gastric mucosal thickness of the corpus/fundus region without prominent cellular changes, in all dogs, while none of the control dogs, or dogs at lower doses had these findings. At 28 mg/kg/day (80 µmole/kg) of omeprazole, one of 3 female dogs had moderate muscular dystrophy, as well as cataract in the right eye (with ruptured capsule, pronounced uveitis and retinal degeneration), this was not noted in the control dogs, or dogs who received omeprazole magnesium.

Toxicokinetics: These are shown in Table 10. The maximal plasma conc were reached within half hr, and both C_{max} and mean AUC values were similar after single (day 0), and repeated administrations (1-2.5 months). Also, half life of the drug was unchanged after single or repeated dosing. There were no differences in AUC values of omeprazole magnesium (80 µmole/kg) or omeprazole (80 µmole/kg) given at the same dose. After repeated oral administration of omeprazole magnesium, no accumulation was observed after 2.5-months (males + females), at any of the doses (0.7, 5.7 and 29 mg/kg/day), and AUC increased approximately in proportion (or slightly less than proportional) to the dose.

Table 10: Pharmacokinetic parameters of omeprazole magnesium and omeprazole in 3-month oral toxicity studies in dogs.

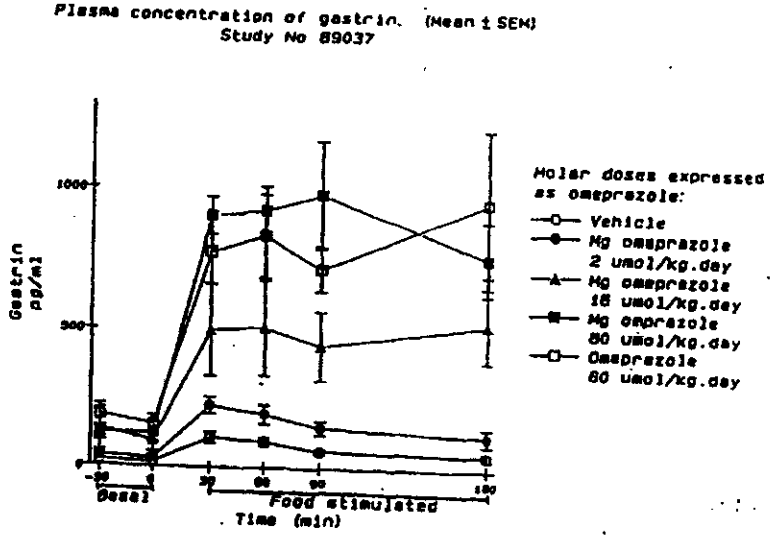
Daily Dose µmol/kg	Day/Months of Dosing	AUC h·µmol/l	$t_{\frac{1}{2}}$ h	C_{max} 0.5 h
2*	Day 0	1.80 ± 0.47	0.66 ± 0.04	1.42 ± 0.41
	1 Month	2.39 ± 0.64	0.73 ± 0.06	1.79 ± 0.52
	2.5 Months	2.11 ± 0.66	0.71 ± 0.04	1.20 ± 0.39
16*	Day 0	16.8 ± 3.3	0.66 ± 0.09	11.6 ± 2.3
	1 Month	10.0 ± 3.5	0.56 ± 0.05	7.4 ± 2.1
	2.5 Months	12.6 ± 3.2	0.53 ± 0.04	9.6 ± 1.7
80*	Day 0	60 ± 23	0.81 ± 0.27	28.4 ± 8.1
	1 Month	63 ± 13	1.05 ± 0.24	32.9 ± 2.8
	2.5 Months	66 ± 13	0.95 ± 0.11	32.4 ± 3.7
80	Day 0	89 ± 19	1.17 ± 0.34	32.2 ± 9.8
	1 Month	57 ± 17	0.75 ± 0.06	36.9 ± 9.3
	2.5 Months	53 ± 14	0.67 ± 0.09	36.1 ± 4.7

* = Daily dose expressed as µmol/kg of omeprazole but administered as magnesium salt.

Basal and Food-Stimulated Plasma Gastrin Levels in Dogs:

In control dogs the basal and food stimulated (30-180 min) gastrin levels were 16 and 49-106 pg/mL, respectively. Plasma levels of gastrin increased in all dogs, as a result of gastric acid inhibition, following administration of the drug (with omeprazole magnesium values at 2, 16, and 80 µmol/kg/day or 0.71, 5.7 and 29 mg/kg/day were 118-233, 444-526, 764-982 respectively, and with omeprazole 80 µmol/kg/day were 722-958 pg/mL). At 0.71, 5.7 and 29 mg/kg/day of omeprazole magnesium, plasma levels of gastrin levels increased in a dose related manner by 2, 6, and 11 times respectively (mean of 30-180 min values). No differences in plasma gastrin levels of omeprazole magnesium or omeprazole (80 µmol/kg) were observed, Figure 6 (reproduced from volume 1.7, page 191 of the submission).

Figure 6. Plasma conc of gastrin with omeprazole magnesium, and omeprazole in 3-month oral toxicity studies in dogs.



These studies indicate that oral administration of omeprazole magnesium (0.71, 5.7 and 29 mg/kg/day) or omeprazole (28 mg/kg/day, or 80 µmole/kg) for 3-months, led to similar exposure levels in dogs (AUC 63-66, and 53-57 h·µmol/L resp, at 1-2.5 months). Similarly, no differences in the plasma gastrin levels of the 2 drugs (80 µmol/kg) were noted at the end of 3-months in dogs. These doses did not cause any toxicity in dogs, except there was a slight increase in gastric mucosal thickness, without prominent cellular changes, at 29 mg/kg/day (80 µmole/kg) of omeprazole magnesium, and at 28 mg/kg/day (80 µmole/kg) of omeprazole. However, these effects may be due to the exaggerated pharmacological action of the drug. One of 3 female dogs, at 28 mg/kg/day of omeprazole had cataract in the eyes and muscular dystrophy, which were not observed in controls or with omeprazole magnesium. These studies indicate that in general, the effects of both drugs (omeprazole magnesium, and omeprazole acid), given at equivalent doses, are similar. The 'tolerated dose' of omeprazole magnesium in 3-month dog toxicity studies was 29 mg/kg/day (80 µmol/kg).

Addendum:

Gross Pathology:

Gross pathological changes for dogs that received omeprazole magnesium at doses of 0.71, 5.7, and 29 mg/kg/day or omeprazole (free acid) at 28 mg/kg/day for 3 months.

Organ/Tissue	Vehicle		Omeprazole Magnesium						Omeprazole	
	0 mg/kg/day		0.71 mg/kg/day		5.7 mg/kg/day		29 mg/kg/day		28 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
Stomach -mucosal thickening (corpus-fundus)	0	0	0	0	0	0	3	3	2	2
-mucosal thickening (antrum)	0	0	0	0	0	0	2	1	2	1
-antral polypous mucosal fold	0	0	0	0	0	0	0	0	0	1

Histopathology: Tissues samples were collected from all dogs for microscopic examination as follows: brain, optic nerve, sciatic nerve, eye, trachea, lung, tongue, submandibular gland, esophagus, stomach, duodenum, jejunum, ileum, colon, liver, gallbladder, pancreas, heart, aorta (+), bone marrow, cervical lymph node, mesenteric lymph node, spleen, thymus, pituitary gland, thyroid gland, parathyroid, adrenal gland, kidneys, urinary bladder, testes, epididymides, prostate, ovaries, oviduct (+), uterus, vagina, female mammary gland, skin, skeletal muscle, and any gross abnormalities. Specimens from all tissues, except those marked with a plus (+), and from any gross abnormalities were embedded in Paraplast®, sectioned at 4-6 µm, and stained with hematoxylin and eosin. Smears of the femoral bone marrow were prepared and stained according to May-Grunvald-Giemsa. Sections from the stomach were additionally stained with Alcian blue/PAS.

Plasma Gastrin Levels: Blood samples for analyses of basal and food-stimulated gastrin levels in plasma were taken after approximately 3 months of treatment. The blood samples were taken 24 hr after dosing. Two samples were at a 30-minute interval before food was given, i.e. basal gastrin (-30 and 0 min), and four samples were taken 30, 60, 90, and 180 min after the food was offered, i.e. stimulated gastrin. Plasma gastrin levels reported in the review were food-stimulated gastrin levels at 30 to 180 min. Basal gastrin levels, prior to presentation of food, are shown in the table below.

Basal gastrin levels (pg/mL) in dogs prior to the presentation of food.

