

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-689

PHARMACOLOGY REVIEW

06/24/04

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:	21-689
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	Original - 09/10/03 Amendment #000BP - 04/09/04
DRUG NAME:	Esomeprazole Injection
INDICATION:	Treatment of gastroesophageal reflux disease
SPONSOR:	Astra Zeneca LP, Wilmington, DE
DOCUMENTS REVIEWED:	Original - EDT Module 4 Amendment #000BP Dated April 9, 2004
REVIEW DIVISION:	Division of Gastrointestinal & Coagulation Drugs Products (HFD-180)
PHARM/TOX REVIEWER:	Yash M. Chopra, MD, Ph.D.
PHARM/TOX SUPERVISOR:	Jasti Choudary, B.V.Sc, Ph.D.
DIVISION DIRECTOR:	Robert Justice, MD, M.S.
PROJECT MANAGER:	Melissa Furness
REVIEW COMPLETION DATE:	June 24, 2004

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Executive Summary

I. Recommendations

A. **Recommendation on Approvability:** From pharmacology standpoint, the approval of the application is recommended.

B. **Recommendation for nonclinical studies:** None

C. **Recommendations on labeling:** None

II. Summary of nonclinical findings

A. Pharmacologic Activity:

Esomeprazole, the S-enantiomer of the omeprazole inhibited the gastric acid secretion by acting on gastric parietal cell H^+/K^+ -ATPase proton pump. It inhibited histamine induced acid formation in the isolated gastric glands in rabbits ($IC_{50} = 0.162 \pm 0.031$) and showed gastric acid anti-secretory effect in rats with chronic gastric fistula and the esomeprazole and omeprazole exerted similar activities.

The plasma concentration of intravenously administered esomeprazole was declined rapidly and its terminal half-life was about 11 to 12 min.

B. Toxicological Findings:

In the acute toxicity studies in rats, the minimal iv lethal dose of H199/18 was 310 mg/kg. Salivation, dyspnea, reduced motor activity, increased or decreased respiration rate, ataxia, tremors, convulsions and cyanosis were the major treatment related toxicity in the animals. In the 28-day continuous intravenous toxicity studies in rats and dogs, intravenously administered esomeprazole produced a non-proportional plasma concentration and the identified target organs of toxicity in rats were central nervous system, site of injection and stomach and, the target organs of toxicity in dogs were the CNS, stomach, thyroid and site of injection. The highest tolerable doses in male and female rats were 48 and 26 mg/kg/day, respectively and, 35 mg/kg/day in dogs.

C. **Nonclinical Safety Issues Relevant to Clinical Use:** None

III. Administrative:

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____

(See memo attached)

C. C.:

Original NDA

HFD-180

HFD-181/CSO

HFD-180/Dr.Chopra

HFD-180/Dr.Choudary

HFD-048/Dr. Viswanathan

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW OF NDA 21-689

2.6.1 INTRODUCTION AND DRUG HISTORY

Esomeprazole an s-enantiomer of omeprazole had earlier been approved in capsules formulation for the short-term treatment gastroesophageal reflux disease (GERD), for the healing and symptomatic relief of erosive esophagitis and, it was also recommended in the maintenance of symptom-healing of erosive esophagitis. It is a proton pump inhibitor that acts by the inhibition of final step of acid secretion of H^+/K^+ -ATPase activity in the gastric parietal cells. The present application of 20 or 40 mg esomeprazole sterile injection preparation, is for the short term 10 days treatment

The esomeprazole injection 20 mg is administered as a single slow iv injection (no less than 3 minutes) or over 30 minutes.

NDA number: 21-689

Review number: 001

Sequence number/date/type of submission: 000/September 10, 2003 (Original);
000BP/April 9, 2004 (Amendment)

Information to sponsor: Yes () No ()

Sponsor and/or agent: Astra Zeneca LP, Wilmington, DE.

Manufacturer for drug substance: Astra Zeneca LP, Wilmington, DE.

Reviewer name: Yash M. Chopra, M.D., Ph.D.

Division name: Division of Gastrointestinal and Coagulation Drug Products, HFD-180

Date of submission: (i) 000/September 10, 2003;
(ii) 000BP/April 9, 2004

Date of HFD180 Receipt: (i) 000- September 12, 2003
(ii) 000BP/April 9, 2004

Review completion date: June 24, 2004

Drug:

Trade name: Nexium[®] Injection 5 ml Vial
Generic name (list alphabetically): Esomeprazole

Code name: H199/18

Chemical name: (S)-5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium

International Non-Proprietary Name Modified: Esomeprazole sodium

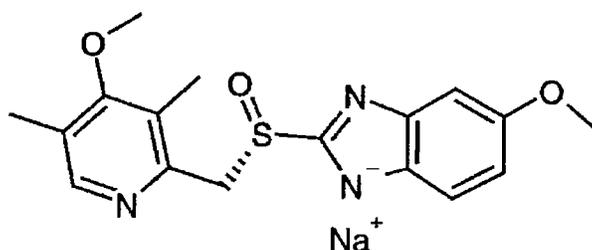
CAS registry number: 161796-78-7

Mole files number: NA

Molecular Formula: $C_{17}H_{18}N_3O_3SNa$

Molecular Weight: 367.4 (sodium salt), 345.4 (parent compound)

Structural Formula:



Relevant INDs and NDA: NDA 19-810 (Omeprazole – Merck Sharp and Dohme Research Laboratories),

NDA 21-153 (Esomeprazole Capsules - AstraZeneca);

IND 23,284 (Omeprazole – AstraZeneca)

IND 53,733 (Omeprazole – AstraZeneca)

IND 64,865 (AstraZeneca – Esomeprazole Injection)

Route of administration: Intravenous

Drug class: Gastric Parietal cell Proton Pump H^+/K^+ -ATPase Inhibitor

Intended Clinical Population: Patients with erosive esophagitis or with the symptoms of gastroesophageal reflux disease (GERD)

Clinical formulation:

Each 5 ml vial contains _____ freeze-dried _____ esomeprazole sodium 21.3 mg or 42.5 mg (equivalent to 20 mg or 40 mg esomeprazole), edetate disodium 1.5 mg and _____ sodium hydroxide solution for pH adjustment.

Marketing Indication and Dose:

The present submission is for the short-term intravenous treatment (up to 10 days) of _____ (GERD) _____

_____ is not possible or appropriate. When the oral dosing is possible, the intravenous therapy will be discontinued. Esomeprazole is recommended at the a single intravenous daily dose of 20 or 40 mg (0.4 or 0.8 mg/kg/day) in gastroesophageal reflux disease (GERD) patients up to 10 days as an alternative to oral therapy.

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

List of Preclinical Studies:

Sponsor referred to NDA 21-153 for esomeprazole magnesium delayed release capsules in the present application.

a. The studies conducted by an intravenous route of administration and submitted in delayed release esomeprazole capsules (NDA 21-153) were reviewed earlier (Pharmacology review of dated August 23, 2000). These were incorporated in the present review: 1. Pharmacokinetic study of omeprazole sodium, H199/18 sodium and H199/19 sodium following single intravenous and intraduodenal administration in rats (study #4151) and, 2. The comparison of acute oral and intravenous toxicity of study of omeprazole sodium, H199/18 sodium and H199/19 sodium in rats.

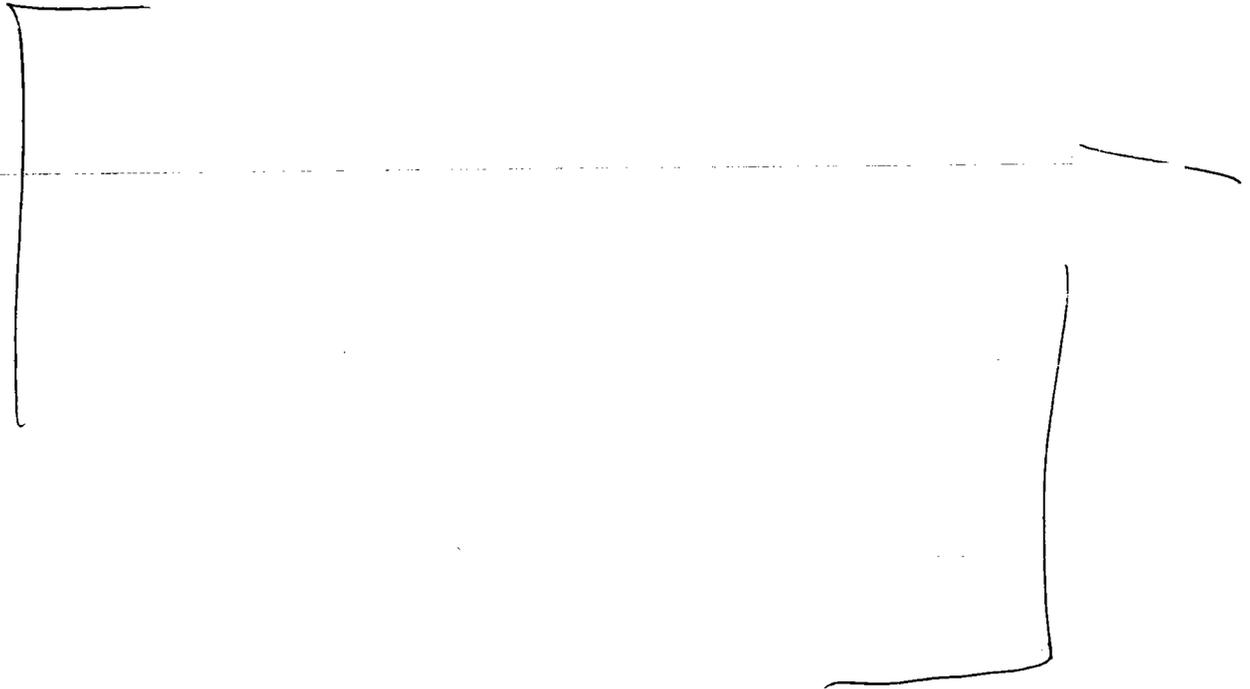
b. List of Studies reviewed in the present application.

PRECLINICAL STUDIES AND TESTING LABORATORIES				
Type of Study	Study Report #	Testing Laboratory	Drug Batch #	Study Page #
Pharmacology				-
Absorption, Distribution, Metabolism and Excretion:				10
Pharmacokinetic study of omeprazole sodium, H199/18 sodium and H199/19 sodium following single intravenous and intraduodenal administration in rats	#4151	-	-	11
Identification of Metabolites of Esomeprazole and Omeprazole in vitro by Microsomal Preparations from Animals and Man	24988	AstraZeneca AB, Sodertalje, Sweden	Radioc: 1:111 (Esomep) 8:2121 (Omepr) Non-Radioc: 1:111 (Esomep) 15(01) (omep.)	12
Chemical Characteristics of Degradants Products/Potential Impurities	A commentary	„	-	18
ACUTE TOXICITY STUDIES:				
1. Comparison of Acute Oral & Intravenous Toxicity of H199/18 Sodium, H199/19 Sodium and Omeprazole Sodium in Rats	#2816& T2821	Astra Safety Assessment, Sweden Sodertalje (Sweden)	HT530-10-1-27 – Eso 600/93 – H199/18; 600/93 – H199/19; 51 – Omeprazole	20
2. A Single Intravenous Dose Toxicity Study in Sprague- Dawley rat. A comparison of the effects of the test compound with and without	#02054/#D9615/826 21	Astra-Zeneca	105/01- With degradants 113/1 – Without degradants	22

degradation products.				
SUBACUTE TOXICITY STUDIES:				
3. 28-Day IV Toxicity Study in SD Rats	SR01334-01; 82621CTBR56771		153/00- HT 1139-01-01, HT 1141- 01-01 HT 1119-01-01 HT 1137-01-01 HT 1136-01-01 HT 1140- 01-01	27
4. 28-Day Intravenous Toxicity Study in Sprague Dawley Rats in the presence of degradants	#CTBR57465/SR 03003-01		105/01 – With Degradant 113/01-Without Degradants	34
5. Estimation of Maximum Tolerated Dose (M TD) in 3 Day Continuous Intravenous Infusion Toxicity Study in Dogs followed by a 14 Day Continuous Infusion Period	#TDD1316.	AstraZeneca R& D, UK, Cheshire (UK)	109/01 (HT1249-01-01-01- Form.)	40
6. 28-Day IV Toxicity Study in Beagle Dogs	SR01333-01; CTBR56859		163/00(Drug) Inject. Form #: HT 1164-01-01-01, HT 1165-01-01-01, HT 1166- 01-01-01	45
7. 1-Month Continuous Intravenous Infusion Study in Dogs	# 0140AD	AstraZeneca UK Limited Safety Assessment UK Alderley (UK)	Esomeprazole (8.0 mg/ml): Esomeprazole	50
8. New 28-Day Continuous Intravenous Infusion Toxicity Study in the Dog:	CTBR#500204/0278 AD	Astra Zeneca Canada Inc., Mississauga (Canada)	117.01	57
9. Hepatic Cytochrome P450 Induction in 1-Month Continuous Intravenous Infusion Toxicity Study in the Dog	1040AD- G10000.019-076-2D	AstraZeneca UK Limited, Alderley Park Macclesfield, Cheshire (England)	Esomeprazole (8.0 mg/ml): Esomeprazole	66
GENOTOXICITY:				
1. Genetic toxicity evaluation using the Ames Test (Salmonella/E.coli reverse mutation test). A comparison of effects of the test compound with and without degradation products.	#02209/SR-0220201- 1/D9615-82621)	AstraZeneca R& D, Sweden	a. Esomeprazole sodium #105/01/ Formulation # HT 1216-01-01-01 Batch #113/ 01 – for Degrad. Formul prod; b. Batch # 113/01 – for Formul without Degrad. Formulation Batch #H 1516- 03-01-01	68
2. Genetic toxicity evaluation using the Ames Test (Salmonella/E. coli reverse mutation test). A comparison of effects of the test compound with and without degradation products (Repeat Study)	#02052/SR-02052- 01/D9615-82621	AstraZeneca R& D, Sweden	Batch #HT 1311-01-01-03	70
3. Genetic Toxicity Evaluation Using an Ames Salmonella/E.coli test of a degradation product of Esomeprazole	#0137BV	AstraZeneca R& D, (Sweden)	Batch #H 431/41-709/02	72
4. Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test of H193/61 – (Interim Study)	# 0306BV/GI. 000- 049-664)	AstraZeneca, Sodertalje, Sweden	Batch #193/61 – 136/32	73
5. Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test of H153/95	Interim # 0307BV/ GI.000-049-701	AstraZeneca, Sodertalje, Sweden	# H 153/95 – 136/46	75
6. Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes:	#24840-0-449OECD		#/	79

7. Induction of Chromosome Aberrations in the Bone Marrow of Mice. A comparison of the effects of Esomeprazole with and without Degradation Products	#1889/39-D6172		#: 105/01 Nexium injection with degrad. Products; 113/01 – for Formul without Degrad. Products Batch #H 1516-03-01-01 #HT 1216-01-01-01 (with degradants)	82
SPECIAL TOXICITY STUDIES:				
1. Vaso- and Tissue Irritation Study in Dogs after Intravenous and subcutaneous Administration for 5 Days	#99073/#82621	Astra AstraZeneca R& D (Sweden)	H199/18 - 600/93	86
2. 10-Day Intravenous Vascular and Perivascular Irritation Study in Dogs. A comparison of the effects of test compound with and without Degradation Products:	02096/D9615-82621	„	#: 105/01 Nexium injection with degrad. Products; 113/01 – for Formul without Degrad. (Injec Batch #H 1516-03-01-01 without degradants & #HT 1216-01-01-01 (with degradants)	89
3. Blood Hemolysis & Protein Flocculation in Human Blood Study in vitro. A Comparison of the Effects of the Test Compound With and Without Degradation Products	#SR02013-01/Study #02013/D9615 (82621)	„	HT1216-01-01-01 – 105/01; HT1516-03-01-01-113/01; HT1216-01-01-01 – 105/01; HT 1516-03-0101- 113/01	91

List of the Studies not reviewed in the present submission:



GOOD LABORATORY PRACTICE & QAU REGULATIONS:

Sponsor submitted a statement of compliance with GLP and QAU regulations with each of the toxicity studies.

