

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-101

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: 22,101

SERIAL NUMBER: 000

DATE RECEIVED BY CENTER: September 27, 2006

DRUG NAME: Nexium / Esomeprazole, Delayed-Release Granules for oral suspension.

INTENDED CLINICAL POPULATION:

NEXIUM (esomeprazole magnesium) is proposed for the short-term treatment of gastroesophageal reflux disease (GERD) and erosive esophagitis (EE) in pediatric patients 1 to 11 years of age.

SPONSOR: AstraZeneca LP
Wayne, PA

DOCUMENTS REVIEWED: EDR - Module 4

REVIEW DIVISION: Division of Gastroenterology Products
(HFD-180)

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Date of review submission to Division File System (DFS):

June 4, 2007

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Executive Summary

1. Recommendations

1.1 Recommendation on approvability

From a preclinical standpoint, approval of NEXIUM (esomeprazole magnesium) is recommended for the short-term treatment of gastroesophageal reflux disease (GERD) and erosive esophagitis (EE) in pediatric patients 1 to 11 years of age.

1.2 Recommendation for nonclinical studies: None

1.3 Recommendation on labeling: None

2. Summary of nonclinical findings:

The results of the 1-month oral toxicity study in neonatal rats (#900404) and 3-month oral toxicity study in neonatal dogs (#900186) indicated that treatment with esomeprazole increased gastrin levels and stomach weight in the neonatal rats and dogs and increased the volume and number of ECL-cells in the neonatal rats. These findings were also identified in the previous toxicity studies with esomeprazole in the adult animals including 1-month and 3-month oral toxicity studies in rats and 3-months oral toxicity study in dogs. Re-examination of the heart tissues from the 3-month oral toxicity study in neonatal dogs (#900186) revealed minimal chronic epicardial inflammation in one control female, one male treated at 28 mg/kg/day, and one male treated at 55 mg/kg/day.

In the complementary 3-month oral toxicity study in neonatal dogs (900715), the chronic epicardial inflammation was identified in all dogs treated with esomeprozole at dose of 28 mg/kg twice a day (55 mg/kg/day). This was not seen in the control and 28 mg/kg once a day groups in this study. It appears that treatment with esomeprozole significantly increased the incidence of the chronic epicardial inflammation at 55 mg/kg/day but not at 28 mg/kg/day in neonatal dogs. The dose of 55 mg/kg/day was substantially higher than the proposed clinical dose of 10 or 20 mg/day and thus, the potential risk to pediatric patients is minimal.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22,101

Review number: 01

Sequence number/date/type of submission: June 29, 2006

Information to sponsor: Yes () No (x)

Sponsor and/or agent: AstraZeneca LP
Wayne, PA

Manufacturer for drug substance:

AstraZeneca AB
Södertälje, Sweden

Reviewer name: Ke Zhang

Division name: Division of Gastroenterology Products

HFD #: 180

Review completion date:

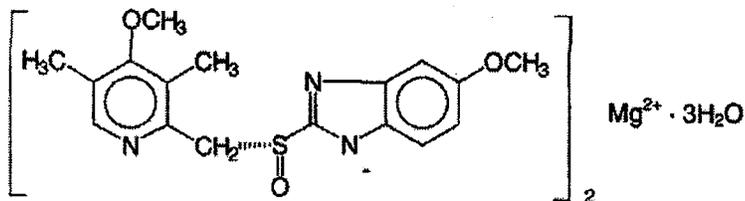
Drug:

Trade name: Nexium Delayed-Release Granules for oral
Suspension

Generic name: Esomeprazole

Chemical name: Bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate.

Structure:



Molecular Formula: $C_{34}H_{36}N_6O_6S_2Mg \cdot 3H_2O$

MW: 767.2

Relevant INDs/NDAs/DMFs: IND 53,733

Drug class: Gastric parietal cell H⁺/K⁺-ATPase inhibitor.

Indication: NEXIUM suspension (esomeprazole magnesium) is proposed for the short-term treatment of gastroesophageal reflux disease (GERD) and erosive esophagitis (EE) in pediatric patients 1 to 11 years of age.

Clinical formulation: Esomeprazole Sachets consist of esomeprazole pellets and excipient granules filled into single-use sachets in strengths 10, 20, and 40 mg. The total contents of a sachet should be added to water to form a viscous suspension prior to use. The suspension is suitable for administration by spoon, drinking or through an enteric tube.

b(4)

Table 1 Composition of Esomeprazole Sachets

Components (name according to AstraZeneca)	Quantity (mg/sachet)	Quantity (mg/sachet)			Function	Standard
		10 mg strength	20 mg strength	40 mg strength		
Esomeprazole pellets*						
Esomeprazole (corresponding to esomeprazole magnesium trihydrate)		10	20	40	Active substance	AstraZeneca
Glycerol monostearate 40-55			22.3	44.5		
Hydroxypropyl cellulose						
Hydroxypropyl methylcellulose						
Magnesium stearate						
Methacrylic acid copolymer type C						
Polysorbate 80						
Sugar spheres						
Talc						
Triethyl citrate						
Weight of esomeprazole pellets		42.7	85.8	171.6		

b(4)

Table I **Composition of Esomeprazole Sachets**

Components (name according to AstraZeneca)	Quantity (mg/sachet)			Function	Standard
	10 mg strength	20 mg strength	40 mg strength		
Excipient granules					
Glucose anhydrous					
Xanthan gum					
Polyvinylpyrrolidone crosslinked					
Citric acid					
Iron oxide					
Hydroxypropyl cellulose					
Weight of excipient granules					
Total weight in sachet					

b(4)

Route of administration: Oral suspension.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Any information or data necessary for approval of NDA 22,101 that AstraZeneca LP does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that AstraZeneca LP does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22,101.

Studies reviewed within this submission:

(1) A 3-Month Oral (Gavage) Toxicity and Toxicokinetic Study in the Neonatal Beagle Dog (a Second Complementary Study)

(2) The amendments of the 3-month oral toxicity study in neonatal dogs (#900186) and 8-week oral toxicity study in neonatal and young adult dogs (#900544).

Following studies were submitted to IND 53,733: pharmacokinetic studies and toxicity studies in neonatal rats (1-month oral toxicity studies in neonatal rats, study # 900404 and #900127) and dogs (3-month oral toxicity study in neonatal dogs study #900186 and 8-week oral toxicity study in neonatal and young adult dogs study #900544). These studies were reviewed on June 16, 2006. The pharmacology review of this amendment is attached in Appendix I.

Studies not reviewed within this submission: None.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Esomeprazole Magnesium: A 3-Month Oral (Gavage) Toxicity and Toxicokinetic Study in the Neonatal Beagle Dog (a Second Complementary Study)
(900715)

Testing Laboratories:

b(4)

Study Start and Completion Date: September 25, 2005 and August 29, 2006

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

Animals:

Male: 0.7-0.9 kg, 10 days old
Female: 0.6-0.8 kg, 10 days old
Beagle dogs (2/sex/group).

Drug Batch No.: 800/02.

Methods: The main objectives of this complementary toxicity study were to investigate the toxicokinetics of esomeprazole, cytochrome P450 activities, and *in vitro* metabolism of esomeprazole following different dosing regimens at various ages of dogs. Esomeprazole was given to the neonatal dogs orally at 0 and 80 $\mu\text{mol/kg}$ (0 and 28 mg/kg) once daily for 92 days. Another group received oral dose of 80 $\mu\text{mol/kg}$ (28 mg/kg) once a day from days 1-27 and then twice a day from days 28-92 (160

µmol/kg/day or 55 mg/kg/day). The parameters assessed were summarized in Table 5 in this report and this table is attached below.

Table 5 **Summary of main investigations**

Group Nos	No/group and sex	Assessments (all animals in each group)	Days pp
1 to 4	n= 4 (2M + 2F)	Day 1 of dosing: a) Toxicokinetics 20 MAD and 1 HAD only in controls, 20 MAD, 1, 4 and 7 HAD in esomeprazole treated animals	10
		Day 14 of dosing: a) Toxicokinetics 20 MAD and 2 HAD only in controls, 20 MAD, 1, 2, 3 and 4 HAD in esomeprazole treated animals	23
		Day 27 of dosing: a) Serum iron, TIBC, UIBC b) Hematology	36

Table 5 **Summary of main investigations**

Group Nos	No/group and sex	Assessments (all animals in each group)	Days pp
		Day 28 of dosing (first day of increased dose for Gr 2 and 4):	37
		a) Toxicokinetics (after both daily doses in Groups 2 and 4) 20 MAD and 2 HAD in Group 1 (vehicle controls, once daily) 20 MAD 1, 2 HAD 1, 4H 20 MAD 1 ^a and 6 HAD 1 ^a in Group 2 (vehicle controls, twice daily) 20 MAD, 1 HAD, 2 HAD, 3 HAD and 4 HAD in Group 3 (esomeprazole, once daily) 20 MAD 1, 1 HAD 1, 2 HAD 1, 3 HAD 1, 3H 55 MAD 1 ^b , 4H 20 MAD 1 ^a , 5 HAD 1 ^a , 6 HAD 1 ^a , 7 HAD 1 ^a and 8 HAD 1 ^a in Group 4 (esomeprazole, twice daily)	
		b) Serum Gastrin 2 HAD (after the first daily dose only in Groups 2 and 4) ^c	
		Day 42 of dosing:	51
		a) Toxicokinetics (after both daily doses in Groups 2 and 4) 20 MAD and 2 HAD in Group 1 (vehicle controls, once daily) 20 MAD 1, 2 HAD 1, 4H 20 MAD 1 ^a and 6 HAD 1 ^a in Group 2 (vehicle controls, twice daily) 20 MAD, 1 HAD, 2 HAD, 3 HAD and 4 HAD in Group 3 (esomeprazole, once daily) 20 MAD 1, 1 HAD 1, 2 HAD 1, 3 HAD 1, 3H 55 MAD 1 ^b , 4H 20 MAD 1 ^a , 5 HAD 1 ^a , 6 HAD 1 ^a , 7 HAD 1 ^a and 8 HAD 1 ^a in Group 4 (esomeprazole, twice daily)	
		Day 55 of dosing	64
		a) Serum iron, TIBC, UIBC	
		b) Hematology	
		Day 56 of dosing	65
		a) Toxicokinetics (after both daily doses in Groups 2 and 4) 20 MAD and 2 HAD in Group 1 (vehicle controls, once daily) 20 MAD 1, 2 HAD 1, 4H 20 MAD 1 ^a and 6 HAD 1 ^a in Group 2 (vehicle controls, twice daily) 20 MAD, 1 HAD, 2 HAD, 3 HAD and 4 HAD in Group 3 (esomeprazole, once daily) 20 MAD 1, 1 HAD 1, 2 HAD 1, 3 HAD 1, 3H 55 MAD 1 ^b , 4H 20 MAD 1 ^a , 5 HAD 1 ^a , 6 HAD 1 ^a , 7 HAD 1 ^a and 8 HAD 1 ^a in Group 4 (esomeprazole, twice daily)	
		b) Serum Gastrin 2 HAD (after the first daily dose only in Groups 2 and 4) ^c	
		Day 91 of dosing (after 3 months' dosing):	100
		a) Toxicokinetics (after both daily doses in Groups 2 and 4) 20 MAD and 2 HAD in Group 1 (vehicle controls, once daily) 20 MAD 1, 2 HAD 1, 4H 20 MAD 1 ^a and 6 HAD 1 ^a in Group 2 (vehicle controls, twice daily) 20 MAD, 1 HAD, 2 HAD, 3 HAD and 4 HAD in Group 3 (esomeprazole, once daily) 20 MAD 1, 1 HAD 1, 2 HAD 1, 3 HAD 1, 3H 55 MAD 1 ^b , 4H 20 MAD 1 ^a , 5 HAD 1 ^a , 6 HAD 1 ^a , 7 HAD 1 ^a and 8 HAD 1 ^a in Group 4 (esomeprazole, twice daily)	
		b) Serum Gastrin 2 HAD (after the first daily dose only in Groups 2 and 4) ^c	

