CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-056

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-056**
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/20/2006
PRODUCT:	Prilosec (omeprazole magnesium, 2.5 mg or 10 mg) for Delayed Release Oral Suspension
INTENDED CLINICAL POPULATION:	Treatment of patients with [redacted] gastroesophageal reflux disease (GERD), [redacted] healing erosive esophagitis [redacted] conditions in patients 0 to 2 years of age.
SPONSOR:	Astra Zeneca LP, Wilmington, DE.
DOCUMENTS REVIEWED:	Electronic submission of the NDA
REVIEW DIVISION:	Division of Gastroenterology Products (HFD-180)
PHARM/TOX REVIEWER:	Sushanta Chakder, Ph.D.
PHARM/TOX SUPERVISOR:	Jasti B. Choudary, B.V.Sc., Ph.D.
ACTING DIVISION DIRECTOR:	Daniel Shames, M. D.
PROJECT MANAGER:	Ryan Barraco

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Date of review submission to Division File System (DFS): 8/9/07

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Executive Summary

I. Recommendations

A. **Recommendation on Approvability:** From a preclinical standpoint, the NDA application is approvable.

B. **Recommendation for Nonclinical Studies:** None.

C. **Recommendations on Labeling:** No changes in the preclinical section of the current labeling of Prilosec are recommended.

II. Summary of Nonclinical Findings

A. Brief overview of nonclinical findings:

Omeprazole belongs to a class of compounds, the substituted benzimidazoles, that suppress gastric acid secretion by specific inhibition of the H^+/K^+-ATPase (the proton pump), resulting in an inhibition of gastric acid secretion. H^+/K^+-ATPase is located in the secretory membranes of the parietal cells in the gastric oxyntic mucosa. When omeprazole reaches the canalicular space of the gastric parietal cell, it is protonated, activated to the reactive sulfonamide, and covalently bonded to the luminal surface of the proton pump to inhibit the pump activity. The primary pharmacodynamic effects of omeprazole have been demonstrated in both *in vitro* and *in vivo* studies. The binding of omeprazole to H^+/K^+-ATPase is irreversible in nature, and effectively inhibits acid secretion until new enzyme is synthesized.

Omeprazole (Prilosec) is approved in the U.S since 1988, and indicated for the treatment of variety of gastric acid-related disorders in adults and children aged 2 years and older. Under NDA 22-056, the sponsor is seeking approval of a new oral formulation of omeprazole, Prilosec (omeprazole magnesium) For Delayed-Release Oral suspension (2.5 mg and 10 mg; also called the sachet formulation or omeprazole sachet) for use in children 0 to 2 years of age. The omeprazole sachet formulation was developed to fulfill the post-marketing commitment to develop an age-appropriate formulation for pediatric patients 0 to 2 years of age (July 12, 2002 approval letter for NDA 19-810/S-074). The sponsor conducted several toxicology studies in juvenile animals with omeprazole in support of the pediatric use of the drug. The studies include a single-dose toxicity study, repeat dose toxicity and toxicokinetic studies in rats, and a carcinogenicity study in $p53^{+/-}$ heterozygous mice. In addition, in a recent toxicity/toxicokinetic study in neonatal/juvenile rats with esomeprazole (study # 900404, 2006), a group of animals received a high dose (140 mg/kg) of omeprazole as a comparator.

In a single-dose toxicity oral study in juvenile rats (10 to 13-day-old) with omeprazole (190 to 2100 mg/kg), the clinical signs observed included reduced motor activity, reduced respiratory frequency, dyspnea, cyanosis, reduced or absent righting reflex, tremor and convulsions. Similar clinical signs were observed in previous toxicology studies with omeprazole in adult animals. In a 1-month oral toxicity study in juvenile rats (14 to 15 days

old), omeprazole was administered at doses of 14, 31, 69 or 140 mg/kg/day. High dose animals had slightly lower body weight gain, and slightly decreased hemoglobin and hematocrit value and an increase in reticulocyte levels were observed in all omeprazole-treated groups. White blood cell counts were decreased between 15 and 30% for all male treatment groups and females receiving 69 and 140 mg/kg/day doses. Liver hyperplasia, lymphoid cell infiltration and congestion were observed at 140 mg/kg/day. A dose-related increase in the stomach weight was observed in the treated animals; however, no changes in the ECL cells in the gastric mucosa were observed in any group. In a 1-month oral toxicity study in neonatal rats (age, 7 days) with esomeprazole, one group of animals received omeprazole at a dose of 140 mg/kg. No treatment-related effects on the functional observation or behavioral performance were observed. Increased stomach weights, increased serum gastrin levels and increased ECL cell volume fraction and profile density were observed in animals receiving omeprazole. Thus, the toxicological effects observed in neonatal rats were similar to those observed in adult animals, and no additional target organs of toxicity were identified.

B. Pharmacologic Activity:

Omeprazole suppresses gastric acid secretion by specific inhibition of the enzyme, H^+, K^+-ATPase at the surface of the gastric parietal cells. Nonclinical pharmacology studies show that omeprazole is a potent and long-lasting inhibitor of gastric acid secretion. The binding of omeprazole to H^+/K^+-ATPase is irreversible in nature, and effectively inhibits acid secretion until new enzyme is synthesized.

C. Nonclinical Safety Issues Relevant to Clinical Use: None

PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

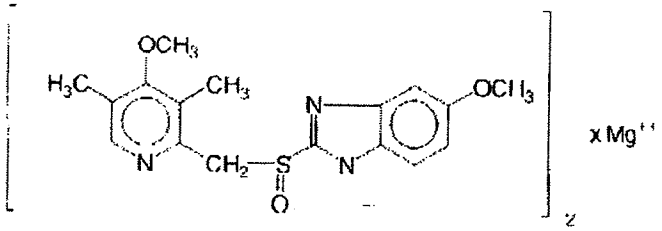
NDA number: 22-056
Review number: 01
Sequence number/date/type of submission: 000/Original/December 20, 2006
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Astra Zeneca LP, Wilmington, DE.
Manufacturer for drug substance: Astra Zeneca LP.

Reviewer name: Sushanta Chakder, Ph.D.
Division name: Division of Gastroenterology Products
HFD #: 180
Review completion date: August 09, 2007

Drug:

Trade name: Prilosec for Delayed Release Oral Suspension
Generic name: Omeprazole magnesium
Code name: N/A
Chemical name: Di-(R,S)-5-methoxy-2[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium.
CAS registry number: 73590-58-6
Molecular formula/molecular weight: $C_{34}H_{36}N_6O_6S_2Mg/713.1$

Structure:



Relevant INDs/NDAs/DMFs:
IND —— Astra Zeneca LP, Wayne, PA. **b(4)**
IND 53,733, Astra Zeneca LP, Wayne, PA.
NDA 19-810, Astra Zeneca LP, Wayne, PA.
NDA 21-229, Astra Zeneca LP, Wayne, PA.

Drug class: Gastric parietal cell H^+, K^+-ATPase (Proton pump) inhibitor.

Intended clinical population: Omeprazole magnesium delayed release suspension is indicated for short term treatment of patients with [redacted], gastroesophageal reflux disease GERD), [redacted] healing of erosive esophagitis [redacted]. b(4)

Clinical formulation: Prilosec Sachets (2.5 mg and 10 mg) consist of omeprazole pellets and excipient granules filled into single use aluminium sachets. The total content of a sachet, i.e., one dose is added to water to form a viscous suspension prior to use. The composition of 2.5 mg and 10 mg sachets is shown in the Table below.

Table 1 Composition of Prilosec Sachets

Components (name according to AstraZeneca)	Quantity (mg/sachet) 2.5 mg strength	Quantity (mg/sachet) 10 mg strength	Function	Standard
Omeprazole pellets[a]				
Omeprazole (corresponding to omeprazole magnesium[b])	2.5	10	Active substance	AstraZeneca
Glyceryl monostearate	[redacted]			
Hydroxypropyl cellulose				
Hydroxypropyl methylcellulose				
[redacted]				
Magnesium stearate				
Methacrylic acid copolymer type C				
Polysorbate	[redacted]			
[redacted]				
Sugar spheres,				
[redacted]				
Talc				
Triethyl citrate				
[redacted]				
Weight of omeprazole pellets				
Excipient granules				
[redacted]				
Xanthan gum				
[redacted]				
Citric acid	[redacted]			
Iron oxide	[redacted]			
Hydroxypropyl cellulose				
[redacted]				
Weight of excipient granules				
Total weight in sachet				

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Route of administration: Oral

Studies reviewed within this submission: The sponsor did not submit any preclinical study reports under the current NDA. However, the sponsor provided the summaries of previously submitted studies with omeprazole in neonatal/juvenile animals. These studies include: (a) ADME studies in juvenile rats, (b) single-dose oral toxicity study in juvenile rats, (c) preliminary dose finding study in juvenile rats, (d) one-month oral toxicity study in juvenile rats, (e) one-month oral toxicity study in neonatal rats with esomeprazole in which omeprazole was used as a comparator, and (f) carcinogenicity study in p53+/- heterozygous mice. The studies were reviewed under earlier submissions and the reviews of these studies are incorporated. In addition, the sponsor provided toxicological qualification of the potential degradation products of omeprazole.

2.6.2 PHARMACOLOGY

No Pharmacology study reports were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:

Plasma Concentrations of Omeprazole in Young Rats After Oral Administration (Report No. T2757/Study No. 92072).

Methods: This study examined omeprazole plasma levels following oral administration to 12-14 day old rats (immature animals), 23-25 day old rats (newly weaned animals), and 44-46 day old rats (young adults). Each of the three age groups was treated with 14 and 69 mg/kg (40 and 200 μmole/kg) omeprazole for 2 days. Blood samples were collected before drug administration, and at 15 and 60 min after dosing on the second day of treatment. Controls were

included for each age group; although, blood samples were only collected at 15 min after dosing on the second day of treatment requiring groups composed of 3 rats/sex. The vehicle used for test formulations was a hydroxypropyl methyl cellulose solution, and the drug was administered by oral gavage. Plasma concentrations of omeprazole in young rats were determined by liquid chromatography. The limit of quantitation for this assay was 25 ng/mL (0.080 μmole/L) of plasma.

Results: One female in the 44-46 day old age group that received 69 mg/kg, died in connection with blood sampling taken at 15 min after the dose. No clinical signs or adverse effects were observed in any other animals during dosing or blood sampling. No significant changes in body weight or weight gain were observed between control and treatment groups. Omeprazole plasma levels were significantly higher in 12-14 day old rats as compared with older rats (23-25 and 44-46 days of age). This age-related effect may be related to differences in metabolism and/or absorption of omeprazole. Plasma concentrations were proportional to dose for the 12-14 and 44-46 day old rats; although, this was not found for the 23-25 day old rats for unknown reasons.

Table 1. Mean omeprazole plasma levels (μg/mL) in rats of different ages treated with 14 mg/kg omeprazole administered by oral gavage.