Dose, mg/kg/day	-30 min	0 min
0 (Omeprazole Mg)	19	16
0.71 (Omeprazole Mg)	36	29
5.7 (Omeprazole Mg)	131	93
29 (Omeprazole Mg)	116	123
28 (Omeprazole, free acid)	181	152

Toxicokinetics: Plasma AUC and C_{max} values have been expressed in units of mg*hr/L and mg/L, respectively.

Toxicokinetic parameters of omeprazole magnesium and omeprazole in 3 month oral toxicity studies in dogs.

Dose, mg/kg/day	Day/Month of Dosing	AUC		C_{max}	
		µmole*hr/L	mg*hr/L	µmole/L	mg/L
0.71 (Omeprazole Mg)	Day 0	1.80	0.62	1.42	0.49
	1 month	2.39	0.83	1.79	0.62
	2.5 months	2.11	0.73	1.2	0.41
5.7 (Omeprazole Mg)	Day 0	16.8	5.80	11.6	4.00
	1 month	10.0	3.45	7.4	2.56
	2.5 months	12.6	4.35	9.6	3.32
29 (Omeprazole Mg)1.80	Day 0	60	30.74	28.4	9.81
	1 month	63	19.69	32.9	11.36
	2.5 months	66	22.8	32.4	11.19
28 (Omeprazole, free2.39 acid)	Day 0	89	30.7	32.2	11.12
	1 month	57	19.69	36.9	12.75
	2.5 months	53	18.31	36.1	12.47

H199/18 Magnesium: 3 Month Oral (Gavage) Toxicity Study in the Dog - A Comparison with Omeprazole Magnesium (Study No. 97103).

Testing Laboratories: Sponsor's laboratories:

Safety Assessment, Leics, England
Safety Assessment, Sodertalje, Sweden

Study Start and Completion Date: June 9, 1997 and
September 21, 1998

GLP Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Male: 9.0-14.6 kg, 7-10 months old
Female: 7.3-11.8 kg, 7-10 months old
Beagle dogs

Drug Batch No.: 602/97

Methods: To evaluate the toxicity of H 199/18 magnesium in dogs, dogs (3/sex/group) were given H 199/18 at 0, 0.65, 5.5 and 28 mg/kg/day for 3 months by oral gavage. Omeprazole magnesium at 28 mg/kg/day was also given as positive control. Both H 199/18 and omeprazole were given in suspension in 0.5% hydroxy-propylmethyl cellulose. The basis of dose selection was not provided in this submission. Clinical signs of toxicity were observed daily. Body weights were recorded weekly. Food consumption was recorded daily. Ophthalmic examinations were conducted before

treatment started and during week 12. ECGs were performed pretest, on day 2 and during weeks 5 and 12. All recordings were taken -20 minutes after dosing. Hematology, clinical chemistry, and urinalysis were determined before treatment and during weeks 4 and 12. The blood samples were taken before each dosing during week 4 and 12. Gastrin levels were determined before treatment and during week 13. All animals were necropsied at termination and organs were weighed. Histopathological examination was conducted in all animals necropsied at termination. Plasma levels of test drug were determined on days 1, 29 and 84.

Results:

1. **Clinical Signs:** Head nodding, unsteady gait and subdued behavior were observed in the high dose males. Red/brown urine was noted in the high dose animals and the animals treated with omeprazole.

2. **Mortality:** There were no deaths.

3. **Body Weight:** The mean initial and final body weights in the control animals were 12.7 kg and 13.5 kg (males) or 9.1 and 11.5 kg (females), respectively. There were no treatment-related changes.

4. **Food Consumption:** There were no treatment-related changes.

5. **Ophthalmoscopy:** There were no treatment-related alterations observed during the study.

6. **ECG:** There were no treatment-related changes.

7. **Hematology:** There were no clear treatment-related changes.

8. **Clinical Chemistry:** Cholesterol level was increased by 25-40% mainly in the mid and high dose groups and omeprazole group. Serum gastrin level was increased in all treatment groups as compared to the control and this information was summarized in a table on page 23. This table is attached below.

Gastrin Levels at Week 13								
Group	Compound	Dose Level ($\mu mol \cdot kg^{-1} \cdot day^{-1}$) ($mg \cdot kg^{-1} \cdot day^{-1}$)	Basal ($pg \cdot ml^{-1}$)		Food Stimulated ($pg \cdot ml^{-1}$)			
			-30 min	0 min	30 min	60 min	90 min	180 min
1	Vehicle	0	a	a	53 ±16	a	a	a
2	H 199/18	1.9 [0.65]	a	a	170 ±140	130 ±90	110 ±90	120 ±60
3	H 199/18	16 [5.5]	320 ±300	310 ±270	700 ±210	650 ±390	610 ±360	500 ±270
4	H 199/18	80 [28]	210 ±210	140 ±110	620 ±290	500 ±230	460 ±230	520 ±380
5	Omeprazole	80 [28]	180 ±150	160 ±140	470 ±210	410 ±160	370 ±110	340 ±140

Individual gastrin levels <50 $pg \cdot ml^{-1}$ are set at 25 $pg \cdot ml^{-1}$ in calculation of the means.
a = No mean levels were estimated when more than 2 out of 6 concentrations were <50 $pg \cdot ml^{-1}$.

9. **Urinalysis**: There were no treatment-related changes.

10. **Organ Weights**: The relative stomach and liver weights were increased in the treatment groups and this information was summarized in a table on 24. This table is attached below.

Relative Mean Organ Weights (% of body weight)										
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Stomach	0.99	1.15	1.48	1.36	1.61	1.02	1.14	1.34	1.23	1.46
(% difference)	-	+16	+49	+37	+63	-	+12	+31	+21	+43
Liver	3.42	3.52	3.66	3.80	3.88	3.49	3.58	3.62	3.77	4.12
(% difference)	-	+3	+7	+11	+13	-	+3	+4	+8	+18

11. **Gross Pathology**: There were no treatment-related changes.

12. **Microscopic Pathology**: Histopathological changes were limited in the stomach and the incidence and severity of these changes were summarized in a table on page 25. This table is attached below.

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
No. Animals	3	3	3	3	3	3	3	3	3	3
Mucosal hyperplasia	0	1	3	2	2	0	0	3	3	3
Mean severity	-	1.0	1.7	1.5	2.0	-	-	1.0	1.3	1.7
Chief cell atrophy	0	1	3	3	3	0	1	3	2	3
Mean severity	-	1.0	1.3	1.3	2.0	-	1.0	1.7	1.0	2.0
Mucosal fibrosis	0	1	3	3	3	1	0	2	2	3
Mean severity	-	1.0	1.0	1.0	2.0	1.0	-	1.0	1.5	1.0
Inflammatory cells Lamina propria	0	1	0	2	3	0	0	0	0	1
Mean severity	-	1.0	-	1.0	2.3	-	-	-	-	1.0
Focal necrosis, gastric glands	0	0	0	1	2	0	0	1	0	1
Mean severity	-	-	-	1.0	2.0	-	-	1.0	-	2.0
Sum of mean severity scores	0	4.0	4.0	5.8	10	1.0	1.0	4.7	4.8	7.7
Total severity scores	0	4	12	13	27	1	1	11	9	17

13. Plasma Level of Test Drug: The maximum plasma levels (C_{max}) of the test drug were 2.93 ± 1.7, 17.9 ± 6.3 and 75.4 ± 24.1 µmol/L in the low, mid and high dose groups, respectively. The AUC values were 169 ± 85, 1100 ± 410 and 5140 ± 1890 µmol·min/L in the low, mid and high dose groups, respectively. The C_{max} and AUC of omeprazole were 65.5 ± 16.8 µmol/L and 5310 ± 2030 µmol·min/L, respectively.

In summary, H 119/18 was tested in dogs at 0, 0.65, 5.5, and 28 mg/kg/day for 3 months by oral gavage. The major treatment related changes were histopathological changes in the stomach including mucosal fibrosis and hyperplasia, chief cell atrophy and focal necrosis in all treatment groups including omeprazole. Serum gastrin level was increased in all treatment groups. No effect dose was not identified and the stomach was the target organ of toxicity.

Addendum:

Drug Batch: H199/18 magnesium, batch 602/97
Omeprazole (H168/68) magnesium, batch 600/97

Observed Effects: Head nodding for short periods after dosing (~2 hr) was observed for 2 male dogs that received H199/18 at 28 mg/kg/day. Animal 20M displayed this transient sign for almost the entire duration of the treatment period, whereas Animal 19M displayed this effect on day 73 only. Unsteady gait was also observed for both these dogs. Subdued behavior was observed for male dogs after dosing with H199/18 at 28 mg/kg/day.