Table 5 **Summary of main investigations**

Group Nos	No/group and sex	Assessments (all animals in each group)	Days <i>pp</i>
		Terminal investigations (after 3 months' dosing): a) Serum iron, TIBC, UIBC b) Hematology c) Organ weights, gross pathology d) CYP enzyme investigations + <i>in vitro</i> metabolism in liver microsomes	102
5 to 8	n= 4 (2M + 2F)	Untreated animals: a) CYP enzyme investigations + <i>in vitro</i> metabolism in liver microsomes	10, 23, 37 and 65, in Groups 5, 6, 7 and 8, respectively

pp= post partum

MAD 1 or 2= Minutes after the first or second daily dose

HAD 1 or 2= Hours after the first or second dose

- a From Day 28 of dosing, the TK samples after the second daily dose were taken 20 MAD 2 and 2 HAD 2 in the controls treated twice daily (Group 2), and 20 MAD, 1, 2, 3 and 4 HAD 2 in the esomeprazole animals treated twice daily (Group 4). However, these sampling times were expressed as 4H 20 MAD 1, 5 HAD 1, 6 HAD 1, 7 HAD 1 and 8 HAD 1 (as relevant in the different groups) in the raw data and on the sampling sheets, to assist TK calculation
- b From Day 28 of dosing, the 5th sample after Dose 1 was taken 3 H 55 MAD, instead of 4 HAD in Group 4, to enable the second dose to be given exactly 4 hours after the first dose in Group 4
- c Samples for gastrin were taken at the same time as the sample taken for TK evaluation, 2 HAD 1

Results:

1. Clinical Signs: One male given 80 µmol/kg esomeprazole had convulsions, slight tremors, increased vocalization, slight incoordination, skin pallor and lied on its side for a short period of time post dosing on Dose Day 72. Vomiting was noted in the twice daily dosing group.

2. Mortality: There were no deaths in this study.

3. Body Weight: The mean initial and final body weights in the control animals were 652 g and 5.2 kg (males) or 583.5 g and 5 kg (females), respectively. Slightly higher body weight gain was noted in the treatment groups as compared to the control. The results were presented in Table 11 in this report. This table is attached below.

Table 11 Summary of body weight gains (kg) for neonatal/juvenile dogs given esomeprazole once or once/twice daily for 3 months

Sex Group Dose ($\mu\text{mol/kg}$)	Males				Females			
	1 0	2 0 ^a	3 80	4 80/160 ^a	1 0	2 0 ^a	3 80	4 80/160 ^a
Dose Days 1-28 (10-37 <i>pp</i>)	1.0	1.4	1.3	1.5	1.0	1.1	1.4	1.2
Dose Days 28-50 (37-59 <i>pp</i>)	1.1	1.2	1.1	1.5	1.3	0.7	1.2	0.9
Dose Days 50-91 (59 to 100 <i>pp</i>)	2.6	2.5	2.7	2.3	2.3	1.4	2.4	1.7
Dose Days 1-91 (10-100 <i>pp</i>)	4.7	5.1	5.1	5.3	4.6	3.2	5.0	3.8
(% of relevant control)			(109)	(104)			(109)	(119)

a Commencing on Day 28 of dosing (Day 37 *post partum*), the given dose in this group was administered twice daily, at approximately 4 hours apart

4. **Growth Measurement and developments:** Growth (height and length) at the end of treatment was not clearly affected by the treatment. The results were summarized in Table 12 in this report and this table is attached below.

Table 12 Summary of growth (height and length) for neonatal/juvenile dogs given esomeprazole once or once/twice daily for 3 months

Sex Group Dose ($\mu\text{mol/kg}$)	Males				Females			
	1 0	2 0 ^a	3 80	4 80/160 ^a	1 0	2 0 ^a	3 80	4 80/160 ^a
Height (cm)								
Dose Days -3 to 26 (Days 7 to 35 <i>pp</i>)	7.0	7.0	7.8	8.0	7.5	6.2	6.8	8.2
(% of equivalent control)			(111)	(114)			(91)	(132)
Dose Days 26 to 89 (Days 35 to 98 <i>pp</i>)	12.2	10.8	10.7	9.2	10.7	9.0	11.7	8.0
(% of equivalent control)			(88)	(85)			(109)	(89)
Length (cm)								
Dose Days -3 to 26 (Days 7 to 35 <i>pp</i>)	13.3	8.5	8.5	14.5	12.3	9.7	10.5	13.3
(% of equivalent control)			(64)	(171)			(85)	(137)
Dose Days 26 to 89 (Days 35 to 98 <i>pp</i>)	18.7	20.5	22.5	16.2	17.7	13.8	21.3	14.7
(% of equivalent control)			(120)	(79)			(120)	(107)

a Commencing on Day 28 of dosing (Day 37 *post partum*), the given dose in this group was administered twice daily, at approximately 4 hours apart

Nursing behavior, auricular startle, pupillary closure, gait and behavior were unaffected. The day of development of vaginal opening and preputial separation was not clearly affected by the treatment.

The parameters measured in the qualitative observational battery were not affected and the results were presented in Table 32 in this report. This table is attached below.

Table 32 Observational battery - Group summary (Groups 1 to 4, only)

Week 6 - Males

Category	Group 1 Vehicle Control		Group 2 Vehicle Control 0 (2 x 0) ^a		Group 3 Esomeprazole		Group 4 Esomeprazole 160 (2 x 80) ^a		
	0 µmol/kg/day		µmol/kg/day		80 µmol/kg/day		µmol/kg/day		
	No.	%	No.	%	No.	%	No.	%	
2	2	100.0	2	100.0	2	100.0	2	100.0	
Home cage									
Body position									
4	Lying on side/curled Up	0	0.0	2	100.0	2	100.0	0	0.0
5	Sitting/standing	2	100.0	0	0.0	1	50.0	2	100.0
Locomotor activity level: (spatial movement)									
0	None; resting	0	0.0	1	50.0	0	0.0	0	0.0
2	Slight movement	0	0.0	1	50.0	2	100.0	0	0.0
4	Moderate movement	2	100.0	0	0.0	1	50.0	2	100.0
6	Vigorous movement, sudden darting	0	0.0	0	0.0	0	0.0	0	0.0
ET	Eating	0	0.0	0	0.0	0	0.0	1	50.0
Arena									
Body position									
2	Lying down on ventral surface	0	0.0	0	0.0	1	50.0	0	0.0
5	Sitting/standing	2	100.0	2	100.0	2	100.0	2	100.0
Locomotor activity level: (spatial movement)									
4	Moderate movement	2	100.0	2	100.0	2	100.0	2	100.0
6	Vigorous movement, sudden darting	0	0.0	0	0.0	2	100.0	1	50.0
Bizarre/stereotypic behavior									
0	None	2	100.0	2	100.0	2	100.0	2	100.0

No treatment effects by the neurological examination were noted in all animals during Weeks 6, 9 and 12.

5. Hematology: Slight decreases in red blood cell counts, hemoglobin and hematocrit values were noted in males treated at 80/160 µmol/kg esomeprazole on Days 27 and 55. These changes were not noted at the end of the treatment period. In females treated at 80/160 µmol/kg esomeprazole, slight reductions in red blood cell counts, hemoglobin and hematocrit values were also noted on Dose Day 55. The results were presented in Table 13 in this report. This table is attached below.

Table 13 Summary of noteworthy haematology findings in neonatal/juvenile dogs given esomeprazole once or once/twice daily for 3 months

Parameter measured and sampling occasion	Dose ($\mu\text{mol/kg}$)							
	0	0 ^a	80	80/160 ^a	0	0 ^a	80	80/160 ^a
Group	Males				Females			
	1	2	3	4	1	2	3	4
Red blood cell count ($10^6/\mu\text{L}$)								
Dose Day 27 (Day 36 <i>pp</i>)	4.5	4.8	4.2	3.7	4.4	4.3	4.3	4.2
Dose Day 55 (Day 64 <i>pp</i>)	5.4	5.8	4.9	4.2	5.6	5.7	5.0	4.1
End of dosing (Day 101 <i>pp</i>)	5.9	6.1	6.2	6.4	6.5	6.2	5.7	5.9
Haemoglobin (g/dL)								
Dose Day 27 (Day 36 <i>pp</i>)	9.6	10.4	9.4	8.1	9.4	9.2	9.6	9.5
Dose Day 55 (Day 64 <i>pp</i>)	11.0	12.6	8.9	6.8	11.8	11.6	9.8	7.4
End of dosing (Day 101 <i>pp</i>)	11.9	13.2	10.7	11.3	13.4	13.2	11.7	11.1
Haematocrit (%)								
Dose Day 27 (Day 36 <i>pp</i>)	31	34	32	26	31	30	33	31
Dose Day 55 (Day 64 <i>pp</i>)	34	39	31	23	37	37	32	24
End of dosing (Day 101 <i>pp</i>)	37	40	35	37	41	40	37	36
Reticulocyte count ($10^9/\text{L}$)								
Dose Day 27 (Day 36 <i>pp</i>)	187	187	282	103	218	173	224	120
Dose Day 55 (Day 64 <i>pp</i>)	137	98	222	101	112	103	158	107
End of dosing (Day 101 <i>pp</i>)	107	110	216	83	91	139	130	115

a Commencing on Day 28 of dosing (Day 37 post partum), the given dose in this group was administered twice daily, at approximately 4 hours apart
Numbers in bold indicate treatment-related changes

6. Clinical Chemistry: Slight increase in total iron binding capacity (TIBC) was noted in the treatment groups. The results were presented in Table 14 in this report. This table is attached below.

Table 14 Summary of serum iron, UIBC and TIBC in neonatal/juvenile dogs given esomeprazole once or once/twice daily for 3 months

Parameter measured and sampling occasion	Dose ($\mu\text{mol/kg}$)								
	0	0 ^a	80	80/160 ^a		0	0 ^a	80	80/160 ^a
	Males				Females				
Group	1	2	3	4	1	2	3	4	
Serum Iron ($\mu\text{g}/\mu\text{L}$)									
Dose Day 27 (Day 36 <i>pp</i>)	110	132	135	52	184	71	154	71	
Dose Day 55 (Day 64 <i>pp</i>)	212	116	214	63	150	101	259	65	
End of dosing (Day 101 <i>pp</i>)	190	204	219	146	190	100	237	381	
UIBC ($\mu\text{g}/\text{dL}$)									
Dose Day 27 (Day 36 <i>pp</i>)	132	126	196	310	73	227	213	302	
Dose Day 55 (Day 64 <i>pp</i>)	146	219	236	332	201	228	177	305	
End of dosing (Day 101 <i>pp</i>)	207	151	212	263	186	263	176	148	
TIBC ($\mu\text{g}/\text{dL}$)									
Dose Day 27 (Day 36 <i>pp</i>)	242	258	331	362	257	297	366	373	
Dose Day 55 (Day 64 <i>pp</i>)	357	335	450	395	351	329	436	370	
End of dosing (Day 101 <i>pp</i>)	396	354	431	409	375	362	413	455	

a Commencing on Day 28 of dosing (Day 37 post partum), the given dose in this group was administered twice daily, at approximately 4 hours apart
Numbers in bold indicate treatment-related changes

7. **Serum gastrin:** Serum gastrin levels were increased in the esomeprazole groups as compared to the controls. The results were summarized in Table 15 in this report. This table is attached below.