2.6.2 PHARMACOLOGY:

2.6.2.1. Brief Summary:

No pharmacology/pharmacodynamic study was submitted with the submission and the preclinical studies with esomeprazole submitted under NDA 21-153 were reviewed earlier.

2.6.2.2 Primary Pharmacodynamics: No new studies are submitted with the present application.

Mechanism of Action: Esomeprazole inhibited both basal and, histamine- and db-cAMP-stimulated maximal acid output in animals and man by acting on gastric parietal cell H^+/K^+ -ATPase (proton pump). The compound was concentrated in the acidic-secretory canaliculus of the parietal cells, protonated rapidly, binds to its membrane and inhibits the acid output. The administered compound was transformed in the active form in isolated gastric cells preparation and in buffer solution. The transformed compound was a pre-requisite for the inhibition of gastric parietal cell H^+ , K^+ -ATPase activity.

Drug Activity related to proposed Indication: No studies were submitted.

2.6.2.3. Secondary pharmacodynamics: No new study on the safety pharmacology was submitted.

2.6.2.4 Safety pharmacology: No new study on the secondary pharmacology was submitted.

2.6.2.5 Pharmacodynamic drug interactions

Sponsor did not conduct any study on the drug interaction with the present application.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Intraduodenally administered esomeprazole maximum plasma concentrations attained the peak plasma concentration within 3 minutes after in rats and within 18- 26 minutes after gastric intubation in dogs. After intravenous administration of omeprazole, H199/18 and H199/19, the distribution of at the steady state (VDss) and the blood clearance for the 3

compounds were similar (V_{Dss} = 0.7 to 0.9 l/kg, CL= 66 to 75 ml/min/kg). The intraduodenal bioavailability of H199/ 18 and omeprazole were 33% and 39%, respectively, in rats. In humans, the plasma levels of H199/18 and omeprazole declined slowly than in rats and dogs with half-life of 1.0 to 1.5 hr in GERD patients. In the vitro liver microsomal preparations of rat, dog, rabbit and man, different metabolites of esomeprazole (M1 to M13) were isolated and their structures elucidated. M1 was detected in only rabbit microsome preparation and M2 to M5 were common metabolites in mouse, rat and rabbit microsomes. The metabolites M6 to M8 and M10 to M13 were formed in liver microsomes preparation of all animals in a variable amounts in mouse, dog and human liver microsomes.

1. Pharmacokinetic Study of Omeprazole Sodium, H199/ 18 Sodium, and H199/ 19 Sodium Following Single Intravenous and Intraduodenal Administration in the Rat (study # 4151, Report # 3222- 0336)

Methods: In male Sprague- Dawley rats (n= 5), omeprazole sodium (batch # 51), H199/ 18 sodium (batch # 600/ 93), and H199/ 19 sodium (batch # 600/93) were given intraduodenally (id) or intravenously (iv) at a dose of 40 mM/kg (~ 15 mg/ kg). Blood samples were collected (by cannulation of the left carotid artery) at 0, 2, 5, 10, 20, 40, 60, 90, and 120 min after dosing. Both H199/ 18 and H199/ 19 were measured in plasma using an established non enantio-selective normal phase liquid chromatography and UV detection method (since earlier study showed that no racemization of 2 enantiomers occurs in rats), with limit of quantitation of 0.05 and 0.025 m M/ l in 0.25 and 0.5 ml of blood respectively.

Results: In male rats, after id administration, all 3 drugs declined in a similar manner with the terminal half life of ~ 10 min. The maximum plasma concentration of omeprazole sodium, H199/ 18 sodium, and H199/19 sodium were reached within 5 min, and were 22, 19 and 25 mM/l respectively. The bioavailability (F) of omeprazole sodium, H199/18 sodium, and H199/ 19 sodium were 39%, 33% and 49% respectively. The bioavailability of H199/ 19 was significantly higher than the bioavailability of its S-enantiomer (H199/18). After iv, the volume of distribution at steady state (V_{dss}) and total blood clearance (Cl) for all 3 compounds (omeprazole sodium, H199/ 18 sodium, and H199/ 19 sodium) were similar (V_{dss} = 0.7- 0.9 l/ kg, CL = 66- 75 ml/ min/ kg). The results are summarized in the following table.

Comparative pharmacokinetic parameters of omeprazole sodium, H199/18 sodium, and H199/19 sodium, after single id and iv administration in male rats.

Parameters	ID (mean)			IV (mean)		
	Omeprazole	H199/18	H199/19	Omeprazole	H199/10	H199/19
V _{ss} (l/kg)	--	--	--	0.9	0.8	0.7
Cl (ml/min/kg)	--	--	--	75	75	66
AUC (μmol.min/l)	220	169	293	555	545	605
T _{1/2} (min)	10	9	10	12	11	11
T _{max} (min)	3	3	2	--	--	--
C _{max} (μmol/l)	21.5	19.1	24.9	--	--	--
F (%)	39	33	49	--	--	--

F% - The bioavailability was calculated as the AUC ratio of each drug after id administration to that after iv administration. 1 μmol = 0.35 mg

2. Identification of Metabolites of esomeprazole and omeprazole formed in Vitro by Liver microsomal preparations from Animals and Man: (Study Report #24988/Document#1869-01)

Name of Laboratory: AstraZeneca R & D Molndal, Molndal (Sweden)

Batch #: ¹⁴C-esomeprazole (H 199/18) – 1:111
 _____ - 8:2121
 _____ - 15(01)

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Table 1: Tabulated product ion spectra of synthetic reference compounds of potential metabolites of esomeprazole.

Compound code	L ₁	MH ⁺	a	b	c	d	e	Fragment ions (%)					
								f	g	h	i	j	
Sulphoxides													
Esomeprazole or Esomeprazole	14.6	345	329		198B	120	179		168	166	151	150	149
H 199/18	10.8	332	344		214D	156	172		134	182	157		149
H 213/12	10.2	332	344		198B	120	165		162	166	151	150	145
H 153/13	10.8	362			231		179	195	184		167	169	145
									155		151	150B	
Sulphides													
H 108/12	11.5	334	297		182B						151	150	149
H 153/12	14.7	345	313		198B		179				157	166	149
H 183/12	14.0	314	283		182B						151	150	145
H 153/12	11.5	315	283		182B						137	136	142

Procedure: Liver microsomes were obtained from male and female mouse (CD-1), rat (Sprague-Dawley), dog (Beagle) and human. The microsomes of male rabbits were also used in the study. The microsomal preparations (0.5 ml) at the concentration of 3 mg

microsomal protein/ml were used and mixed with NADPH and allowed to react for 60 min, reaction stopped and preserved at _____ till used. The incubates were mixed with LC mobile phase and analyzed for total radioactivity by LSC analysis in duplicate. These microsomal preparations were analyzed by liquid chromatography (_____) mass spectrometry (_____) and on-line radioactivity monitoring (_____) procedures. The recovery of the radioactivity was _____. No metabolite outside the range of _____ m/z was detected during LC/MS analysis. The recovery of radioactivity from LC column was _____. The metabolites were identified by mass spectrum covered by radioactivity monitoring (RAM) peak.

Results: The amounts of esomeprazole and omeprazole were decreased in a similar pattern in the system for the duration of the study of 60 min showing that the compounds were similar in their metabolism. The compounds/metabolites formed at different intervals are shown in the tables below.

Table 4 Decrease in amount of parent esomeprazole and omeprazole during 60 min incubation.

Species/Sex	Esomeprazole		Omeprazole	
	% in control sample ^a	% metabolised ^b (+ NADPH)	% in control sample ^a	% metabolised ^b (+ NADPH)
Mouse/M	93	45	92	57
Mouse/F	94	41	94	53
Rat/M	94	64	93	53
Rat/F	94	70	90	53
Rabbit/M	94	66	91	54
Dog/M	95	26	92	22
Dog/F	94	37	91	31
Human/M	94	17	92	17
Human/F	93	24	92	24

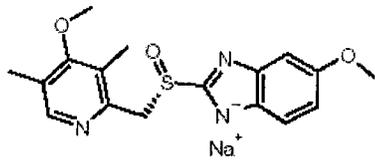
^a Given as the % radioactivity allocated to the LC/RAM peak of the parent in the controls (without NADPH; n=1).

^b Given as the difference between the % radioactivity allocated to the LC/RAM peak of the parent in the controls (without NADPH; n=1) and those of the incubated samples (with NADPH; mean, n=2).

According to sponsor, these compounds were characterized structurally and their structures are shown in the following tables. The synthetic reference compounds used in the study were racemate forms of sulfoxide metabolites, i.e., H 195/80; H215/02, H153/73. Several metabolites (M1 to M15) were structurally characterized and their amounts in the microsomes were estimated as shown in the following sponsor tables (table # 3-8). The structures of esomeprazole and omeprazole are given as reference:

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Figure 1 Chemical structure of esomeprazole sodium



H199/19

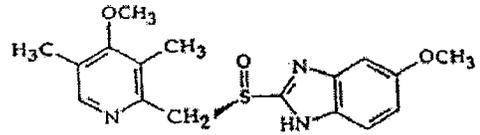


Table 3 Metabolism of esomeprazole and omeprazole: Structures and amounts of metabolites formed in liver microsomal incubations given as % of metabolised parent during 60 min.

Metabolite No.	t _{1/2} (min)	Structure	Species (Sex): Amount of formed metabolites ^a									
			Mouse (M)	Mouse (F)	Rat (M)	Rat (F)	Rabbit (M)	Dog (M)	Dog (F)	Human (M)	Human (F)	
M1	4.4		Esomeprazole									
			-	-	-	-	3.0	-	-	-	-	-
			Omeprazole									
			-	-	-	-	2.4	-	-	-	-	-
M2	5.1		Esomeprazole									
			0.9	0.4	14	11	1.0	-	-	-	-	-
			Omeprazole									
			1.3	1.1	12	11	1.4	-	-	-	-	-
M3	5.2		Esomeprazole									
			0.4	0.5	0.5	0.4	2.6	-	-	-	-	-
			Omeprazole									
			0.5	0.5	0.6	0.5	2.3	-	-	-	-	-
M4	8.2		Esomeprazole									
			2.0	2.5	0.3	0.5	2.6	-	-	-	-	-
			Omeprazole									
			1.8	1.5	0.6	0.8	2.5	-	-	-	-	-
M5	8.2		Esomeprazole									
			2.2	2.5	0.2	0.1	2.6	-	-	-	-	-
			Omeprazole									
			1.4	1.5	0.3	0.2	2.0	-	-	-	-	-

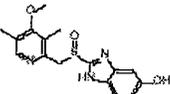
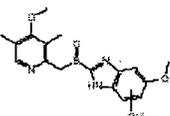
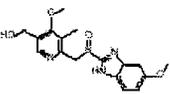
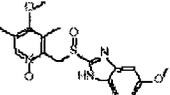
^a calculated as % of metabolised parent (= % of total metabolites formed)

^b below LOQ (RAM) but presence established by MS

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Table 3. Metabolism of esomeprazole and omeprazole (continued)

Metabolite No.	t _{1/2} (min)	Structure	Species (Sex): Amount of formed metabolites ^a									
			Mouse (M)	Mouse (F)	Rat (M)	Rat (F)	Rabbit (M)	Dog (M)	Dog (F)	Human (M)	Human (F)	
M6	10.3		Esomeprazole									
			27	17	37	65	25	18	18	15	13	
			Omeprazole									
			14	9.6	34	53	23	17	23	19	13	
M7	10.3		Esomeprazole									
			7.2	5.7	7.4	6.5	8.5	6.4	5.4	4.2	3.8	
			Omeprazole									
			4.6	3.2	6.8	7.6	8.4	8.4	5.8	2.7	2.7	
M8	10.7		Esomeprazole									
			30	30	34	13	39	37	42	25	18	
			Omeprazole									
			58	55	38	21	42	37	40	39	34	
M9	10.7		Esomeprazole									
			1.6	3.4	- ^b	0.3	-	-	-	1.3	5.9	
			Omeprazole									
			-	6.1	-	-	-	0.8	0.8	-	1.0	

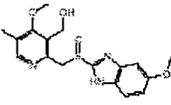
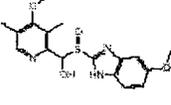
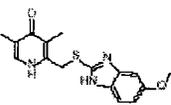
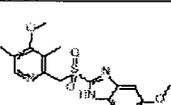
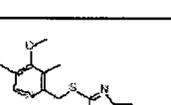
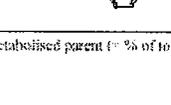
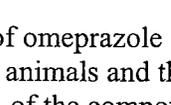
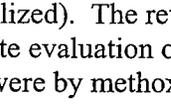
^a calculated as % of metabolised parent (= % of total metabolites formed)

^b below LOQ (RAM) but presence established by MS

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Table 3. Metabolism of esomeprazole and omeprazole (continued)

Metabolite No.	t _n (min)	Structure	Species (Sex): Amount of formed metabolites *									
			Mouse (M)	Mouse (F)	Rat (M)	Rat (F)	Rabbit (M)	Dog (M)	Dog (F)	Human (M)	Human (F)	
M10	11.3		Esomeprazole									
			5.2	8.8	0.5	0.2	1.4	4.3	5.4	8.9	0.6	
			Omeprazole									
			5.1	7.6	1.4	0.5	1.9	7.1	6.5	11	11	
M11	11.6		Esomeprazole									
			0.7	3.3	1.4	0.8	7.1	6.4	5.4	8.9	0.6	
			Omeprazole									
			1.0	1.1	1.4	1.6	9.6	7.1	6.5	4.4	7.6	
M12	13.1		Esomeprazole									
			21	24	2.1	1.0	4.6	28	22	34	40	
			Omeprazole									
			10	11	1.8	0.9	3.1	20	16	22	26	
M13	14.1		Esomeprazole									
			0.5	0.3	0.6	0.7	0.5	0.4	0.6	1.1	0.7	
			Omeprazole									
			0.5	0.4	0.8	0.9	0.6	1.0	0.7	0.9	1.0	

* calculated as % of metabolised parent (= % of total metabolites formed)

In general, 13 metabolites of omeprazole and esomeprazole were identified in liver microsomes from the study animals and the metabolism in the rabbit liver preparation was more efficient as 41 to 70% of the compound was metabolized than those from dogs and humans (17 to 37% metabolized). The retention time, molecular weight and structures of the metabolites and complete evaluation of product ion spectra were analyzed. Three major metabolites formed were by methoxybenzimidazole demethylation (M6), 5'-methylpyridinyl hydroxylation (M8) and sulfoxidation (M12). In rats and rabbits, M6 and M8 were major metabolites whereas M12 was a minor. Metabolite M1 to M5 were minor ones in mouse, rat and rabbit microsomes and not identified in dog and human microsomes. The metabolites structures identified and characterized by sponsor are shown below.