Body Weight and Food Consumption: Body weight gains and food consumption for female dogs that received H199/18 at 5.5 and 28 mg/kg/day or omeprazole magnesium at 28 mg/kg/day were slightly suppressed as compared to the female control. Body weight gains for male and female controls, expressed as percentage of initial body weights prior to start of treatment, were 4.7 and 22.75%, respectively. Body weight gains for male dogs that received 0.65 mg/kg/day H199/18, 5.5 mg/kg/day H199/18, 28 mg/kg/day H199/18, and 28 mg/kg/day omeprazole magnesium, expressed as percentage of initial body weight prior to start of treatment, were 0, 1.04, 1.24, and

3.39%, respectively. Body weight gains for male dogs that received H199/18 displayed no dose response relationship. Body weight gains for female dogs that received 0.65 mg/kg/day H199/18, 5.5 mg/kg/day H199/18, 28 mg/kg/day H199/18, and 28 mg/kg/day omeprazole magnesium, expressed as percentage of initial body weight prior to start of treatment, were 23.15, 17.4, 13.15, and 15.4%, respectively. Food consumption for female dogs that received H199/18 at 5.5 and 28 mg/kg/day or omeprazole magnesium at 28 mg/kg/day were reduced to 96.3, 94.6, and 93.1% of the female control (394.6 g/day), respectively.

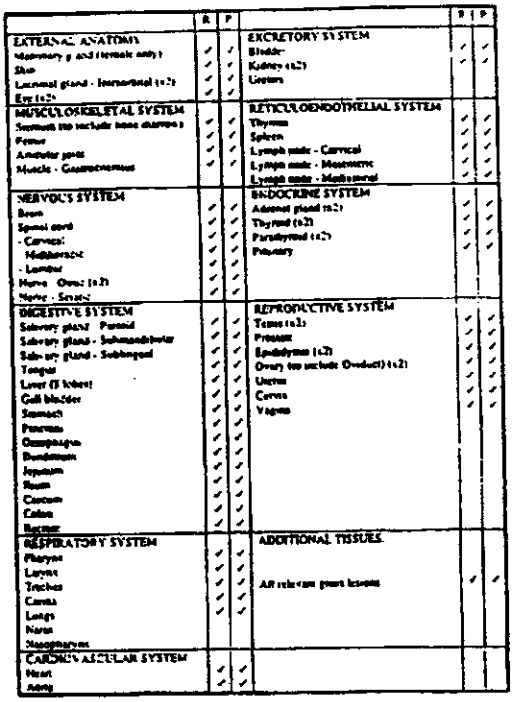
Hematology: Red blood cell counts, hemoglobin levels, and hematocrit during week 12 for female dogs that received H199/18 at 28 mg/kg/day were increased to 116.3, 111.2, and 111% of control values (6.023×10^{12}/L, 13.76 g/dL, and 0.4514), respectively.

Clinical Chemistry and Urinalysis: Observed changes in clinical chemistry and urinalysis parameters for H199/18- or omeprazole magnesium-treated dogs are suggestive of alterations in liver metabolism; although, there were no corresponding histopathological changes. Triglyceride levels for male H199/18 treatment groups at weeks 4 and 12 were increased to 105.4-131.4% and 129.4-164.3% of control values (0.373 and 0.286 mmol/L), respectively. Triglyceride levels at week 4 for female dogs that received 0.65 mg/kg/day H199/18, 5.5 mg/kg/day H199/18, 28 mg/kg/day H199/18, or 28 mg/kg/day omeprazole magnesium were increased to 111, 128, 141, and 154% of the control (0.406 mmol/L), respectively. Triglyceride levels at week 12 for female dogs that received 0.65 mg/kg/day H199/18, 5.5 mg/kg/day H199/18, 28 mg/kg/day H199/18, or 28 mg/kg/day omeprazole magnesium were increased to 116, 150, 176.5, and 152% of the control (0.353 mmol/L), respectively. Total bilirubin levels for male treatment groups that received H199/18 or omeprazole magnesium during weeks 4 and 12 were increased to 130-150% and 115-150% of control values (2.0 µmole/L), respectively. Total bilirubin levels during week 12 for female dogs that received H199/18 at 28 mg/kg/day were increased to 138.5% of the control (2.6 µmol/L). Serum alkaline phosphatase activity for male rats that received omeprazole magnesium at 28 mg/kg/day was increased to 125% of the control (79.6 IU/L). Urinary urobilinogen levels during weeks 4 and 12 for female dogs that received omeprazole magnesium at 28 mg/kg/day were increased to 500 and 367% of control values (3.20 µmole/L), respectively.

Electrocardiographic Examinations: Heart rate for female dogs that received H199/18 at 28 mg/kg/day during weeks 1, 5, and 13 were elevated to 143, 122.5, and 158% of control values (110, 120, and 103 beats/min), respectively.

Histopathology: Samples of all tissues collected from animals at necropsy were processed into paraffin blocks. Any remaining tissue was retained in fixative. Sections were cut at approximately 4-5 µm and stained with hematoxylin and eosin and submitted to microscopic examination. Additional sections of the stomach were prepared and stained with hematoxylin and eosin and with periodic acid-Schiff/Alcian blue.

Samples of tissues retained at necropsy were as follows:



R = Retained
P = Processed

Plasma Drug Levels: Plasma C_{max} and AUC values on days 1, 29, and 84 for dogs that received H199/18 increased in an approximate dose proportional manner.

Plasma drug levels on days 1, 29, and 84 for dogs that received H199/18 at 0.65, 5.5, and 28 mg/kg/day or omeprazole magnesium at 28 mg/kg/day.

Dose, mg/kg/day	Day	C_{max},		AUC,	
		µmole/L	mg/L	µmole·min/L	mg*hr/L
0.65 mg/kg/day	1	2.81	0.97	154	0.89
	29	2.73	0.94	178	1.02
	84	3.24	1.12	174	1.00
5.5 mg/kg/day	1	17.4	6.01	1170	6.74
	29	21.4	7.39	1270	7.31
	84	15.0	5.18	855	4.92
28 mg/kg/day	1	73.5	25.39	5890	33.91
	29	69.8	24.11	4400	25.33
	84	82.9	28.64	5140	29.59
28 mg/kg/day omeprazole magnesium	1	63.3	21.86	6490	37.36
	29	66.8	23.1	4400	25.33
	84	66.6	23.0	5030	28.96

IMMUNOTOXICOLOGY:

Lymphocyte Transformation Test of H168/68 (Study No. 86041).

Testing Laboratory: Astra

Safety Assessment
S-151 85 Sodertalje, Sweden

Date Started: Unknown

Date Completed: July 9, 1986

GLP Compliance: Unknown

Drug Batch: Unknown

Methods: A lymphocyte transformation test was conducted to assess omeprazole for its ability to activate peripheral lymphocytes from patients suspected to have an allergic reaction to this substance. Activation is determined by measurement of incorporation of radiolabeled thymidine into DNA and by the morphological evaluation of lymphocyte transformation. Cells were incubated with omeprazole (34-1633 µmole/mL for 2 days or 8.5-544 µmole/mL for 5 days) or the positive control (i.e., tuberculin purified protein derivative) for 2 or 5 days. Following exposure to omeprazole or the positive control for 2 or 5 days, incorporation of radiolabeled thymidine into DNA was determined. Following treatment for 5 days, the number of transformed lymphocytes was determined. This test cannot differentiate between test substances that are mitogens and antigens or between allergenic and non-allergenic antigens. Differentiation between these types of compounds can be performed by conducting the lymphocyte transformation test with lymphocytes from subjects that have been exposed to the substance and shown allergenic reactions, subjects that have been exposed without allergic reactions, and non-exposed subjects. Five human subjects were used in this test. Lymphocytes were obtained from 5 human subjects. Subjects 1, 2, 3, and 5 were exposed to H168/68 in their daily work. Subjects 2 and 5 experienced no allergic symptoms. Subjects 1 and 3 developed eczema on the upper eyelid, when working with H168/68. Subject 4 had never been exposed to H168/68.