Table 15 Mean (range) serum gastrin levels (pg/mL) in neonatal/juvenile dogs given esomeprazole once/twice daily for 3 months

Dose Group	Males		Females	
	2 0 ^a	4 80/160 ^a	2 0 ^a	4 80/160 ^a
Dose Day 28 (Day 37 <i>pp</i>)	71 (60-82) ^b	408 (401-414) ^b	70 (68-71) ^b	95 (85-105) ^b
Fold change compared to controls	-	\uparrow 5.7	-	\uparrow 1.4
Dose Day 56 (Day 65 <i>pp</i>)	<LLOQ ^c	1719 (1364-2075) ^b	<LLOQ ^c	1439 (1426-1453) ^b
Fold change compared to controls	-	\uparrow 104	-	\uparrow 87
Dose Day 91 (Day 100 <i>pp</i>)	51 ^d	1288 (1115-1461) ^b	<LLOQ ^c	1610 (1341-1879) ^b
Fold change compared to controls	-	\uparrow 25	-	\uparrow 98

<LLOQ= lower limit of quantitation (33.1 pg/mL)

a Commencing on Day 28 of dosing (Day 37 post partum), the given dose was administered twice daily, at approximately 4 hours apart

b n= 2

c Both of the values were <LLOQ. Half of the LLOQ (ie, 16.5 pg/mL) has been used for the control value in the comparison between the esomeprazole-treated and control animals

d n= 1; one of the 2 values was <LLOQ

Numbers in bold indicate treatment-related changes

8. Cytochrome P450 enzyme: The total P450 enzyme activity and the activity of each of individual enzymes were either slightly increased or slightly decreased at the end of the treatment period. The results were summarized in the following table.

Table 2 Mean cytochrome P450 and b₅ content and CYP enzyme activities in dog liver microsomes after 3 months' oral administration of the vehicle or esomeprazole

Group	1	2	3	4
Treatment	Vehicle	Vehicle	Esomeprazole	Esomeprazole
Dose (µmol/kg)	0	0/0 ^a	80	80/160 ^a
Age at sampling (Day <i>pp</i>)	102	102	102	102
Total P450 ^{b,c}	899 ±70.3	901±80	793 ±168	1089 ±249
Fold change ^d	-	-	↓ 0.88	↑ 1.2
Total b ₅ ^{b,c}	499 ±67	507 ±32	445 ±61	495 ±94
Fold change ^d	-	-	↓ 0.88	↓ 0.98
EROD activity ^{b,e} [CYP1A1/2]	417 ±101	246 ±19	623 ±89	759 ±100
Fold change ^d	-	-	↑ 1.9	↑ 2.3
BROD activity ^{b,e} [CYP2B11]	464 ± 78	409 ±34	299 ±58	341 ±63
Fold change ^d	-	-	↓ 0.68	↓ 0.78
CZXH activity ^{b,e} [CYP2E1]	2080 ±486	1676 ±376	2142 ±438	3105 ±1069
Fold change ^d	-	-	↑ 1.1	↑ 1.7
TESH activity ^{b,e} [CYP3A12]	1441 ±205	1648 ±315	908 ±156	954 ±211
Fold change ^d	-	-	↓ 0.59	↓ 0.62
DEMD activity ^{b,e} [CYP2D15]	176 ±32	139 ±14	105 ±3.9	103 ±17
Fold change ^d	-	-	↓ 0.67	↓ 0.65
DIFH activity ^{b,e} [CYP2C21]	104 ±3.1	108 ±13	69 ±16	77 ±15
Fold change ^d	-	-	↓ 0.65	↓ 0.73

Day *pp*= Day *post partum*

n = 2 males and 2 females in each group

- The dose of vehicle or esomeprazole was increased to twice daily (approximately 4 h apart) from Dose Day 28 (Day 37 *post partum*) in Groups 2 and 4
- Values are expressed as the mean ±SD (males and females combined)
- The units for the P450 and b₅ content are pmol/mg protein
- Fold change compared to the combined controls (↑ = increase, ↓ = decrease)
- The units for the CYP enzyme activities are pmol/mg protein*min

The total and individual P450 enzyme activities were increased with age in the untreated animals. The results were summarized in the following table.

Table 3 Mean cytochrome P450 and b₅ content and CYP enzyme activities in liver microsomes from untreated neonatal/juvenile dogs of different ages

Group	5	6	7	8	1 + 2 ^a
Treatment	Untreated	Untreated	Untreated	Untreated	Controls ^a
Age at sampling (Day <i>pp</i>)	10	23	37	65	102
Total P450 ^{b,c}	527 ±200	581 ±40	678 ±187	892 ±129	900 ±70
Fold change ^d	-	↑ 1.1	↑ 1.3	↑ 1.7	↑ 1.7
Total b ₅ ^{b,c}	322 ±84	396 ±32	385 ±61	518 ±101	503 ±49
Fold change ^d	-	↑ 1.2	↑ 1.2	↑ 1.6	+↑ 1.6
EROD activity ^{b,e} [CYP1A1/2]	49 ±20	40 ±8	199 ±77	431 ±74	331 ±114
Fold change ^d	-	↓ 0.82	↑ 4.1	↑ 8.8	↑ 6.8
BROD activity ^{b,e} [CYP2B11]	44 ±15	150 ±29	291 ±33	361 ±42	437 ±63
Fold change ^d	-	↑ 3.4	↑ 6.6	↑ 8.2	↑ 9.9
CZXH activity ^{b,e} [CYP2E1]	434 ±83	760 ±109	1426 ±353	2128 ±546	1878 ±457
Fold change ^d	-	↑ 1.8	↑ 3.3	↑ 4.9	↑ 4.3
TESH activity ^{b,e} [CYP3A12]	1466 ±745	2002 ±404	1749 ±457	1848 ±356	1544 ±270
Fold change ^d	-	↑ 1.4	↑ 1.2	↑ 1.3	↑ 1.1
DEMD activity ^{b,e} [CYP2D15]	211 ±47	158 ±16	167 ±17	169 ±26	158 ±30
Fold change ^d	-	↓ 0.75	↓ 0.79	↓ 0.80	↓ 0.75
DIFH activity ^{b,e} [CYP2C21]	32 ±8.8	44 ±4.1	64 ±9.6	91 ±6.7	106 ±9.2
Fold change ^d	-	↑ 1.4	↑ 2.0	↑ 2.8	↑ 3.3

Day *pp* = Day post partum

n = 2 males and 2 females in each group

- a These are the combined results from the 2 vehicle control groups (Groups 1 and 2), representing untreated pups at 102 days of age
- b Values are expressed as the mean ±SD (males and females combined)
- c The units for the P450 and b₅ content are pmol/mg protein
- d Fold change compared to the values in the youngest age group of pups [i.e. 10 days post partum] (↑ = increase, ↓ = decrease)
- e The units for the CYP enzyme activities are pmol/mg protein*min.

9. Organ Weights: Increased liver and stomach weights and decreased spleen and thymus weights were noted in the treatment groups as compared to the controls. The results are presented in the following table.

Table 19 Treatment-related changes in absolute and relative (% brain) organ weights in neonatal/juvenile dogs given esomeprazole once or once/twice daily for 3 months

Group	Dose (µmol/kg)	Males				Females			
		0	0 ^a	80	80/ 160 ^a	0	0 ^a	80	80/ 160 ^a
Brain weight	Absolute (g)	64	67	67	70	71	60	66	65
	(% of relevant control)			(105)	(104)			(93)	(108)
Stomach weight	Absolute (g)	48	59	89	102	47	37	87	72
	(% of relevant control)			(185)	(173)			(185)	(195)
	Relative to brain weight (%)	76	87	132	147	66	62	133	110
	(% of relevant control)			(174)	(169)			(202)	(177)
Liver weight	Absolute (g)	168	177	209	251	159	135	190	167
	(% of relevant control)			(124)	(142)			(119)	(124)
	Relative to brain weight (%)	262	264	311	361	225	225	289	256
	(% of relevant control)			(119)	(137)			(128)	(114)
Spleen weight	Absolute (g)	22.4	30.5	20.2	19.1	30.1	25.2	18.9	15.8
	(% of relevant control)			(90)	(63)			(63)	(63)
	Relative to brain weight (%)	35.1	45.5	30.0	27.4	43.1	42.0	28.7	24.0
	(% of relevant control)			(85)	(60)			(67)	(57)
Thymus weight	Absolute (g)	8.8	8.2	6.4	13.6	9.7	9.7	5.8	7.3
	(% of relevant control)			(73)	(166)			(60)	(75)
	Relative to body weight (%)	13.7	12.3	9.5	19.5	14.0	16.2	8.9	11.1
	(% of relevant control)			(69)	(159)			(64)	(69)

a Commencing on Day 28 of dosing (Day 37 post partum), the given dose was administered twice daily at approximately 4 hours apart
Numbers in bold indicate treatment-related changes

10. **Gross Pathology:** There were no treatment related changes.

11. **Microscopic Pathology:** Chronic minimal inflammation of the epicardium localized on the left atrium and ventricles (at the base, adjacent to the atrium) was noted in all 4 pups treated at 80/160 µmol/kg. The inflammation was characterized by formation of fibrous papillary projections covered by enlarged mesothelial cells. These animals were from the same litter. The chronic inflammation was not seen in the control groups and in the group of 80 µmol/kg once a day.

No ECL-cell hyperplasia was noted in any of the animals examined on light microscopic examination of the Sevier-Munger stained stomach specimens.

12. **Toxicokinetics:** The toxicokinetic results were summarized in the following table.

Table 1 Summary of median (range) C_{max1} , C_{max2} , $AUC_{(0-4h)}$ and AUC for esomeprazole in neonatal/juvenile dogs following oral administration of esomeprazole for 3 months

Daily dose ($\mu\text{mol/kg}$)	Group	Dose Day	Day <i>pp</i>	C_{max1} ($\mu\text{mol/L}$)	C_{max2} ($\mu\text{mol/L}$)	$AUC_{(0-4h)}$ ($\mu\text{mol}\cdot\text{h/L}$)	AUC ($\mu\text{mol}\cdot\text{h/L}$)
80	3	1	10	29 (17-75)	NA	67 (43-119)	72 (47-122)
80	3	14	23	27 (22-48)	NA	26 (15-40) ^a	22 (15-40)
80	3	28	37	72 (57-88)	NA	53 (46-75)	53 (46-75)
80	3	42	51	4.5 (3.0-71)	NA	6.2 (2.6-37)	6.9 (2.7-37) ^b
80	3	56	65	12 (5.7-14)	NA	11 (10-11) ^c	11 (3.7-11)
80	3	91	100	6.3 (2.2-7.1)	NA	5.1 (4.3-5.5)	5.1 (4.4-5.5)
80	4	1	10	43 (37-62)	NA	64 (53-108)	91 (68-113) ^c
80	4	14	23	21 (9.2-74)	NA	15 (10-49) ^a	17 (10-49)
80+80	4	28	37	59 (21-98)	33 (29-37)	47 (29-80)	82 (59-120)
80+80	4	42	51	9.0 (4.8-12)	24 (14-35)	8.5 (3.8-10)	33 (26-54)
80+80	4	56	65	10 (8.3-21)	26 (17-38)	13 (9.4-18)	58 (36-65) ^b
80+80	4	91	100	3.4 (3.0-9.1)	30 (5.4-61)	3.5 (2.2-7.0)	29 (6.6-47)

pp = post partum

NA = not applicable

N = 2M+2F except where noted

a 2M+1F

b 1M+2F

c 0M+2F

In summary, Esomeprazole was given to the neonatal dogs orally at 0 and 80 $\mu\text{mol/kg}$ (0 and 28 mg/kg) once daily for 92 days. Another group received oral dose of 80 $\mu\text{mol/kg}$ (28 mg/kg) once a day from days 1-27 and then twice a day (160 $\mu\text{mol/kg/day}$ or 55 mg/kg/day) from days 28-92. Convulsion, slight tremor, increased vocalization, in-coordination, and skin pallor were noted in one low dose male. As expected, serum gastrin and stomach weights were increased in treatment groups. Histopathological examination revealed chronic inflammation of the epicardium localized on the left atrium and ventricles in all animals treated at 80/160 $\mu\text{mol/kg}$ (none in the control and 28 mg/kg once a day groups). The control animals had a small but clear increase in some CYP enzyme activities and in total cytochrome P450 and b5 content with age.