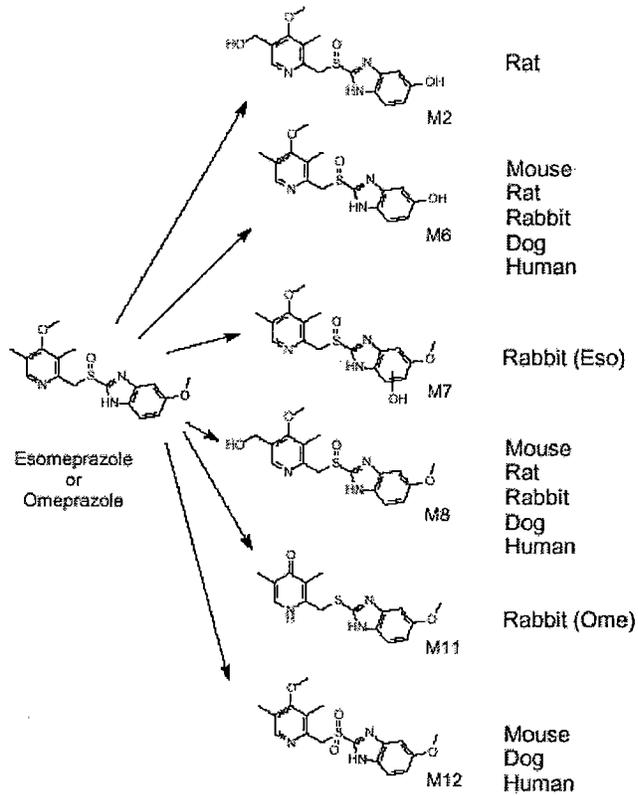


Figure 14 Summarised metabolic scheme for esomeprazole and omeprazole including the three main metabolites from each species tested.

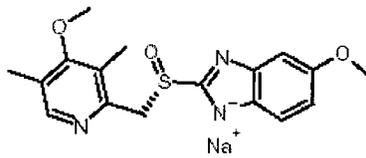
In summary, esomeprazole and omeprazole showed similar metabolism in liver microsomes of animals and 3 major metabolites were formed by methoxybenzimidazole demethylation (M6), 5'-methylpyridinyl hydroxylation (M8) and sulphoxidation (M12). The rate of metabolite formation was greater in the rabbit liver than those from dogs and humans.

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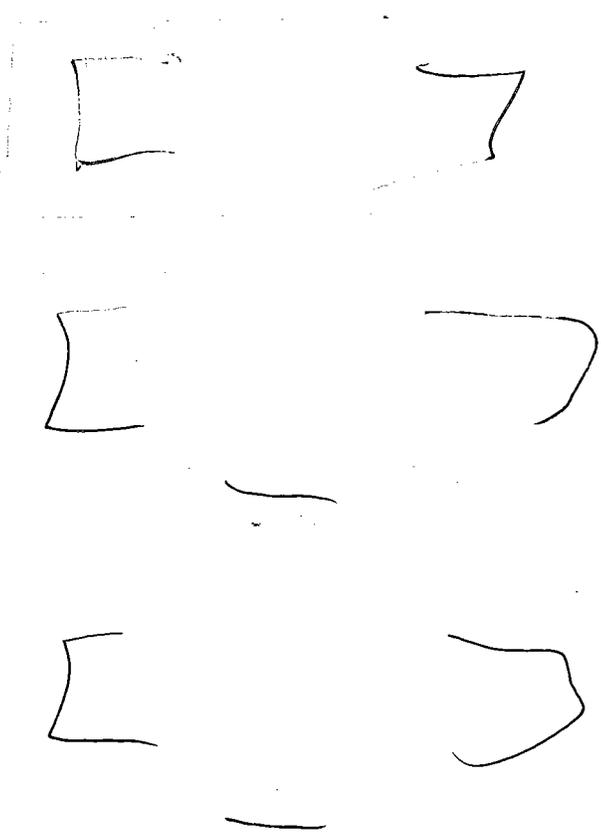
2. **Chemical Characteristics of Degradation Products/Potential Impurities of**

Esomeprazole: Sponsor identified and characterized the main degradation products from the drug substance (esomeprazole sodium) or the proposed commercial drug powder for solution for injection/infusion 20/40 mg or the constituted esomeprazole solution during the storage or in use of the product. The degradation compounds in the final drug product are (i) _____ a metabolite and a potential impurity in the final product; (ii) _____ potential degradation product of esomeprazole sodium and known from degradation of omeprazole magnesium and esomeprazole magnesium and (iii) _____. The impurity isolated _____ was isolated was pH dependent as it had half-lives of 2.5 and 1.5 hr at pH 10.5 and 9.6. It was formed during the storage and the proportion of the proposed specific impurity. These two impurities were present up to _____, respectively. The structural formulae of these substances are given in sponsor's Figure 1 and scanned here.

Figure 1: Esomeprazole sodium and potential degradation products in the final product



Esomeprazole sodium



The toxicity of total degradation products in esomeprazole solution was evaluated in toxicology studies. Two additional degradation products _____ formed during the storage of the proposed injection solution, were specified in the amount of _____ in the solution. _____ was proposed as degradation product of _____, which was further seen to be degraded to _____

The safety margin of the total degradation products administered with the clinical IV dose of 0.8 mg/kg esomeprazole was determined from the 28-day intravenous toxicity study in rats and 1-month continuous toxicity study in dogs. The total degradants present in the esomeprazole used in 28-day intravenous toxicity study in rats (study #574655R/82621) was estimated as _____ and a dose of 4 mg/kg/day was the identified 'no effect dose'. The amount of degradants injected with this dose was computed as _____ in rats and the amount of total degradants administered with a clinical dose of 40 mg/day (0.8 mg/kg) would be _____. This provides a safety margin of about _____. The total amount of degradants administered in the 1-month continuous intravenous toxicity study in dogs was _____ and the dose of 35 mg/kg/day was the highest tolerable dose. The estimated total amounts of degradants administered with 35 mg/kg/day esomeprazole dose was _____ and this provided the safety margin of _____ to the total degradants administered with the recommended clinical 40 mg/day (0.8 mg/kg). The 1-month continuous intravenous infusion toxicity study in dogs provided the safety to the total amounts of degradation compounds. The mutagenicity of the compound was not affected by the degradants as the Ames tests and in vivo chromosome aberration study showed no additional mutagenicity.

2.6.6 TOXICOLOGY:

2.6.6.1 Overall toxicology summary

After iv administration, the 3 drugs (H199/18 sodium, H199/ 19 sodium and omeprazole sodium) showed clinical signs reduced motor activity, increased or decreased respiratory frequency, abdominal respiration, clonic convulsions and the minimum lethal were 310 mg/kg for all three drugs except for the males after omeprazole (240 mg/kg) and a dose of 180 mg/kg was not lethal. In the 28-day rat toxicity study at the intravenous doses of 48, 86 and 160 mg/kg/day in males and, 26, 52 and 100 mg/kg in females, a treatment related and non-proportional plasma concentrations, gastric chief cell hypertrophy, chronic nephropathy and inflammatory and other adverse reactions at the site of injection were seen. The identified organ of toxicity were CNS, stomach, kidney and site of injection and the 'highest tolerable dose' was 48 and 26 mg/kg/day in males and females of the study. In another 28-day rat toxicity study, esomeprazole at 4, 80 (with degradants) and 80 (without degradants) in the presence of the degradants produced similar toxicity in rats indicating degradants did not interfere with esomeprazole toxicity. In 14-day iv toxicity study in dogs, the only 2 doses of 120 and 240 mg/kg/day were used and these were lethal. In 28-day continuous toxicity studies in the presence and absence of degradants, the

highest tolerable dose was 35 mg/kg/day in both the studies. The study provided the safety margin to the total degradants administered with the recommended clinical 40 mg/day (0.8 mg/kg). The mutagenicity of the compound was not affected by the degradants as the Ames tests and in vivo chromosome aberration study showed no additional mutagenicity.

2.6.6.2 **Single Dose Toxicology Studies:**

The following single dose toxicity studies were conducted by using esomeprazole iv preparation.

1. **The Comparisons of Acute Oral and Intravenous Toxicity Of H199/ 18 Sodium, H199/ 19 Sodium and Omeprazole Sodium in Rats:** (Study # T2816 and T2821)

Methods: The acute toxicity of H199/ 18 sodium (batch # 600/ 93), H199/ 19 sodium (batch # 600/ 93) and omeprazole sodium (batch # 51) after oral (gavage) and iv administrations were examined in male and female Wistar rats. All 3 compounds were dissolved in distilled water (at concentration of 70, 140 and 270 m M/ ml, pH 10.6- 11.3) and saline (pH 10.7- 10.9, and given in volumes of 20 ml/kg) for oral and iv administrations, respectively. Additional 2 groups of rats (n= 5/ sex) were given omeprazole sodium (310 mg/kg) dissolved in buffered polyethylene glycol 400 (PEG400, pH 10.3) or PEG400 alone (control animals) by iv administration. The dosing information is summarized in a table in the result section. All animals were observed for toxic signs and mortality daily for 14 days. At the end of observation period, all animals were necropsied and subjected to standard examinations. In addition esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum were examined microscopically.

Results: After oral administration of H199/18, deaths were noted at 510 (2 females died), 990 (one male + 3 females), and 2000 (4 males died) mg/kg. Death with H199/19 were found at 990 (1 M + 1 F died) and 2000 (4 M + 5 F died) mg/ kg. Deaths after omeprazole were 1 male and 5 females at 990 mg/kg and 3 males at 2000 mg/ kg. All 3 drugs (at all oral doses) caused similar clinical signs (reduced activity to unconsciousness, coupled with reduced respiratory frequency and abdominal respiration). Also, repeated clonic convulsions (associated with tremors, salivation, dyspnea, cyanosis, ataxia and/ or reduced motor activity) were observed at all doses of these 3 compounds, except at 510 mg/ kg of H199/ 19 sodium. Convulsions (which lasted for 5 seconds) were usually first observed within few min and continued intermittently for up to 1- 3 hrs. Females in general had clinical signs (also had lacrimation and dark colored urine) with greater intensity and duration, as well as increased mortality ratios. All surviving animals recovered from these abnormalities within 23 hrs after treatment. Since no macroscopic changes were noted, no histopathology in GI tracts were examined.

After iv administration, all 3 drugs (H199/ 18 sodium, H199/ 19 sodium and omeprazole sodium) showed similar clinical signs and the minimum lethal were 310 mg/ kg for all

three drugs except for the males after omeprazole (240 mg/kg) and a dose of 180 mg/kg was not lethal. Major clinical signs were reduced motor activity, increased or decreased respiratory frequency, abdominal respiration, clonic convulsions in association with tremors, salivation, dyspnea, cyanosis, and ataxia observed after both oral and iv doses. Clinical signs in females in general were of greater intensity and duration. All surviving animals recovered from these effects within 22- 23 hrs after treatment.

The minimum oral lethal doses was 990 mg/kg and clinical signs including piloerection and lacrimation at higher doses) as noted after oral administration. A marked local reaction at the injection site was observed in the tail in all drug treated animals and incidence and severity were increased into ulcerations. With omeprazole sodium given in PEG400, a more pronounced reaction was observed with death within 4 min (all 10 rats died, vs none with the vehicle PEG400 alone). The vehicle PEG400 itself caused reduced motor activity and increased respiratory frequency in rats. Some animals displayed pulmonary congestion due to agonal circulatory insufficiency. The mortality rate is summarized in the following table along with the dosing information.

Mortality rate in oral dose study in rats

Species (strain) Wistar Rats	No./Sex/Group	Doses (mg/kg)	Mortality rate	Minimum Lethal Doses (mg/kg)
H199/18 Sodium				
Males	5	510	0	990
		990	1	
		2000	4	
Females	5	260	0	510
		510	3	
		990	3	
H199/19 Sodium				
Males & Females	5	510	0	990

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		990	1 male, 1 female	
		2000	4 males, 5 females	
Omeprazole Sodium				
Males & Females	5	510	0	990
		990	1 male, 5 females	
		2000 (males)	3 males,	
Mortality rate in the intravenous dose study in rats				
Species (strain)	No./Sex/Group	Doses (mg/kg)	Mortality rate	Minimum Lethal Doses (mg/kg)
Wistar Rats				
H199/18 Sodium				
Males & Females	5	100	0	310
		310	1 male, 2 females	
H199/19 Sodium				
Males & Females	5	180	0	310
		310	5 males, 1 female	
Omeprazole Sodium				
Males & Females in saline	5	100	0	240 for males
		240	1 male	310 for females
		310	2 males, 2 females	
In PEG400		310	5 males, 5 females	

2. A Single Intravenous Dose Toxicity Study in Sprague- Dawley rat. A comparison of the effects of the test compound with and without degradation products. (Study #02054/Project #D9615/82621)

Testing Facility: _____

Dates of Initiation and Completion: May 13, 2002 and January 10, 2003

GLP and QAU Requirements: A statement of compliance was attached.

Batch #: 105/01 –with degradation compounds; 113/012 – without degradation compounds.

These studies injection alone and the drug substance (the same batch # was stored under accelerated favorable degradation conditions prior to use). The analysis of the substance after storage indicated the presence of the following products: _____

Methods: Three groups (6/sex) of rats were treated with a single 30-minute intravenous infusion dose of esomeprazole and an additional 4th group (6/sex) was for blood sampling for the TK evaluation only. Two different batches of the proposed commercial product, (5 ml vial containing 42.5 mg esomeprazole- a freeze- dried formulation) were used in this

study. One of these batches “esomeprazole with degradation products” was prepared by storing the _____ This batch was used in groups 1 and 4 in this study. The animals in group 2 were administered the same doses of esomeprazole without degradation products and, group 3 animals were administered esomeprazole with degradation product administered directly after reconstituting the solution.

The high dose of 160 mg/kg was administered in males and, dose of 100 mg/kg dose was administered in females of each of the group 1, 2 and 3 as shown in the table. The study was conducted from May 8, 2002 to May 29, 2002 in 4 groups (6/dose) of animals. The animals in group 4 were used for the collection of blood for TK data estimation of the compound. The blood samples from group 4 animals were used for estimating TK data. The dose schedule and formulation concentrations etc. is shown in the table below (Sponsor’s table).