Age (Days)	15 min after dosing		60 min after dosing	
	M	F	M	F
12 to 14	7.59	4.90	3.81	3.62
23 to 25	0.39	0.55	0.09	0.062
44 to 46	0.15	-	0.47	0.028

Table 2. Mean omeprazole plasma levels (μg/mL) in rats of different ages treated with 69 mg/kg omeprazole administered by oral gavage.

Age (Days)	15 min after dosing		60 min after dosing	
	M	F	M	F
12 to 14	30.08	36.86	13.53	17.90
23 to 25	0.275	0.887	0.036	0.038
44 to 46	0.981	2.97	0.086	0.459

Plasma drug levels were examined following oral administration of 14 or 69 mg/kg/day omeprazole for 2 days to 12-14 day old rats (immature animals), 23-25 day old rats (newly weaned animals), and 44-46 day old rats (young adults). Omeprazole plasma levels were significantly higher in 12 to 14 day old rats as compared with older rats (23-25 and 44-46 days of age). This age-related effect may be related to differences in metabolism and/or absorption of omeprazole. Young rats (12-14 days) may have immature liver function and are unable to significantly metabolize omeprazole as contrasted to adult rats, where the drug undergoes a significant first pass effect.

2.6.5 TOXICOLOGY

Single Dose Toxicity of Omeprazole in Young Rats after Oral Administration (Report No. T2755/Study No. 92021).

Testing Laboratory: ——— b(4)

Study Started: March 25, 1992

Study Completed: January 13, 1994

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

Animals: 10 to 13 day old male and female Sprague Dawley rats at the initiation of dosing. Initial mean body weights were 25.5 to 29.2 grams for males and 25.4 to 27.7 grams for females.

Drug Batch: Omeprazole, Batch 599.

Methods: This study examined the single dose toxicity of omeprazole administered by oral gavage to young rats (10-13 days of age). Dose levels were 0, 190, 260, 380, 520, and 2100 mg/kg (0, 540, 750, 1100, 1500, and 6000 μmole/kg). The vehicle used for test formulations was a hydroxypropyl methyl cellulose solution. The volume administered was 10 mL/kg. Pregnant females were obtained and allowed to give birth in the laboratory. Animals were treated when they were 10-13 days of age. Each group consisted of 6 males and 6 males from 3 different litters (2 males and 2 females from each litter). Animals were observed for clinical signs and mortality at least once daily for 21 days after dosing. Animals were weighed prior to dosing and surviving animals were weighed daily throughout the study. Dams were removed 4 to 6 hr prior to dosing, thus animals were fasted before dosing. The dams were returned to the young animals at 0.5 to 1 hr after treatment. Food consumption was not measured. The behavior of the mother towards the young was monitored following treatment. All animals were autopsied following death/sacrifice or at termination. The autopsy encompassed abdominal, thoracic, and cranial cavities, with their respective organs and coatings.

1. **Observed Effects**: Reduced motor activity, reduced respiratory frequency, tremor, and/or intermittent clonic/tonic were observed at all dose levels. In conjunction with convulsions or a marked reduction in motor activity, dyspnea and cyanosis were noted at all dose levels. The incidence, degree, and duration of these clinical signs increased with increasing dose. Reduced motor activity and reduced respiratory frequency were observed within 30 min for the 380, 520, and 2100 mg/kg groups and 3 to 5 hr for the 190 and 260 mg/kg groups. These signs continued for 1 to 2 days after treatment. Convulsions and/or tremors were found within 30 min for the 2100 mg/kg group and 2 to 5 hr at other dose levels. These signs continued for 6 to 12 hr, or until death.

2. **Mortality**: Mortality was observed at 260, 380, 520, and 2100 mg/kg as noted in Table 3 below. Death for several animals in the 380, 520, and 2100 mg/kg groups occurred within 3 to 22 hr after treatment. One male in the 260 mg/kg group and several others in the 380 and 520 mg/kg group were sacrificed on days 1 and 2.

Table 3. Incidence of mortality and convulsion for 10 to 13 day old rats treated with a single oral dose of omeprazole (Adapted from Sponsor's Table on page 15 in Study No. 92021).

Dose mg/kg	Number of animals with convulsions		Number of animals that died or were sacrificed.	
	M	F	M	F
0	0/6	0/6	0/6	0/6
190	1/6	0/6	0/6	0/6
260	0/6	0/6	1/6	0/6
380	5/6	3/6	6/6	6/6
520	3/6	5/6	6/6	6/6
2100	6/6	6/6	6/6	6/6

3. **Body weight**: Mean body weights for male controls on days 1 and 21 were 29.2 and 137 grams, respectively. Weight gain for the male 190 and 260 mg/kg groups were 94 and 91.9% of the control, respectively. Mean body weights for female controls on days 1 and 21 were 27.7 and 126 grams, respectively. Weight gain for the female 190 and 260 mg/kg groups were 97.2 and 88.4% of the control, respectively.

4. Histopathology: Pale livers were observed for several animals in the 260, 380, and 520 mg/kg groups that died or were sacrificed on days 0 to 2. For the 2100 mg/kg group, all animals died within 6 hr of treatment with no macroscopic indications of effects on the liver. Microscopic examination of these livers from animals in the 260, 380, and 520 mg/kg groups found slight atrophy with decreased amounts of hepatocellular cytoplasm. The cytoplasm had a slightly foam or vacuolated appearance. This effect was probably related to starvation in these animals.

Young rats (10-13 days old at the initiation of the study) were treated with a single dose of omeprazole administered by oral gavage at dose levels of 0, 190, 260, 380, 520, and 2100 mg/kg. Mortality occurred at the 260, 380, 520, and 2100 mg/kg. Reduced motor activity, reduced respiratory frequency, tremor, and/or intermittent clonic/tonic were observed at all dose levels. The minimum lethal dose for 10-13 day old rats was 260 mg/kg as compared to 2600 mg/kg for adults. The maximum nonlethal dose for 10-13 day old rats was 190 mg/kg as compared with 2210.7 mg/kg for adult rats. These differences in toxicity between young and adult rats may be related to the significantly higher plasma drug levels found in young animals as compared with adults receiving identical dosages.

Dose Finding Study of Omeprazole Given Orally to Young Rats for Up to 8 days (Report No. T2756/Study No. 92037).

Testing Laboratory: ——— b(4)

Study Started: May 26, 1992

Study Completed: January 12, 1994

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

Animals: Male and female Sprague Dawley rats with age ranges of 11 to 12 days and 13 to 14 days. Mean body weights for males ranged from 23.9 to 26.9 grams and females ranged from 24.3 to 25.8 grams.

Drug Batch: Omeprazole, Batch 599.

Methods: This study examined the toxicity of omeprazole given by oral administration to young rats (11-12 days of age) at doses of 69 and 140 mg/kg/day (200 and 400 μmole/kg/day) for 8 days. A further two groups of rats (13-14 days of age) were treated with 140 and 210 mg/kg (400 and 600 μmole/kg/day) for 2 days. The rats from the two dosing experiments were observed for 7 and 6 days following the last day of drug administration. This study was intended to identify dosages for use in a subsequent 1 month toxicity study with young rats. The vehicle used for test

formulations was a hydroxypropyl methyl cellulose solution. The volume administered was 5 mL/kg. For the 210 mg/kg group, the administration volume was 7.5 mL/kg. Pregnant females were obtained and allowed to give birth at the laboratory. Animals were treated when they were 11-14 days of age. Each group consisted of 6 rats/sex from 3 different litters (2 rats/sex from each litter). Animals were observed frequently during the first 2 to 3 days of treatment for clinical signs, and then daily for the remaining period of the study. All animals were weighed immediately prior to the start of dosing and then daily throughout the remaining period of the study. Food and water consumption were not measured. All animals were autopsied following death or at termination. All animals were autopsied following death/sacrifice or at termination. The autopsy encompassed abdominal, thoracic, and cranial cavities, with their respective organs and coatings.

Results:

1. **Observed Effects**: One animal in the 69 mg/kg/day group displayed signs of respiratory distress and convulsions for approximately 2 min after treatment on day 1. No other effects were found in 69 mg/kg/day group for the remaining period of the study. One animal (11-12 days old) in the 140 mg/kg/day group was found with reduced motor activity and intermittent clonic convulsions within 3 hr of dosing on the first day of treatment. Reduced motor activity as well as reduced respiratory frequency persisted in this animal for another 4 hr.

2. **Mortality**: One male (11-12 days old) in the Control group was found dead on day 2. Histopathological analysis found pleuritis and pericarditis in this animal. One male (13-14 days old) in the 140 mg/kg/day group, following treatment on days 0 and 1, was found dead on day 3. Histopathological analysis found bronchopneumonia in this animal. One male and one female (13-14 days old) in the 210 mg/kg/day group, both displaying significant clinical signs, were found dead on day 0 (5 hr after treatment) and day 1 (23 hr after treatment), respectively. Clinical signs for these two animals in the 210 mg/kg/day group included reduced motor activity and reduced respiratory frequency occurring from 1.5 to 3 hr after treatment until death.

3. **Body Weight**: Mean body weights for the male control group on days 0 and 8 were 26.9 and 48.0 grams, respectively. Weight gains for the male 69 and 140 mg/kg/day groups following 8 days of treatment were 105.7 and 93.4% of the control, respectively. Mean body weights for the female control group on days 0 and 8 were 25.8 and 45.7 grams, respectively. Weight gains for the female 69 and 140 mg/kg/day groups following 8 days of treatment were 110.0 and 89.4% of the control, respectively.

Young rats (11-12 days of age) received omeprazole by oral administration at doses of 69 and 140 mg/kg/day for 8 days. Further, two groups of rats (13-14 days of age) were treated with 140 and 210 mg/kg/day for 2 days. Treatment-related mortality was observed for 2 animals in the 210 mg/kg/day group. One animal (11-12 days old) in the 140 mg/kg/day group was found with reduced motor activity and intermittent clonic convulsions within 3 hr of dosing on the first day of treatment as well as reductions in motor activity and respiratory frequency that persisted for 4 hr; however, no further signs were found in this or any other animal of this group. One animal in the 69 mg/kg/day displayed brief signs of respiratory distress and convulsions on day 1; however, no further signs were found in this or any other animal of this group. Based upon these results, the sponsor chose doses of 14, 31, 69, and 140 mg/kg/day for the 1 month study.

General Toxicity of Omeprazole Given Orally to Young Rats for 1 Month (Report No. T2793/Study No. 92119).

Testing Laboratory: ——— b(4)

Study Started: February 11, 1993

Study Completed: April 11, 1994

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

Animals: Male and female Sprague Dawley rats were 14 to 15 days old at the initiation of treatment. Mean body weights for males ranged from 36.0 to 37.2 grams and females ranged from 34.4 to 36.5 grams.