Amendments for the 3-month oral toxicity study in neonatal dogs (#900186) and the 8-week oral toxicity study in neonatal and young adult dogs (#900544)

Due to the chronic inflammation identified in the epicardium of the left atrium and ventricles in dogs treated with esomeprazole

in the second complementary 3-month oral toxicity study in neonatal dogs, the sponsor re-examined histopathologically the heart tissues from the previous 3-month and 8-week toxicity studies in neonatal dogs (#900186 and #900544).

3-month oral toxicity study in neonatal dogs (#900186)

Table 1 **Groups and dose levels**

Group	Animals	Animal reference numbers	Treatment	Daily dose level $\mu\text{mol/kg}$	mg/kg
Phase II (Main dose phase)^a					
1	5M	101-105	Vehicle	0	0
	4F	151-154			
2	5M	201-205	Esomeprazole	80	28
	3F	251-253			
3	4M	301-304 ^b	Esomeprazole	160/120 ^b	55/41
	5F	351-355 ^b			
Recovery animals^a					
1	2M	101, 105	Vehicle	0	0
	2F	153, 154			
2	3M	201, 204, 205	Esomeprazole	80	28
	1F	253			
3	1M	304	Esomeprazole	160/120 ^b	55/41
	2F	353, 354			

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

- a Phase II: Animals were dosed for 3 months (Day 10 to Day 101, 102, or 103 *pp*), followed by a 3 month recovery period.
- b One male (302) and 1 female (355) from Group 3 were euthanised as moribund and the dose was lowered for the surviving animals in this group, from Day 53/54 to termination of dosing

Sections of heart were examined for all animals. Minimal chronic epicardial inflammation was found in the following dogs:

- Dog No 151 (Group 1 female, control: necropsy after 3 months' dosing)
- Dog No 201 (Group 2 male, 80 $\mu\text{mol/kg}$ esomeprazole: necropsy after 3 months' dosing + 3 months' recovery)
- Dog No 301 (Group 3 male, 160/120 $\mu\text{mol/kg}$ esomeprazole: necropsy after 3 months' dosing)

In summary, the minimal chronic epicardial inflammation was identified in one control female, one low dose male (28 mg/kg), and one high dose male (55 mg/kg).

8-week oral toxicity study in neonatal and young adult dogs (#900544)

Table 1 Groups and dose levels

Group/ Age ^a	No. of Animals	Animal reference numbers	Days of treatment	Treatment	Daily dose level	
					µmol/kg	mg/kg
1 - Pups	3M 1F	101-103 151	Days 10-65 <i>pp</i>	Vehicle	0	0
2 - Pups	2M 2F	201-202 251-252	Days 10-65 <i>pp</i>	Esomeprazole	80	28
3 - Pups	2M 2F	301-302 351-352	Days 10-65 <i>pp</i>	Esomeprazole	80/120 ^b	28/41 ^b
4 - Pups	2M 2F	401-402 451-452	Days 10-60 <i>pp</i> ^c	Esomeprazole	120/180 ^d	41/62 ^d
5 - Pups	2M 2F	501-502 551-552	Days 10, 23, 37, 51 & 65 <i>pp</i>	Esomeprazole	80 ^e	28 ^e
6 - Young adult dogs	2M 2F	601-602 651-652	Dose Days 1-56	Esomeprazole	80	28

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

- a The animals in Groups 1 to 5 were 10 days old at the start of dosing. The animals in Group 6 were approximately 6 to 8 months old at the start of dosing.
- b The doses were increased by 50% on Dose Day 28, from 80 to 120 µmol/kg.
- c The animals in this group were terminated on Days 58 or 60 *post partum*.
- d The doses were increased by 50% on Dose Day 28, from 120 to 180 µmol/kg.
- e Group 5 was dosed intermittently, once every 14 days, on Days 10, 23, 37, 51 and 65 *post partum* (Dose Days 1, 14, 28, 42 and 56 in the other groups of pups).

Sections of heart were examined for all animals. Minimal chronic epicardial inflammation was found in the following dog:

-Dog No 351 (Group 3 female, 80/120 µmol/kg esomeprazole: necropsy after 2 months' dosing)

In summary, the minimal chronic epicardial inflammation was identified in one female treated at 28/41 mg/kg but not at higher dose of 41/62 mg/kg.

LABELING:

The preclinical portion of the proposed labeling is consistent with the Nexium labeling. Therefore, no change in the current labeling is recommended.

3.6 OVERALL conclusions and recommendations

Esomeprazole magnesium is a S-enantiomer of the racemic proton pump inhibitor, omeprazole, which inhibits H⁺/K⁺-ATPase activity in the gastric parietal cells and thus blocks the final step of the gastric acid secretion. In the present NDA, the sponsor is seeking approval to market esomeprazole magnesium for the short-term treatment of gastroesophageal reflux disease (GERD) and erosive esophagitis (EE) in pediatric patients 1 to 11 years of age. The proposed dose is 10 or 20 mg once daily for up to 8 weeks.

According to the Amended Written Requests for pediatric studies for Nexium® (esomeprazole magnesium) Delayed-Release Capsules issued on December 20, 2005 under IND 53,733, the sponsor is required to conduct (1) a 4-week repeated dose toxicity study in neonatal rats and (2) a 90-day repeated dose toxicity study in neonatal dogs. The reports of these studies were submitted to IND 53,733 and reviewed on June 16, 2006. The pharmacology review of this amendment is attached in Appendix I.

In the 1-month oral toxicity study in neonatal rats (#900404), rat pups were given esomeprazole (0, 93 or 280 mg/kg) or omeprazole (140 mg/kg) by oral gavage, once daily, for 28 days, from Day 7 to Day 34 post partum. 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 appearance. 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 central nervous system and the stomach were the target organs of toxicity.

In the 3-month oral toxicity study in neonatal dogs (#900186), esomeprazole was given to 10-day old Beagle pups orally at 0, 80 and 160 µmol/kg (0, 28 and 55 mg/kg) once daily for 92 days. The higher dose was reduced to 120 µmol/kg (41 mg/kg) after 53/54 days of dosing due to clinical signs of toxicity. Treatment with esomeprazole induced clinical signs of toxicity (convulsions, head shaking, eye discharge, salivation, and oily fur), decreased body weight gain and growth, and increased gastrin secretion. Treatment-related increases in the

stomach weight, stomach reference (tissue) volume and height of the gastric mucosa were observed in both esomeprazole-treated pups. The plasma levels of the test drug were much lower at the end of the treatment than those on day 1. The central nervous system and the stomach were the target organs of toxicity. Re-examination of the heart tissue revealed that three dogs (one control female, one male treated at 28 mg/kg/day at the end of recovery period, and one male treated at 55 mg/kg/day at the end of 3 month dosing) had minimal chronic epicardial inflammation.

In the current submission, the sponsor also submitted a complementary 3-month oral toxicity study in dogs (900715). In this study, esomeprazole was given to the neonatal dogs orally at 0 and 80 $\mu\text{mol/kg}$ (0 and 28 mg/kg) once daily for 92 days. Another group received oral dose of 80 $\mu\text{mol/kg}$ (28 mg/kg) once a day from days 1-27 and then twice a day from days 28-92 (160 $\mu\text{mol/kg/day}$ or 55 mg/kg/day). Convulsion, slight tremor, increased vocalization, in-coordination, and skin pallor were noted in one low dose male. As expected, serum gastrin and stomach weights were increased in treatment groups. Histopathological examination revealed chronic inflammation of the epicardium localized on the left atrium and ventricles (at the base, adjacent to the atrium) in all animals treated at 80/160 $\mu\text{mol/kg}$. This was not seen in the control group and in the 28 mg/kg/day group. The control animals had a small but clear increase in some CYP enzyme activities and in total cytochrome P450 and b5 content with age.

Esomeprazole is approved for treatment of GERD in adult patients. In the current submission, the sponsor is seeking approval to market esomeprazole magnesium for short-term treatment of GERD in patients 1-11 years old. The results of the 1-month oral toxicity study in neonatal rats (#900404) and 3-month oral toxicity study in neonatal dogs (#900186) indicated that treatment with esomeprazole increased gastrin levels and stomach weight in the neonatal rats and dogs and increased the volume and number of ECL-cells in the neonatal rats. These findings were also identified in the previous toxicity studies with esomeprazole in the adult animals including 1-month and 3-month oral toxicity studies in rats and 3-months oral toxicity study in dogs.

In the complementary 3-month oral toxicity study in neonatal dogs (900715), the chronic epicardial inflammation was identified in all dogs treated with esomeprozole at dose of 28 mg/kg twice a day (55 mg/kg/day). This was not seen in the control and 28 mg/kg once a day groups in this study. Re-

Appendix I: Pharmacology review of Amendment #351 of IND 53,733 dated March 6, 2006.

PHARMACOLOGIST'S REVIEW of IND 53,733
(Amendment #351 Dated March 6, 2006)

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Sponsor and Address: AstraZeneca LP
Wilmington, DE

Dates of Submission: March 6, 2006

Date of HFD-180 Receipt: March 7, 2006

Date of Review: June 16, 2006

Drug: Nexium / H 199/18, delayed release capsules

Category: Gastric parietal cell H⁺/K⁺ ATPase Inhibitor.
H 199/18 is an enantiomer of omeprazole.

Submission Contents: (1) pharmacokinetic studies, (2) 1-month oral toxicity study in neonatal rats, (3) 3-month oral toxicity study in neonatal dogs, (4) 8-week oral toxicity study in neonatal and young adult dogs (a complementary study).

Background: Amended Written Requests for pediatric studies for Nexium® (esomeprazole magnesium) Delayed-Release Capsules were issued on December 20, 2005. In the requests, the sponsor is asked to conduct (1) a 4-week repeated dose toxicity study in neonatal rats and (2) a 90-day repeated dose toxicity study in neonatal dogs. The sponsor submitted the reports of these studies in the current submission.

Pharmacokinetics:

Esomeprazole: Comparison of the *In Vitro* Plasma Protein Binding of [14C]Esomeprazole in Neonatal, Juvenile and Adult Rat and Dog (Zen/12)

Methods: To determine the *in vitro* plasma protein binding of esomeprazole in neonatal, juvenile and adult rats and dogs,

plasma samples were collected from previous studies (CTBR study references 900404 and 900186). The plasma protein binding of esomeprazole-14C was determined at 2 concentrations (5 and 50 μM) by equilibrium dialysis techniques in neonatal, juvenile and adult rat and dog plasmas. These concentrations represent the plasma levels observed in the low to intermediate range in the toxicity studies in neonatal and adult animals. Plasma had been collected from animals of the following ages:

Rat: Day 7, Day 34 and Day 122 \pm 2 *post partum*

Dog: Day 23, Day 37, Day 71/75, Day 101 and 191/192 *post partum*

Results: The results were presented in Tables 1 and 2 in this report. These tables are attached below.

Table 1 Plasma protein binding of esomeprazole in neonatal, juvenile and adult rat

Nominal Esomeprazole Conc.			Day 7 <i>pp</i>	Day 34 <i>pp</i>	Day 122 \pm 2 <i>pp</i>
Rat	5 μM	Mean	94.7	90.6	92.8
		\pm SD	\pm 0.4	\pm 0.7	\pm 0.8
	50 μM	Mean	88.8	89.6	91.8
		\pm SD	\pm 1.0	\pm 0.6	\pm 0.6

pp = *post partum*

Table 2 Plasma protein binding of esomeprazole in neonatal, juvenile and adult dog

Nominal Esomeprazole Conc.			Day 23 <i>pp</i>	Day 37 <i>pp</i>	Day 71/75 <i>pp</i>	Day 101 <i>pp</i>	Day 191/192 <i>pp</i>
Dog	5 μM	Mean	90.3	89.6	91.6	91.3	91.7
		\pm SD	\pm 1.3	\pm 0.8	\pm 0.5	\pm 0.5	\pm 0.6
	50 μM	Mean	83.8	82.4	85.9	85.1	87.0
		\pm SD	\pm 1.4	\pm 0.6	\pm 1.0	\pm 1.3	\pm 0.9

pp = *post partum*

The results indicated that there were no clear differences in the degree of protein binding at the different age in both rats and dogs. The degree of protein binding was slightly lower (~6%) at 50 μM than that at 5 μM on at Day 7 *post partum* in rats. In dogs, the degree of protein binding was ~6% lower at 50 μM than at 5 μM in all age groups.