Details of dosing

Group no.		Dose level		Dose volume mL/kg	Infusion rate mL/kg/min
		mg/kg ¹	µmol/kg		
1 and 4.	Esomeprazole with degradation products and “degradation storage” of the constituted solution				
	Males	160	460	20	0.67
	Females	100	300	13	0.43
2.	Esomeprazole without degradation products				
	Males	160	460	20	0.67
	Females	100	300	13	0.43
3.	Esomeprazole with degradation products administered directly after reconstitution of the solution				
	Males	160	460	20	0.67
	Females	100	300	13	0.43

¹ Expressed as the neutral form of the test compound, although, esomeprazole sodium was used to prepare the solutions.

Results: The median values and ranges for TK parameters as shown in sponsor table were summarized below.

TABLE 1 Median (range) plasma concentrations of esomeprazole in male and female rats (n=6)
Single dose: intravenous infusion for 30 minutes of esomeprazole sodium with degradation products

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Daily dose $\mu\text{mol/kg}$	Sex	n	Cmax $\mu\text{mol/L}$	AUC $\mu\text{mol} \times \text{h/L}$
460	M	6	197(168- 237)	123 (104-151)
300	F	6	318(303- 336)	512 (468- 651)

The plasma concentrations (calculated AUC value) in males injected with degradation products were lower than females, even though a higher dose of 160 mg/kg was given to male rats. Decrease in motor activity, closure of eyelids, ataxia and increase in respiration depth were observed in both male and female animals. Piloerection, ploughing, decreased respiration rate and staining around the nostrils were also reported in a dose-related manner. None of the animals died during the study. The toxicity of the esomeprazole without degradation products (group#2) was similar to esomeprazole with degradants. Sponsor did not provide the plasma concentrations of groups of animals administered esomeprazole without degradation products and claimed that those animals treated with esomeprazole alone had similar concentrations. These claims could not be confirmed. The body weight gain of animals was similar in all study groups animals and no treatment/dose related gross pathology changes were seen. The maximum dose of the study, 160 mg/kg by an iv infusion route was tolerated by the animals and was not lethal in rats and the presence of degradants in esomeprazole solution did not affect the toxicological effects of the compound in this study.

2.6.6.3 REPEAT DOSE TOXICITY STUDIES:

a. Rats:

4. 28-Day Intravenous Toxicity Study in SD Rats: (Study # 56771/Project #82621; SR01334-01)

Name of Laboratory:  

Dates of Initiation and Completion of Study: November 8, 2000 and January 14, 2002

GLP and QAU Requirements: A statement of compliance was attached.

Animal Species: SD rats 255.1 to 264.7 g (males) and 217.9 to 226.6 g (females) about 10 weeks old.

Batch #: 153/00 (pure) & 245/00 (pure),

Procedure: Eighty albino rats (40/sex) were divided into 4 groups (10/sex/group) and administered 0, 48(140), 86(250) or 160 (450) mg/kg/day (umol/kg) esomeprazole in males and, 0, 26 (75), 52 (150) or 100 (300) mg/kg/day (umol/kg) esomeprazole in females (vol = 10 ml/kg) as a slow infusion via surgically implanted catheters for 30 min. Additionally, 3 groups (9/sex/group) of animals were included in toxicokinetic part of the study. The dose selection was based on a 5-day iv toxicity study in rats (study #00160/Project #D9615 (82621). The intravenous doses of 120 and 160 (350 and 450 umol/kg) mg/kg/day males and 86 (250 and 300 umol/kg/day) and 100 mg/kg/day esomeprazole in females produced a dose related and dose proportional plasma concentration, and CNS depression, decreased motility, rigidity, ataxia and convulsions. The CNS, respiratory system and site of injection were identified target organs of toxicity and 'the highest tolerated doses' in males and females were 160 and 100 mg/kg/day, respectively. The doses used and # animals used in study are shown below in the following table (scanned from sponsor submission).

Text Table 4 Dosage and Dose Groups

Group Number	Dose Level				Dose Concentration				Number of Animals			
	umol/kg-day		mg/kg-day		umol/mL		mg/mL		Main Study		TK Animals	
	M	F	M	F	M	F	M	F	M	F	M	F
1	0	0	0	0	0	0	0	0	10	10	-	-
2	140	75	48	26	14	7.5	4.8	2.6	10	10	9	9
3	250	150	86	52	25	15	8.6	5.2	10	10	9	9
4	450	300	160	100	45	30	16	10	10	10	9	9

Text Table 5 Individual Animal Identification

Group Number	Animal Reference Number			
	Main Study		TK Animals	
	M	F	M	F
1	1011, 1021, 1031, 1041	1511, 1521, 1531, 1541		
	1051, 1062, 1072, 1082	1651, 1562, 1572, 1582		
	1092, 1102	1692, 1602		
2	2014, 2024, 2034, 2044	2611, 2621, 2631, 2641	2061, 2071, 2081, 2091	2561, 2571, 2581, 2591
	2054, 2102, 2112, 2222	2571, 2662, 2612, 2622	2162, 2172, 2182, 2192	2632, 2662, 2672, 2682
	2132, 2142	2632, 2642	2252	2692
3	3011, 3021, 3031, 3041	3511, 3521, 3531, 3541	3061, 3071, 3081, 3091	3561, 3571, 3581, 3591
	3051, 3102, 3112, 3122	3651, 3662, 3642, 3622	3162, 3172, 3182, 3192	3652, 3662, 3672, 3682
	3232, 3242	3632, 3642	3252	3692
4	4011, 4021, 4031, 4041	4611, 4521, 4531	4061, 4071, 4081, 4091	4561, 4571, 4581, 4591
	4051, 4202, 4112, 4122	4541, 4551, 4602, 4612	4152, 4162, 4172, 4182	4652, 4662, 4672, 4682
	4132, 4142	4622, 4632, 4642	4192	4692

Prior to dosing on Day 1, Animal 4511 was noted to have a kinked catheter which required surgery to repair, therefore Animal 4611 replaced 4511 on Day 1.

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The study animals were observed twice daily for changes in clinical signs and death among the animals on days 1, 3, 7, 14, 21 and 28 at 10 and 30 minutes after the start of the infusion and 5, 10 and 30 min and, 1 and 2 hr after the end of the infusion. The body weights and food consumption were recorded weekly during the study. The ophthalmic

examination was done prior to the start of treatment and during week 4. The hematological parameters and chemistry parameters were estimated on the blood samples collected from aorta of 24-hr fasted rats. The blood sample (1 ml) from all animals were drawn during week 3 at 2 and 24 hr after the end of the infusion for gastrin estimation (double antibody gastrin radioimmunoassay). The transferrin receptors in bone marrow were determined to determine the relationship between the iron deficiency anemia and number of the receptors. The bone marrow cells were stained to find relative percentage of erythroid cells (CD71 positive, CD45 negative to intermediate positive) and lymphoid/myeloid (CD71 negative (dim)/CD45 positive) cells. The 0.5 ml/animal blood samples were collected from low and mid dose TK animals on days 1 and 28 at 10 min after the start of infusion, immediately prior to the end of infusion and, 5 min, 1 and 2 hr after the end of the infusion. The blood samples from high dose treatment group animals were collected at the same intervals excepting the additional blood sample was collected at 6 hr interval instead of 1 hr interval. The toxicokinetic parameters were evaluated in the samples. The animals of all group were sacrificed and necropsy on each of the animals was performed, the examination of each of the organs, orifices and cavities of each animal separated and cleaned. The adrenals*, aorta, brain*, cecum, colon, duodenum, injection site, jejunum, esophagus, heart*, ileum, kidneys*, liver*, lungs, mammary glands (only females), pancreas, pituitary*, spleen*, prostate*, stomach*, seminal vesicles, eye, femur, spinal cord, testes*/ovaries* with epididymides, thymus, thyroid*+parathyroid*, urinary bladder, mesenteric and mandibular lymph nodes, vagina/uterus* were separated, cleaned and preserved for histopathological examination. The organs marked with asterisk (*) were weighed after the necropsy. The histopathological examination of the tissues of control and high dose treatment groups were examined by histopathological examination. The chief cell eosinophilia was observed in all groups of animals but the ECL morphology was not studied during the study. The liver, kidneys, spleen, sternum, stomach and gross lesions of low and mid dose treatment group animals were microscopically examined.

Results:

- a. **Observed Effects:** Treatment related decreased activity was seen in 0, 0, 8 and 10 males and, 0, 1, 5 and 10 out of 10 females/group of 0, 48, 86 and 160 mg/kg/day treatment groups. Tremors and convulsions were seen in 1 male of 160 mg/kg/day treatment group and, 1 female of the 100 mg/kg/day (high dose group) also showed convulsions. Partial closure of the eyes was noted in 2, 1, 6 and 10 males and, 0, 3, 4 and 10 females of 4 study treatment groups.
- b. **Mortality:** None of the animals of the main and satellite study groups died during the study.
- c. **Body Weight/Food Consumption/Water Consumption Changes:** During the study, the body weight changes in the males and females belonging to treatment groups were not

statistically different from the control group animals. The initial body weights of study males were 255.1, 264.7, 260.2 and 257.6 g and, females were 225.3, 217.9, 226.3 and 226.6 g, respectively of control, low, mid and high dose treatment groups. The final body weights were 353.9, 341.9, 330.4 and 339.0 g among males and, 256.4, 254.3, 265.2 and 265.8 g among females of the study. The food consumption changes in control and esomeprazole treatment groups were similar in males and females animals. The initial food consumption of the control group animals was 15.2 and 17.6 g among males and females, respectively. The final daily food consumption was 21.9, 21.0, 20.9 and 22.1 g among males and, 17.8, 18.2, 18.9 and 18.6 g among females of study groups.

d. Hematology/Coagulation/Bone Marrow Changes: There was a dose related increase in monocytes counts was reported in males and females but it was only significant in males of high dose treatment group ($P < 0.05$). No significant difference in erythroid, myeloid and lymphoid cells of bone marrow were seen in both animals of study groups. No other changes in the hematology parameters were seen in the study animals. The changes in coagulation parameters were not recorded during the study. No changes of bone marrow cytology were seen in animals.

e. Blood Chemistry/Urinalysis Changes: An increase in the serum calcium and phosphate was reported in high dose treatment group males but these were only of statistical significant ($p < 0.05-0.01$) and of no toxicological significance because the changes were within the normal ranges. A slight decrease in serum triglycerides was seen in males and females. A dose related increase in mean serum gastrin was noted in males and females at 2 and 24 hr after the dosing as shown in the following table of sponsor.

Text Table 10 Summary of Gastrin Alterations During Week 3 of the Treatment Period

Group	2 h post Rx		24 h post Rx	
	M	F	M	F
1	103.0	70.2	84.4	71.2
2	568.2	527.5	292.9	212.3
3	562.0	530.2	353.9	402.7
4	510.4	512.4	464.7	453.2

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Toxicokinetics:

The plasma concentration of esomeprazole (H 199/8) on day 1 in male and female rats were dose proportional with $t_{1/2}$ of 12.7 to 14.1 min in males and, 17.2 to 33.7 min in

females. On day 28, the AUC values in males and females were dose proportional and sponsor did not calculate the t1/2 of the compound in the animals. The plasma exposure of the metabolite, , was linear in both males and females as shown below in the table (sponsor table 7b, pp27).

Median Pharmacokinetic parameters of esomeprazole in rats

Daily dose ($\mu\text{mol/kg}$)	Group	Sex	Day	n	C_{max}	AUC	t _{1/2}
					($\mu\text{mol/l}$)	($\mu\text{mol}\cdot\text{h/l}$)	(min)
					esomeprazole	esomeprazole	esomeprazole
140	2	M	1	4	93.4 (80.3-129)	64.9 (57.5-81.3)	14.1 (13.9-14.8)
250	3	M	1	4	189 (177-218)	119 (103-132)	12.7 (12.7-13.6)
450	4	M	1	4	359 (310-368)	243 (216-299)	nc
75	2	F	1	4	48.2 (33.3-62.6)	36.7 (29.3-43.8)	17.2 (14.7-21.2)
150	3	F	1	4	149 (137-156)	146 (123-175)	23.8 (20.0-27.4)
300	4	F	1	4	356 (230-391)	551 (267-601)	33.7 (30.5-41.8)
140	2	M	28	4	85.1 (48.0-114)	56.5 (35.7-73.3)	nc
250	3	M	28	5	154 (86.2-181)	120 (69.5-158)	nc
450	4	M	28	4	300 (243-371)	213 (172-252) ^a	nc
75	2	F	28	5 ^b	60.3 (2.99-73.3)	44.1 (36.6-49.7)	nc
150	3	F	28	5	140 (99.1-163)	116 (93.0-137)	nc
300	4	F	28	5	283 (244-310)	311 (279-359) ^a	nc

nc = not calculated ^a AUC_(0-2h) ^b n=4 (AUC)

Median Pharmacokinetic parameters of esomeprazole metabolite in rats (Day 28)

Daily dose ($\mu\text{mol/kg}$)	Group/ Sex	Day	n	C_{max} ($\mu\text{mol/l}$)	AUC _(0-2h) ($\mu\text{mol}\cdot\text{h/l}$)
140	2/M	28	4 ^c	5.24 (3.61-7.51)	5.74 (5.39-6.08)
250	3/M	28	5	11.9 (10.7-16.5)	12.2 (10.7-21.9)
450	4/M	28	4	25.4 (24.0-26.9)	25.2 (21.5-32.3)
75	2/F	28	4 ^d	1.55 (1.39-2.95)	2.33 (2.07-4.61)
150	3/F	28	5	5.83 (5.31-8.51)	9.98 (8.79-13.1)
300	4/F	28	5	16.7 (13.6-20.3)	33.5 (22.7-40.4)

^c n=2 (AUC) ^d n=3 (AUC)

The plasma exposure (AUC_{0-2hr}) of esomeprazole was increased dose proportionately and in linearly with the doses in both males and in females. The half-life of the compound was not calculated on day 28. A single clinical dose of 40 mg/day (0.8 mg/kg) intravenous dose of esomeprazole achieved an AUC value of 7.10 umol.h/l in man and the AUC values in 3 treatment groups of animals were 9.1, 16.8 and 34 times the clinical exposure.

h. Physical Examination and Ophthalmic Test: On study day 28, the incidences of nuclear and central corneal opacity were similar in treated and control group animals.

i. Organ Weight Changes: The absolute weight of thymus was decreased in a dose related manner by 52.7, 31.7 and 53.5% in males and 6.7, 13.5 and 21.1% in females included in 3 treatment groups. The absolute weights of liver and stomach was increased (p<0.01) in males included in high dose treatment group. In females, the absolute liver and stomach weights were also increased (p<0.01). The organ weight changes in tissues of study animals are shown below:

Text Table 12 Selected mean organ weights and percentage change compared to the control group for males