Results: In subjects 1 and 3, an increase in DNA synthesis was found with the 2 and 5-day treatments in the presence of H168/68 and the number of transformed lymphocytes was increased in the 5-day culture. For subjects 2, 4, and 5, no increases in DNA synthesis were found in the 2 and 5 (or 4) day treatments in the presence of H166/68 and the number of transformed lymphocytes was increased in the 5-day culture. A positive control confirmed that DNA synthesis in lymphocytes from all subjects could be stimulated. Results of the study confirm the diagnosis of allergy to H168/68 in subjects observed with allergic symptoms (i.e., subjects and 1 and 3).

ASSAYS AND RESULTS - SUBJECT NO. 1 - 5-DAY TREATMENT

Footnote
†toxic
*Only a part of cytological preparation could be evaluated.
dpm disintegrations - mean values of triplicate aliquots per culture.
SI stimulation index
PPD tuberculin purified protein derivative

ASSAYS AND RESULTS - SUBJECT NO. 3 - 5-DAY TREATMENT

Footnote

†toxic
*Only a part of cytolcgical preparation could be evaluated.
dpm disintegrations - mean values of triplicate aliquots per culture
SI stimulation index
PPD tuberculin purified protein derivative

GENETIC TOXICOLOGY:

H199/18 Sodium, H199/19 Sodium, Omeprazole Sodium, H225/20 (Lansoprazole): Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (Study No. 524/12).

Testing Facility: [redacted]

Date Started: December 20, 1999

Date Completed: March 2, 2000

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

Drug Batch: H199/18 sodium, Batch number 600/93
H199/19 sodium, Batch number 600/93
Omeprazole sodium, Batch number 13
H225/20 (Lansoprazole), Batch number 512/91

Methods: The clastogenic activity of omeprazole sodium (racemic mixture), the S-enantiomer of omeprazole (H199/18 sodium), the R-enantiomer of omeprazole (H199/19 sodium), and lansoprazole (H225/20; reference compound) were assessed using the in vitro human lymphocyte chromosomal aberration assay. The negative control was DMSO. The positive control in the absence of metabolic activation was 4-nitroquinoline 1-oxide (NQO). No studies were conducted in the presence of metabolic activation. Lymphocytes were obtained from the peripheral circulation of healthy, non-smoking volunteers. Whole blood cultures were established and treated with 10 µg/mL phytohemagglutinin to stimulate lymphocyte division over a 48-hr incubation period at 37°C. Cultures were treated with the test article for 3 hr, followed by washing the cells and an additional incubation for 17 hr. For experiment 1, test article concentrations were as follows: H199/18, H199/19, and omeprazole, 0.05342 to 0.6219 mg/mL (precipitate at 0.4975 and 0.6219 mg/mL for H199/18 and H199/19, and at 0.6219 mg/mL for omeprazole); and lansoprazole, 0.03655 to 0.4256 mg/mL (precipitate at concentrations ≥0.2179 mg/mL). For experiment 2, test article concentrations were as follows: H199/18, H199/19, and omeprazole, 0.2246 to 0.6219 mg/mL (precipitate at concentrations ≥0.5183 mg/mL for H199/18 and H199/19, and at 0.6219 mg/mL for omeprazole); and lansoprazole, 0.1663 to 0.3510 mg/mL (precipitate at concentrations ≥0.2032 mg/mL). For experiment 3, test article concentrations were as follows: H199/18, H199/19, and omeprazole, 0.4319 to 0.6219 mg/mL (precipitate at concentrations ≥0.5010 mg/mL for H199/18, ≥0.5183 mg/mL for H199/19, and ≥0.4319 mg/mL for omeprazole); and lansoprazole, 0.1293 to 0.2955 mg/mL (precipitate at

concentrations ≥0.2032 mg/mL). At 2 hr prior to harvest, colchicine was added to cultures at a final concentration of 1 µg/mL to arrest dividing cells in metaphase. After an incubation period of 20 hr, cells were collected, fixed, and stained with Giemsa stain. Where possible, 100 metaphases from each treatment were analyzed for chromosomal aberrations. Only cells with 44-46 chromosomes were considered acceptable for analysis of structural aberrations. Cells with >46 chromosomes (i.e., polyploid, endoreduplicated, and hyperdiploid cells) were recorded separately. A test compound was considered positive in this assay if: (1) the proportions of cells with structural aberrations at one or more concentrations exceeds the normal range in both replicates, (2) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurs at these doses, and (3) the results are reproduced between independent experiments.

Results: The highest dose for chromosome analysis from cultures sampled at 20 hr was one at which at least 50% mitotic inhibition was observed. Otherwise, the highest tested dose was analyzed. Slides from cultures treated with heavily precipitating doses were checked to confirm that the presence of the precipitate did not preclude analysis. Slides from the highest selected dose and two lower doses, with cytotoxicity ranging from approximately to 50% to little or none, were analyzed. Analysis of slides from highly cytotoxic concentrations was avoided. Based upon cytotoxicity, concentrations selected for analysis of chromosomal aberrations for each test compound were as follows: H199/18 sodium, 0.4492, 0.4664, 0.4837, and 0.5010 mg/mL (mitotic inhibition at 0.5010 mg/mL was 64%); H199/19 sodium, 0.4837, 0.5010, and 0.5183 mg/mL (mitotic inhibition at 0.5355 mg/mL was 54%); omeprazole sodium, 0.4664, 0.4837, and 0.5010 mg/mL (mitotic inhibition at 0.5010 mg/mL was 54%); and lansoprazole, 0.1847, 0.2032, and 0.2216 mg/mL (mitotic inhibition at 0.2216 mg/mL was 42%). Omeprazole, the S-enantiomer of omeprazole, the R-enantiomer of omeprazole, and lansoprazole were found to produce an increased incidence of chromosomal aberrations in human lymphocytes as compared to the solvent control. H199/18 and lansoprazole produced dose-related increases in the number of cells with aberrations excluding gaps. H199/19 and omeprazole produced an increased incidence of cells with aberrations excluding gaps. The positive control produced expected responses.

S-enantiomer of omeprazole (H199/18 sodium), Experiment 2: Cells with Structural Aberrations

3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	4	3.9
0.3628	1.050	200	4	3.9
0.4146	1.200	200	7*	3.8
0.4664	1.350	200	9*	3.4
0.5183	1.500	200	11*	2.8
NQO, 1.25 µg/mL	-	192	25*	

* p ≤0.01

S-enantiomer of omeprazole (H199/18 sodium), Experiment 3: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	2	11.2
0.4492	1.300	200	8*	10.5
0.4664	1.350	200	21*	6.8
0.4837	1.400	200	19*	6.4
0.5010	1.450	200	24*	4.0
NQO, 2.5 µg/mL	-	200	22*	

* $p \leq 0.01$

R-enantiomer of omeprazole (H199/19 sodium), Experiment 2: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	4	3.9
0.3628	1.050	200	4	4.3
0.4146	1.200	200	7*	3.6
0.4664	1.350	200	13*	2.4
0.5183	1.500	200	18*	3.3

* $p \leq 0.01$

R-enantiomer of omeprazole (H199/19 sodium), Experiment 3: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	2	11.2
0.4837	1.400	200	27*	4.5
0.5010	1.450	200	18*	6.5
0.5183	1.550	200	20*	5.2

* $p \leq 0.01$

Omeprazole sodium, Experiment 2: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	4	3.9
0.3628	1.050	200	1	3.5
0.4146	1.200	200	5*	3.1
0.4664	1.350	200	1	3.1
0.5183	1.500	200	17*	2.2

* $p \leq 0.01$

Omeprazole sodium, Experiment 3: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	2	11.2
0.4664	1.350	200	16*	6.3
0.4837	1.400	200	24*	4.9
0.5010	1.450	200	16*	5.2

* $p \leq 0.01$

Lansoprazole, Experiment 2: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	4	3.9
0.1663	0.450	200	1	3.0
0.2032	0.550	200	2	2.1
0.2402	0.650	200	11*	1.5

* $p \leq 0.01$

Lansoprazole, Experiment 3: Cells with Structural Aberrations
3 hr treatment + 17 hr recovery

Treatment		Cells Scored	Cells with aberrations excluding gaps	Mitotic Index
mg/mL	mmol/L			
0	0	600	2	11.2
0.1847	0.500	200	4*	8.8
0.2032	0.550	200	14*	8.5
0.2216	0.600	200	17*	6.6

* $p \leq 0.01$

Omeprazole sodium (racemic mixture), the S-enantiomer of omeprazole (H199/18 sodium), the R-enantiomer of omeprazole (H199/19 sodium), and lansoprazole (H225/20) were found to be positive for clastogenic activity in the in vitro human lymphocyte chromosomal aberration assay. These studies were conducted in the absence of metabolic activation. No studies were conducted in presence of metabolic activation.