Esomeprazole: In vitro Metabolism Studies using Liver Microsomes
from Esomeprazole Treated Neonatal and Young Adult Dogs

(26154)

Methods: To investigate the metabolism of esomeprazole in the liver of neonatal and young adult dogs, the livers obtained from the dogs in the repeat-dose toxicity studies (studies 900544 and 500204). The liver samples were collected from the dogs at 66 days old (neonatal) and at 9 to 10 months old (young adult). The neonatal dogs were treated orally with the vehicle or 80 $\mu\text{mol/kg}$ (28 mg/kg) esomeprazole daily. The neonatal dogs were also treated intermittently (once every 14 days) with 80 $\mu\text{mol/kg}$ esomeprazole. The treatment was initiated at Day 10 *post partum* for 8 weeks. The young adult dogs were treated orally with 80 $\mu\text{mol/kg/day}$ esomeprazole for 8 weeks.

Esomeprazole was incubated at an initial concentration of 2 $\mu\text{mol/L}$ and a liver microsomal protein concentration of 0.5 mg/mL for 30 minutes. The half-life ($t_{1/2}$) of esomeprazole consumption and the capacity of the dog liver microsomes to metabolize esomeprazole (the intrinsic clearance, CL_{int}) were determined.

Results: The results were summarized in Table 1 in this report. This table is attached below.

Table 1 Metabolic stability of esomeprazole in dog liver microsomes (obtained from Study Nos 900544 and 500204, as described by CTBR)

Age and sex	Group No - Identification	Dose level ($\mu\text{mol/kg}$)	$t_{1/2}$ ^a (min)	CL_{int} ^a ($\mu\text{L}/\text{min}/\text{mg}$)
Study No 900544				
Neonatal dogs (3M+1F)	1- Vehicle control, daily oral treatment	0	25.0 \pm 6.1	58.8 \pm 17.6
Neonatal dogs (2M+2F)	2- Esomeprazole, daily oral treatment	80	25.4 \pm 4.4	55.9 \pm 9.3
Neonatal dogs (2M+2F)	5- Esomeprazole, intermittent oral treatment ^b	80	27.4 \pm 8.4	53.7 \pm 13.3
Young adult dogs (2M+2F)	6- Esomeprazole, daily oral treatment	80	35.0 \pm 5.9	40.4 \pm 6.8
Study No 500204				
Young adult dogs (3M+3F)	1- Vehicle control, continuous IV infusion	0	47.2 \pm 13.5	31.3 \pm 8.0

^a Mean \pm SD

^b Dosed once every 14 days

The data indicated that there were no differences in the half-life or intrinsic clearance of esomeprazole in liver microsomes obtained from the vehicle and esomeprazole-treated neonatal dogs, suggesting that repeated oral daily or intermittent dosing with esomeprazole (28 mg/kg) did not alter the hepatic metabolic clearance rate of esomeprazole in neonatal dogs. Slightly lower intrinsic clearance was noted in the esomeprazole-treated young adult dogs as compared to that in the

neonatal dogs. In the control animals, a longer half-life and lower intrinsic clearance of esomeprazole were noted in the young adults as compared to the neonatal animals.

TOXICITY:

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

Testing Laboratories:

F

b(4)

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 26
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 <i>post partum</i>
		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 ^a	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 ^a	As appropriate
a	Target number of animals, including dosed reserves in subsets C and D			
b	Samples for investigation of the <i>in vitro</i> 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, 9025-9029	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 <i>post partum</i>
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		
Groups 8-10	28M+28F	Toxicokinetics (on first day of dosing) 7 time points	12M+12F ^c	7
			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	4M	1F	2F	3F	4F
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 ($\mu\text{mol/kg}$)	Males			Females		
	Dose Day 1 Day 7 <i>pp</i>	Dose Day 15 Day 21 <i>pp</i>	Gain (% control)	Dose Day 1 Day 7 <i>pp</i>	Dose Day 15 Day 21 <i>pp</i>	Gain (% control)
0	13.9 \pm 0.7	52.9 \pm 2.0	39.0	13.4 \pm 0.6	50.8 \pm 1.9	37.4
270 (Eso)	14.4 \pm 1.3	53.7 \pm 2.9	39.3 (100)	13.5 \pm 1.3	49.9 \pm 3.0	36.4 (97)
800 (Eso)	14.1 \pm 1.6	43.8 \pm 6.8**	29.7 (76)	13.7 \pm 1.4	43.7 \pm 4.2***	30.0 (80)
400 (Ome)	14.6 \pm 1.6	53.2 \pm 2.3	38.6 (99)	13.8 \pm 1.3	49.9 \pm 2.7	36.1 (97)

** $p \leq 0.01$

*** $p \leq 0.001$

N= 10 litters in the vehicle control, esomeprazole 270 $\mu\text{mol/kg}$ and 400 $\mu\text{mol/kg}$ omeprazole groups, 16 litters in the esomeprazole 800 $\mu\text{mol/kg}$ 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	Day 24	Day 35	Day 24	Day 35	Day 24	Day 35	Day 24	Day 35
(μmol/kg)	pp	pp	pp	pp	pp	pp	pp	pp
0	64 \pm 4	118 \pm 8	63 \pm 4	138 \pm 10	62 \pm 5	102 \pm 6	59 \pm 4	118 \pm 8
270 (Eso)	65 \pm 4	116 \pm 8	63 \pm 4	136 \pm 7	60 \pm 6	100 \pm 8	58 \pm 4	114 \pm 8
800 (Eso)	53 \pm 8**	98 \pm 11***	51 \pm 13*	119 \pm 21**	53 \pm 7**	88 \pm 10**	50 \pm 5**	104 \pm 7***
(% control)	(83)	(83)	(81)	(86)	(85)	(86)	(85)	(88)
400 (Ome)	64 \pm 5	115 \pm 8	65 \pm 5	141 \pm 9	59 \pm 6	100 \pm 8	58 \pm 7	114 \pm 6
Body weight gains (g/animal)								
Dose	24-35	35-119	24-35	35-119	24-35	35-119	24-35	35-119
(μmol/kg)	pp	pp	pp	pp	pp	pp	pp	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

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

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 ($\mu\text{mol/kg}$) / parameter Test Compound	Males				Females			
	0 Con	270 Eso	800 Eso	400 Ome	0 Con	270 Eso	800 Eso	400 Ome
RBC ($10^6/\mu\text{L}$)								
Day 35 <i>pp</i>	6.3	6.7**	6.3	6.7**	6.5	6.9	6.6	6.8
Recovery (70 \pm 2 <i>pp</i>)	8.5	8.6	8.3	8.5	8.1	8.5	8.2	8.4
Recovery (122 \pm 2 <i>pp</i>)	9.3	9.3	9.2	9.5	8.5	8.9	8.7	8.8
Hb (g/dL)								
Day 35 <i>pp</i>	12.9	12.6	10.2***	11.9*	13.5	12.9	10.7***	12.4
Recovery (70 \pm 2 <i>pp</i>)	16.4	16.3	15.9	16.0	15.8	16.0	15.0	16.2
Recovery (122 \pm 2 <i>pp</i>)	16.3	16.3	16.0	16.6	15.6	16.2	15.8	16.4
HT (%)								
Day 35 <i>pp</i>	41	42	36***	41	43	43	37*	42
Recovery (71 \pm 2 <i>pp</i>)	52	52	50	51	49	50	46	50
Recovery (122 \pm 2 <i>pp</i>)	51	51	50	52	48	50	48	50
MCV (fL)								
Day 35 <i>pp</i>	66	63	57***	61*	66	63	56***	61*
Recovery (71 \pm 2 <i>pp</i>)	61	60	60	60	60	59	57*	59
Recovery (122 \pm 2 <i>pp</i>)	55	55	55	55	57	57	56	56
MCH (pg)								
Day 35 <i>pp</i>	20	19	16***	18**	21	19*	16***	18**
Recovery (71 \pm 2 <i>pp</i>)	19	19	19	19	19	19*	18***	19
Recovery (122 \pm 2 <i>pp</i>)	18	18	17	17	19	18	18	19
MCHC (g/dL)								
Day 35 <i>pp</i>	31	30**	28***	29***	32	30***	29***	30***
Recovery (71 \pm 2 <i>pp</i>)	31	31	32	31	32	32	32	33
Recovery (122 \pm 2 <i>pp</i>)	32	32	32	32	33	32	33	33
RDW (%)								
Day 35 <i>pp</i>	15.1	17.9	21.4***	18.3*	14.9	16.8	19.7***	17.3*
Recovery (71 \pm 2 <i>pp</i>)	10.7	11.8**	12.5***	12.2***	10.4	11.6*	13.7***	12.5***
Recovery (122 \pm 2 <i>pp</i>)	12.0	12.3	12.4	12.6	10.8	10.8	10.9	11.2
Platelet ($10^3/\mu\text{L}$)								
Day 35 <i>pp</i>	1705	1768	1955	1964	1724	1708	2091*	1905
Recovery (71 \pm 2 <i>pp</i>)	1161	1203	1265	1244	1162	988	1208	1194
Recovery (122 \pm 2 <i>pp</i>)	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 ($\mu\text{mol/kg}$) / parameter Test Compound	Males				Females			
	0 Con	270 Eso	800 Eso	400 Ome	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 \pm 2 pp)	8.5	8.1	8.2	9.1	8.8	9.0	8.1	9.0
Recovery (122 \pm 2 pp)	6.8	7.0	7.3	7.1	7.9	7.6	7.4	7.4
Reticulocyte ($10^9/\text{L}$)								
Day 35 pp	613	798**	937***	752*	564	775	818	686
Recovery (71 \pm 2 pp)	194	193	225	210	175	152	209	216
Recovery (122 \pm 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 \pm 2 pp)	2.3	2.3	2.7	2.5	2.2	1.8	2.6	2.6
Recovery (122 \pm 2 pp)	1.8	1.9	2.2	2.0	1.9	1.9	2.0	2.1
WBC ($10^3/\mu\text{L}$)								
Day 35 pp	3.1	2.6	2.2	2.9	3.0	3.0	2.3	2.7
Recovery (71 \pm 2 pp)	9.6	9.9	10.3	9.8	6.8	7.3	7.2	6.4
Recovery (122 \pm 2 pp)	8.6	8.1	9.1	8.6	5.6	5.6	5.3	5.4
Lymphocytes ($10^3/\mu\text{L}$)								
Day 35 pp	2.7	2.3	1.8*	2.6	2.7	2.7	1.9	2.4
Recovery (71 \pm 2 pp)	8.2	8.5	9.0	8.3	5.8	6.2	6.1	5.4
Recovery (122 \pm 2 pp)	6.9	6.5	7.5	6.9	4.9	4.7	4.3	4.6