Drug Batch: Omeprazole, Batch 599.

Methods: This study examined the oral toxicity of omeprazole given by daily oral gavage to young rats (14-15 days old when dosing commenced) at 0, 14, 31, 69, and 140 mg/kg/day (0, 40, 90, 200, and 400 μmole/kg/day) for 1 month. The vehicle used for test formulations was a hydroxypropyl methyl cellulose solution. The volume administered was 5 mL/kg. Each group consisted of 12 rats/sex from 6 different litters. Pregnant females were allowed to give birth at the laboratory. The litters (standardized to 2 rats/sex before the initiation of treatment) were housed with their mothers until 25 days of age. Animals were observed for clinical signs frequently during the initial 3 days of treatment and then daily throughout the remaining period of the study. A comprehensive clinical examination of each animal was performed once a week. Animals were monitored for mortality twice a day. Animals were weighed daily during the initial 14 days of the study and then three times a week for the remaining period of the study.

Measurement of food consumption started on experimental day 14 (3 days after separation of the young from their mothers) and consisted of recording consumption two to three times per week for the animals in each cage. Water consumption was measured 2-3 times per week beginning on experimental day 14. The mean daily food and water consumption per animal was calculated for each group and sex. Urine and blood samples were collected after 3 weeks of treatment, when animals were 36 to 38 days old. Urine samples were collected overnight with 2 animals from each cage placed in a metabolism cage. Water consumption was recorded and urine was collected under refrigeration. Blood samples were collected the following day from the orbital plexus. At the end of the treatment period, rats were anesthetized with pentobarbital and exsanguinated. Autopsy encompassed the abdominal, thoracic, and cranial cavities with their respective organs and coatings. Tissue samples for microscopic analysis were taken from all rats.

Additional groups treated with 14 and 69 mg/kg/day were included for toxicokinetic analysis. These groups consisted of 4 rats/sex from 2 different litters. Blood samples were collected from animals in the toxicokinetic groups following 10 days of treatment when animals were 24-25 days old. Samples were taken 15 min after dosing. In addition, blood samples for measurement of plasma drug levels were collected from all primary study animals following 1 month of treatment, when animals were 42-43 days old. These samples were collected at 0 and 15 min after dosing from 4 rats/sex of each group. Plasma concentrations of omeprazole in young rats were determined by liquid chromatography. The limit of quantitation was 39.05 ng/mL (125 nmole/L) of plasma.

Results:

1. **Observed Effects**: One male in the 140 mg/kg/day group showed piloerection, decreased motor activity, and abdominal respiration, 30 min after treatment on day 1. No other signs were observed throughout the remaining period in the study.

2. **Mortality**: Two male controls (day 22 for blood chemistry), 1 male in the 69 mg/kg/day group (day 28 for plasma drug levels), and 1 male in the 140 mg/kg/day group (day 28 for plasma drug levels) died during anaesthesia for blood sampling. One male in the 31 mg/kg/day was sacrificed on day 8 (22-23 days of age), and 1 female in the 31 mg/kg/day was found dead on day 12 (26-27 days of age), about 10 min after treatment. The deaths of these two animals in the 31 mg/kg/day group appeared to be related to gavage error. One male in the 140 mg/kg/day group was found dead on day 3 at an age of 17-19 days; the cause of death was not explained by the sponsor. One female in the 14 mg/kg/day satellite group was found dead on day 3; the cause of death was not established.

3. Body Weight, Food Consumption, and Water Consumption: There were no significant effects on body weight gain, food consumption, or water consumption for male and female rats treated with omeprazole for 1 month. Body weights for male controls on days 0 and 28 were 36.3 and 207.7 grams, respectively. Body weight gains for the male 14, 31, 69, and 140 mg/kg/day groups were 102.9, 95.9, 96.3, and 94.2% of the male control, respectively. Body weights for the female controls on days 0 and 28 were 35.5 and 165.2 grams, respectively. Body weight gains for the female 14, 31, 69, and 140 mg/kg/day groups were 101.3, 100.4, 99.7, and 96.3% of the female control, respectively. Slight increases of food consumption (<10%) during weeks 2 and 3 were observed for the male 14 mg/kg/day group and the female 14 and 31 mg/kg/day groups. Water consumption for the female 14 mg/kg/day group was increased to 115% of the control (23.8 mL/animal/day).

4. Hematology:

A. Males: White blood cell counts for the male 14, 31, 69, and 140 mg/kg/day groups were decreased in a dose-related manner to 84.6, 74.3, 70.5, and 67.9% of the control (7.8 x 10^9 cells/L), respectively.

Red blood cell counts for the male 31, 69, and 140 mg/kg/day groups were increased to 104.9, 108.2, and 104.9% of the control (6.1 x 10^{12} cells/L), respectively; however, changes were small and no dose response relationship was evident. Decreases of hematocrit, hemoglobin content, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC) were observed sporadically in all male treatment groups; however, changes were generally <10% and the dose response relationship was relatively flat. The sponsor has suggested that these changes may be indicative of a hypochromic, microcytic anemia. This would be classified as a very mild anemia.

B. Females: White blood cell counts for the female 69 and 140 mg/kg/day were both decreased to 73.1% of the control (7.8 x 10^9/L).

As noted for males above, decreases of hemoglobin content, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC) were observed sporadically in all female treatment groups; although, changes were generally <10% and the dose response relationship was relatively flat. These changes may be indicative of a hypochromic, microcytic anemia.

5. Blood Chemistry and Urinalysis:

A. Male Blood Chemistry: Urea levels for the male 14, 31, 69, and 140 mg/kg/day were increased to 124.3, 117.1, 125.7, and 114.3% of the control (7.0 mmol/L), respectively; although, the dose response relationship was relatively flat. Creatinine levels for the male 140 mg/kg/day group were decreased to 92.1% of the control (38 µmole/L). Alkaline phosphatase activity for the male 140 mg/kg/day group was increased to 118.6% of the control (4.3 µkat/L). Phosphate levels for the male 14, 31, 69, and 140 µmole/kg/day groups were all decreased to 89.7-92.3% of the control (3.9 mmol/L). Magnesium levels for the male 14, 31, 69, and 140 mg/kg/day groups were all increased to 107.2-111.3% of the control (0.97 mmol/L). Small and sporadic changes (<10%) of alanine aminotransferase activity, total protein, the albumin to globulin ratio, Na^+, Cl^-, and Ca^{2+} were observed for the male treatment groups. Changes of urea and electrolytes could be reflective of kidney damage. Changes in alkaline phosphatase and alanine aminotransferase may be indicative of liver damage observed in the 140 mg/kg/day group.

B. Female Blood Chemistry: Glucose levels for the female 69 and 140 mg/kg/day groups were increased to 115.1 and 114.0% of the control (8.6 mmole/L), respectively. Urea levels for the female 14, 31, 69, and 140 mg/kg/day groups were increased to 120.9, 132.8, 122.4, and 137.3% of the control (6.7 mmole/L), respectively; although, the dose response relationship was relatively flat. Total protein levels for the female 31, 69, and 140 mg/kg/day groups were increased to 103.3-105% of the control (60 g/L). Albumin levels for the female 31, 69, and 140 mg/kg/day group were all increased to 103.4% of the control (29 g/L); although, only the 140 mg/kg/day group was different. Potassium levels for the female 14, 31, 69, and 140 mg/kg/day groups were increased to 106.4-108.5% of the control (4.7 mmole/L); although, only the 69 and 140 mg/kg/day groups were different. Magnesium levels for the female 14, 31, 69, and 140 µmole/kg/day groups were increased to 103.8-111.4% of the control (1.05 mmole/L), respectively; although, the 69 mg/kg/day was not different. Changes of urea and electrolytes could be reflective of kidney damage.

C. Male Urinalysis: There were no significant differences in urinalysis (i.e., volume, pH, solute particles, protein, glucose, methylketone, hemoglobin, bilirubin, erythrocytes, leukocytes, epithelial cells, and casts) between male control and treatment groups.

D. Female Urinalysis: There were no significant differences in urinalysis between female control and treatment groups.

6. Vital Signs, Physical Examination, and Ophthalmic Examinations: No measurements were reported.

7. Organ Weights: Changes in absolute and relative organ weights were observed for several organs (i.e., the heart, stomach, thymus, lungs, and kidneys); however, histopathological changes were principally confined to the liver, thymus, and lungs in the 140 mg/kg/day group.

Liver: The relative liver weight for the male 140 mg/kg/day group was increased to 108% of the control (5.0%).

Thymus: Absolute thymus weight for the male 69 and 140 mg/kg/day groups were decreased to 87.4 and 83.0% of the control (0.887 g); although, only the 140 mg/kg/day group was different. Absolute thymus weight for the female 69 and 140 mg/kg/day groups were decreased to 81.6 and 91.1% of the control (0.678 g); although, only the 140 mg/kg/day group was different. The relative thymus weight for the 140 mg/kg/day group was decreased to 85.7% of the control (0.42%).

Lung: Relative lung weights for the female 31, 69, and 140 mg/kg/day groups were increased to 114.5, 109.7, and 111.3% of the control (0.62 g), respectively.

Heart: Relative heart weights for the male 31, 69, and 140 mg/kg/day groups were increased to 111.4, 113.6, and 111.4% of the control (0.44%), respectively. Relative heart weights for the female 14, 31, 69, and 140 mg/kg/day groups were increased to 108.7, 108.7, 115.2, and 110.9% of the control (0.46%), respectively. Increased heart weights may be related to an iron deficient anemia; however, there were no correlative histological changes.

Stomach: Absolute stomach weights for the male 69 and 140 mg/kg/day groups were increased to 106.8 and 107.4% of the control (1.48 g), respectively; although, only the 140 mg/kg/day group was significantly different. Relative stomach weights for the male 69 and 140 mg/kg/day groups were increased to 111.1 and 115.3% of the control (0.72%), respectively. Absolute stomach weights for the female 14, 31, 69, and 140 mg/kg/day groups were increased to 106.2-110.2% of the control (1.28 g); although, only the 140 mg/kg/day was significantly different. Increased stomach weights were probably caused by an omeprazole-induced increased in gastrin release as observed in adult rat studies.

Kidney: Relative kidney weights for the male 14, 31, 69, and 140 mg/kg/day groups were increased to 104.7, 109.4, 109.4, and 107.1% of the control (0.85%), respectively.

8. **Gross Pathology:** For the male 140 mg/kg/day group, 1 of 12 animals was reported with solidifications in the lung and another was reported with a pale discoloration of the liver. For the female 31 mg/kg/day group, 1 of 12 animals was reported with a kidneys of reduced size that had a pale discoloration.