OVERALL SUMMARY AND EVALUATION:

Omeprazole is a substituted benzimidazole that suppresses gastric acid secretion by specific inhibition of the H^+,K^+-ATPase enzyme system at the secretory surface of the gastric parietal cell. Given that omeprazole is acid labile and undergoes rapid degradation in the acid environment of the stomach, the drug is administered as an enteric-coated granule formulation (Note: In animal toxicology studies, omeprazole

was administered in an alkaline vehicle to prevent degradation in the acid environment of the stomach). Following intestinal absorption, the drug reaches the gastric mucosa by way of blood circulation. Omeprazole has a pK_a of approximately 4.0 leading to accumulation in the acidic secretory canaliculus of the stimulated parietal cell. In this acidic environment, omeprazole undergoes conversion to a cationic sulfenamide. The sulfenamide interacts in a covalent manner with cysteine residues at critical sites in the extracellular (lumenal) domain of the membrane-spanning H^+,K^+-ATPase. Full inhibition occurs with two molecules of inhibitor bound per molecule of enzyme. Since this enzyme system is regarded as the acid (proton) pump within the gastric mucosa, omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. In studies with both animals and humans, this effect has been found to be dose-related and leads to inhibition of both basal and agonist-stimulated acid secretion. Administration of omeprazole results in permanent inhibition of enzyme activity in vivo. Given the short plasma half-life of omeprazole (i.e., less than 1 hr), inhibition of acid secretion persists for significant periods after the drug has been eliminated from the plasma. With an enzyme half-life of 50 hr, approximately 25% of available H^+,K^+-ATPase is synthesized over a 24 hr period. This is the predominant mechanism for recovery of acid secretion following covalent inhibition of the pump by benzimidazoles. Given the short half-life of omeprazole, a significant quantity of newly synthesized H^+,K^+-ATPase, that has not been exposed to omeprazole, will be present 24 hr after dosing. With repeated once-daily treatment regimens using therapeutic doses (i.e., 20 to 40 mg/day in humans), achievement of steady-state inhibition of acid secretory capacity, defined as greater than 70% inhibition, could be characterized as delayed in nature, occurring at 2 to 3 days after the start of dosing in rats or humans and 5 to 7 days in dogs. Long-term treatment of rats and dogs with omeprazole for 3 and 12 months, respectively, produced profound, dose-related inhibitory effects on both basal and stimulated gastric acid secretion; however, within a few days after the cessation of treatment, a rebound phenomenon of increased acid secretion was observed in both species. Basal and/or agonist-stimulated acid secretion were found to be elevated in both rats and dogs that had received omeprazole, as compared to corresponding untreated controls, for periods up to 3 months after completion of treatment. This effects was attributed to an increased acid secretion capacity as no changes in agonist dose response relationships were found. Rebound acid hypersecretion has been observed in humans following cessation of omeprazole treatment.

In the present application, the sponsor proposes to market the magnesium salt of omeprazole (Prilosec 1®; i.e., omeprazole magnesium) as an over-the-counter drug product. Omeprazole magnesium will be supplied in tablet form, which the sponsor claims is more suitable to the consumer environment. Each tablet will contain 20.6 mg of omeprazole magnesium, equivalent to 20 mg of omeprazole, in the form of enteric-coated pellets. Proposed indications for omeprazole magnesium are as follows: (1) for **relief** of heartburn, acid indigestion, and sour stomach and (2) for **prevention** of heartburn, acid indigestion, and sour stomach brought on by consuming food and beverages, or associated with events such as stress, hectic lifestyle, lying down, or exercise.

Proposed uses of omeprazole magnesium (i.e., Prilosec 1) for adults and children 12 years of age and older are as follows: (1) for relief of symptoms, swallow 1 tablet with a glass of water, and (2) for prevention of symptoms for 24 hours, swallow 1 tablet with a glass of water anytime during the day, or if preferred, one hour before those events associated with occasional heartburn, such as consuming food and beverages, stress, hectic lifestyle, lying down, or exercise. Not more than one tablet should be taken per day. This product should not be used for more than 10 days in a row unless directed by a doctor. The tablets should not be chewed or crushed.

Omeprazole has been an approved prescription drug for more than 10 years. Omeprazole is supplied as delayed-release capsules for oral administration. Each delayed-release capsule contains either 10, 20, or 40 mg of omeprazole in the form of enteric-coated granules. During this period it has been marketed for indications that include treatment of duodenal ulcer, treatment of gastric ulcer, treatment of symptomatic gastroesophageal reflux disease (GERD), treatment of heartburn and other symptoms associated with GERD, treatment of erosive esophagitis, maintenance of healing of erosive esophagitis, and treatment of pathological hypersecretory conditions.

In support of the over-the-counter use of omeprazole magnesium tablets by humans, the sponsor has referenced preclinical pharmacology and toxicology studies with omeprazole submitted to NDA 19,810. In accordance with Division recommendation, the sponsor submitted reports of preclinical studies to IND [redacted] (Amendment #376 dated February 18, 2000 and Amendment #383 dated May 25, 2000) and NDA 19,810 (Correspondence dated March 29, 2000), which had not been previously submitted to NDA 19,810, IND [redacted] or IND [redacted] These studies were considered in the present review. In the present application, the sponsor has submitted preclinical pharmacology and toxicology studies in support of omeprazole magnesium as follows: pharmacology; acute toxicity studies with omeprazole magnesium in mice and rats; a comparative 3-month oral toxicity study of omeprazole magnesium and omeprazole in dogs; a comparative 3-month oral toxicity study of H199/18 magnesium (i.e., magnesium salt of S-enantiomer of omeprazole) and omeprazole magnesium in dogs; and a human lymphocyte transformation test with omeprazole. A 12-month oral toxicology study in rats (submitted to IND [redacted] Amendment #376 dated February 18, 2000) and a human lymphocyte chromosomal aberration assays with H199/18 sodium [i.e., sodium salt of S-enantiomer of omeprazole], H199/19 [i.e., sodium salt of R-enantiomer of omeprazole], and omeprazole sodium [i.e., sodium salt of racemic mixture of omeprazole] (submitted to NDA 19,810 as correspondence dated March 29, 2000) were incorporated into the present review.

Absorption, distribution, metabolism, and excretion studies have been conducted with omeprazole in rats, dogs, and humans. Absorption is rapid in rats, dogs, and humans, with peak plasma levels of omeprazole occurring within 0.25 to 3 hr after dosing. Oral bioavailability of omeprazole is low in rats and dogs due to a high first-pass effect, which is saturable. In humans, peak plasma concentrations of omeprazole and

AUC are approximately proportional to doses up to 40 mg, but because of a saturable first-pass effect, a greater than linear response in peak plasma concentration and AUC occurs with doses greater than 40 mg. In fed and fasted rats, oral bioavailability of omeprazole administered is 5% and 15-20%, respectively. In dogs, oral bioavailability is 15%. In humans, oral bioavailability is 30-40% at doses of 20-40 mg. In rats, dogs, and humans, the plasma half-life of omeprazole is approximately 0.5 to 1 hour. The volume of distribution in male rats, female rats, dogs, and humans was 9.7, 4.5, 0.56, and 0.3 L/kg, respectively. In rats, significant amounts of drug-related radioactivity were distributed to the gastric mucosa, biliary tree, intestinal contents, urinary bladder, liver, and kidney. Total body clearance in rats, dogs, and human was 280-440, 1, and 10-12 mL/min/kg, respectively. Plasma protein binding in rats, dogs, and humans is approximately 87.5, 90, and 95.7%, respectively. Omeprazole is extensively metabolized with metabolites rapidly eliminated by biliary and renal pathways. Following oral administration of a single dose of omeprazole to rats, dogs, or humans, the drug was extensively metabolized with <0.1% of the unchanged drug excreted in urine or bile. In rats, urinary and fecal excretion accounted for 43 and 49% of an administered dose, respectively. In dogs, urinary and fecal excretion accounted for 32 and 54.7% of an administered dose, respectively. For rats and dogs, at least 7 urinary metabolites have been identified. Metabolic pathways were complex. Omeprazole underwent aromatic hydroxylation at position 6 in the in the benzimidazole ring followed by glucuronidation, oxidative O-dealkylation of both methoxy groups, and aliphatic hydroxylation of a pyridine methyl group followed by oxidation to the corresponding carboxylic acid. For humans, approximately 77% of an oral dose was eliminated in urine as at least six metabolites. Two were identified as hydroxyomeprazole and the corresponding carboxylic acid. The remainder of the dose was recoverable in feces. Biliary excretion of the metabolites of omeprazole plays a significant role in rats, dogs, and humans. For humans, three plasma metabolites have been identified, the sulfide and sulfone derivatives of omeprazole, and hydroxyomeprazole. These metabolites possess little or no antisecretory activity. Omeprazole inhibited drug metabolism in rat liver microsomal preparations and prolonged hexobarbital sleeping time and aminopyrine half-life in rats. After oral administration of omeprazole to animals or humans, the onset of the antisecretory effect of omeprazole occurred within 1 hr, and maximal effect occurred within 2 hr. For humans at 24 hr after dosing, inhibition of acid secretion had declined to 50% of the maximal effect observed at 2 hr. These periods were shorter in rats, which possess a significant first pass effect, although, comparable in dogs. The antisecretory effect persists longer than predicted from the short (<1 hr) plasma half-life, due to irreversible binding of the active moiety to the gastric parietal cell H^+,K^+-ATPase enzyme. For rats, dog, and humans, when the drug treatment is discontinued, secretory activity returns gradually over a 3 to 5 day period. For rats, dogs, or humans, the inhibitory effect of therapeutic doses of omeprazole on acid secretion increases with repeated once-daily treatment, reaching a steady-state at 2 to 3 days in rats or humans and 5-7 days in dogs. Steady-state inhibition may be achieved more rapidly with supra-therapeutic doses.