* $p < 0.05$

Con= Control

** $p < 0.01$

Eso= Esomeprazole

*** $p < 0.001$

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

* $p \leq 0.05$

Con= Control

** $p \leq 0.01$

Eso= Eesomeprazole

*** $p \leq 0.001$

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 Group Test compound Dose (µmol/kg)	Males				Females			
	1 Con	2 Eso	3 Eso	4 Ome	1 Con	2 Eso	3 Eso	4 Ome
	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 Group Test compound Dose (µmol/kg)	Males				Females			
	1 Con	2 Eso	3 Eso	4 Ome	1 Con	2 Eso	3 Eso	4 Ome
	0	270	800	400	0	270	800	400
Absolute stomach weight (g), Day 35 pp (subset A)	1.0 ±0.11	1.2** ±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	83*** ±9.8	81** ±6.5	67 ±7.0	79*** ±6.4	81*** ±8.1	80*** ±5.4
% of control value (relative wt)	-	121	124	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 ±0.19	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 ±6.7	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 ($\mu\text{mol/kg}$)	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 <i>pp</i> (subset A)	760 (710-990)	750 (680-1000)	810 (590-1100)	630 (570-830)	780 (550-900)	740 (680-860)
% change vs controls	-	-3.9	6.6	-	24	17
ECL-cell volume fraction $\times 10^3$, Day 35 <i>pp</i> (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 <i>pp</i> (subset A)	77 (53-110)	140* (83-170)	100 (73-110)	92 (74-110)	100 (50-170)	95 (65-120)
% change vs controls	-	82	30	-	8.7	3.2
Gastric mucosal height (μm), Day 35 <i>pp</i> (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 <i>pp</i> (subset A)	710 (570-990)	750 (550-1000)	750 (590-1100)			
% change vs controls	-	5.6	5.6			
ECL-cell volume fraction $\times 10^3$, Day 35 <i>pp</i> (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 <i>pp</i> (subset A)	88 (53-110)	120* (50-170)	96 (65-120)			
% change vs controls	-	36	9.1			
Gastric mucosal height (μm), Day 35 <i>pp</i> (subset A)	420 (220-560)	470 (250-750)	450 (160-790)			
% change vs controls	-	12	7.1			
* $p < 0.05$	** $p < 0.01$	*** $p < 0.001$				
C Control	E Esomeprazole	O Omeprazole				
<i>pp</i> <i>post partum</i>						

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 ($\mu\text{mol/kg}$)	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 122 \pm 2 <i>pp</i> (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 $\times 10^3$, Day 122 \pm 2 <i>pp</i> (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 \pm 2 <i>pp</i> (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 \pm 2 <i>pp</i> (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 \pm 2 <i>pp</i> (subset B)	1300 (1000-1700)	1500 (1300-1800)	1700 (1100-2200)			
% change vs controls	-	15	31			
ECL-cell volume fraction $\times 10^3$, Day 122 \pm 2 <i>pp</i> (subset B)	24 (18-31)	27 (11-31)	31** (22-44)			
% change vs controls	-	13	29			
ECL-cell profile density (mm^{-2}), Day 122 \pm 2 <i>pp</i> (subset B)	160 (140-240)	200 (95-260)	210** (190-290)			
% change vs controls	-	25	31			
Gastric mucosal height (μm), Day 122 \pm 2 <i>pp</i> (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 ($\mu\text{mol/kg}$)	Dose Day	Days post <i>partum</i>	Sex	Analysed compound	C_{max} ($\mu\text{mol/L}$)	AUC ($\mu\text{mol} \cdot \text{h/L}$)
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 ($\mu\text{mol/kg}$)	Dose Day	Days post partum	Sex	Analysed compound	C_{max} ($\mu\text{mol/L}$)	AUC ($\mu\text{mol}\cdot\text{h/L}$)
400	1	7	M+F	Omeprazole	83.2	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 urogenital 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.

Esomeprazole Magnesium: A Repeat-Dose Toxicity Study with Up to 3Months' Oral (Gavage) Administration in the Neonatal Beagle Dog (900186)

Testing Laboratories:

b(4)

Study Start and Completion Date: October 30, 2003 and
February 28, 2006

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

Animals:

Male: 615 g, 10 days old
Female: 730 g, 10 days old
Beagle dogs

Drug Batch No.: 800/02

Methods: The toxicity of H 199/18 magnesium was characterized in 10-day old Beagle pups. Esomeprazole was given to the neonatal dogs orally at 0, 80 and 160 $\mu\text{mol/kg}$ (0, 28 and 55 mg/kg) once daily for 92 days. The high dose was reduced to 120 $\mu\text{mol/kg}$ (41 mg/kg) after 53/54 days of dosing (Day 62/63 *post partum*) due to severe adverse clinical signs of toxicity. About half the animals in each group were necropsied and examined directly following completed dosing, while the remaining animals were allowed a 3-month recovery period after cessation of dosing and prior to necropsy. The following parameters were assessed: clinical signs of toxicity, length (nose to tail) and height measurements, body weight, haematology, clinical biochemistry, serum gastrin, plasma concentrations of esomeprazole, lymphocyte transformation test, organ weights and gross and microscopic pathology (including gastric morphology and morphometry). Blood samples were collected for TK analysis on Dose Days 14, 28 and 92 in all surviving pups.

Results:

1. **Clinical Signs:** Convulsions and head shaking were noted in the 55 mg/kg group. The majority of the pups at the reduced dose of 41 mg/kg showed a clear eye discharge, salivation, oily fur and a skin rash on the forelimbs, hindlimbs, ventral region, muzzle, and pinnae. The results were presented in Table 9 in this report. This table is attached below.

Table 9 Summary of noteworthy clinical signs during the dosing period

Clinical sign	Number of animals in which sign observed (on total number of days)					
	Group No and sex			Group No and sex		
	1M	2M	3M	1F	2F	3F
Dose level ($\mu\text{mol/kg}$)	0	80	160/120	0	80	160/120
No of animals per group	5	5	4	4	3	5
Convulsions	0	0	0	0	0	1(1)
Head-shaking	0	0	2(2)	0	0	0
Tremors	0	0	1(1)	0	0	0
Uncoordinated/abnormal gait	0	0	1	0	0	0
Decreased activity/hunched posture	0	1	1	0	0	1
Retching/vomitus			1			
Salivation post dosing	1	0		0	0	1
	0	0	2	0	0	3
Thin/backbone prominent/weak	0	0	2	0	0	3
Dehydration	0	0	1	0	0	1
Ptosis	0	0	1	0	0	0
Eye discharge, clear	0	0	3	0	0	4
Fur oily	0	0	1	0	0	3
Fur thin cover	0	2	2	2	2	3
Skin rash, lesions or scratching on forelimbs/hind limbs/ventral region/muzzle/pinna(e)	0	0	2	2	0	4

2. Mortality: One male and one female in the 55 mg/kg group were sacrificed on Dose Day 53 or 52 due to severe toxicity. The male pup had weight loss, tremors, retching, incoordination, head shaking, and ptosis. The female pup had weight loss and was weak, thin and dehydrated with decreased activity.

3. Body Weight: The mean initial and final body weights in the control animals were 615 g and 6 kg (males) or 730 g and 6.2 kg (females), respectively. Treatment with esomeprazole decreased terminal body weight gain by 14% (males) and 21% (females) in the 28 mg/kg group and by 26% (males) and 37% (females) in the 55 mg/kg group. The results were presented in Table 10 in this report. This table is attached below.

Table 10 Body weight and weight gain (mean±SD) in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Dose ($\mu\text{mol/kg}$)	Males			Females		
	Day 10 <i>pp</i>	Day 56 <i>pp</i>	Gain (% control)	Day 10 <i>pp</i>	Day 56 <i>pp</i>	Gain (% control)
Pre-weaning (g, Dose Days 1 to 47, Days 10 to 56 post partum)						
0	615±121	3180±477	2565	730±57	3420±208	2690
80	789±72	2830±210	2041 (80)	677±45	2754±73	2077 (77)
160/120	613±96	2230±465	1617 (63)	540±186	1854±380	1314 (49)
Post-weaning (kg, Dose Days 48 to 92, Days 57 to 101 post partum)						
	Day 59 <i>pp</i>	Day 101 <i>pp</i>	Gain (% control)	Day 59 <i>pp</i>	Day 101 <i>pp</i>	Gain (% control)
0	3.3±0.5	6.0±0.9	2.7	3.5±0.2	6.2±0.5	2.7
80	2.9±0.3	5.4±0.2	2.5 (93)	2.9±0.1	5.0±0.1	2.1 (78)
160/120	2.3±0.6	4.6±0.6	2.3 (85)	1.9±0.4	4.0±0.6	2.1 (78)
Recovery (kg, Days 102 to 189 post partum)						
	Day 105 <i>pp</i>	Day 189 <i>pp</i>	Gain (% control)	Day 105 <i>pp</i>	Day 189 <i>pp</i>	Gain (% control)
0	7.1±1.0	12.4±1.8	5.3	6.5±0.8	11.6±1.4	5.1
80	5.9	11.7±1.1	5.8 (109)	5.2	10.4	5.2 (102)
160/120	5.4	11	5.6 (106)	3.7±0.7	7.6±0.9	3.9 (76)

pp = post partum

The values in bold indicate a treatment-related effect

4. Growth measurements: Growth length and height were decreased in a dose-related manner. Final length was 98% and 90% (males) and 90% and 86% (females) of the control in the 28 and 55/41 mg/kg groups, respectively. The final heights were 94% and 88% (males) and 86% and 83% (females) of the control in the 28 and 55/41 mg/kg groups, respectively. The results were summarized in Tables 11 and 12 in this report and these tables are attached below.

Table 11 Growth (length) (mean±SD) in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Dose ($\mu\text{mol/kg}$)	Males			Females		
	Day 14 pp	Day 56 pp	Growth ^a (% control)	Day 14 pp	Day 56 pp	Growth ^a (% control)
Pre-weaning (cm, Dose Days 5 to 47, Days 14 to 56 post partum)						
0	23±1.9	41±2.3	18	26±1.0	41±1.6	15
80	24±1.1	39±0.7	15 (83)	24±0.5	36±1.0	12 (80)
160/120	23±0.7	36±2.2	13 (72)	23±2.4	35±2.7	12 (80)
Post-weaning (cm, Dose Days 47 to 89, Days 56 to 98 post partum)						
	Day 56 pp	Day 98 pp	Growth ^b (% control)	Day 56 pp	Day 98 pp	Growth ^b (% control)
0	41±2.3	51±4.1	10	41±1.6	51±1.3	10
80	39±0.7	50±0.7	11 (110)	36±1.0	46±1.3	10 (100)
160/120	36±2.2	46±2.6	10 (100)	35±2.7	44±3.0	9.0 (90)
Recovery (cm, Days 105 to 189 post partum)						
	Day 105 pp	Day 189 pp	Growth ^c (% control)	Day 105 pp	Day 189 pp	Growth ^c (% control)
0	56±5.3	68±7.8	12	52±0.7	63±0.0	11
80	51	65±2.1	14 (117)	48	61	13 (118)
160/120	49	66	17 (142)	44±2.1	61±2.1	17 (155)

a Growth between Days 14-56 post partum (pp)

b Growth between Days 56-98 post partum

c Growth between Days 105-189 post partum

The values in bold indicate a treatment-related effect

Table 12 Growth (height) (mean±SD) in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Dose ($\mu\text{mol/kg}$)	Males			Females		
	Day 14 pp	Day 56 pp	Growth ^a (% control)	Day 14 pp	Day 56 pp	Growth ^a (% control)
Pre-weaning (cm, Dose Days 5 to 47, Days 14 to 56 post partum)						
0	14±0.5	25±1.7	11	14±0.7	26±1.3	12
80	15±0.7	25±0.8	10 (91)	14±0.3	24±0.3	10 (83)
160/120	14±0.7	23±0.9	9 (82)	13±1.3	22±1.6	9 (75)
Post-weaning (cm, Dose Days 47 to 89, Days 56 to 98 post partum)						
	Day 56 pp	Day 98 pp	Growth ^b (% control)	Day 56 pp	Day 98 pp	Growth ^b (% control)
0	25±1.7	34±1.5	9	26±1.3	35±0.9	9
80	25±0.8	32±0.6	7 (78)	24±0.3	30±0.8	6 (67)
160/120	23±0.9	30±1.5	7 (78)	22±1.6	29±2.9	7 (78)
Recovery (cm, Days 105 to 189 post partum)						
	Day 105 pp	Day 189 pp	Growth ^c (% control)	Day 105 pp	Day 189 pp	Growth ^c (% control)
0	34±1.8	43±3.2	9	35±1.1	42±2.5	7
80	33	42±0.8	9 (100)	33	40	7 (100)
160/120	31	41	10 (111)	29±3.5	38±2.5	9 (129)

a Growth between Days 14-56 post partum (pp)

b Growth between Days 56-98 post partum

c Growth between Days 105-189 post partum

The values in bold indicate a treatment-related effect

5. Hematology: Slight decreases (17-21%) in the red blood cell counts, haemoglobin, and haematocrit in the high dose esomeprazole group on Dose Days 29 as compared to the control. The results were presented in Table 24 in this report. This table is attached below.