9. **Histopathology:** Bone marrow hyperplasia was observed in the 31, 69, and 140 mg/kg/day groups. For the 31 mg/kg/day group, only 1 of 24 animals was found to have bone marrow hyperplasia and the grade was only 1. Bone marrow hyperplasia may have been a compensatory response to alterations in white and red blood cell parameters. For the liver at 140 mg/kg/day, hyperplasia, lymphoid cell infiltration, and congestion were observed, and may correspond to elevations of alkaline phosphatase and alanine aminotransferase. There were no significant pathological changes in the gastric mucosa, which have been reported in adult rat studies.

Table 4. Percentage Incidence of Histopathological Findings for Rats treated with 0, 14, 31, 69, and 140 mg/kg/day omeprazole by oral gavage for 28 days.

Histopathological Findings	0		14		31		69		140	
	M	F	M	F	M	F	M	F	M	F
Bone marrow, sternal										
-hyperplasia/granulocytic	0	0	0	0	0	1	2	0	2	2
-hyperplasia/erythroid	0	0	0	0	0	1	2	0	2	2
-hemorrhage	0	0	0	0	0	0	0	1	0	0
Stomach										
-parietal cell degeneration	0	0	0	0	0	0	1	0	0	0
-lymphoid follicular enlargement	0	1	0	0	0	0	2	0	0	1
-congestion	0	1	0	0	0	0	0	0	0	0
Liver										
-hyperplasia	0	0	0	0	0	0	0	0	1	0
-lymphoid cell infiltration	1	0	0	0	0	0	0	0	2	0
-congestion	0	0	0	0	0	1	0	0	1	0
Thymus										
-Atrophy	0	0	0	0	1	0	0	0	1	0
Kidney										
-pelvic dilation	0	3	0	0	0	1	3	1	1	0
-congestion	2	0	0	0	0	1	0	0	1	0
-tubular hyperplasia	0	0	0	0	0	0	0	1	0	0
-renal hypoplasia	0	0	0	0	0	1	0	0	0	0
Lungs										
-lymphoid hyperplasia	0	0	0	0	0	0	0	0	1	0
-bronchopneumonia	0	0	0	0	0	0	0	0	1	0
-granuloma	0	0	0	0	0	0	0	0	1	0
-atelectasis	2	0	0	0	0	0	1	0	0	0
-congestion	2	0	0	0	0	1	1	0	1	0

10. Drug Plasma Levels: Plasma levels of omeprazole were approximately proportional to dose on experimental days 10 and 28, 15 min after dosing. Plasma levels tended to be higher in females than males. Analysis of plasma drug levels in 42-43 day old rats on experimental day 28, 15 min before dosing found no detectable levels of omeprazole.

Table 5. Plasma levels of omeprazole in young rats (24-25 days old) on experimental day 10, 15 minutes after dosing.

Dose mg/kg/day	Plasma omeprazole levels, μg/mL (mean ± standard deviation)	
	Male	Female
14	290.5 ± 174.9	343.6 ± 203
69	1387 ± 1334	4467 ± 4623

Table 6. Plasma levels of omeprazole in young rats (42-43 days old) on experimental day 28, 15 minutes after dosing.

Dose mg/kg/day	Plasma omeprazole levels, μg/mL (mean ± standard deviation)	
	Male	Female
0	0	0
14	187 ± 106	265 ± 78.1
31	240 ± 87.5	1118 ± 787
69	1484 ± 587	1921 ± 1403
140	2109 ± 1709	4873 ± 3155

Omeprazole was given by oral gavage to young rats (14 to 15 days old when dosing commenced) at 0, 14, 31, 69, and 140 mg/kg/day for 1 month. The no effect level was 31 μmole/kg/day. One male in the 140 mg/kg/day group died on day 3 of unexplained causes. White blood cell counts were decreased between 15 and 30% for all male treatment groups and the female 69 and 140 mg/kg/day groups. Bone marrow hyperplasia was found in the 31, 69, and 140 mg/kg/day groups and may have been a compensatory response to changes in white and red blood cell parameters. Absolute and relative thymus weights were decreased in the 69 and 140 mg/kg/day groups and may be indicative of a stress response to omeprazole treatment. Liver hyperplasia, lymphoid cell infiltration, and congestion were observed at 140 mg/kg/day. Plasma levels of omeprazole were approximately proportional to dose on experimental days 10 and 28, 15 min after dosing. Plasma levels tended to be higher in females than males.

Esomeprazole Magnesium: 1-Month Oral (Gavage) Toxicity Study in the Neonatal Sprague-Dawley Rat
(900404)

Testing Laboratories: [redacted] b(4)

[redacted]

Study Start and Completion Date: May 17, 2004 and February 28, 2006

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

Animals:
Male: 13.9-14.6 g, 7 days old
Female: 13.4-13.8 g, 7 days old
Harlan Sprague-Dawley (Hsd:SPRD.SD®)

Drug Batch No.: 800/02

Methods: The toxicity of H 199/18 magnesium was characterized in neonatal rats. There are three phases in this study. In the phase I study, three groups of rat pups were given esomeprazole (0, 93 or 280 mg/kg) or omeprazole (140 mg/kg) orally, once daily, for 28 days, from Day 7 to Day 34 *post partum*. The study design for phase I was summarized in Tables 3-4 in this report. These tables are attached below.

Table 3 **Groups and dose levels: Phase I, main study and recovery animals**

Group	Subset[a]	No of animals	Animal reference numbers	Treatment[b]	Daily dose levels	
					µmol/kg	mg/kg
Main study animals (subset A)						
1	A	10M	1001-1010	Vehicle control	0	0
		10F	1501-1510			
2	A	10M	2001-2010	Esomeprazole	270	93
		10F	2501-2510			
3	A	11M[c]	3001, 3003, 3005, 3007-3010, 3052-3055[d]	Esomeprazole	800	280
		11F[c]	3501, 3503, 3505, 3507-3510, 3552-3555[d]			
4	A	10M	4001-4010	Omeprazole	400	140
		10F	4501-4510			
Recovery animals (subset B)						
1	B	10M[e]	1011-1020	Vehicle control	0	0
		10F	1511-1520			
2	B	10M	2011-2020	Esomeprazole	270	93
		10F	2511-2520			
3	B	10M	3011, 3013, 3015, 3017-3020, 3062-3064[d]	Esomeprazole	800	280
		10F[e]	3511, 3513, 3515, 3517-3520, 3562-3564[d]			
4	B	10M	4011-4020	Omeprazole	400	140
		10F	4511-4520			

a One pup/sex/litter from 10 separate litters was randomly assigned to subsets A, B, C and D as illustrated in Appendix A (Assignment to groups). The animals in subset A were treated for 1 month and then euthanised the day after the final dose (on Day 35 *post partum*). Those in subset B were also dosed for 1 month, but were allowed a 3-month recovery period prior to necropsy (on about Day 125 *post partum*)

b All doses and concentrations referred to in this report are expressed in terms of the parent form of the test compound, ie, neutral esomeprazole or omeprazole

c Rat No 3055 was found dead on Day 27 *post partum* (after 21 days of treatment), ie, prior to the intended date of necropsy. An additional male animal was therefore included in this subset. Rat No 3554 was euthanised as moribund on Day 23 *post partum* (after 17 days of treatment), ie, prior to the intended date of necropsy. An additional female animal was therefore included in this subset

d Because of excessive mortality noted for the Group 3 pups at the beginning of the study, additional litters were added to the study to ensure that a sufficient number of pups would be available to populate the various subsets. This is the reason for the non-sequential numbers in this group

e Rat No 1017 (male) was found dead on Day 123 *post partum* (near the end of the recovery period), rat No 3517 (female) was found dead on Day 71 *post partum* (after about 5 weeks of recovery), and rat No 3562 (female) was found dead on Day 122 *post partum* (near the end of the recovery period). None of these rats were replaced

Table 4 **Groups and dose levels: Phase I, toxicokinetic evaluation Dose Day 28**

Group	Subset[a]	No of animals[b]	Animal reference numbers	Treatment[c]	Daily dose levels	
					µmol/kg	mg/kg
Satellite animals for toxicokinetic evaluation on Day 28 of dosing (subsets C and D)						
1	C	7M	1021-1027[d]	Vehicle control	0	0
		7F	1521-1527[d]			
	D	7M	1031-1037[d]			
		7F	1531-1537[d]			
2	C	8M	2021-2028	Esomeprazole	270	93
		7F	2521-2527			
	D	8M	2031-2038			
		7F	2531-2537			
3	C	6M	3002, 3022-3024, 3026, 3027[e]	Esomeprazole	800	280
		7F	3502, 3504, 3527, 3529, 3530, 3571, 3573[e]			
	D	8M	3012, 3016, 3031-3035, 3037[e]			
		7F	3531, 3533, 3535, 3537, 3538, 3583, 3587[e]			
4	C	7M	4021-4027	Omeprazole	400	140
		7F	4521-4527			
	D	7M	4031-4037			
		7F	4531-4537			

a One pup/sex/litter from 10 separate litters was randomly assigned to subsets A, B, C and D as illustrated in Appendix A (Assignment to groups). The animals in subsets C and D were treated for 1 month and blood samples for toxicokinetic evaluation were taken on the final day of dosing (on Day 34 *post partum*)

b Blood samples for TK evaluation were taken from 4 rats/sex and group at 2 different time points after dosing in the control group (Group 1), and from 6 to 8 rats/sex at 7 different timepoints after dosing in the groups treated with esomeprazole or omeprazole (Groups 2 to 4). A maximum of 2 samples was taken from each individual rat

c All doses and concentrations referred to in this report are expressed in terms of the parent form of the test compound, ie, neutral esomeprazole or omeprazole

d In the control group the blood samples taken from the first 4 animals/sex and subset were used to confirm the absence of the test compounds in the plasma of the control animals. The blood samples taken from the last 3 animals/sex and subset were used in an *in vitro* investigation of the plasma protein binding of esomeprazole in various ages of rats

e Because of excessive mortality noted for the Group 3 pups at the beginning of the study, additional litters were added to the study to ensure that a sufficient number of pups would be available to populate the various subsets. This is the reason for the non-sequential numbers in this group

The parameters monitored in phase I were summarized in Table 5. This table is attached below.