The acute toxicity of omeprazole magnesium was examined in mice and rats. The minimum lethal oral doses of omeprazole magnesium and omeprazole in male

mice were 1900 and 1300 mg/kg, respectively. Minimum lethal doses for omeprazole magnesium and omeprazole in female mice were not identified. The minimum lethal oral doses of omeprazole magnesium in male and female rats were 1800 and 930 mg/kg, respectively. Minimum lethal doses for omeprazole in male and female rats were not identified. Clinical signs for both omeprazole magnesium and omeprazole in mice and rats consisted of reduced motor activity, reduced reaction to external stimuli, prostration, and piloerection. The intensity and duration of these clinical signs increased in a dose-related manner for both omeprazole magnesium and omeprazole in mice and rats. These studies suggest that the acute toxicities of omeprazole magnesium and omeprazole in mice and rats were similar.

In four 13-week oral toxicology studies, Sprague-Dawley received omeprazole at doses ranging from 0.016 to 500 mg/kg/day [redacted] (Study #86-85; [redacted] Study #88006 [redacted] Studies #82062 and #83032). Doses less than 0.4 mg/kg were subtherapeutic (i.e., 20 mg/50 kg = 0.4 mg/kg). A no effect dose was established at 0.08 mg/kg/day. Red blood cell counts, hematocrit, and hemoglobin levels were decreased for rats at 500 mg/kg/day. Reticulocyte percentages for male rats at 8, 32, 125, and 500 mg/kg/day were increased. These hematological changes appeared to be indicative of a hypochromic, microcytic anemia. Omeprazole has been demonstrated to impair the absorption of iron by increasing the stomach pH. The target organs of toxicity were the stomach, adrenal glands, kidneys, lungs, and pancreas. Hyperplasia and hypertrophy of enterochromaffin-like (ECL) cells (Grimelius positive) were observed for male and female rats at doses greater than or equal to 0.4 mg/kg/day. Eosinophilia of secretory granules of chief cells with hypertrophy and pyknosis were observed for male and female rats at doses greater than or equal to 8 and 32 mg/kg/day, respectively. Dilatation of gastric glands were observed in all male treatment groups at doses greater than 0.4 mg/kg/day. Heterotypic squamous epithelium (i.e., polyps) were observed for 1 male rat at 32 mg/kg/day. For the adrenal gland, an increase of pale cells (i.e., poorly stainable ground-glass-like or bulla-like cytoplasm) in the zona glomerulosa was observed for male and female rats at doses greater than 0.4 mg/kg/day. For the kidneys, deposits of crystalloid material (i.e., drug-related material) were observed for male and female rats at doses greater than or equal to 125 mg/kg/day. Tubular epithelial clearing was observed for male and female rats at doses greater than or equal to 8 and 125 mg/kg/day, respectively. Degeneration of medullary tubular epithelium was observed for female rats at 500 mg/kg/day. For male and female treatment groups at doses greater than or equal to 8 mg/kg/day, increased incidences of tubular atrophy, dilation of the lumen, protein casts, and marked subcapsular hemorrhage and subsequent organization were observed. Water consumption was increased for male treatment groups at doses greater than 8 mg/kg/day, which may correspond with observed histopathological changes in the kidneys. For the lungs, there was hemorrhage for male rats at doses greater than or equal to 32 mg/kg/day, and there were increased incidences of congestion, edema, and hemorrhage for female rats at 500 mg/kg/day. For the pancreas, adenomatous hyperplasia of the exocrine region was observed for 1 male rat at 500 mg/kg/day. Gastrin levels were found to be elevated for rats at 32 mg/kg/day; however, other dose levels were not examined.

Histopathological changes in the stomach were generally not observed at the end of the 13-week recovery period for rats that received doses less than or equal to 32 mg/kg/day.

In a 6-month oral toxicology study, rats received omeprazole by oral gavage at doses of 0, 14, 43, or 138 mg/kg/day (Astra Toxicology Laboratories; Report No. T1347). The dose of 43 mg/kg/day could be considered a tolerated dose. There was no treatment-related mortality. The target organs of toxicity were the stomach, bone marrow, lungs, and liver. There was a dose-related eosinophilia of the zymogen granules of chief cells in all treatment groups. These effects in stomach are most likely an exaggerated pharmacological response due to elevated serum gastrin levels. There was a slight atrophy of pepsinogen-secreting cells in the 138 mg/kg/day group. Bone marrow hyperplasia was observed for 2 of 25 males in the 138 mg/kg/day group. Microscopic analysis of the lung for the 138 mg/kg/day group identified an increased incidence of peribronchiolar lymphoid hyperplasia with or without lymphocyte infiltration. Microscopic analysis of the liver for the 138 mg/kg/day group identified an increased incidence of periportal leukocyte infiltration with and without microfocal necrosis.

In a 52-week oral toxicology study, Sprague-Dawley rats received omeprazole at doses of 0, 0.4, 2, and 16 mg/kg/day. A no effect dose was not established. There was no treatment-related mortality. The target organs of toxicity were the stomach, kidneys, adrenal glands, and liver. Hyperplasia/hypertrophy of ECL (Grimelius positive) cells was observed in all male and female treatment groups. Thickening of the gastric mucosa was observed for male and female rats at doses ≥2 mg/kg/day. An increase of eosinophilic granules in chief cells (focal) was observed for all male and female treatment groups. For the kidneys, an increased incidence of chronic nephropathy was observed for male and female rats at 16 mg/kg/day. For the adrenal glands, an increase of pale cells of zona glomerulosa was observed for male and female rats at 16 mg/kg/day. For the liver, an increased incidence of altered cell foci was observed for male treatment groups; however, increased incidences were not observed for female treatment groups. For the brain, astrocytomas were observed for male rats at 0.4, 2, and 16 mg/kg/day with an incidence of 1 of 23 (4.3%), 1 of 23 (4.3%), and 2 of 24 (8.3%), respectively. There was no reported spontaneous incidence rate for brain astrocytoma in 12-13 month studies (Charles River Laboratories, February 1992). Bronchiolar/alveolar adenomas were observed for 1 of 24 (4.2%) female rats at 16 mg/kg/day and 1 of 23 (4.3%) male rats at 2 mg/kg/day. There was no reported spontaneous incidence rate for bronchiolar/alveolar adenoma in 12-13 month studies (Charles River Laboratories, February 1992).

In a 3-month oral toxicology study, dogs received omeprazole magnesium at doses of 0.71, 5.7, and 29 mg/kg/day or omeprazole at a dose of 28 mg/kg/day. The dose of omeprazole magnesium at 29 mg/kg/day could be considered a tolerated dose. The target organ of toxicity was the stomach. For omeprazole magnesium at 29 mg/kg/day or omeprazole at 28 mg/kg/day, a slight increased in the gastric mucosal thickness without prominent cellular changes was observed. These changes in the stomach may be attributed to the exaggerated pharmacological action of omeprazole.

appearance of eosinophilic granulated cells, decrease of chief cells in number, degeneration and necrosis of glandular cells (i.e., chief cells and parietal cells) in the fundus, and inflammatory cell infiltration in the lamina propria of the fundus were observed. Edema in the lamina propria of the fundus was observed for male dogs at 50 mg/kg/day and female dogs at 5 and 50 mg/kg/day. The incidence of formation of lymphocytic follicles in the basal region of the lamina propria of the fundus and vacuolation of parietal cells in fundal glands were increased in omeprazole-treatment groups. Omeprazole-induced histopathological changes in the stomach appeared to be reversible as most changes were not observed following a 13-week recovery period.