Group No.	1	2	3	4
Adrenal gland				
Absolute (g)	0.054	0.054	0.057	0.066
Relative (% brain weight)	3.099	2.999	3.195	3.642*
% Increase - absolute (relative)	-	0 (-3)	6 (5)	22 (18)
Liver				
Absolute (g)	8.138	8.360	8.208	9.340*
Relative (% brain weight)	468.434	463.894	462.526	515.866
% Increase - absolute (relative)	-	3 (-1)	1 (-1)	15 (10)
Stomach				
Absolute (g)	2.058	2.350	2.339	2.640**
Relative (% brain weight)	118.181	130.536	131.737	145.694**
% Increase - absolute (relative)	-	14 (10)	14 (11)	28 (23)
Thymus				
Absolute (g)	0.319	0.270	0.237*	0.199**
Relative (% brain weight)	19.066	15.029	13.298	10.978**
% Decrease - absolute (relative)	-	15 (21)	26 (30)	38 (42)

* P<0.05 (Dunnett's) ** P<0.01 (Dunnett's)

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Text Table 13 Selected mean organ weights and percentage change compared to the control group for females

Group No.	1	2	3	4
Adrenal gland				
Absolute (g)	0.068	0.071	0.076	0.089**
Relative (% brain weight)	3.946	4.166	4.466	5.197**
% Increase - absolute (relative)	-	5 (6)	12 (13)	31 (32)
Kidney				
Absolute (g)	1.480	1.553	1.689**	1.703**
Relative (% brain weight)	85.844	90.904	99.308**	99.539**
% Increase - absolute (relative)	-	5 (6)	14 (16)	15 (16)

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Liver				
Absolute (g)	5.970	6.047	6.636	7.379**
Relative (% brain weight)	346.442	353.375	390.433	431.323**
% Increase - absolute (relative)	-	1 (2)	11 (13)	24 (25)
Stomach				
Absolute (g)	1.793	2.164**	2.231**	2.373**
Relative (% brain weight)	103.987	126.493**	130.965**	138.718**
% Increase - absolute (relative)	-	21 (22)	24 (26)	32 (33)
Thymus				
Absolute (g)	0.221	0.207	0.191	0.183
Relative (% brain weight)	12.834	12.093	11.180	10.652
% Decrease - absolute (relative)	-	6 (6)	14 (13)	17 (17)
Thyroid				
Absolute (g)	0.014	0.017*	0.017*	0.017*
Relative (% brain weight)	0.832	0.978*	0.982*	0.980*
% Increase - absolute (relative)	-	21 (18)	21 (18)	21 (18)

* D=0/15 (Examinations) ** D=0/01 (Examinations)

j. **Gross Pathology Findings:** Thickening at the site of injection, small thymus in 1/sex animal was seen high dose treatment group. Enlargement of spleen was seen in 1 female of high dose treatment group. The scab formation and thickening at the site of injection was common among control and treated females and males.

k. **Histopathological Changes:** Slight cortical adrenal hypertrophy and hemorrhage were noted in 1 and 2 out of 10 females in each of control and high dose treatment groups. This was limited only in study females. A decrease in hemosiderin deposits (Perl's staining) in liver and spleen was noted. Corneal inflammation was seen in 1 and 2 out of 10 males and, 2 and 1 females out of 10 in each of 0 and high dose treatment groups. Thrombosis of slight to mild nature (grade 1 or 2) at the site of injection was seen in 3/10, 0/0, 1/2 and 6/10 males and, 2/10, 0/1, 1/2 and 4/10 females in control and 3 treatment groups. No significant difference in erythroid, myeloid and lymphoid cells of bone marrow were seen in both animals of study groups. Vascular inflammation in 3, 0, 1 and 8 out of 10 males and 5/10, 0/1, 1/2 and 6/10 females in each of control, and 3 treatment groups. Perivascular fibrin exudate of grade 1 was present in 1/10 males of control group and, 1/10 females of high dose treatment group. Chronic progressive nephropathy of grade 1 was observed in 7, 7, 5 and 6 males and, 5, 4, 6 and 7 females of control and 3 treatment groups. Chronic progressive nephropathy of grade 2 was in 1 and 3 males and, 1 and 0 females of mid and high dose treatment groups. Chief cell eosinophilia incidences were 0, 6, 6 and 10 males and, 0, 6, 10 and 10 out 10 females out of 10/sex/group in each of control and 3 treatment groups. The intensity was of grade 1 (slight) in most of the animals.

Tissue Examined	Treatment Groups (# animals Examined) 1 (10)			
	1 (10)	2 (10)	3 (10)	4 (10)
Adrenal Hypertrophy (grade 1) M	-	-	-	-

	F	1	-	-	2
Corneal Inflammation	M	1	-	-	2
	F	2	-	-	1
Site of Injection - Thrombosis	M	3	-	1	6
	F	2	-	1	4
„ - Vascular Inflammation	M	3	-	1	8
	F	5	-	1	6
Perivascular Fibrin Exudate (Grade 1)	M	1	-	-	-
	F	-	-	-	1
Chronic Progr. Nephropathy	M	7	7	6	9
	F	5	4	7	7
Stomach - Chief Cell Eosinophilia	M	0	6	6	10
	F	0	6	10	10

In summary, esomeprazole at intravenous doses of 48 to 160 mg/kg/day in males and, 26 to 100 mg/kg/day in female rats produced treatment related increase in the plasma concentrations. The gastric chief cell hypertrophy, chronic nephropathy and inflammatory and other adverse reactions at the site of injection were observed in a treatment related manner in treatment group animals. The identified organ of toxicity were CNS, stomach, kidney and site of injection and the 'highest tolerable doses' were 48 and 26 mg/kg/day in males and females of the study.

5. Esomeprazole sodium: A 28 day intravenous toxicity study in the Sprague-Dawley rat. A comparison of effects of the test compound with and without degradation products.

(Study # 57465SR/D9615(82621)/03003-01)

Conducting Laboratory




Dates of Initiation and Completion of Study: April 18, 2002 and December 20, 2002

GLP and QAU Requirements: Statement of compliance were attached.

Animal Species: Beagle dogs weighing between 8.8 to 9.9 kg (males) and 6.7 to 8.6 kg (females)

Batch #: Used in the study groups:

Used in Groups	Batch no. esomeprazole Sodium	Batch no. freeze-dried powder	Concentration esomeprazole in solutions used for dosing	pmol/mL
II	105/01	HT 1216-01-01-01*	1.2	0.4
III	105/01	HT 1216-01-01-01	23	8
IV	113/01	H 1516-03-01-01**	23	8

• Batch HT 1216-01-01-01 - esomeprazole with degradation products stored _____, prior to use. This gave a mixture of relevant degradation products _____ **Batch H 1516-03-01-01 - esomeprazole without degradation products (stored _____) the solution was also prepared from batch HT 1216-01-01-01 which was subjected to a storage period at room temperature that generously exceeded the proposed in-use period for clinical use. This produced additional degradation products and actual amounts and composition of the degradation products in the solutions.

Procedure: Eighty rats (40/sex) were divided in to 4 groups (10/sex/group) and administered 0, 4 (12), 80(230) or 80(230) mg/kg/day (umol/kg) esomeprazole (vol = 10 ml/kg) as a slow injection via surgically implanted catheters for 30 min/day. The doses used of esomeprazole (without and with degradation products) and # animals used in study are shown in the following tables (scanned from sponsor submission):

The esomeprazole was administered at the doses of 0 (vehicle), 4 (esomeprazole with degradation products) and 80 mg/kg (esomeprazole without degradation product but contained "degradation storage" products) in a volume of 0.4 mg/ml. The degradants administered in Group II animals with the doses were (mg/kg/day): _____

Text Table 1 Dosage and Dose Groups

Group Number	Dose Volume (mL/kg-day)	Infusion Rate (mL/kg-h)	Dose Level		Dose Concentration	
			µmol/kg-day	mg/kg-day	µmol/mL	mg/mL
I	10	20	0	0	0	0
II ^a	10	20	12	4	1.2	0.4
III ^a	10	20	230	80	23	8
IV ^b	10	20	230	80	23	8

^a esomeprazole with degradation products; ^b esomeprazole without degradation products



The amount of degradants injected were _____ in group II, III and IV animals.

The study animals were observed daily for changes in clinical signs and death, the body weights and food consumption were recorded weekly during the study the ophthalmic examination was done prior to the start of treatment and during week 4. The hematological parameters and chemistry parameters were estimated on the blood samples collected at autopsy. The blood samples from group 3 (with degradation products) and group 4 (without degradation products) were collected for the toxicokinetic parameters estimation for esomeprazole 25 min, 10 and 25 min and, 3 hr after the start of infusion in group 1, III and IV animals. Samples were assayed for plasma concentrations of esomeprazole by liquid chromatography and UV detection by a non-enantioselective analytical method (TK evaluation conducted by _____). All surviving animals of all group were sacrificed and necropsy on each of the animals was performed, the examination of each of the organs, orifices and cavities of each animal separated and cleaned. The adrenals*, aorta, brain*, cecum, colon, duodenum, injection site, jejunum, esophagus, heart*, ileum, kidneys*, liver*, lungs*, mammary glands (only females), pancreas, pituitary*, spleen*, prostate*, stomach*, seminal vesicles, eye, femur, spinal cord, testes*/ovaries* with epididymides, thymus*, thyroid*+parathyroid*, urinary bladder, mesenteric and mandibular lymph nodes, vagina/uterus* were separated, cleaned and preserved in formalin for histopathological examination. The organs marked with asterisk (*) were weighed (paired organs weighed together) after the necropsy. The histopathological examination of all the tissues of group 1, 3 and 4 and liver, kidney, spleen, sternum, stomach and gross lesions of group 2 animals was performed. Numerical data obtained were used to obtain mean values and SD values.

Results:

- a. **Observed Effects:** Dose related hunched posture in 1, 0, 4 and 3 males and, 0, 0, 3 and 10 females, decreased activity in 2, 0, 0, 0 and, 0, 0, 4 and 10 females and partial eye closure in 2, 1, 7 and 8 males and, 4, 4, 6 and 8 females of control, 4, 80 (with degradation products) and 80 (without degradation products) mg/kg/day treatment groups respectively.
- b. **Body weight/Food Consumption Changes:** The body weight gain in the treated animals was greater by 68.2, 94.7 and 118.0% in males and 24.3, 76.0 and 21.1% in females than control group animals of the study. The initial body weights of male and female rats were 273.8 and 193.8 g respectively. On day 28, the final body weight of the animals were 332.4, 322.8, 324.3 and 343.2 g among males and, 219, 213.4, 228.4 and 224.3 g in females of control, 12, 230 (with degradation products) and 230 (without degradation products) umol/kg/day treatment groups respectively. The daily food consumption was not affected during the study.

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c. Hematology/Coagulation/Bone Marrow Changes: No treatment related changes in the hematology parameters were seen in the study males and females. The coagulation parameters of the animals were affected by treatment and were similar to control group animals during the study.

d. Blood Chemistry/Urinalysis Changes: An statistically significant increase in the serum cholesterol and calcium, unsaturated iron binding capacity and total iron content was observed in these 2 groups of animals. The total iron binding capacity of females of 230 umol/kg/day treatment group with and without degradants was increased in a statistical significant manner in only females and the increase was not significant in males.

Text Table 11 Summary of Serum Iron, TIBC, UIBC, and Cholesterol

Group	Iron (ug/dL)		TIBC (ug/dL)		UIBC (ug/dL)		Cholesterol (mg/dL)	
	M	F	M	F	M	F	M	F
I	106.7	189.6	410.6	430.3	304.1	240.9	78.9	70.9
II	115.4	199.6	402.3	446.5	286.9	246.9	76.5	60.6
III	100.2	223.9	414.2	478.8**	314.0	254.8	86.5	81.7
IV	92.1	222.2	423.7	481.9**	331.8	259.6	93.4***	99.9***

* = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001 (Dunnett)

e. Toxicokinetics:

The plasma concentrations of esomeprazole were determined in animals treated with 230 umol/kg/day treatment groups, i.e., group 3 and 4 with and without degradation products were determined. The plasma concentration (C_{max} and AUC values) of esomeprazole in the animals of group of animals without degradation products was only slightly lower than the animals treated with esomeprazole containing degradation products. The plasma concentrations in males were lower than in females. The data is shown in the following table of the sponsor (document # 57465, pp 12(47)).

Text Table 2 C_{max} and AUC values in rats treated intravenously with esomeprazole for 28 days

Dose (umol/kg)	Degradation products	Group	Sex	n	C _{max} (umol/L)	AUC (umol·h/L)
230	Yes	III	M	3	155 (136-156)	97.0 (93.1-105)
230	No	IV	M	4	108 (95.7-184)	66.0 (59.4-90.0)
230	Yes	III	F	4	224 (214-249)	240 (222-247)
230	No	IV	F	4	180 (133-229)	207 (135-236)

h. Physical Examination and Ophthalmic Test: On study day 28, there were no treatment related ophthalmic changes in animals treated with esomeprazole in the absence and presence of degradation products.

i. Organ Weight Changes: The absolute and relative to brain weight of adrenals, liver and kidney were increased in females included in esomeprazole without and with degradation products. The absolute and relative brain weight of liver was increased in male these treatment groups. The absolute weight of liver, adrenal and kidney in control animals were 7.4, 0.05574 and 2.112 g in males and, 5.11, 0.0609 and 1.339 g in females of control, 12, 230 (with degradant) and 230 (without degradants) mg/kg/day treatment groups, respectively. The percent increase is shown below in the text table 15 of sponsor at pp 46(47):

Text Table 15 Organ weight changes in the adrenal glands, liver, and kidney

Esomeprazole-Related Organ Weight Changes: Approximate Percent Difference from Control Mean						
Dose Group ($\mu\text{mol/kg-day}$):	12 (Esomeprazole with degradation products)		230 (Esomeprazole with degradation products)		230 (Esomeprazole without degradation products)	
	Sex:	M	F	M	F	M
Organ	Percent Difference from Concurrent Control Mean (%)					
Adrenal glands						
-Absolute weight				+16.20		+13.65
-Relative weight ^a				+13.94		+14.71
Liver						
-Absolute weight ^b			+11.64 ^b	+12.22	+19.72	+14.07
-Relative weight ^a			+12.45	+10.11	+18.83	+15.12
Kidneys						
-Absolute weight				+11.53	+10.66	+11.35
-Relative weight ^a				+9.36	+9.89	+12.41

a: Relative to brain weight. b: Not statistically significant.

j. Gross Pathology Findings: The dilatation, pale discoloration and thickening of the stomach were seen in 230 $\mu\text{mol/kg/day}$ treatment groups. The incidences were similar or more in animals treated with esomeprazole without degradation products. Dark colored areas in lungs were seen in 0, 1, 2 and 2 males and, not in females of 230 $\mu\text{mol/kg/day}$ treatment groups with or without degradation products. Enlargement of lymph node in 1, 1, 3 and 3 females and foci of dark color in 0, 0, 1 and 3 females of study groups.

k. Histopathological Changes: Corneal inflammation of only slight grade was seen in 3 of 10 rats of esomeprazole without degradation products. Corneal epithelium inflammation of

grade 1 was noted in only 1/10 males included in each of 230 umol/kg/day treatment group with and without degradation products, respectively. These were considered as not treatment related effects. Intimal proliferation of grade 1 at the site of injection was seen in 1 and 2 of 10 males and, 2 and 2 females of 230 umol/kg/day treatment groups with and without degradation products. Perivascular inflammation of slight to mild nature at the site of injection was seen in 0, 0, 3 and 2 males and, 2, 1, 2 and 3 females in control and 3 study treatment groups. Subacute inflammation of lungs was seen in 2, 1, 2 and 5 males of study groups. Chronic progressive nephropathy of grade I was noted in 7, 7, 5 and 6 males 2, 5, 8 and 5 females of study groups. Grade II nephropathy was noted in 1 and 3 males and 0 and 1 female of 230 mg/kg/day treatment groups with and without degradants, respectively. Lymph node erythrocytosis/hemorrhage in 1, 2, 3 and 4 males and, 0, 0, 1 and 3 females out of 10/sex/group was noted. Chief cell eosinophilia incidences were 0, 6, 6 and 10 males and, 0, 1, 10 and 10 out 10 females out of 10/sex/group in each of control and 3 treatment groups. The intensity was of grade 1 (slight) in most of the animals. Liver necrosis (grade 1 or 2) was seen in 1/sex in high dose treatment group animals.