Table 5 **Summary of main investigations: Phase I, main study, and recovery animals**

Groups & subset	Total No animals[a]	Assessment	No per assessment	On days *post partum*
Groups 1-4, subset A (main toxicity study, 1 month's dosing)	10M+10F	Developmental parameters (during the dosing period):		
		Observational battery	10M+10F	10, 14, 17, 21, 34
		Ophthalmoscopy	10M+10F	32±1
		"E" water maze	10M+10F	28±2
		Clinical pathology (at end of dosing):		
		Haematology	10M+10F	35
		Biochemistry (including serum gastrin 24 hours after dosing)	10M+10F	35
		Urinalysis (overnight)	10M+10F	34-35
		Necropsy (at end of dosing):		
		Organ weights	10M+10F	35
		Gross pathology	10M+10F	35
		Histopathology (as appropriate + gastric ECL-cells Groups 1, 3 & 4)	10M+10F	35
Groups 1-4, subset B (main toxicity study, 1 month's dosing + 3 months' recovery)	10M+10F	Developmental parameters (during both the dosing and recovery periods):		
		Eye opening	10M+10F	From Day 12
		Auricular startle response	10M+10F	From Day 12
		Observational battery	10M+10F	10, 14, 17, 21, 34, 117±2
		Vaginal opening	10F	From Day 36
		Preputial separation	10M	From Day 35
		Ophthalmoscopy	10M+10F	32±1, 117±2
		Locomotor activity	10M+10F	28±1, 60±2
		Auditory startle habituation	10M+10F	30±1, 55±2
		"E" water maze	10M+10F	65±3
		Clinical pathology (during recovery):		
		Haematology	10M+10F	70±2, 122±2
		Biochemistry (including serum gastrin 24 hours after dosing)	10M+10F	70±2, 122±2
		Urinalysis (overnight)	10M+10F	69-70±2, 121-122±2
		Plasma protein binding (at end of recovery):		
		1 time point (Group 1 only)	10M+10F[b]	122±2

Table 5 **Summary of main investigations: Phase I, main study, and recovery animals**

Groups & subset	Total No animals[a]	Assessment	No per assessment	On days *post partum*
		Necropsy (at end of recovery):		
		Organ weights	10M+10F	About Day 125
		Gross pathology	10M+10F	About Day 125
		Histopathology (as appropriate + gastric ECL-cells Groups 1, 3 & 4)	10M+10F	About Day 125
Group 1, subsets C + D (toxicokinetic/ reserve rats, 1 month's dosing)	20M+20F[c]	Toxicokinetics (at end of dosing)		
		2 time points	8M+8F[d]	34
		Plasma protein binding (at end of dosing):		
		1 time point	6M+6F[b]	34
		Reserve animals	6M+6F[e]	As appropriate
Groups 2-4, subsets C + D (toxicokinetic/ reserve rats, 1 month's dosing)	20M+20F[c]	Toxicokinetics (at end of dosing)		
		7 time points	14M+14F[d]	34
		Reserve animals	6M+6F[e]	As appropriate

a Target number of animals, including dosed reserves in subsets C and D

b Samples for investigation of the *in vitro* plasma protein binding of esomeprazole were taken from the control animals only (Group 1, subsets C and D at the end of the dosing period and subset B at the end of the recovery period)

c Total number of animals in subsets C and D together

d Samples for toxicokinetic evaluation were taken from 4M+4F animals per time point. A maximum of 2 samples were taken from each individual rat

e The last 3M+3F per group and subset in subsets C and D served as dosed spares, and were used as appropriately to replace other animals in Phase I

In the phase II study, a group of 20 male and 20 female rat pups were given omeprazole 400 µmol/kg (140 mg/kg) orally once daily for 8 days, from Day 7 to Day 14 *post partum*. Control animals (4/sex) received the vehicle. The following parameters were monitored: mortality and clinical observations, body weights, TK and serum gastrin. The animals were terminated following completed blood collection on Dose Day 8 (Day 14 *post partum*). The study design and the parameters monitored for phase II were summarized in Tables 6 and 7 in this report. These tables are attached below.

Table 6 **Groups and dose levels: Phase II, toxicokinetic evaluation Dose Day 8**

Group	No of animals[a]	Animal reference numbers[b]	Treatment[c]	Daily dose levels	
				µmol/kg	mg/kg
Satellite animals for toxicokinetic evaluation on Day 8 of dosing					
5	4M	5001-5004	Vehicle control	0	0
	4F	5501-5504			
6	19M	6001-6019	Omeprazole	400	140
	18F	6501-6510, 6512-6519			

a Blood samples for TK evaluation were taken from 4 rats/sex and group at a single time point after dosing in the control group (Group 5), and at 5 different time points after dosing in the group treated with omeprazole (Group 6). A single sample was taken from each individual rat

b Some additional animals were bled in Group 6, but these samples were missing from the analysis (due to technical problems) and therefore these rats are not included in this table. The intended number of rats in Group 6 was 20M and 20F. However, when samples were missing, no replacement animals were available

c All doses and concentrations referred to in this report are expressed in terms of the parent form of the test compound

Table 7 **Summary of main investigations: Phase II, toxicokinetic evaluation Dose Day 8**

Group	Total No animals[a]	Assessment	No per assessment	On days *post partum*
Group 5	4M+4F	Toxicokinetics (at end of dosing) 1 time point	4M+4F[b]	14
		Serum gastrin (at end of dosing) 1 time point	4M+4F[c]	14
Group 6	20M+20F[c]	Toxicokinetics (at end of dosing) 5 time points	4M+4F[b]	14
		Serum gastrin (at end of dosing) 1 time point	4M+4F[c]	14

a Target number of animals

b Samples for toxicokinetic evaluation were taken from 4M+4F animals per time point. A single sample was taken from each individual rat

c Samples for serum gastrin analysis were taken from 4M+4F per group, at the same time as blood was sampled for toxicokinetic evaluation, 2 hours after dosing

In phase III study, three groups of rat pups, each consisting of up to 28 males and 28 females, were given esomeprazole or omeprazole orally once, on Day 7 *post partum*. The dose levels were 270 or 800 µmol/kg (93 or 280 mg/kg) for esomeprazole, and 400 µmol/kg (140 mg/kg) for omeprazole. A control group consisting of 20 males and 20 females received the vehicle. The animals in phase III were used for blood sampling for toxicokinetic evaluation only. Four pups/sex were bled at each TK sampling time point. The study design and the parameters monitored for phase III were summarized in Tables 8 and 9 in this report. These tables are attached below.

Table 8 **Groups and dose levels: Phase III, toxicokinetic evaluation Dose Day 1**

Group	No of animals[a]	Animal reference numbers[b]	Treatment[c]	Daily dose levels	
				µmol/kg	mg/kg
Satellite animals for toxicokinetic evaluation on Day 1 of dosing					
7	20M	7001-7011, 7013, 7015-7022[d]	Vehicle control	0	0
	20F	7501-7516, 7518-7521[d]			
8	26M	8001-8006, 8008-8015, 8018-8022, 8024-8026, 8029-8032	Esomeprazole	270	93
	25F	8502, 8503, 8505-8514, 8517-8529			
9	25M	9001-9009, 9012-9020, 9022, 9023, 9025-9039	Esomeprazole	800	280
	28F	9501-9507, 9509-9514, 9517-9521, 9523, 9524, 9526, 9528-9534			
10	25M	10001-10004, 10006-10008, 10012-10021, 10023-10027, 10029-10031	Omeprazole	400	140
	28F	10501-10506, 10508-10529			

a Blood samples for TK evaluation were taken from 4 rats/sex and group at 2 different time points after dosing in the control group (Group 7), and at 7 different time points after dosing in the groups treated with esomeprazole or omeprazole (Groups 8 to 10). A single sample was taken from each individual rat

b Some additional animals were bled in Groups 8, 9 and 10, but these samples were either missing from the analysis (due to technical problems) or the sample tubes were empty or contained an insufficient quantity for analysis, and therefore these rats are not included in this table. The intended number of rats in Groups 8 to 10 was 28M and 28F. However, when samples were missing, replacement animals were not always available

c All doses and concentrations referred to in this report are expressed in terms of the parent form of the test compound

d In the control group the blood samples taken from the first 8 animals/sex were used to confirm the absence of the test compounds in the plasma of the control animals. The blood samples taken from the last 12 animals/sex were used in an *in vitro* investigation of the plasma protein binding of esomeprazole in various ages of rats

4.1.3.2 Summary of main investigations and number of animals per assessment

This Summary is given for the Phase III animals only, in Table 9 (see below).

Table 9 **Summary of main investigations: Phase III, toxicokinetic evaluation Dose Day 1**

Group	Total No. animals[a]	Assessment	No per assessment	On days *post partum*
Group 7	20M+20F	Toxicokinetics (on first day of dosing)		
		2 time points	8M+8F[b]	7
		Plasma protein binding (on first day of dosing):		
		1 time point	12M+12F[c]	7
Groups 8-10	28M+28F	Toxicokinetics (on first day of dosing)		
		7 time points	28M+28F[b]	7

a Target number of animals

b Samples for toxicokinetic evaluation were taken from 4M+4F animals per time point. A single sample was taken from each individual rat

c Samples for investigation of the *in vitro* plasma protein binding of esomeprazole were taken from the control animals only (Group 1). A single sample was taken from each individual rat

The dose selection was based on the results of the previous toxicity studies for esomeprazole (a 1-month oral toxicity study in neonatal rats, #900127) and the racemate omeprazole (GI.000-012-011, 1996) in young rats. The two doses of esomeprazole tested in the current study are the same as the mid and high doses used in Study No 900127. The dose of esomeprazole (800 µmol/kg or 280 mg/kg) resulted in mortalities (about 19%) in the neonatal pups during the first days of dosing in Study No 900127. This is the high dose used in adult rats using esomeprazole in earlier studies. The dose of omeprazole (400 µmol/kg or 140 mg/kg) was the high dose tested in both young and adult rats in earlier studies.

Results:

1. Clinical Signs: Following clinical signs of toxicity were observed prior to death: cold to touch, labored breathing, decreased activity, weak and/or thin. Clinical signs of toxicity were summarized in Table 15 in this report. This table is attached below.

Table 15 **Summary of preweaning, treatment-related clinical observations in neonatal rat pups given esomeprazole or omeprazole for up to 28 days**

Clinical signs	Group No and sex				Group No and sex			
	1M	2M	3M	4 M	1F	2F	3F	4 F
Dose level (µmol/kg)	0	270	800	400	0	270	800	400
Test Compound	Con	Eso	Eso	Ome	Con	Eso	Eso	Ome
No of animals per group	40	40	64	40	40	40	64	40
Activity decreased	0	0	10	0	0	0	6	0
Cold to touch	0	0	4	0	0	0	9	0
Thin	0	0	7	0	0	0	5	0
Fur staining, muzzle/cranium	0	0	3	0	0	0	5	0
Fur staining, urogenital region	0	0	50	0	0	0	42	0
Fur staining, thoracic region(s)	0	0	8	0	0	0	10	0
Fur staining, abdominal/lumbar/ sacral region(s)	0	0	3	0	0	0	12	0
Fur staining, hindpaw(s)/hindlimb(s)	0	0	22	0	0	0	29	0
Skin red anus	0	0	7	0	0	0	3	0
Found dead	0	1	14	0	0	0	23	0

Con= Control Eso= Esomeprazole Ome= Omeprazole

2. Mortality: A total of 37 (out of 128) dosed pups was found dead during the pre-weaning period (up to Day 18 *post partum)* in the neonatal pups given 280 mg/kg esomeprazole. The majority of these deaths occurred during the first 4 days of dosing. Six litters were added to this group due to the high mortality in this group. During the post-weaning treatment period, one male and one female given 280 mg/kg esomeprazole were found dead on Dose Day 21 and 17 (Days 27 and 23 *post partum*) respectively.