In a 12-month oral toxicology study, beagle dogs received omeprazole by the oral route using gastric intubation at doses of 0, 0.7, 5.5, and 28 mg/kg/day (Astra Toxicology Laboratories; Report No. T1371). The dose of 5.5 mg/kg/day could be considered a tolerated dose. The target organ of toxicity was the stomach. Histopathological examination found a dose-dependent rugal hypertrophy for the 5.5 and 28 mg/kg/day groups. This effect may be related to a prolonged inhibition of gastric acid secretion and a secondary hypergastrinemia. A separate 12 month study in dogs (Report #222-0054) found hypergastrinemia with doses of 0.68 and 27.6 mg/kg/day. Microscopic examination also found atrophic chief cell changes in the 28 mg/kg/day group. Atrophy, a sign of chief cell inactivity, appeared to be due to the pharmacological inhibition of gastric acid secretion. After a 4-month recovery period for the 28 mg/kg/day group, chief cells were restored to a normal appearance; however, rugal hypertrophy and a discrete fibrosis of the lamina propria persisted.

The current approved labeling for carcinogenicity study results with omeprazole are as follows: *"In two 24-month carcinogenicity studies in rats, omeprazole at daily doses of 1.7, 3.4, 13.8, 44.0 and 140.8 mg/kg/day (approximately 4 to 352 times the human dose, based on a patient weight of 50 kg and a human dose of 20 mg) produced gastric ECL cell carcinoids in a dose-related manner in both male and female rats; the incidence of this effect was markedly higher in female rats, which had higher blood levels of omeprazole. Gastric carcinoids seldom occur in the untreated rat. In addition, ECL cell hyperplasia was present in all treated groups of both sexes. In one of these studies, female rats were treated with 13.8 mg omeprazole/kg/day (approximately 35 times the human dose) for one year, then followed for an additional year without the drug. No carcinoids were seen in these rats. An increased incidence of treatment-related ECL cell hyperplasia was observed at the end of one year (94% treated vs 10% controls). By the second year the difference between treated and control rats was much smaller (46% vs 26%) but still showed more hyperplasia in the treated group. An unusual primary malignant tumor in the stomach was seen in one rat (2%). No similar tumor was seen in male or female rats treated for two years. For this strain of rat no similar tumor has been noted historically, but a finding involving only one tumor is difficult to interpret. A 78-week mouse carcinogenicity study of omeprazole did not show increased tumor occurrence, but the study was not conclusive."*

Assessment of the carcinogenic potential of omeprazole remains incomplete at present, with specific reference to the 78-week mouse carcinogenicity study. The

Division and CDER Carcinogenicity Assessment Committee have both reviewed the 78-week mouse carcinogenicity study with omeprazole and concluded that this study was critically flawed and unacceptable. The CDER CAC strongly recommended that an acceptable mouse carcinogenicity study of omeprazole should be conducted given the potential widespread use of this drug. In a letter to the sponsor, Merck Sharp and Dohme Research Laboratories, dated November 30, 1990, the Division communicated the recommendations of the CDER CAC and reiterated the necessity to conduct a valid mouse carcinogenicity study as part of a Phase IV program to assess the long term safety and tumorigenic potential of omeprazole. Omeprazole was demonstrated to be tumorigenic in the rat carcinogenicity study; however, to date, the preclinical assessment of the carcinogenic potential in a second species continues to be incomplete.

The current approved labeling for genotoxicity test results with omeprazole is as follows: *"Omeprazole was not mutagenic in an in vitro Ames Salmonella typhimurium assay, an in vitro mouse lymphoma cell assay and an in vivo rat liver DNA damage assay. A mouse micronucleus test at 625 and 6250 times the human dose gave a borderline result, as did an in vivo bone marrow chromosome aberration test. A second mouse micronucleus study at 2000 times the human dose, but with different (suboptimal) sampling times, was negative."*

The in vivo mouse micronucleus tests and in vivo bone marrow chromosome aberration test described in the current approved labeling suggests that omeprazole may possess clastogenic activity. For the first assay for chromosomal aberrations in mouse bone marrow conducted by Astra (Study No. 85095), an increased incidence of chromosomal aberrations was observed at the 24 hr sampling interval. However, in the second assay conducted by Merck, methodology deviated significantly from that used in the Astra study as well as published standards in the scientific literature (Mutation Research 189: 157-165, 1987) and the company's own standard operating procedure (see letter from Division to sponsor dated November 30, 1990). The Merck study was deficient with regard to the following parameters: use of weanling animals rather sexually mature animals; use of male animals only rather than animals of both sexes given that gender-related differences in plasma drug levels were known to occur (i.e., plasma drug levels in females were twice those observed in males); and use of less than optimal sampling times (i.e., 6, 24, and 48 hr) given the emphasis on a 12 hr sampling time in standardized methodology.

Additional studies conducted with omeprazole by sponsors, Merck Sharp and Dohme Research Laboratories and AstraZeneca LP, as well as by independent laboratories, described below, confirm the clastogenic activity of this drug as well as possible mutagenic and aneugenic activity. It should be noted that this information is not described in current approved labeling for the prescription drug product.

An in vitro human lymphocyte chromosomal aberration assay was conducted by AstraZeneca LP with omeprazole and it R- and S-enantiomers and submitted to NDA 19,810 in correspondence dated March 29, 2000 (Official Review Date, August 9,

2000). Omeprazole sodium (racemic mixture), the S-enantiomer of omeprazole (H199/18 sodium), the R-enantiomer of omeprazole (H199/19 sodium), and lansoprazole (H225/20) were found to be positive for clastogenic activity in the in vitro human lymphocyte chromosomal aberration assay. These studies were conducted in the absence of metabolic activation. No studies were conducted in presence of metabolic activation. In vitro human lymphocyte chromosomal aberration tests conducted at earlier dates by the sponsor with omeprazole [racemic mixture; IND [redacted] Amendment #185 dated February 13, 1992 (Official Review Date, March 23, 1992)] and its S-enantiomer [IND [redacted] Amendment #059 dated November 10, 1998 (Official Review Date, February 9, 1999)] obtained similar positive results for clastogenic activity.

Published studies in the scientific literature have reported drug-related genotoxic activity as follows: omeprazole bound with DNA in vivo to form either a chemically-labile covalent or non-covalent adduct (Mutagenesis 7: 277-283, 1992); omeprazole produced a positive response with an in vivo unscheduled DNA synthesis assay examining fundic mucosal cells obtained from treated rats, suggesting drug-induced DNA damage (Mutation Research 262: 73-76, 1991; Mutagenesis 6: 3-9, 1991; Mutagenesis 6: 11-18, 1991); omeprazole increased the in vitro frequency of micronucleated cells with two human lymphoblastoid $TK^{+/-}$ cell lines, suggesting clastogenic activity (Mutagenesis 8: 363-372, 1993); omeprazole increased the in vivo frequency of nuclear anomalies, characterized by pyknosis and karyorrhexis (i.e., shrunken nucleus with increased basophilia due to clumping of chromatin and fragmentation of the nucleus), in the forestomach mucosa and descending colon of rats when administered as a single oral dose of 100 mg/kg (Mutagenesis 8: 379-386, 1993); omeprazole increased the incidence of in vivo liver γ-glutamyl transpeptidase positive foci in female rats, consistent with possible promotional activity, when administered by the oral route at 100 mg/kg/day for 14 days following treatment with N-nitrosodiethylamine, a hepatocellular carcinogen activated by CYP2A6 (Mutagenesis 8: 379-386, 1993); omeprazole induced in vivo DNA single-strand scission in the pyloric mucosa of rats when administered as single oral doses ranging from 30 to 500 mg/kg (Mutation Research 368: 1-6, 1996); omeprazole increased the in vitro frequency of micronucleated cells in primary hepatocyte cultures prepared from rats or 1 of 2 human donors, suggesting clastogenic activity (Toxicology 130: 29-41, 1998); omeprazole increased the in vivo frequency of micronucleated cells in hepatocytes obtained from female rats when administered as a single oral dose of 1000 mg/kg (Toxicology 130: 29-41, 1998); omeprazole acted as a promoter to increase the number of aberrant crypt foci, a putative preneoplastic lesion, in the colon of female rats when administered as an oral dose of 10 mg/kg on alternate days over an 8 week period following treatment with azoxymethane, a colon carcinogen; and omeprazole produced an increase in the sister chromatid exchange frequency of peripheral blood lymphocytes when administered to healthy human volunteers at a dose of 20 mg/day for 30 days suggesting possible in vivo clastogenic activity (Gastroenterology 102[4, part 2]: A177, 1992). It should be noted that for some of these published studies, there is significant debate concerning methodologies used and reproducibility of results.