Table 13 Summary of noteworthy haematology findings (mean±SD) in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Parameter measured and sampling occasion	0	80	Dose (µmol/kg)			160/120
			160/120	0	80	
	Males			Females		
	No animals/group					
Main Study	5	5	4	4	3	5
Recovery	2	3	1	2	1	2
Red blood cell count (10⁶/µL)						
Dose Day 29	4.2±0.3	3.8±0.1	3.5±0.7	4.4±0.3	3.9±0.1	3.6±0.7
Dose Day 93	5.9±0.3	5.5±0.6	5.1±0.6	5.9±0.2	6.0±0.7	5.3±0.5
Recovery	6.7±0.3	6.4±0.2	6.4	7.2±0.1	6.5	6.8±0.4
Haemoglobin (g/dL)						
Dose Day 29	9.1±0.6	8.2±0.3	7.6±2.1	10.0±0.5	8.9±0.2	8.1±2.0
Dose Day 93	13.2±0.5	10.6±1.8	10.8±1.2	13.1±0.2	10.5±1.9	11.1±0.7
Recovery	15.7±0.3	14.5±0.9	14.7	16.8±0.3	15.4	15.7±0.7
Haematocrit (%)						
Dose Day 29	30±1.6	27±1.0	25±5.9	33±2.1	29±0.6	26±6.1
Dose Day 93	40±1.8	33±5.0	33±4.0	40±1.3	35±5.6	35±2.6
Recovery	47±1.7	44±2.4	45	50±0.6	46	47±1.9
MCV (fL)						
Dose Day 29	72±1.3	72±1.7	70±2.3	74±0.3	74±0.9	72±3.4
Dose Day 93	67±0.8	61±5.2	65±1.6	67±0.6	58±3.6	65±2.4
Recovery	71±0.6	69±2.2	70	69±0.3	71	69±1.7
MCH (pg)						
Dose Day 29	22±0.6	22±0.6	21±1.8	23±0.7	23±0.3	22±1.4
Dose Day 93	22±0.4	19±2.3	21±0.2	22±0.5	18±1.5	21±1.0
Recovery	24±0.6	23±1.0	23	23±0.3	24	23±0.5
MCHC (g/dL)						
Dose Day 29	30±0.5	31±0.5	30±1.6	31±0.8	31±0.2	31±0.8
Dose Day 93	33±0.3	32±1.4	33±0.6	33±0.7	30±0.8	32±0.8
Recovery	33±0.6	33±0.4	33	34±0.2	34	33±0.1

Table 13 Summary of noteworthy haematology findings (mean±SD) in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Parameter measured and sampling occasion	Dose (µmol/kg)					
	0	80	160/120		80	160/120
	Males			Females		
Main Study	5	5	4	4	3	5
Recovery	2	3	1	2	1	2
Reticulocyte count (10⁹/µL)						
Dose Day 29	174±49	132±24	86±46	251±43	154±37	92±41
Dose Day 93	91±24	121±16	127±6	79±34	151±78	153±26
Recovery	80±32	109±17	82	93±20	85	82±5
Platelet count (10⁹/µL)						
Dose Day 29	266±40	599±92	608±44	290±59	482±141	659±176
Dose Day 93	302±39	704±167	604±172	324±57	633±145	662±63
Recovery	239±11	386±66	269	306±45	259	313±25

Dose Days 29 and 93 correspond to Days 38 and 102 *post partum*, respectively. The recovery sampling was performed on Day 191 or 192 *post partum*.

The values in bold indicate a treatment-related effect

6. Clinical Chemistry: Slight increase (11-14%) in total iron binding capacity (TIBC) was noted in the high dose group. The results were presented in Table 20 in this report. This table is attached below.

Table 14 Summary of serum iron, UIBC and TIBC (mean±SD) in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Parameter measured and sampling occasion	Dose (µmol/kg)					
	0	80	160/120		80	160/120
	Males			Females		
Main Study	5	5	4	4	3	5
Recovery	2	3	1	2	1	2
Serum Iron (µg/µL)						
Pre-dose	253±47	334±46	305±50	342±47	270±70	257±59
Dose Day 13	194±33	182±74	234±68	227±32	324±21	254±73
Dose Day 29	124±106	98±52	143±107	288±58	134±25	115±85
Dose Day 93	148±41	104±59	119±56	153±21	158±49	73±47
Recovery	135±25	119±37	133	208±64	156	164±36
UIBC (µg/dL)						
Pre-dose	0±0.0	0±0.0	0±0.0	0±0.0	37±64	2±4.5
Dose Day 13	145±65	146±85	137±92	62±47	3±6	101±73
Dose Day 29	221±122	258±64	251±145	71±38	215±43	264±133
Dose Day 93	234±41	304±105	316±93	247±27	285±41	371±50
Recovery	235±54	254±52	209	217±59	205	215±102
TIBC (µg/dL)						
Pre-dose	253±47	334±46	305±50	342±47	307±17	259±58
Dose Day 13	338±34	328±33	370±33	288±18	327±21	354±23
Dose Day 29	346±23	356±35	394±59	359±30	349±24	379±54
Dose Day 93	382±14	408±48	435±38	400±11	444±35	444±16
Recovery	370±29	373±23	342	425±5	361	379±66

The values in bold indicate a treatment-related effect

7. Serum gastrin: Serum gastrin levels were increased in the esomeprazole groups (18 and 25 folds in males and 48 and 23 folds in females) as compared to the controls. The results were summarized in Table 21 in this report. This table is attached below.

Table 15 Serum gastrin concentrations (mean±SD) 2 h after dosing in Beagle pups during 3 months' oral esomeprazole treatment and 3 months' recovery

Dose (µmol/kg)	Pre-dose (Day 9 pp)	Day 14 (Day 23 pp)	Day 28 (Day 37 pp)	Day 92 (Day 101 pp)	End Recovery (Day 190 pp)
Concentration, pg/mL (fold difference compared to controls)					
Males					
0	121±64	114±73	62±19	91±18	<LLOQ
80	156±63	303 ±196 (3)	683 ±586 (11)	1656** ±846 (18)	55, <LLOQ
160/120	89±29	387 ±54 (3)	1373 ±705 (22)	2285*** ±450 (25)	43
Females					
0	91±34	155±87	86±46	66±12	<LLOQ
80	133±40	257 ±111 (2)	843 ±357 (10)	3158*** ±1020 (48)	<LLOQ
160/120	113±29	281 ±95 (2)	1031 ±817 (12)	1538*** ±634 (23)	42, <LLOQ

** p≤0.01

*** p≤0.001 (t-test)

Data shown are mean values for pre-dose, Days 14, 28 and 92 of treatment. For the end of recovery, individual values are shown, as the number of animals was low and many values were <LLOQ

The number of samples analysed during the dosing period varied between 3 and 5 for each group and sex

Number in parenthesis are x fold of the corresponding control

LLOQ= lower limit of quantification

The samples taken during the dosing period were taken 2 h post-dose. The pre-dose and recovery samples were taken in the morning (as dosing was not performed on these occasions)

The values in bold indicate a treatment-related effect

8. Lymphocyte transformation test: There were no treatment related changes.

9. 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 16 Absolute and relative (to brain weight) stomach weights (mean±SD) in Beagle pups following 3 months' oral esomeprazole treatment and 3 months' recovery

Dose (µmol/kg)	Males			Females		
	0	80	160/120	0	80	160/120
Dosing period						
No/group examined	3	2	2	2	2	2
Terminal body weight (kg)	5.4±0.4	5.1±0.4	4.3±0.8	6.1±0.1	4.8±0.0	4.3±0.2
Absolute stomach weight (g)	54±2.7	94±6.8	77±18.9	61±5.8	69±0.4	85±7.7
Relative wt (% brain weight)	80	148***	114***	82	107**	129***
	±4.2	±6.4	±12	±7.0	±0.9	±1.7
% of control value (relative wt)	-	185	143	-	130	157
Recovery period						
No/group examined	2	3	1	2	1	2
Terminal body weight (kg)	12.2±1.8	11.6±1.0	10.7	11.3±1.6	10.2	7.4±0.9
Absolute stomach weight (g)	76±9.4	102±5.2	97	73±7.0	74	75±8.5
Relative wt (% brain weight)	87±13.6	135±11.7	146	91±2.5	113	105±14.2
% of control value (relative wt)	-	155	168	-	124	115

* p≤0.05

** p≤0.01

*** p≤0.001

The values in bold indicate a treatment-related effect

10. **Gross Pathology:** Dark, discoloration of the skin and/or scabs on the paws, limbs and/or head (lower jaw/pinnae) were noted in the high dose animals.

11. **Microscopic Pathology:** There no treatment-related changes.

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

There were increases in the reference volume and mucosal height of the stomach and in the total number of ECL-cell profiles per stomach in the esomeprazole-treated groups. The results were summarized in Table 17 in this report. This table is attached below.

Table 17 Results of the gastric morphometry (median and range) in juvenile dogs after oral administration of esomeprazole for 3 months, followed by 3 months' recovery

Necropsy time Test compound Dose ($\mu\text{mol/kg}$)	End dose	End dose	End dose	End rec	End rec	End rec
	C	E	E	C	E	E
	0	80	160/120	0	80	160/120
Stomach reference volume (cm^3)	54 (51-67)	79* (68-170)	76 (27-94)	68 (64-76)	93 (67-97)	71 (65-79)
% change vs controls	-	46	41	-	37	4.4
Total number ECL-cell profiles/stomach	4400 (3300-7000)	5200 (3500-7000)	5400 (2900-8000)	8500 (5400-9100)	7100 (6500-9100)	8200 (6300-8300)
% change vs controls	-	18	23	-	-16	-3.5
Number ECL-cell profiles/stomach vol (cm^3)	83 (59-120)	70 (20-92)	88 (62-110)	120 (76-140)	87 (74-97)	110 (88-130)
% change vs controls	-	-16	6.0	-	-28	-8.3
Gastric mucosal height (μm)	870 (740-890)	1100* (980-1200)	1100** (1100-1400)	1100 (990-1200)	1200 (1000-1300)	1100 (910-1300)
% change vs controls	-	26	26	-	9.0	0

Rec= Recovery C= Control E= Esomeprazole
* $p \leq 0.05$ ** $p \leq 0.01$

13. **Toxicokinetics:** The results were presented in Table 8 in this report. This table is attached below.

Table 8 Summary of C_{max} and AUC values for esomeprazole in Beagle pups after oral dosing with esomeprazole for up to 3 months

Study Phase	Daily dose ($\mu\text{mol/kg}$)	n and sex	Dose Day	Day pp	C_{max}^a ($\mu\text{mol/L}$)	AUC ^a ($\mu\text{mol}^2\text{h/L}$)
I	40	1M+1F	1	10	8.9, 20	28, 40
I	40	1M+1F	7	16	4.3, 2.7	6.2, 4.4
I	80	1M+1F	1	11	30, 46	62, 83
I	80	1M+1F	7	17	27, 6.7	23, 14
II	80	5M+3F	14	23	33 (23-86)	30 (21-60)
II	80	5M+3F	28	37	17 (1.9-29)	18 (2.2-20) ^b
II	80	5M+3F	92	101	7.8 (3.3-29)	5.8 (2.7-28)
I	160	2M+2F	1	10/11	64, 60, 72, 86	194, 197, 256, 246
I	160	2M+2F	7	16/17	62, 46, 56, 29	124, 58, 84, 76
II	160	4M+5F	14	23	116 (43-161)	100 (44-138)
II	160	4M+5F	28	37	88 (29-132)	64 (21-87)
II	120 ^c	3M+4F	92	101	12 (1.6-17)	7.8 (2.1-12)

a The median and range are given for sampling during Phase II. However, the number of animals in Phase I was limited, and thus individual values are given.

b n= 5M+2F

c The dose in Group 3 was reduced from 160 to 120 $\mu\text{mol/kg}$ after about 7 weeks' treatment (62/63 days pp). At the same time that the dose was reduced, 2 animals in this group were removed from the study, due to poor clinical condition.