One pup given 93 mg/kg esomeprazole was found dead on Dose Day 4 (Day 10 *post partum*). The deaths were considered as treatment related since the majority of deaths were in the high dose group. However, the cause of death could not be determined.

During the recovery period, one control male was found dead on Day 123 *post partum*. Two females in the 280 mg/kg esomeprazole-treated group were found dead on Days 71 and 122 *post partum*.

3. Body Weight: The mean initial and final body weights in the control animals were 13.9 g and 118 g (males) or 13.4 and 102 g (females), respectively. Mean terminal body weight gain was decreased by 19% in males or 16% in females in pups given 280 mg/kg esomeprazole as compared to the controls. The body weights for the 93 mg/kg esomeprazole-treated pups and the 140 mg/kg omeprazole-treated pups were similar to those of the control group. The results were summarized in Tables 16 and 17 in this report. These tables are attached below.

Table 16 **Summary of pre-weaning body weights and weight gains in neonatal rats given esomeprazole or omeprazole once daily**

Dose	Males			Females		
(µmol/kg)	Dose Day 1 Day 7 *pp*	Dose Day 15 Day 21 *pp*	Gain (% control)	Dose Day 1 Day 7 *pp*	Dose Day 15 Day 21 *pp*	Gain (% control)
0	13.9±0.7	52.9±2.0	39.0	13.4±0.6	50.8±1.9	37.4
270 (Eso)	14.4±1.3	53.7±2.9	39.3 (100)	13.5±1.3	49.9±3.0	36.4 (97)
360 (Eso)	14.1±1.6	43.8±6.8**	29.7 (76)	13.7±1.4	43.7±4.2***	30.0 (80)
400 (Ome)	14.6±1.6	53.2±2.3	38.6 (99)	13.8±1.3	49.9±2.7	36.1 (97)

** $p \leq 0.01$ *** $p \leq 0.001$

N= 10 litters in the vehicle control, esomeprazole 270 µmol/kg and 400 µmol/kg omeprazole groups, 16 litters in the esomeprazole 360 µmol/kg group

Eso: Esomeprazole Ome: Omeprazole

Table 17 **Summary of post-weaning and recovery body weights and weight gains in juvenile rats given esomeprazole or omeprazole once daily for 1 month, followed by 3 months of recovery**

	Males (Subset A)		Males (Subset B)		Females (Subset A)		Females (Subset B)	
Body weights (g/animal)								
Dose (µmol/kg)	Day 24 *pp*	Day 35 *pp*	Day 24 *pp*	Day 35 *pp*	Day 24 *pp*	Day 35 *pp*	Day 24 *pp*	Day 35 *pp*
0	64±4	118±8	63±4	138±10	62±5	102±6	59±4	118±8
270 (Eso)	65±4	116±8	63±4	136±7	60±6	100±8	58±4	114±8
800 (Eso)	53±8**	98±11***	51±13*	119±21**	53±7**	88±10**	50±5**	104±7***
(% control)	(83)	(83)	(81)	(86)	(85)	(86)	(85)	(88)
400 (Ome)	64±5	115±8	65±5	141±9	59±6	100±8	58±7	114±6
Body weight gains (g/animal)								
Dose (µmol/kg)	24-35 *pp*	35-119 *pp*	24-35 *pp*	35-119 *pp*	24-35 *pp*	35-119 *pp*	24-35 *pp*	35-119 *pp*
0	54	NA	75	300	40	NA	59	152
270 (Eso)	51	NA	73	326	40	NA	56	152
800 (Eso)	45	NA	68	343	35	NA	54	177
400 (Ome)	51	NA	76	338	41	NA	56	166

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$
Eso= Esomeprazole Ome= Omeprazole
pp= post partum NA= not applicable

4. **Food Consumption**: The average food consumption in the control group was 11-18 g/animal/day for males or 11-16 g/animal/day. Food consumption was lower in the high dose group (9-17 g/animal/day) as compared to the control. The food consumption for the 93 mg/kg esomeprazole and the 140 mg/kg omeprazole groups was not affected.

5. **Ophthalmoscopy**: Anterior suture cataracts were found in 1 control male, 6 females in the 280 mg/kg esomeprazole group, and 3 males and 3 females in the 140 mg/kg omeprazole group. The lenticular opacities were usually faint and unilateral and no longer present during the re-examination on Day 117.

6. **Developmental Parameters**: There were no treatment related changes in eye opening, auricular startle, and vaginal opening. The mean day of development of preputial separation for the 280 mg/kg esomeprazole-treated males was Day 47 *post partum*, as compared to Day 45 *post partum* in the control group. The mean day of development for preputial separation was not affected in the 93 mg/kg esomeprazole-treated males and the 140 mg/kg omeprazole treated males.

7. **Functional Observational Battery**: There were no treatment related changes.

8. **Behavioral Performance:** There were no treatment-related effects on locomotor activity and learning/memory-"E" water maze. A statistically significant increase in mean maximum startle was noted for the 280 mg/kg/day esomeprazole-treated females and the 140 mg/kg/day omeprazole-treated females. This was not seen during the recovery period.

9. **Hematology:** Slight decreases (10-24%) in hemoglobin, hematocrit, mean red cell volume (MCV), mean red cell haemoglobin (MCH), and mean red cell haemoglobin concentration (MCHC) values were noted in the high dose group as compared to the control.

Slight increases in platelet count (15-21%), mean platelet volume (19-26%), red cell distribution width (RDW, 32-41%) and reticulocyte counts (45-53%) were also found in the high dose group as compared to the control. These changes were recovered during the recovery period. The results were presented in Table 23 in this report. This table is attached below.

Table 19 **Summary of noteworthy haematology findings in juvenile rats given esomeprazole or omeprazole for 1 month or after 3 months of recovery**

Dose (µmol/kg) / parameter / Test Compound	Males 0 Con	270 Eso	800 Eso	400 Ome	Females 0 Con	270 Eso	800 Eso	400 Ome
RBC (10^6/µL)								
Day 35 *pp*	6.3	6.7**	6.3	6.7**	6.5	6.9	6.6	6.8
Recovery (70±2 *pp*)	8.5	8.6	8.3	8.5	8.1	8.5	8.2	8.4
Recovery (122±2 *pp*)	9.3	9.3	9.2	9.5	8.5	8.9	8.7	8.8
Hb (g/dL)								
Day 35 *pp*	12.9	12.6	10.2***	11.9*	13.5	12.9	10.7***	12.4
Recovery (70±2 *pp*)	16.4	16.3	15.9	16.0	15.8	16.0	15.0	16.2
Recovery (122±2 *pp*)	16.3	16.3	16.0	16.6	15.6	16.2	15.8	16.4
HT (%)								
Day 35 *pp*	41	42	36***	41	43	43	37*	42
Recovery (71±2 *pp*)	52	52	50	51	49	50	46	50
Recovery (122±2 *pp*)	51	51	50	52	48	50	48	50
MCV (fL)								
Day 35 *pp*	66	63	57***	61*	66	63	56***	61*
Recovery (71±2 *pp*)	61	60	60	60	60	59	57*	59
Recovery (122±2 *pp*)	55	55	55	55	57	57	56	56
MCH (pg)								
Day 35 *pp*	20	19	16***	18**	21	19*	16***	18**
Recovery (71±2 *pp*)	19	19	19	19	19	19*	18***	19
Recovery (122±2 *pp*)	18	18	17	17	19	18	18	19
MCHC (g/dL)								
Day 35 *pp*	31	30**	28***	29***	32	30***	29***	30***
Recovery (71±2 *pp*)	31	31	32	31	32	32	32	33
Recovery (122±2 *pp*)	32	32	32	32	33	32	33	33
RDW (%)								
Day 35 *pp*	15.1	17.9***	21.4***	18.3*	14.9	16.8	19.7***	17.3*
Recovery (71±2 *pp*)	10.7	11.8**	12.5***	12.2***	10.4	11.6*	13.7***	12.5***
Recovery (122±2 *pp*)	12.0	12.3	12.4	12.6	10.8	10.8	10.9	11.2
Platelet (10^3/µL)								
Day 35 *pp*	1705	1768	1955	1964	1724	1708	2091*	1905
Recovery (71±2 *pp*)	1161	1203	1265	1244	1162	988	1208	1194
Recovery (122±2 *pp*)	1109	1145	1142	1164	969	1048	1096	1140

Table 19 Summary of noteworthy haematology findings in juvenile rats given esomeprazole or omeprazole for 1 month or after 3 months of recovery

Dose (µmol/kg) / parameter / Test Compound	Males 0 Con	270 Eso	800 Eso	400 Ome	Females 0 Con	270 Eso	800 Eso	400 Ome
MPV (fL)								
Day 35 *pp*	8.3	8.3	9.9**	8.5	8.5	8.4	10.7*	8.6
Recovery (71±2 *pp*)	8.5	8.1	8.2	9.1	8.8	9.0	8.1	9.0
Recovery (122±2 *pp*)	6.8	7.0	7.3	7.1	7.9	7.6	7.4	7.4
Reticulocyte (10^9/L)								
Day 35 *pp*	613	798**	937***	752*	564	775	818	686
Recovery (71±2 *pp*)	194	193	225	210	175	152	209	216
Recovery (122±2 *pp*)	169	180	201	188	163	165	172	183
Reticulocyte (%)								
Day 35 *pp*	9.8	11.9*	14.8***	11.2	8.7	11.3	12.3*	11.4
Recovery (71±2 *pp*)	2.3	2.3	2.7	2.5	2.2	1.8	2.6	2.6
Recovery (122±2 *pp*)	1.8	1.9	2.2	2.0	1.9	1.9	2.0	2.1
WBC (10^3/µL)								
Day 35 *pp*	3.1	2.6	2.2	2.9	3.0	3.0	2.3	2.7
Recovery (71±2 *pp*)	9.6	9.9	10.3	9.8	6.8	7.3	7.2	6.4
Recovery (122±2 *pp*)	8.6	8.1	9.1	8.6	5.6	5.6	5.3	5.4
Lymphocytes (10^3/µL)								
Day 35 *pp*	2.7	2.3	1.8*	2.6	2.7	2.7	1.9	2.4
Recovery (71±2 *pp*)	8.2	8.5	9.0	8.3	5.8	6.2	6.1	5.4
Recovery (122±2 *pp*)	6.9	6.5	7.5	6.9	4.9	4.7	4.3	4.6

* p≤0.05 ** p≤0.01 *** p≤0.001
Con= Control Eso= Esomeprazole Ome= Omeprazole

10. Clinical Chemistry:

Increases in the alkaline phosphatase (16-26%), cholesterol (20-29%), blood urea nitrogen (12-31%), serum iron (20-24%), total iron binding capacity (TIBC, 61-63%), and unsaturated iron binding capacity (UIBC, 73-75%) were noted for the 280 mg/kg esomeprazole-treated animals. For the 93 mg/kg esomeprazole-treated animals, increases in the serum iron (34-56%), total iron binding capacity (TIBC, 26-27%) and unsaturated iron binding capacity (UIBC, 16-26%) were found. For the 140 mg/kg omeprazole-treated animals, increases in the cholesterol (15-17%), serum iron (20-69%), total iron binding capacity (TIBC, 29-34%), and unsaturated iron binding capacity (UIBC, 17-38%) were noted. These changes were not seen during recovery period. The results were presented in Table 20 in this report. This table is attached below.