The genotoxic activity of omeprazole raises concerns regarding potential development of tumors. In a 12-month toxicology study with omeprazole in rats, brain astrocytomas were observed in a dose-related manner in male animals, and bronchiolar/alveolar adenomas were found in one male and one female, although, no dose response relationship was evident. The appearance of these tumors within 1 year is extremely unusual in this rat strain suggesting that they are not spontaneous lesions. In the two-year rat carcinogenicity study, ECL cell carcinoids were observed in a dose-related manner in male and female treatment groups. There was no dose threshold for the development of these tumors. ECL cell hyperplasia and the development of gastric carcinoids has been attributed to hypergastrinemia (i.e., an epigenetic mechanism), secondary to inhibition of gastric acid secretion by omeprazole; however, a genotoxic mechanism cannot be completely discounted given the lack of a threshold dose. In addition, an adenocarcinoma, an extremely rare tumor finding, was observed in the stomach for 1 rat in which omeprazole was administered for 1 year followed by a 1-year drug-free recovery period. This finding raises additional concern regarding the genotoxic activity of omeprazole. The mouse carcinogenicity study conducted with omeprazole was judged to be inadequate, as elaborated earlier, and assessment of the carcinogenic potential of omeprazole remains incomplete. Possible development of tumors in tissues other than the stomach remains a significant concern. A lack of a complete understanding of the carcinogenic potential of omeprazole may pose an unacceptable risk to the general public exposed to this drug as an over-the-counter product given its genotoxic activity. The potential for chronic, intermittent usage of this drug product further elevates this risk.

Under current approved labeling for pregnancy, omeprazole is classified as "*Pregnancy Category C*". Current approved labeling is as follows: *"Teratology studies conducted in pregnant rats at doses up to 138 mg/kg/day (approximately 345 times the human dose) and in pregnant rabbits at doses up to 69 mg/kg/day (approximately 172 times the human dose) did not disclose any evidence for a teratogenic potential of omeprazole. In rabbits, omeprazole in a dose range of 6.9 to 69.1 mg/kg/day (approximately 17 to 172 times the human dose) produced dose-related increases in embryo-lethality, fetal resorptions and pregnancy disruptions. In rats, dose-related embryo/fetal toxicity and postnatal developmental toxicity were observed in offspring resulting from parents treated with omeprazole 13.8 to 138.0 mg/kg/day (approximately 35 to 345 times the human dose). There are no adequate or well-controlled studies in pregnant women. Sporadic reports have been received of congenital abnormalities occurring in infants born to women who have received omeprazole during pregnancy. Omeprazole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus."*

Current pregnancy labeling is based upon a Segment I fertility and reproductive performance study in rats, a Segment II teratology studies conducted in rabbits, and a Segment III perinatal and postnatal development study in rats, which were submitted to NDA 19,810 (Official review dates, May 25, 1989 and May 26, 1989) and are described below.

In a Segment I study, the effects of omeprazole administered by the oral route at doses of 0, 13.8, 43.1, and 138 mg/kg/day were assessed on fertility and reproductive performance in male and female Sprague-Dawley rats. Fertility and reproductive performance were apparently unaffected at oral doses ≤ 138 mg/kg/day in rats. However, there was evidence for dose-related fetotoxicity and developmental toxicity with F_1 pups from all treatment groups. There was no evidence of maternal toxicity at any dose.

In a Segment II teratology study, pregnant female rabbits received omeprazole by the oral route of administration at doses of 0, 6.9, 27.6, 69.1, and 138.2 mg/kg/day from days 6 to 18 of gestation. Treatment at 138.2 mg/kg/day had to be discontinued due to severe signs of clinical toxicity. Omeprazole at doses equal to or less than 69.1 mg/kg/day was not teratogenic in female rabbits. Omeprazole at maternally nontoxic doses equal to or less than 69.1 mg/kg/day was disruptive to pregnancy. Further, omeprazole treatment at doses equal to or less than 69.1 mg/kg/day was embryotoxic and fetotoxic as it produced dose-related increases in embryonic deaths/dam and in percent fetal losses, and a dose-related decrease in the number of viable fetuses/dam.

In a Segment III perinatal and postnatal development study, omeprazole was administered by the oral route at doses of 0, 13.8, 43.1, or 138 mg/kg/day to female F_0 Sprague-Dawley rats from day 15 of gestation to day 20 postpartum. Omeprazole produced a dose-related developmental toxicity for F_1 pups in all treatment groups as evidenced by decreased body weights on day 21 postpartum.

In NDA 19,810 Supplement #SE8-058 dated October 7, 1998, the sponsor submitted additional reproductive toxicology studies conducted in rats and rabbits with the intention of providing support for a change in the Pregnancy Category Label from C to B. These additional reproductive toxicology studies with omeprazole revealed evidence of adverse effects not observed in earlier studies as well as confirming previously observed toxic effects (Official Review Date, August 11, 1999). In a Segment II teratology study with rats, omeprazole at oral doses less than or equal to 320 mg/kg/day produced no structural teratogenic effects; however, toxic effects with regard to behavioral development were evident, which had not been described in earlier studies. A Segment III perinatal and postnatal development study conducted in rats using the oral route of administration confirmed earlier observations of postnatal developmental toxicity in offspring resulting from maternal treatment with omeprazole. Two additional Segment III studies, conducted by the intravenous route, also confirmed these earlier observations. These additional studies in the supplement did not change the conclusions of 1989 reviews of reproductive toxicology studies with omeprazole submitted under NDA 19,810. From a preclinical standpoint, it was recommended that omeprazole should remain under Pregnancy Category C.

Prilosec 1 (omeprazole magnesium) is proposed as an over-the-counter drug product available to the general public. Under self-directed use (i.e., without physician supervision), omeprazole magnesium tablets will be administered by the oral route for periods up to 10 days. Preclinical toxicology studies submitted to NDA 19,810, IND

[redacted] IND [redacted] and IND [redacted] as well as the present application were considered in the preparation of this review. A 3-month bridging study using beagle dogs, submitted to the present application, demonstrated that there were no differences in the toxicity profiles between omeprazole magnesium and omeprazole. In 3-month oral toxicology studies, rats received omeprazole and dogs received either omeprazole magnesium or omeprazole. In both rats and dogs, hematological changes were observed, that were indicative of a hypochromic, microcytic anemia. This anemia was related to an impairment of iron absorption produced by the action of omeprazole to increase stomach pH. The target organs of toxicity in rats were the stomach, adrenal glands, kidneys, lungs, and pancreas, and in dogs were the central nervous system and stomach as described in detail earlier.

The proposed treatment regimen with Prilosec 1 will mostly likely result in chronic, intermittent usage of drug for relief and/or treatment of heartburn under circumstances where there is no physician supervision. Omeprazole possesses both in vitro and in vivo genotoxic activity (i.e., clastogenic effects in the in vitro human lymphocyte chromosomal aberration assay, in vivo mouse micronucleus test, and in vivo bone marrow chromosomal aberration assay). Omeprazole was tumorigenic in rats. Preclinical assessment of the carcinogenic potential of omeprazole in a second species remains incomplete. Present human clinical assessment of the tumorigenic potential of omeprazole is insufficient in duration, given the potential latency for development of cancer that may exceed 20 years or more. Further, these human clinical studies were generally focused on assessing development of cancer within the gastrointestinal tract and possessed limited ability for assessment of other tissues. Chronic, intermittent usage of this over-the-counter product is of particular concern given the genotoxicity and tumorigenicity of omeprazole. As compared to over-the-counter indications, use of omeprazole under the prescription setting appears to involve treatment of more serious disorders (i.e., ulcer, gastroesophageal reflux disease, erosive esophagitis, pathological hypersecretory conditions) and requires physician supervision.

RECOMMENDATION:

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9-12-2000
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Comments: Concur

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