Tissue Examined		Treatment Groups (# animals Examined)			
		1 (10)	2 (10)	3 (10)	4 (10)
Adrenal Hypertrophy (grade 1)	M	1	-	-	2
	F	1	1	-	-
Corneal Inflammation	M	1	-	1	3
	F	-	-	-	1
Site of Injection - Intimal Prolif.	M	-	-	1	2
	F	-	-	2	2
- Perivascular Inflammation	M	-	-	3	2
	F	2	1	2	3
Vascular Inflammation (Grade 1)	M	3	-	1	8
	F	5	-	1	6
Cardiac Mononucle cell infiltr.	M	-	-	-	1
	F	-	-	-	1
Subacute Lung Inflammation	M	2	1	1	5
	F	2	0	0	0
Chronic Progr. Nephropathy	M	7	7	6	9
	F	2	5	8	6
Stomach - Chief Cell Eosinophilia	M	-	2	1	3
	F	0	1	10	10

Liver	M	0	0	1	1
	F	0	0	1	1

In summary, esomeprazole at intravenous doses of 4 to 80 mg/kg/day produced treatment related non-proportional plasma concentrations. The amounts of total esomeprazole degradants administered with the study doses were _____ in treated group II, II and IV animals. These degradants did not interfere with activity of the compound. The increase in serum iron binding was only slight and in the study animals treated with or without degradants. The chief cell eosinophilia incidences in stomach of animals included in 80 mg/kg/day esomeprazole with and without degradants were similar, thus degradants may not interfere drug toxicity. A dose of 4 mg/kg/day was a 'no effect dose' in the study and site of injection, stomach and kidney were the site of injection. The presence of degradants of the compound produced significant decrease in vascular inflammation. The degradation products did not contribute to the toxicity but the efficacy of the compound could be reduced in the presence of degradation product.

DOGS:

6. Esomeprazole Sodium: Maximum Tolerated Dose (MTD) 3 Day Continuous Intravenous Infusion Toxicity Study in Dogs followed by a 14 Day Continuous Infusion Period (Study #TDD1316).

Sponsor: AstraZeneca R& D,
Mölndal (Sweden)

Test Facility: AstraZeneca UK Limited (Safety Assessment),
Cheshire (England)

Date of Start and Completion: February 11, 2002 and June 18, 2003

GLP and QAU Compliance: A statement of compliance was attached.

Batch #: Compound - 109/ 01 (purity _____, enantiomeric purity > _____),
Formulation - HT1249-01-01-01 (The formulation containing 120 mmol/ml (40 mg/ml) esomeprazole was prepared in 0.9% sterile saline solution by sponsor. Esomeprazole sodium 367.4 g = 345.4 g esomeprazole)

Procedure: The study was divided in to Phase 1 (MTD) and Phase 2 (dose finding). In the 3-day continuous iv infusion toxicity study (Phase I) in dogs, esomeprazole was administered in at the dose of 690 or 1200 umol/kg (240 or 414 mg/kg) in 1/sex dog (concentration = 8 mg/ml). The TK of the compound was determined in phase 1 and phase

2 of the study. The blood samples were collected 0, 2, 4 and 6 hr after the start of infusion on day 1 and, at 24 and 30 hr (day 2), at 48 and 56 hr (day 3) and at 72 hr (day 4) after the start of infusion. The doses administered in the study are shown below in sponsor's table 4:

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Table 4 Study number TDD1316. Study design - Phase 1

Group	Sex and animal no.	Day number	Dose volume (mL/kg/day)	Dose concentration mg/mL	Nominal dose level* $\mu\text{mol/kg}$	Nominal dose level mg/kg
1	M303, F6	1 to 4	30	8.0	690	240
2	M391, F28	1, 1 to 4	52	8.0	1200	414

*Actual mean dose levels for M303, F6 and F28 were estimated to be 680, 610 and 870 $\mu\text{mol/kg}$ respectively (see Table 2, Section 2.1.4)

The 3 groups of dogs (3/sex/group) with surgically implanted with access ports were used in 14-day continuous iv toxicity study (phase 2). Three dogs/sex were administered a continuous IV infusion dose of either 350 (120) or 690 (240) $\mu\text{mol/kg/day}$ (mg/kg/day). The dose schedule, estimated dose range on day 1, 7 and 14 (termination day) and the group mean estimated dose level are shown in the following table (sponsor's table, vol. 5 of 14, page 1382):

Table 5 Study number TDD1316. Study design - Phase 2

Group	Sex and animal no.	Day number	Dose volume (mL/kg/day)	Dose concentration		Nominal dose level*	
				mg/mL	$\mu\text{mol/kg}$	$\mu\text{mol/kg}$	mg/kg
3	M106	1 to 15	30	0	0	0	0
3	M143	1 to 15	30	0	0	0	0
3	M400	1 to 15	30	0	0	0	0
3	F9	1 to 15	30	0	0	0	0
3	F10	1 to 15	30	0	0	0	0
3	F31	1 to 15	30	0	0	0	0
4	M55	1 to 10	15	8.0	350	120	120
4	M132	1 to 15	15	8.0	350	120	120
4	M137	1 to 15	15	8.0	350	120	120
4	F17	1 to 15	15	8.0	350	120	120
4	F19	1 to 7	15	8.0	350	120	120
4	F162	1 to 15	15	8.0	350	120	120
5	M131	1 to 15	30	8.0	690	240	240
5	M138	1 to 15	30	8.0	690	240	240
5	M144	1 to 15	30	8.0	690	240	240
5	F18	1 to 2	30	8.0	690	240	240
5	F165	1 to 15	30	8.0	690	240	240
5	F167	1 to 15	30	8.0	690	240	240

* Actual mean dose levels for groups 4 and 5 were estimated to be 320 and 670 $\mu\text{mol/kg}$ respectively (see Table 3, Section 2.1.4).

Table 5 Study number TDD1316. Study design - Phase 2

Group	Sex and animal no.	Day number	Dose volume (mL/kg/day)	Dose concentration	Nominal dose level*	
				mg/mL	µmol/kg	mg/kg
NDA 21-689 Page 40 of 98	M106	1 to 15	30	0	0	0
	M143	1 to 15	30	0	0	0
	M400	1 to 15	30	0	0	0
	F9	1 to 15	30	0	0	0
	F10	1 to 15	30	0	0	0
	F31	1 to 15	30	0	0	0
4	M55	1 to 10	15	8.0	350	120
4	M132	1 to 15	15	8.0	350	120
4	M137	1 to 15	15	8.0	350	120
4	F17	1 to 15	15	8.0	350	120
4	F19	1 to 7	15	8.0	350	120
4	F162	1 to 15	15	8.0	350	120
5	M131	1 to 15	30	8.0	690	240
5	M138	1 to 15	30	8.0	690	240
5	M144	1 to 15	30	8.0	690	240
5	F18	1 to 2	30	8.0	690	240
5	F165	1 to 15	30	8.0	690	240
5	F167	1 to 15	30	8.0	690	240

* Actual mean dose levels for groups 4 and 5 were estimated to be 320 and 670 µmol/kg respectively (see Table 3, Section 2.1.4).

All animals were observed twice daily throughout the study for abnormal signs and mortality, the body weights were taken on day -7, -1, 1, 2, 3, 4, 8, 11 and 14 and the food consumption were recorded daily. Ophthalmic examination was done in rats of phase 2 of the study. During Phase 1, EKGs were recorded once pre-dosing, 2, 4 and 6 hr of the start of dosing and, in Phase 2 animals, EKG recordings (lead I, II, III and aVR, aVL, aVF) were recorded 2 hr after the start and 2 hr after the changing the infusion bag on the morning of day 14. The hematology and blood chemistry parameters were performed during the both phases. At necropsy, the pathological and histopathology abnormalities in all of the animals were recorded. The organ/tissue weights of adrenal glands, brain, epididymides, heart, kidneys, liver, lungs including bronchus, ovaries, pituitary gland, prostate gland, spleen, stomach, submaxillary glands, testes, thymus, thyroid glands and uterus of all animals were recorded. In phase 2 animals, the blood samples were collected 0, 2, 4 and 6 hr after the start of infusion on day 1 and, at noon on day 2, 3, 4, 8, 11 and 14 and 15 (prior to termination of infusion). The control group animals samples were collected at noon on day 14.

Results:

a. Plasma Concentrations and Toxicokinetics:

The plasma concentrations of a single dose of the compound on day 1 varied from 46.2 to 51.5 µmol/l in male and, 29.3 to 40.4 µmol/l in female dogs. The blood concentrations on day 2 to 4 in male and female dogs as expected, were more uniform and lower than on day 1 in both study males and females (as seen in table 6).

Table 6 Study number TDD1316. Individual plasma concentrations of esomeprazole in dogs, Phase 1 (MTD). Daily dose: 690 µmol/kg of esomeprazole sodium - Group 1

Sampling time	Sampling day	Sampling date (yr mo da)	Esomeprazole (µmol/l) Dog no./sex	
			M303	F6
PS	1	020211	—	—
2h	1	020211	46.2	40.4
4h	1	020211	51.4	36.0
6h	1	020211	49.5	29.3
24h	2	020212	19.4	16.5
30h	2	020212	23.6	17.4
48h	3	020213	28.6	22.4
54h	3	020213	25.2	17.7
72h	4	020214	22.1	19.7

PS = prior to the start of infusion
— = < 0.125 µmol/l

The median plasma concentrations in animals of continuous infusion part of the study from day 2 to 15 were similar in males and females during the study. During the Phase 2 (14-days treatment period), the mean concentrations were 21.4 and 21.2 µmol/l in males and, 11.6 and 33.9 µmol/l in females included in 350 (120) and 690 (240) µmol/kg/day (mg/kg/day) treatment groups, respectively. The blood concentration of the compound in the female animals was erratic during this phase.

b. **Clinical Observations:** In MTD phase (Phase 1) of the study, male #391 was killed on day 2. The animal had increased salivation, trembling, ataxia, and head shaking and subdued behavior after 2 hours on day 1. Another male #303 had soft feces and dark colored urine.

In phase 2, a dose related emesis in 0, 2 and 3 males and 1, 1 and 3 females, soft to fluid feces in 0, 1, and 2 males and, 1, 1, 0 females, decreased activity and subdued behavior in 0, 2 and 2 males and, 0, 0 and 2 females, ataxia in 0, 2, and 1 male and, 0, 0 and 1 female dogs, red colored urine in 0, 3 and 3 males and, 0, 0 and 2 females were seen animals belonging to control (0), 350 (120 mg/kg/day) and 690 (240 mg/kg/day) µmol/kg/day treatment groups.

c. **Mortality:** Male animal #391 of 414 mg/kg treatment group in 3-day phase 1 study was killed on day 1 after 2 hr of the administration of the compound. This animal had pronounced CNS signs of increased salivation, trembling, ataxia, head shaking and inactivity and the plasma levels of the compound were 3 times greater than the samples taken from animal of the high dose group. The respective initial and final body weights were 12.5 and 12.6 kg in males and 11.2 and 11.0 kg in females. Three animals (1 males

and 2 females) of phase 2 group died and male #M55 was sacrificed on day 10 and it looked pale, subdued and showed decreased activity. Female #18 had decreased activity, which followed noisy breathing, ataxia and collapse and was killed on day 2. Another female #19 was killed on day 7 due to the development of dermal lesions/scabs on the forelimbs, neck and right flank.

Body weight and Food Consumption Changes: In 14-day continuous iv infusion phase animals, a body weight loss of 0, 12.9 and 2.7% in males and, 1.2, 9.1 and 5.7% in females belonging to 0, 120 and 240 mg/kg/day treatment groups was observed. There was a dose related reduction in food consumption from study day 1 to 5 both in male and female animals but no significant difference in mean food consumption was seen among males on day 14 and food consumption was 350, 331.0, 306.0 and 329 g/male/day. Among females, it was reduced, and was 350, 195 and 135.5 g/kg/female, respectively in animals included in 0, 120 and 240 mg/kg/day treatment groups.

Hematology/Urinalysis Changes: No significant changes in hematology parameters were seen in animals included in phase 1 and phase 2 of the study. The urinalysis of the study treatment groups animals was similar to the control group values and the changes were not of clinical/statistical importance.

Blood Chemistry Changes: The blood enzymes and electrolytes analysis of the animals treated with esomeprazole were similar to the control group values. No treatment/dose related changes were seen.

Changes in Physical Examination (Cardiovascular parameters and EKG): In phase 1 study animal, an increase in heart rate was seen in an animal at 414 mg/kg dose for up to 4 hr of the study. The observed heart rate was limited only for 1 animal, therefore data was not considered. In phase 2 group animals, increase in heart rate was from 121 (mean control) to 149 and 170 b/min on day 1 and 14 respectively. The animals included in 240 mg/kg/day treatment group also showed increase in heart rate from 117 (mean control) to 129 on day 1. On day 14, the heart rate was increased from 117 to 138. The mean QTc interval (in msec) was not affected in 120 and 240 mg/kg/day treatment groups animals and, QTc intervals were 233, 235 and 238 msec in control, 120- and 240- mg/kg/day treatment groups.

On day 1, the heart rate of the animals included in 120 mg/kg/day group was increased by 51.8 and 40% in male 2 dogs. This heart rate increase was insignificant, as this effect was not seen in animals of high dose of 240 mg/kg/day treatment group. In females, the increase was 16 and 21.2% on day 1 and 36.4% (only 1 female) on study day 14. The QTc interval was not affected in female animals.

Organ Weight Changes: The organ weight changes of phase 2 of the 14-day continuous intravenous infusion study in rats were calculated and are shown below in the table. The absolute weight of stomach in males included in 240 mg/kg/day treatment group increased while in females there was no change/reduced during the study. The spleen weight in both male and female was decreased during the study (see table below). The absolute liver weight was increased in only male and not in female animals. The effects on the organ weight of animals included in treatment groups are shown in the table below.