Table 20 **Summary of noteworthy blood biochemistry changes in juvenile rats given esomeprazole or omeprazole for 1 month or after 3 months of recovery**

Dose (μmol/kg)/ parameter Test compound	Males 0 Con	270 Eso	800 Eso	400 Ome	Females 0 Con	270 Eso	800 Eso	400 Ome
ALP								
Day 35 *pp*	188	204	237**	213	162	169	188	186
Recovery (71±2 *pp*)	124	133	144	134	98	105	104	97
Recovery (122±2 *pp*)	91	88	100	89	69	72	81	64
BUN								
Day 35 *pp*	17	17	19	17	16	17	21*	19
Recovery (71±2 *pp*)	18	19	19	21	18	17	19	19
Recovery (122±2 *pp*)	19	20	21	22	19	20	20	21
Cholesterol								
Day 35 *pp*	91	99	117***	105*	112	125	134**	131**
Recovery (71±2 *pp*)	100	101	104	116	106	106	99	119
Recovery (122±2 *pp*)	111	131	140	180*	116	112	125	133
Iron								
Day 35 *pp*	86	115	103	103	98	153	121	166
Recovery (71±2 *pp*)	154	146	141	141	310	338	315	272
Recovery (122±2 *pp*)	152	147	156	132	361	391	376	316
TIBC								
Day 35 *pp*	403	513*	658***	541**	404	507*	649***	522**
Recovery (71±2 *pp*)	528	520	504	496	504	532	487	486
Recovery (122±2 *pp*)	519	479	476	435	535	536	525	487
UIBC								
Day 35 *pp*	317	398*	555***	437***	305	354	528***	356
Recovery (71±2 *pp*)	374	374	363	355	194	194	172	214
Recovery (122±2 *pp*)	367	331	321	304*	174	145	149	171

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$
Con= Control Eso= Esomeprazole Ome= Omeprazole

11. **Serum gastrin:** Serum gastrin levels were increased 24 hours after dosing in both the esomeprazole- and omeprazole-treated animals as compared to the controls. These increases were not clearly seen during the recovery period. The results were summarized in Table 21 in this report. This table is attached below.

Table 21 **Serum gastrin levels (mean±SD) in juvenile rats 24 h after oral administration of esomeprazole or omeprazole**

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Test compound	Con	Eso	Eso	Ome	Con	Eso	Eso	Ome
Dose (μmol/kg)	0	270	800	400	0	270	800	400
Serum gastrin (pg/mL), Day 35 *pp* (dose phase, subset A)	46 ±18	143* ±58	372*** ±86	179** ±84	38 ±6	143* ±42	238*** 117	223** ±132
% of control value	-	311	809	389	-	376	626	587
Serum gastrin (pg/mL), Day 70±2 *pp* (subset B, recovery)	56 ±28	53 ±29	62 ±32	56 ±31	41 ±4	49 ±14	71 ±25	37 ±3
Serum gastrin (pg/mL), Day 122±2 *pp* (subset B, recovery)	43 ±11	54 ±31	47 ±12	53 ±17	36 ±1	41 ±6	49 ±14	35 ±4

* p≤0.05 ** p≤0.01 *** p≤0.001
Con= Control Eso= Esomeprazole Ome= Omeprazole

12. **Urinalysis:** There were no treatment related changes.

13. **Organ Weights:** The absolute and relative stomach weights (to brain weight) were increased in both esomeprazole and omeprazole-treated groups as compared to the controls. The results were presented in Table 19 in this report. This table is attached below.

Table 22 **Stomach weights (mean±SD) in juvenile rats after oral administration of esomeprazole or omeprazole**

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Test compound	Con	Eso	Eso	Ome	Con	Eso	Eso	Ome
Dose (μmol/kg)	0	270	800	400	0	270	800	400
Absolute stomach weight (g), Day 35 *pp* (subset A)	1.0 ±0.11	1.1** ±0.08	1.1 ±0.15	1.2* ±0.11	0.99 ±0.11	1.1** ±0.11	1.1 ±0.09	1.1** ±0.08
Relative stomach weight (%), Day 35 *pp* (subset A)	67 ±7.4	81** ±5.9	85*** ±9.8	81** ±6.5	67 ±7.0	79*** ±6.4	81*** ±8.1	80*** ±5.4
% of control value (relative wt)	-	121	126	121	-	118	121	119
Absolute stomach weight (g), Day 122±2 *pp* (subset B)	2.4 ±0.26	2.4 ±0.18	2.4 ±0.22	2.5 ±0.20	1.7 ±0.13	1.7 ±0.10	1.7 ±0.25	1.8 ±0.27
Relative stomach weight (%), Day 122±2 *pp* (subset B)	127 ±12.5	130 ±9.4	138 ±10.6	134 ±12.0	96 ±8.7	101 ±11.0	106 ±15.0	108 ±13.8

* p≤0.05 ** p≤0.01 *** p≤0.001
Con= Control Eso= Esomeprazole Ome= Omeprazole

14. **Gross Pathology:** Increased incidence of renal pelvic dilatation and surface irregularity in animals given 280 mg/kg esomeprazole or 140 mg/kg omeprazole was noted.

15. **Microscopic Pathology:** There were no treatment-related histopathological findings.

16. **Morphological and morphometrical examination of the gastric mucosa:**

There were no statistically significant differences in the reference volume of the stomach and mucosal height between the controls and the esomeprazole treated group. However, an increase in the ECL-cell volume fraction and profile density was noted in the esomeprazole treated group (mainly in males) at the end of the 1 month dosing period. The median increase in the ECL-cell volume fraction for males and females combined was about 50%, and that for the ECL-cell profile density was about 36%. These were not seen at the end of the 3 month recovery period.

Increases in the ECL-cell volume fraction and profile density were also noted in the omeprazole-treated rats as compared to the control. The stomach reference volume and mucosal height were also increased in the omeprazole-treated males but not females. The results were presented in Tables 23 and 24 in this report. These tables are attached below.

Table 23 **Results of the gastric morphometry (median and range) in juvenile rats at the end of the 1-month dosing period (subset A)**

Test compound Dose (μmol/kg)	C 0	E 800	O 400	C 0	E 800	O 400
Group and sex	1 M	3 M	4 M	1 F	3 F	4 F
Stomach reference vol. (mm^3), Day 35 *pp* (subset A)	760 (710-990)	730 (680-1000)	810 (590-1100)	630 (570-830)	780 (550-900)	740 (680-860)
% change vs controls	-	-3.9	6.5	-	24	17
ECL-cell volume fraction $*10^3$, Day 35 *pp* (subset A)	11 (7.0-18)	23* (12-38)	18 (11-21)	16 (10-18)	20 (7.2-28)	12 (8.4-20)
% change vs controls	-	110	64	-	25	-25
ECL-cell profile density (mm^{-2}) Day 35 *pp* (subset A)	77 (53-110)	140* (83-170)	100 (73-110)	92 (74-110)	106 (50-170)	95 (65-120)
% change vs controls	-	82	30	-	8.7	3.2
Gastric mucosal height (μm) Day 35 *pp* (subset A)	520 (220-560)	520 (290-750)	450 (160-780)	400 (280-480)	390 (250-620)	440 (420-790)
% change vs controls	-	0	-13	-	-2.5	10
Group and sex	1 M + F	3 M + F	4 M + F			
Stomach reference vol. (mm^3), Day 35 *pp* (subset A)	710 (570-990)	750 (550-1000)	750 (590-1100)			
% change vs controls	-	5.6	5.6			
ECL-cell volume fraction $*10^3$, Day 35 *pp* (subset A)	14 (7.0-18)	21* (7.2-38)	13 (8.4-21)			
% change vs controls	-	50	-7.1			
ECL-cell profile density (mm^{-2}) Day 35 *pp* (subset A)	88 (53-110)	120* (50-170)	96 (65-120)			
% change vs controls	-	36	9.1			
Gastric mucosal height (μm) Day 35 *pp* (subset A)	420 (220-560)	470 (250-750)	450 (160-790)			
% change vs controls	-	12	7.1			

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$
C Control E Esomeprazole O Omeprazole
pp *post partum*

Table 24 Results of the gastric morphometry (median and range) in juvenile rats at the end of the 3-month recovery (subset B)

Test compound Dose (µmol/kg)	C 0	E 300	O 400	C 0	E 300	O 400
Group and sex	1 M	3 M	4 M	1 F	3 F	4 F
Stomach reference vol. (mm^3), Day 122±2 *pp* (subset B)	1500 (1300-1700)	1700 (1300-1800)	2100** (1900-2200)	1200 (1000-1300)	1400 (1300-1600)	1400 (1100-1500)
% change vs controls	-	13	40	-	17	17
ECL-cell volume fraction $*10^3$, Day 122±2 *pp* (subset B)	26 (19-31)	24 (21-30)	30 (22-33)	21 (18-30)	29 (11-31)	31* (27-44)
% change vs controls	-	-7.7	15	-	38	48
ECL-cell profile density (mm^{-2}) Day 122±2 *pp* (subset B)	160 (140-210)	170 (170-210)	210* (190-240)	150 (140-240)	220 (95-260)	210* (210-290)
% change vs controls	-	6.3	31	-	47	40
Gastric mucosal height (µm) Day 122±2 *pp* (subset B)	540 (340-670)	470 (380-630)	710* (620-920)	540 (290-830)	560 (410-610)	460 (310-730)
% change vs controls	-	-13	31	-	3.7	-15
Group and sex	**1 M + F**	**3 M + F**	**4 M + F**			
Stomach reference vol. (mm^3), Day 122±2 *pp* (subset B)	1300 (1000-1700)	1500 (1300-1800)	1700 (1100-2200)			
% change vs controls	-	15	31			
ECL-cell volume fraction $*10^3$, Day 122±2 *pp* (subset B)	24 (18-31)	27 (11-31)	31** (22-44)			
% change vs controls	-	13	29			
ECL-cell profile density (mm^{-2}) Day 122±2 *pp* (subset B)	160 (140-240)	200 (95-260)	210** (190-290)			
% change vs controls	-	25	31			
Gastric mucosal height (µm) Day 122±2 *pp* (subset B)	540 (290-830)	540 (380-630)	670 (310-920)			
% change vs controls	-	0	24			

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$
C Control E Esomeprazole O Omeprazole
pp *post partum*

16. Toxicokinetics: The results were presented in Tables 13 and 14 in this report. These tables are attached below.

Table 13 Summary of median C_{max} and AUC for esomeprazole and the metabolite H 168/66 in juvenile rats following oral dosing with esomeprazole

Daily dose (µmol/kg)	Dose Day	Days *post partum*	Sex	Analysed compound	C_{max} (µmol/L)	AUC (µmol*h/L)
270	1	7	M+F	Esomeprazole	52.1	149
	28	34	M+F	Esomeprazole	1.96	2.62
800	1	7	M+F	Esomeprazole	141	956
	28	34	M+F	Esomeprazole	12.7	28.8
800	1	7	M+F	H 168/66	48.2	443
	28	34	M+F	H 168/66	6.32	11.2

Table 14 **Summary of median C_{max} and AUC for omeprazole in juvenile rats following oral dosing with omeprazole**

Daily dose (µmol/kg)	Dose Day	Days *post partum*	Sex	Analysed compound	C_{max} (µmol/L)	AUC (µmol*h/L)
400	1	7	M+F	Omeprazole	83.3	288
	8	14	M+F	Omeprazole	68.2	93.4
	28	34	M+F	Omeprazole	7.16	8.26

The plasma levels were much lower on day 28 than those on day 1.