The plasma levels of the test drug were much lower at the end of the treatment than those on day 1.

In summary, esomeprazole was given to 10-day old Beagle pups orally at 0, 80 and 160 $\mu\text{mol/kg}$ (0, 28 and 55 mg/kg) once daily for 92 days. The higher dose was reduced to 120 $\mu\text{mol/kg}$ (41 mg/kg) after 53/54 days of dosing due to clinical signs of toxicity. Treatment with esomeprazole induced clinical signs of

toxicity (convulsions, head shaking, eye discharge, salivation, and oily fur), decreased body weight gain and growth, and increased gastrin secretion. Treatment-related increases in the stomach weight, stomach reference (tissue) volume and height of the gastric mucosa were in both esomeprazole-treated pups. No effect dose was not identified. The dose of 28 mg/kg was tolerated. The study did not reveal any unexpected toxicity. The central nervous system and the stomach were the target organs of toxicity.

Esomeprazole Magnesium: An 8-Week Oral (Gavage) Toxicity and Toxicokinetic Study in the Neonatal and Young Adult Beagle Dog (a Complementary Study)
(900544)

Testing Laboratories:

b(4)

Study Start and Completion Date: September 10, 2004 and
February 22, 2006

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

Animals:

Male: 615 g, 10 days old
Female: 730 g, 10 days old
Beagle dogs

Drug Batch No.: 800/02

Methods: To compare the toxicokinetic profiles of esomeprazole in the neonatal and adult dogs, the sponsor conducted a complementary study to the 3-month oral toxicity study with in neonatal dogs (Study No 900186). In the current study, the toxicokinetics of esomeprazole in various ages of dogs and after different dosing regimens were performed. Five groups of Beagle pups (10 days old) and a single group of young adult dogs (6 to 8 months old) were included in this study. These animals were given esomeprazole orally at 80, 80/120 or 120/180 $\mu\text{mol}/\text{kg}$ (28, 28/41 or 41/62 mg/kg), once daily for up to 8 weeks (Days 10 to 65 *post partum*). The dose levels in the mid and high dose groups were increased from 80 to 120 $\mu\text{mol}/\text{kg}$ and 120 to 180 $\mu\text{mol}/\text{kg}$, respectively, from Dose Day 28 (Day 37 *post partum*).

Another group was also given a dose of 80 µmol/kg (28 mg/kg) on Days 1, 14, 28, 42 and 56 (Days 10, 23, 37, 51 and 65 *post partum* in the pups). For comparison, a group of young adult Beagle dogs (2 males and 2 females) received 80 µmol/kg (28 mg/kg), once daily for 8 weeks. The plasma levels of esomeprazole and its metabolite H 168/66, liver microsomal cytochrome P450 enzyme activity were determined. The blood samples were collected on Dose Days 1, 14, 28, 42 and 56 (Days 10, 23, 37, 51 and 65 *post partum* in the pups).

Results: The results indicated that the plasma levels of esomeprazole were comparable between males and females at various ages. The results were presented in Table 1 in this report. This table is attached below.

Table 1 Median (range) C_{max} and AUC values for esomeprazole in neonatal dogs given esomeprazole once daily or intermittently for 8 weeks

Dose regimen and Dose (µmol/kg) DD	Daily	C _{max} (µmol/L)				AUC (µmol·h/L)			
		Daily	Daily	Daily	Intermittent	Daily	Daily	Daily	Intermittent
80	80/120 ^a	120/180 ^a	80 ^b	80	80/120 ^a	120/180 ^a	80 ^b	80 ^b	
1	10	23 (11-31)	40 (6.5-51)	43 (37-69)	39 (30-41)	71 ^c (55-72)	60 (19-74)	112 (92-127)	83 (45-93)
14	23	46 (18-53)	26 (7.3-52)	17 (12-25)	19 (12-92)	28 (17-37)	20 (11-33)	20 (11-26)	47 (22-112)
28	37	22 (16-61)	70 (14-104)	65 (32-105)	20 (14-30)	17 (11-45)	54 (12-87)	86 (75-112)	23 (17-24)
42	51	18 (7.9-23)	6.1 (3.1-6.4)	51 (24-97)	22 (4.8-84)	9.3 (4.9-18)	5.2 (2.3-5.4)	54 (24-71)	30 (7.2-64)
56	65	9.7 (3.9-14)	2.9 (2.6-5.9)	NA	5.4 (1.8-7.4)	7.1 (5.1-18)	3.8 (1.9-4.5)	NA	9.4 (3.1-13)

DD= dose day pp= *post partum* NA= Not applicable (group terminated)
a The given doses were increased by 50% from Dose Day 28 (Day 37 *post partum*)

b These animals were dosed once every 14 days, on the days of TK sampling (i.e. Days 10, 23, 37, 51 and 65 *post partum*)

c n= 4 (2M+2F) with the exception of this value, where n= 3 (1M+2F)

The plasma levels of esomeprazole were decreased with the duration of treatment and/or the age of the pups. The plasma levels of esomeprazole in the young adult dogs were also decreased following the first dose, but subsequently remained at a relatively similar level throughout the rest of the 8-week dosing period. The results were presented in Table 2 and this table is attached below.

Table 2 Median (range) C_{max} and AUC values for esomeprazole in young adult dogs given esomeprazole 80 $\mu\text{mol/kg}$ once daily for 8 weeks

Dose Day	C_{max} ($\mu\text{mol/L}$)	AUC ($\mu\text{mol}\cdot\text{h/L}$)
1	48 (44-55)	65 (52-77)
14	14 (8.7-21)	25 (18-37)
28	19 (7.3-30)	38 (14-41)
42	16 (6.8-26)	27 (13-38)
56	24 (10-29)	27 (21-50)

n= 4 (2M+2F)

H 168/66 is one of the main esomeprazole metabolites in adult dogs. Similar to the parent compound, esomeprazole, the plasma levels of H 168/66 were also decreased with the duration of treatment and/or the age of the animals.

The results of the P450 enzyme analysis using livers microsomes indicated that the total cytochrome P450 content (but not the b5 content) of the microsomes, chlorzoxazone hydroxylase activity (CZXH or CYP2E1), and 7-ethoxyresorufin-O-deethylase activity (EROD or CYP1A1/2) were increased in both pups and young adult dogs. The benzoxyresorufin-O-dealkylase activity (BROD or CYP2B11) and testosterone-6 β hydroxylase activity (TESH or CYP3A12) were decreased. The results were summarized in Table 3. This table is attached below.

Table 3 Total amounts of CYP P₄₅₀ and b₅ proteins, and CYP dependent enzyme activities in dog liver microsomes after oral administration of esomeprazole

Age	Dose	Total P ₄₅₀ ^a	Total b ₅ ^a	EROD ^a CYP1A1/2	BROD ^a CYP2B11	CZXH ^a CYP2E1	TESH ^a CYP3A12
	$\mu\text{mol/kg}$	nmol/mg protein	nmol/mg protein	nmol/mg of protein/min	nmol/mg of protein/min	nmol/mg of protein/min	nmol/mg of protein/min
Pups	Vehicle (daily)	0.47±0.23	0.41±0.016	0.15±0.067	0.17±0.031	2.0±0.45	1.8±0.32
Pups	80 (daily)	0.97±0.32 (↑ 2.1x)	0.50±0.16	1.6±0.54 (↑ 11x)	0.063±0.015 (↓ 0.37x)	5.5±1.5 (↑ 2.8x)	1.3±0.32 (↓ 0.72x)
Pups	80 (intermittent)	0.91±0.39 (↑ 1.9x)	0.51±0.18	0.91±0.36 (↑ 6.1x)	0.16±0.050	3.0±1.2	1.1±0.025 (↓ 0.61x)
Young adults	80 (daily)	0.85±0.69 (↑ 1.8x)	0.43±0.18	1.2±0.78 (↑ 8.0x)	0.10±0.039	3.7±2.6	0.95±0.38 (↓ 0.53x)

a Values shown are mean ±SD (males and females combined)
Numbers in parenthesis reflect comparison with vehicle control group

It appears that treatment with esomeprazole increased the total cytochrome P450 content of the microsomes and 7-ethoxyresorufin-O-deethylase activity (CYP1A1/2) in both neonatal and adult dogs. However, no comparison between the enzyme activities in the control neonatal dogs and the control adult dogs was made.

SUMMARY AND EVALUATION:

Amended Written Requests for pediatric studies for Nexium® (esomeprazole magnesium) Delayed-Release Capsules were issued on December 20, 2005. In the requests, the sponsor is required to conduct (1) a 4-week repeated dose toxicity study in neonatal rats and (2) a 90-day repeated dose toxicity study in neonatal dogs. The sponsor submitted the reports of these studies in the current submission.

In the 1-month oral toxicity study in neonatal rats, rat pups were given esomeprazole (0, 93 or 280 mg/kg) or omeprazole (140 mg/kg) by oral gavage, once daily, for 28 days, from Day 7 to Day 34 *post partum*. 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. 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.

In the 3-month oral toxicity study in neonatal dogs, esomeprazole was given to 10-day old Beagle pups orally at 0, 80 and 160 $\mu\text{mol/kg}$ (0, 28 and 55 mg/kg) once daily for 92 days. The higher dose was reduced to 120 $\mu\text{mol/kg}$ (41 mg/kg) after 53/54 days of dosing due to clinical signs of toxicity. Treatment with esomeprazole induced clinical signs of toxicity (convulsions, head shaking, eye discharge, salivation, and oily fur), decreased body weight gain and growth, and increased gastrin secretion. Treatment-related increases in the stomach weight, stomach reference (tissue) volume and height of the gastric mucosa were in both esomeprazole-treated pups. The plasma levels of the test drug were much lower at the end of the

treatment than those on day 1. The results did not reveal any unexpected toxicity. The central nervous system and the stomach were the target organs of toxicity. No effect dose was not identified. The dose of 28 mg/kg was tolerated.

The toxicity profiles of H199/18 were characterized in adult animals in 1-month, 3-month oral toxicity studies in rats and 3-months oral toxicity study in dogs under NDA 21,153. The stomach and kidney were the target organs of toxicity as evidenced by histopathological changes in the stomach (minimal foci of the chief cell eosinophilia, acanthosis, and hyperkeratorosis, mucosal fibrosis, hyperplasia, chief cell atrophy, and focal necrosis) and kidney (basophilic cortical tubules and inflammatory cell infiltration). The results of the 1-month oral toxicity study in neonatal rats and 3-month oral toxicity study in neonatal dogs did not reveal any unexpected toxicity.

The plasma levels of esomeprazole and omeprazole were lower at the end of the treatment period (28 day or 92 days) than those on the first day in both neonatal rats and dogs. Treatment with esomeprazole increased the total cytochrome P450 content of the microsomes and 7-ethoxyresorufin-O-deethylase activity (CYP1A1/2) in both neonatal and adult dogs. However, no comparison between the enzyme activities in the control neonatal dogs and adult dogs was made.

There were no clear differences in the degree of protein binding at the different age in both rats and dogs. Slightly lower intrinsic clearance was noted in the esomeprazole-treated young adult dogs as compared to that in the neonatal dogs. Treatment with esomeprazole (28 mg/kg) did not alter the hepatic metabolic clearance rate of esomeprazole in neonatal dogs. The mechanism of higher plasma levels of esomeprazole in the neonatal animals is not clear. The sponsor may initiate the clinical study with pediatric patients of less than one year old with esomeprazole. However, the clinical dose of esomeprazole should be adjusted accordingly for these patients.

RECOMMENDATION:

1. The sponsor may initiate the clinical study with pediatric patients of less than one year old with esomeprazole.
2. The sponsor should closely monitor the dose and exposure of esomeprazole in the clinical study.

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/s/

Ke Zhang
6/4/2007 10:33:49 AM
PHARMACOLOGIST

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Signed for Dr. Choudary