Group mean Organ/Tissue weights (g) of control group rats (male/female) and % increase as compared to control values in 14-Day Toxicity Study (Phase 2) in rats

Tissue	Group 3 (Saline control) (actual weight in g)	Group 4 (Esomeprazole) 120 mg/kg/day (% change)	Group 5 (Esomeprazole) 240 mg/kg/day (% increase)
Prostate	7.0/-	↓ 45.8/-	↓ 4.0/-
Spleen	112.4/103	↓ 31.4/↓ 48.7	↓ 19.2/↓ 31.5
Kidneys	60.0/49.8	↓ 14.5/↓ 12.0	↓ 9.2/↓ 3.4
Liver	364.8/386.7	↑ 26.2/↓ 9.8	↑ 39.6/↓ 3.5
Stomach	132.2/114.3	↓ 14.5/↑ 11.7	↑ 10.2/↓ 1.4
Ovary	-/0.94	-/47.9	↑ -/49.9

↑ = increase; ↓ = decrease

Histopathology: The parietal cell vacuolation/atrophy was noted in 3 out of 3 males included in 120 and 240 mg/kg/day treatment groups. These included the microscopic findings of the animal sacrificed during the study. Cytoplasmic vacuolization of the parietal cells in the gastric body mucosa was found in 1 and 2 dogs in 120 and 240 mg/kg/day treatment groups. The vacuoles were of varying size, the largest of which appeared as distinct empty circular spaces the cytoplasm. Spiral-shaped bacteria (presumably Helicobacter sp.) were observed within many of the larger cells in one dog in control and 120 mg/kg/day treated dogs. The grade of parietal cell vacuolization was minimal in the males and minimal to slight in the females. One dog in group 4 (# 12) showed scattered parietal cells, homogenous, eosinophilic cytoplasm and condensed nuclear chromatin. Some of these cells were apparently apoptotic.

In summary, esomeprazole at the doses of 120 and 240 mg/kg dose were lethal and animals showed the salivation, CNS depressant effects, noisy breathing and collapse before death. The heart rate and EKG were not affected by the treatment. The overt pharmacodynamic effects in stomach were observed. The target organ of toxicity was CNS.

7. 28-Day Intravenous Toxicity Study in Beagle Dogs:
(Study # —56859/SR 01333-01)

Name of Laboratory: _____

Dates of Initiation and Completion of Study: December 8, 2000 and January 11, 2002

GLP and QAU Requirements: A statement of compliance was attached.

Animal Species: Beagle dogs weighing between 8.8 to 9.9 kg (males) and 6.7 to 8.6 kg (females)

Batch #: Sponsor's table is scanned below:

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Text Table 2 Test Formulations

Used in group	Batch no. Esomeprazole sodium	Batch no. test formulations	Concentration Esomeprazole		Expiry date
			µmol/mL	Mg/mL	
2	163/00	HT 1164-01-01-01	2.8	0.97	8 May 2001
3	163/00	HT 1165-01-01-01	6.0	2.1	8 May 2001
4	163/00	HT 1166-01-01-01	13	4.5	9 May 2001

Procedure: Twenty-four dogs (12/sex) were divided in to 4 groups (3/sex/group) and administered 0, 4.8 (14), 10(30) or 22(65) mg/kg/day (umol/kg) esomeprazole (vol = 3 ml/kg) as a slow injection via surgically implanted catheters for 30 min/day. The doses used and # animals used in study are shown in the following table (scanned from sponsor submission). The dose selection was based on a highest tolerable dose in 30 min

Text Table 3 Dosage and Dose Groups

Group Number	Dose Level		Dose Concentration		Number of Animals	
	µmol/kg-day	mg/kg-day	µmol/mL	mg/mL	M	F
1	0	0	0	0	3	3
2	14	4.8	2.8	0.97	3	3
3	30	10	6.0	2.1	3	3
4	65	22	13	4.5	3	3

continuous iv PD study (the reduction in activity in dogs at this dose was observed). The study animals were observed twice daily for changes in clinical signs and death among the animals on days 2, 3, 4, 7, 14, 21 and 27 at 10 and 30 minutes after the start of the infusion and 5, 10 and 30 min and, 1 and 2 hr after the end of the infusion. The body weights and food consumption were recorded weekly during the study. The body weights of the fasted animals before necropsy were also recorded. The ophthalmic examination was done prior to the start of treatment and during week 4. The EKG (limb leads I, II, III, aVR, aVL and aVF) of the dogs were recorded once prior to the start of the treatment and on day 1 and 28 approximately 30 min before infusion and, 5, 35 min and 4 hr after the infusion. The hematological parameters and chemistry parameters were estimated on the 2

ml blood samples collected from aorta of 24-hr fasted rats. The blood samples (2 ml) from all animals were drawn during week 3 prior to dosing (17-hr fasting animals). Blood samples for basal gastrin were collected 24 hr of the previous day dosing. At approx. 24 hr after the previous day dosing, animals were offered 50 g gastrin stimulating diet _____ for 15 min and, the blood samples (all animals consumed diet in 15 min). Blood samples from jugular vein for gastrin concentrations were collected, centrifuged and preserved for gastrin levels estimation (double antibody gastrin radioimmunoassay). Three ml/animal blood samples were collected from all animals on days 1 and 28 at 10 min after the start of infusion, immediately prior to the end of infusion and, 30 min, 1, 2 and 24 hr after the end of the infusion. In group 4 animals, the blood samples were collected 10 min prior to the infusion, immediately prior to the end of infusion, 30 min, 3, 5 and 24 hr after the end of the infusion. The toxicokinetic parameters for esomeprazole and its metabolite _____ were evaluated in the samples. The animals of all group were sacrificed and necropsy on each of the animals was performed, the examination of each of the organs, orifices and cavities of each animal separated and cleaned. The adrenals*, aorta, brain*, cecum, colon, duodenum, injection site, jejunum, esophagus, heart*, ileum, kidneys*, liver*, lungs*, mammary glands (only females), pancreas, pituitary*, spleen*, prostate*, stomach*, seminal vesicles, eye, femur, spinal cord, testes*/ovaries* with epididymides, thymus*, thyroid*+parathyroid*, urinary bladder, mesenteric and mandibular lymph nodes, vagina/uterus* were separated, cleaned and preserved for histopathological examination. The organs marked with asterisk (*) were weighed after the necropsy. The histopathological examination of the tissues of all the study groups was performed. Numerical data obtained were used to obtain mean values and SD values.

Results:

- a. **Observed Effects:** Treatment related excessive scratching and redness at the site of injection, vomiting and salivation in more incidences than in control group animals were seen in males and females included in mid and high dose treatment groups. One out of 4 males of high dose treatment group had reduced activity and convulsions of mild to severe intensity.
- b. **Mortality:** None of the animals of the main and satellite study groups died during the study.
- c. **Body Weight/Food Consumption/Water Consumption Changes:** During the study, the body weight changes in the males and females belonging to treatment groups were similar to those of control group animals. The initial body weights were 9.1, 9.4, 9.1 and 9.4 kg of males and, 7.8, 7.7, 7.6 and 8.0 kg of females included in control, low, mid and high dose treatment groups. The food consumption changes in control and esomeprazole treatment group animals were similar. The initial food consumption of the control group animals on day -1 were 267 and 221 g in males and females, respectively. The food consumption on

day 27 of study groups of animals were 354, 376, 363 and 371 g in males and, 313, 262, 313 and 297 g in females of study groups.

d. Hematology/Coagulation/Bone Marrow Changes: There were no dose/treatment related changes were seen in both males and females belonging to the study treatment groups. The coagulation parameters of the study animals were not affected, the PT and APTT were similar to those of control group animals during the study.

e. Blood Chemistry/Urinalysis Changes: The blood chemistry and urinalysis parameters of treated study group animals during the study. A dose related increase in mean serum gastrin was noted in males and females as shown in the following tables.

Table No. 6
Project No. 56858.
Concentration of Gastrin - summary - males

		Day 15			% Increase from pre to post-food concentrations*
		Pre food Gastrin (pg/mL)	Post food Gastrin (pg/mL)	Food consumption (g)	
Group I - Vehicle control	Mean	12.0	39.4	50	226%
	S.D.	10.34	7.28	0.0	
Group II - Esomeprazole 14 µmol/kg	Mean	31.8	151.3	50	376%
	S.D.	21.64	93.16	0.0	
Group III - Esomeprazole 30 µmol/kg	Mean	12.2	99.6	50	708%
	S.D.	10.00	14.99	0.0	
Group IV - Esomeprazole 65 µmol/kg	Mean	45.5	101.5	50	321%
	S.D.	22.33	70.63	0.0	

*% increase calculated using mean concentrations, not calculated as a mean of the individual % increases

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Table No. 8
Project No. 56859,
Concentration of Gastrin - summary - females

		Day 15			% Increase from pre to post-food concentrations*
		Pre food Gastrin (pg/mL)	Post food Gastrin (pg/mL)	Food consumption (g)	
Group I - Vehicle control	Mean	3.5	48.0	50	1271%
	S.D.	-	5.63	0.0	
Group II - Esomeprazole 14 µmol/kg	Mean	50.8	194.3	50	282%
	S.D.	31.41	94.57	0.0	
Group III - Esomeprazole 30 µmol/kg	Mean	51.0	191.3	50	275%
	S.D.	24.85	68.23	0.0	
Group IV - Esomeprazole 65 µmol/kg	Mean	139.0	285.5	50	105%
	S.D.	62.39	179.55	0.0	

*% increase calculated using mean concentrations, not calculated as a mean of the individual % increases

f. Toxicokinetics: The plasma exposure levels (AUC values) of esomeprazole after 30 minute infusion were similar on Days 1 and 28 in both sexes (as shown in the table below). The concentrations were increased in a dose proportion manner in the dogs. On day 1, the half-lives of the compound at 14, 30 and 65 umol/kg were 25.2 to 32.6 min in dogs. On day 28, the AUC values and t1/2 were similar. The main metabolites of esomeprazole, at the highest dose of esomeprazole was identified in plasma and the median AUC_(0-5hr) values of _____ were 52 and 46 umol.h/L on Days 1 and 28, respectively. _____ was present in higher concentrations in the samples collected at 2-5 hours after the completion of infusion.

g. Organ Weights Changes: A treatment-related increase in stomach weight was seen in dogs at all dose levels in a non-linear dose response. The increase was treatment related as seen in the following table and was the expected effects with the proton pump inhibitors.

h. Physical Examination and Ophthalmic Test: On study day 28, the ophthalmic

Text Table 9 Mean stomach weights (relative to brain) and percentage change compared to the control group

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Group No.	Males			
	1	2	3	4
Relative weight (% brain)	109.087	146.558	135.406	151.704
% Increase	-	34	24	39

Group No.	Females			
	1	2	3	4
Relative weight (% brain)	101.849	115.089	117.064	139.681
% Increase	-	13	15	37

examination of the dogs did not show a treatment effects in the animals and the incidences of nuclear and central corneal opacity were similar in treated and control group animals.

i. Gross Findings: Raised areas in duodenum, gall bladder was seen in 1 high dose male. Thickening of the site of injection was noted in 1 of 3 males in each of mid and high dose treatment groups. Raised dark/pale area was seen in lungs of females. These observations were considered to be spontaneous by sponsor and of no toxicological importance.

j. Histopathology Changes: Treatment related changes in the stomach of 2 of the 3 male dogs of the high dose treatment group. The reduced size of the parietal cells within the fundic mucosa. Interstitial edema was also seen in these dogs. The cells were dense in eosinophilic cytoplasm suggesting atrophic state of the cells. These effects were the exaggerated pharmacological response of the drug.

In summary, esomeprazole at intravenous doses of 4.8, 10 and 22 mg/kg/day in dogs produced treatment related non-proportional plasma concentrations. The CNS related toxicity of reduced activity, tremor and convulsions and the reduced size parietal cells within the fundic mucosa, interstitial edema and denser eosinophilic cytoplasm were suggestive of CNS and stomach as the possible target organs of toxicity. A dose of 10 mg/kg/day was 'the identified as the 'highest tolerable dose' for the study.

8. 1-Month Continuous Intravenous Infusion Study in Dogs: (Study # 0140AD)

Name of the Laboratory: AstraZeneca UK Limited Safety Assessment UK Alderley Alderley Park Macclesfield Cheshire SK10 4TG England

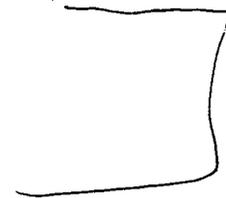
Dates of Initiation and Completion of Study: October 3, 2002 and July 16, 2003

GLP and QAU Requirements: A statement of compliance was attached.

Animal Species: Beagle dogs weighing between 8.8 to 9.9 kg (males) and 6.7 to 8.6 kg (females)

Batch #: The composition of the esomeprazole and its degradants used in the study groups:

Group 2: (esomeprazole 8.0 mg/ml)(in 30 mg/kg/day):



Group 4: (esomeprazole 8.0 mg/ml)(in 170 mg/kg/day):



Procedure: Twenty four dogs (12/sex) were divided in to 4 groups (3/sex/group) and administered 0, 100 (35), 250(86) or 500(170) umol/kg (mg/kg/day) esomeprazole (dose concentration = 8.0 mg/ml) as a slow intravenous continuous infusion via surgically implanted catheters for 28 days. The total amount of degradants injected were 1.28, 2.2 and 5.08 mg/kg/day in the 3 groups of animals. The doses used and # animals used in study are shown in the following table (scanned from sponsor submission). The object of the study was to determine the effects of many possible degradation products in esomeprazole preparation (formed in the proposed commercial preparation of Esomeprazole powder used for injection/ infusion 40 mg and not the dried-freeze esomeprazole powder). Two additional groups were given 64 or 490 umol/kg/day “esomeprazole with degradation products”. The animals in these groups were given esomeprazole as a continuous IV infusion and 30-min IV infusion, respectively. The esomeprazole solutions in infusion bags were stored in the dark at room temperature (20 to 25°C) for 8 days and then transferred to in the light for 3 days to achieve the degradation products. Following this ‘degradation storage’ the bags were stored for up to 7 days at 2 to 8°C prior to use. The dose schedule of the study is shown in the following table.

Summary Table of Animals and Dosage Schedule

Treatm ent Gr	Dose Levels (umol/kg/day) mg/kg/day	Dose Volume Concentration	Dose Volume (ml/kg)	# Animals/Sex
1	0	0	213	3
2	35 (100)	8.0 mg/ml	4.4	3
3	86 (250)	8.0 mg/ml	10.8	3
4	170 (500)	8.0 mg/ml	213	3

NB: One male and 1 female from control group were transferred to group 3 and 4, to replace the animals terminated during the study.