In summary, esomeprazole was lethal at 280 mg/kg. One pup treated with 93 mg/kg esomeprazole was also found dead on Dose Day 4. The clinical signs of toxicity observed prior to death included cold to touch, labored breathing, decreased activity, weak and/or thin. Additional clinical signs of toxicity observed in the 280 mg/kg esomeprazole included fur staining (red, brown and/or yellow) at the urogenital/thoracic/sacral/abdominal regions and/or fore and/or hindpaws/limbs and/or cranium prior to weaning. After weaning, occasional yellow fur staining of the urogential region was noted in a few males.

The gastrin levels and stomach weight were increased in the treatment groups as compared to the control. The small increases in the volume and number of ECL-cells were noted in the esomeprazole/omeprazole-treated rats. The toxicokinetic evaluation indicated that the plasma levels of both esomeprazole and omeprazole were decreased with the duration of treatment and/or the age of the animals. The results did not reveal any unexpected toxicity. The central nervous system and the stomach were the target organs of toxicity. No effect dose and tolerated dose were not clearly identified.

Toxicological Qualification of the Potential Degradation Products:

There is a possibility that during the shelf-life of the omeprazole sachets, there will be degradation of the drug and formation of organic degradants. The sponsor stated that as no individual organic impurity is predicted to increase above the qualification threshold of — of the active compound at the end of the proposed shelf life, any toxicological qualification studies have not been performed on the organic impurities that may form in the omeprazole sachets. b(4)

Low levels of 2 degradation products of omeprazole, ——, were noted after 9 months' storage of the drug product at 25°C/60% RH. However, both metabolites formed from omeprazole and esomeprazole were present in animals and humans. In *in vitro* metabolism studies in human microsomes, incubation with omeprazole resulted in the formation of ——. In an *in vivo* study in human volunteers, — was seen to be a major metabolite in the plasma, but was not detected in the urine. The percent radioactivity in the plasma attributed to — following a radiolabeled dose of omeprazole was — in extensive metabolisers and — in slow b(4)

On August 21, 2007, I faxed an information request letter to George Kummeth with the following request:

> Regarding Study D9586C0002 titled, "A Phase I, Open, Randomized, Three-Way Crossover, Single-Center Biovailability Study Comparing Three Different Formulations of Omeprazole, 20mg Following Single and 5 days Repeated Once Daily Oral Administration in Healthy Male and Female Subjects": please provide the raw data for the freeze-thaw, bench-top, and long-term stability results for omeprazole in the analytical report. In addition, confirm that the stability conditions validated cover the actual analysis conditions.

On August 23, 2007, you e-mailed the following questions in response:

Reference is made to the August 21 fax from the Agency regarding NDA 22-056 requesting information about the bioavailability study D9586C0002; specifically raw data for the freeze-thaw, bench-top, and long-term stability results for omeprazole in the analytical report and confirmation that the stability conditions validated cover the actual analysis conditions. Analytical Method Validation Report 1312-477, Validation of liquid chromatographic methods for omeprazole, [redacted] and metabolites in plasma, Methods No. BA-179, 222, 315, 318, and 323 contains information on the issues you address. Do you have a copy of this report? b(4)

In addition to the report, we need to know what information you need and would appreciate further clarification.

We have the following responses to your questions:

Reference is made to the August 21 fax from the Agency regarding NDA 22-056 requesting information about the bioavailability study D9586C0002; specifically raw data for the freeze-thaw, bench-top, and long-term stability results for omeprazole in the analytical report and confirmation that the stability conditions validated cover the actual analysis conditions. Analytical Method Validation Report 1312-477, Validation of liquid chromatographic methods for omeprazole, [redacted] and metabolites in plasma, Methods No. BA-179, 222, 315, 318, and 323 contains information on the issues you address. Do you have a copy of this report? b(4)

In addition to the report, we need to know what information you need and would appreciate further clarification.

We do not have a copy of the Analytical Method Validation Report 1312-477. If this report includes all information requested in our August 21, 2007 letter, we do not need any additional information. Thanks.

metabolisers. The corresponding values for omeprazole were ——— respectively. — ——— was detected in the urine at ——— of the administered dose of omeprazole, or ——— of the administered dose of esomeprazole, in both cases in extensive and poor metabolisers, respectively. b(4)

Low levels of two other degradation products, ———, ——— was seen after 6 months' storage of the drug product at 40^0C/75% relative humidity. — is an organic impurity (degradation product) that was also present in the Nexium (esomeprazole) intravenous formulation at levels above the qualification threshold. This degradant has been fully toxicologically qualified in both *in vitro* and *in vivo* mutagenicity studies and repeat dose toxicity studies in rats and dogs.

If the levels of the ———, or any other organic impurity increase on continuing storage at 25^0C/60% relative humidity, then these impurities need to be identified and qualified as required by the ICH guidelines Q3B. b(4)

LABELING:

No changes in the preclinical section of proposed labeling of Prilosec are recommended.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Omeprazole belongs to a class of compounds, the substituted benzimidazoles, that suppress gastric acid secretion by specific inhibition of the H^+/K^+-ATPase (the proton pump), resulting in an inhibition of gastric acid secretion. After reaching the canalicular space of the gastric parietal cell, omeprazole is protonated, activated to the reactive sulfonamide, and covalently bonded to the luminal surface of the proton pump to inhibit the pump activity. The primary pharmacodynamic effects of omeprazole have been demonstrated in both *in vitro* and *in vivo* studies. The binding irreversibly to H^+/K^+-ATPase, it effectively inhibits acid secretion until new enzyme is synthesized.

Under NDA 22-056, the sponsor is seeking approval of a new oral formulation of omeprazole, Prilosec (omeprazole magnesium) For Delayed-Release Oral suspension (2.5 mg and 10 mg; also called the sachet formulation or omeprazole sachet) for use in children 0 to 2 years of age. The omeprazole sachet formulation was developed to fulfill the post-marketing commitment to develop an age-appropriate formulation for pediatric patients 0 to 2 years of age (July 12, 2002 approval letter for NDA 19-810/S-074). Under the current NDA, the sponsor did not submit any preclinical study reports with omeprazole. The sponsor conducted several toxicology studies in juvenile animals with omeprazole in support of the pediatric use of the drug, and the studies were reviewed earlier. Summarized reports of these studies were included in the submission. The studies include a single-dose toxicity study in rats, repeat dose toxicity and toxicokinetic studies in rats, and a carcinogenicity study in $p53^{+/-}$ heterozygous mice. In addition, in a recent toxicity/toxicokinetic study in neonatal/juvenile rats with esomeprazole

(study # 900404, 2006), a group of animals received a high dose (140 mg/kg) of omeprazole as a comparator.

Plasma drug levels were determined in rats (12 to 14 days old, 23 to 25 days old, and 44 to 46 days old) following oral administration of 14 or 69 mg/kg/day doses of omeprazole for 2 days. Omeprazole plasma levels were significantly higher in 12 to 14 days old rats as compared with older rats (23 to 25 days old and 44 to 46 days old). This age related effect on the plasma drug levels may be related to the differences in the metabolism and/or absorption of omeprazole. Young rats (12 to 14 days old) may have immature liver function and are unable to effectively metabolize omeprazole as compared to adult rats, in which the drug undergoes a significant first pass effect.

In a single-dose toxicity oral study with omeprazole (0, 190, 260, 380, 520 and 2100 mg/kg) in juvenile rats (10 to 13-day-old), the minimal lethal dose was 260 mg/kg (the minimal lethal dose in adult rats was 2600 mg/kg. The maximum non-lethal dose for 10 to 13 days old rats was 190 mg/kg, as compared to 2210 mg/kg for adult rats. The differences in toxicity between young and adult rats may be related to the higher plasma exposure levels in the juvenile animals as compared with the adult animals. Clinical signs observed included reduced motor activity, reduced respiratory frequency, dyspnea, cyanosis, reduced or absent righting reflex, tremor and convulsions, and the observed clinical signs were similar to those previously seen in adult animals.

In a 1-month oral toxicity study in juvenile rats (14 to 15 days old), omeprazole was administered at doses of 14, 31, 69 or 140 mg/kg/day doses. The no effect dose was 31 mg/kg/day. One male in the 140 mg/kg/day group died on day 3 of unexplained causes. White blood cell counts were decreased between 15 and 30% for all male treatment groups and females receiving 69 and 140 mg/kg/day doses. Bone marrow hyperplasia was found in the 31, 69 and 140 mg/kg/day groups, and may have been a compensatory response to changes in white and red blood cell parameters. Absolute and relative thymus weights were decreased in the 69 and 140 mg/kg/day groups, and may be indicative of a stress response to omeprazole treatment. Liver hyperplasia, lymphoid cell infiltration and congestion were observed at 140 mg/kg/day. A dose-related increase in the stomach weight was observed in the treated animals; however, no changes in the ECL cells in the gastric mucosa were observed in any group.

In a 1-month oral toxicity study in neonatal rats (age, 7 days) with esomeprazole, one group of animals received omeprazole at a dose of 140 mg/kg. No treatment-related effects on the body weight gain and functional observation or behavioral performance were observed in animals treated with omeprazole. Increased stomach weights, increased serum gastrin levels and increased ECL cell volume fraction and profile density were observed in animals receiving omeprazole.

Thus, the toxicological effects observed in neonatal rats treated with omeprazole were similar to those observed in adult animals, and no additional target organs of toxicity were identified. However, the plasma exposure levels of omeprazole were significantly higher in juvenile animals than adult animals.

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/s/

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8/9/2007 01:22:46 PM
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