The study animals were observed 2, 4 and 6 hours after initial infusion start and then twice daily for any abnormal signs. The ophthalmologic examination was performed at the pre-study period and once during week 4. The body weight were recorded weekly prior to feeding and the food consumption was recorded daily during the study. The electrocardiogram (EKG) tracings of all dogs were recorded prior to the start of dosing (pre- study treatment day - 7, - 11, - 12 or - 14) and the leads I, II, III, aVR, aVL and aVF from limb electrodes were measured 2 hours after the daily change of the infusion bag on the second day and towards the end of week 4 of dosing (Day 25, 26 or 27). Two animals of the control vehicle group (Group 1) were used to replace dosed animals that had been terminated early. Blood samples were collected (jugular vein) from all animals twice (before treatment and during the final week) and, the samples from unscheduled sacrificed animals were also collected for blood chemistry and haematology. Iron and total iron binding capacity (TIBC) were estimated on the samples from all surviving animals during

week 2. Additional blood samples from overnight fasting animals for gastrin analysis were taken from all surviving animals on one day, pre and post (30 minutes) feeding of a test meal (100 g of _____) during week 4. Urine samples were collected from the animals before treatment and during study week 4/5. All surviving animals were sacrificed, full necropsy performed and the external, cranial, thoracic and abdominal cavities and contents were examined. The femoral-tibial joint cavity from each animal was examined; bone marrow smear was prepared from each animal and stored but not examined. The liver of the animals were separated and examined for the enzyme induction (reported in a separate study). The organ weights of adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, salivary gland, stomach, testes, thyroid glands, thymus and uterus were taken. The following tissues were separated, cleaned and preserved in 10% buffered formalin except the eyes which were fixed in Davidson's fixative and the testes which were fixed in Bouin's fixative. The organs separated were adrenal glands, aorta (thoracic), bladder (gall), bladder (urinary), brain, cervix, epididymides, eyes, femoral head (bone and marrow), heart (atria, ventricles and papillary muscle), infusion site (jugular vein), intestine - duodenum, intestine - jejunum, intestine - ileum, intestine - cecum, intestine - colon, intestine - rectum, kidneys, lacrimal glands, larynx, liver (right median left lateral and caudate lobes), lungs (caudal lobes), lymph node - axillary, lymph node - mesenteric, muscle (skeletal), nerve (sciatic), skin and mammary gland (abdominal), oesophagus, optic nerves, ovaries, pancreas, parathyroid glands, pituitary gland, prostate gland, salivary gland - parotid, salivary gland - sublingual, salivary gland - submandibular, spinal cord (lumbar and cervical), spleen, sternum (bone and marrow), stomach, testes, thymus, thyroid glands, tongue, trachea, uterus, vagina and any macroscopic abnormalities. The preserved tissues were processed by standard histological techniques, blocked in paraffin wax and sectioned. The tissues from all the control and treatment groups animals were examined by light microscopy.

Results:

1. **Clinical Observations:** One male of each of mid and high dose groups and, 1 female of high dose treatment group showed decreased activity and sore wet lesions were seen in 1 and 2 animals of 250 and 500 umol/kg/day treatment groups. Dark colored urine and soft to liquid feces were seen in 250 and 500 umol/kg/day treatment groups. The males had ataxia and swollen neck and the liquid diarrhoea. Minimal, mild, moderate and severe lung thrombus was found in 1 female of control group, 2 animals (1/sex) of 100 umol/kg/day group, 2 females of 250 umol/kg/day and, 3 (1 male and 2 females) of 500 umol/kg/day treatment groups. Esomeprazole treatment produced the lung thrombus formation in dogs.
2. **Mortality:** Eight animals (5 male and 3 females) died during the study. These were 2 (animal#M1 and M4), 1 (male #M9), 2 (1/sex) and 3 animals (2 males and 1 female) of 0, 35, 86 and 170 mg/kg/day treatment groups. Animal #M9 of 35 mg/kg/day group had bloody vomiting and decreased activity and it was killed during the study and animal #M3

of high dose group showed ataxia, red colored urine and swollen neck and cold extremities. The other animals were terminated early because of infection and other welfare reasons.

Table 6 Study number 0140AD. Mortality

Group	Sex / animal no.	Nominal dose level		Day no.	Reason
		µmol/kg/day	mg/kg/day		
1	M1	0	0	8	Welfare - infection related to dosing procedures
1	M4	0	0	28	Welfare - infection related to dosing procedures
2	9	100	35	25	Welfare - inadvertent apparent subcutaneous dosing
3	M17	250	86	14	Welfare - infection related to dosing procedures
3	F18*	250	86	14	Welfare - infection related to dosing procedures
4	M22*	500	170	19	Welfare - infection related to dosing procedures
4	F24	500	170	23	Welfare - infection related to dosing procedures
4	M3*	500	170	25	Welfare - infection related to dosing procedures and also related to abscess [†] formation after administration of antibiotics by injection on Day 22

NB * Animals M3 and F 6 replaced M22 and F18

[†] See necropsy findings

3. Body Weight/Food Consumption Changes:

The body weight changes were not affected by the treatment, the mean body weights of animals on day 0, were 10.4, 11.2, 10.8 and 12.7 kg in males and, 9.4, 9.2, 9.0 and 10.1 kg in females in control and 3 treatment groups. On day 29, the mean weights were 9.9, 11.7, 9.8 and 12.2 kg in males and, 9.2, 9.0, 9.5 and 9.2 kg in females in control and 3 treatment groups. On study day 28, the food consumption was 259, 294, 204 and 250 g/male and, 216, 202, 190 and 192 g/female, respectively in control and 3 treatment groups.

4. Blood Chemistry & Urinalysis Changes:

The increase of gastrin levels were seen in both the pre- feed and the post- feed observation period in animals of all treatment groups (as shown in the table below). Thus esomeprazole induced increase in gastrin levels if given before or after feed. But the increase was significantly higher during post-feed time than the pre-feed period (as seen in the sponsor's table).

Table 9 Study number 0140AD. Gastrin findings at week 4

Male gastrin	Group 1	Group 2	Group 3	Group 4
Pre-feed	50.8	149.7 ↑	176.8 ↑	216.5 ↑
Post-feed	53.5	213.4 ↑	231.8 ↑	226.5 ↑
Female gastrin	Group 1	Group 2	Group 3	Group 4
Pre-feed	57.0	140.3 ↑	174.0 ↑	164.2 ↑
Post-feed	74.8	219.2 ↑	258.1 ↑	281.7 ↑

↑ = considered to be a significant increase compared to controls

The decrease of ALT was seen in both males and females of high dose treatment group. The decrease of ALT in high dose animals was greater than in other 2 groups animals (15.25 and 17.3 vs 27 and 25 IU/l in control males and females). AST levels were decreased slightly more in males of high dose treatment group (30.8 vs 43.3 IU/l in control; 38.3 vs 33.7 IU/l in females). Urine analysis did not show an abnormality but the amber to brown colored urine was seen in 2 males and 1 female of 100 mmol/ kg/day group and 1/sex animal of 500 umol/kg treatment group.

5. Enzyme Estimations: Liver cytochrome P450 isoform CYP1A1 was increased mutifolds in a treatment related manner in dogs treated at 100 (35), 250(86) or 500(170) umol/kg (mg/kg/day) esomeprazole.

The increase in liver enzyme in 35, 86 and 170 mg/kg/day esomeprazole treated animals was about 10-, 25- and 14-folds in male liver and, about 5, 3 and 7 folds in female liver in comparison to the control animals. The changes in the concentrations of other P450 enzymes were not clinically significant as CYP2B11 enzyme was increased maximum by 2.1, 3.1 and 1.9 times in males and, 0.91, 0.96 and 1.1 times in females included in the 35, 86 and 170 mg/kg/day esomeprazole treatment groups. The amount of other enzymes like CYP2C21 and CYP3A12 was altered in an insignificant manner

6. Toxicokinetics: A treatment related non-dose proportional mean plasma concentrations of esomeprazole were seen between 0 and 8 hours and between day 2 and 28 as shown in the following table (taken from sponsor's submission Table 5).

Table 5 Study number 0140AD. Summary of Toxicokinetic data

Daily dose (µmol/kg)	Group	Sex	n	AUC (days 1-28)	Mean plasma concentrations	Mean plasma concentrations
				(range)	0 to 8 hours	day 2 to 28
				µMol-h/L	(µmol/l)	(µmol/l)
100	2	M	2	6640 (3790-9500)	5.19 (5.10-5.28)	6.04 (5.75-6.33)
		F	3	4220 (2550-5750)	5.74 (3.06-8.34)	6.43 (3.89-8.73)
250	3	M	3	6570 (6190-6950) ^a	14.2 (12.9-14.8)	10.5 (9.36-11.7)
		F	4	9900 (9610-11500) ^b	20.5 (13.2-22.0)	15.1 (14.4-17.7)
500	4	M	4	24600 (17500-31600) ^b	42.1 (30.0-48.2) ^b	24.9 (23.2-48.3)
		F	3	10100 (8910-11300) ^a	22.0 (18.7-22.5)	15.6 (13.4-17.0)

^an=2, ^bn=3

7. Physical Examination & Electrocardiographic Changes: On week 4, the heart rate per minute was decreased only slightly in males and not in females. No statistically significant changes in QT and QT_C intervals were observed in animals included in 100 (35 mg/kg), 250 (86 mg/kg) and 500 (170 mg/kg) umol/kg/day treatment groups. The changes in heart rate were also not significant.

8. Pathological Changes: The incidences of inflammation and redness at the infusion sites or the skin areas adjacent to the site were 1, 1, 3 and 4 in males and, 1, 0, 4 and 2 females of 100 (35 mg/kg), 250 (86 mg/kg) and 500 (170 mg/kg) umol/kg/day treatment groups. Fibrosis of minimal to moderate intensity was seen in 1 and 3 males of control and high dose treatment groups. No fibrosis was seen in low and mid dose treatment groups animals. Lung mass/ nodules were seen in 33.4 and 25% males and females of 250 mmol/kg/day (86 mg/kg/day) treatment group but not in low and high dose treatment groups animals.

9. Organ Weight Changes: Treatment related increase in the absolute weight of liver was observed and the liver weights were 401.1, 468.0, 467.2 in males and 560.2 g and, 342.5, 408.9, 363.0 and 377.8 g in females of control and 3 treatment groups. Spleen organ absolute weights were increased in males and these were 86.3, 97.4, 103.8 and 102.9 g in study males. These were similar in females and were 89.2, 85.6, 76.7 and 87.9 g in control and 3 treatment groups females. The stomach weights were increased, i.e., 109.7, 188.23, 164.7 and 177.9 g in males and, 109.3, 154.8, 123.9 and 110.8 g in females of control and 3 treatment groups, respectively.

10. Histopathology Changes: Gastric chief cell atrophy and parietal cell atrophy were in similar incidences among males and females, i.e., in 3, 3 and 4 males and 3, 4 and 3 females of low, mid and high dose treatment groups (none in the control group). The severity in the stomach changes and inflammation accompanied these changes in males and females included in 250 umol/kg/day (86 mg/kg/day) and 500 umol/kg/day (170 mg/kg/day) treatment groups were treatment related. Thyroid follicular epithelial hypertrophy was reported in 1 and 3 males and, 0 and 3 females of 250 and 500 umol/kg/day treatment groups. Thrombus formation was seen in the lung along with pleural inflammation and fibrosis/haemorrhage in 0, 1, 1 and 2 males and, 1, 1, 3 and 2 females of 0, 100, 250 and 500 umol/kg/day treatment groups. In 1 animal included in 500 umol/kg/day (170 mg/kg/day), axonal degeneration in the spinal cord was seen. At the infusion site, instances of inflammation, oedema, fibrosis, ulceration or abscess formation were noted in animals included in 3 treatments groups of the study.

Table 15 Study #0140AD.
Incidence of salient histological findings in Dogs

INCIDENCE

Organs Lesions	Doses	Males				Females			
		0	100	250	500	0	100	250	500
		umol/ kg/day							
Stomach									
Parietal cell vacuolation									
Mild		0	2/3	1/3	1/4	0/3	3/3	1/4	0/3
Moderate		0	0/3	1/3	1/4	0/3	0/3	1/4	1/3
Severe		0/3	1/3	0/3	0/4	0/3	0/3	0/4	0/3
Total									
Mucosal Inflammation									
Mild		0/3	0/3	0/3	0/4	0/3	0/3	2/4	0/3
Moderate		0/3	0/3	0/3	1/4	0/3	0/3	0/4	1/3
Total		0/3	0/3	0/3	0/4	0/3	0/3	0/4	0/3
Parietal Cell Atrophy									
Mild		0/3	1/3	1/3	0/4	0/3	3/3	1/4	0/3
Moderate		0/3	1/3	2/3	3/4	0/3	0/3	3/4	1/3
Total		0/3	1/3	0/3	1/4	0/3	0/3	0/4	3/3
Chief Cell Atrophy									
Mild		0/3	0/3	0/3	0/4	0/3	1/3	1/4	0/3
Moderate		0/3	1/3	0/3	0/4	0/3	1/3	0/4	1/3
Severe		0/3	1/3	1/3	1/4	0/3	1/3	2/4	1/3
Total		0/3	1/3	2/3	3/4	0/3	0/3	1/4	2/3
Thyroid Gland									
Follicular Epithelial Hypertrophy									
Mild		0/3	0/3	1/3	3/4	0/3	0/3	0/4	1/3
Total		0/3	0/3	1/3	3/4	0/3	0/3	0/4	2/3
Liver									
Extramedullary hematopoiesis									
Minimal									
Mild		2/3	0/3	0/3	3/4	1/3	0/3	1/4	1/3
Total		0/3	0/3	1/3	3/4	1/3	0/3	1/4	1/3
Lungs									

Thrombus									
Minimal	0/3	1/3	0/3	0/4	1/3	1/3	0/4	0/3	
Mild	0/3	0/3	1/3	0/4	0/3	0/3	0/4	0/3	
Moderate	0/3	0/3	0/3	1/4	0/3	0/3	3/4	1/3	
Severe	0/3	0/3	0/3	1/4	0/3	0/3	0/4	1/3	
Total	0/3	1/3	1/3	2/4	1/3	1/3	3/4	2/3	
Pleural Fibrosis (mod. To severe)	0	0	1/3	1/4	1/3	0/3	0/4	0/3	
Skin (Injection Site)									
Folliculitis	0/3	0/3	0/3	0/4	0/3	1/3	0/4	0/3	
Thrombi Rt. Forearm	0/3	0/3	0/3	1/4	0/3	0/3	0/4	0/3	
Fibrosis Rt. Forearm	0/3	0/3	0/3	1/4	0/3	0/3	0/4	0/3	

In summary, the intravenous continuous infusion doses of 100, 250 and 500 umol/kg/day esomeprazole produced a treatment related non-dose proportional plasma concentrations, treatment related inflammatory changes (thrombus formation) at the implantation/ infusion site, gastric chief cell and parietal cell atrophy and vacuolation, thyroid (follicular epithelial hypertrophy), thrombus formation in the lung of animals included in 250 (86 mg/ kg/ day) and 500 umol/ kg/ day (170 mg/ kg/ day) treatment groups. Esomeprazole treatment induced CYP1A1 enzyme from 10 to 25 folds in male and, 2.8 to 6.7 folds in female dog liver microsomes in a treatment related manner in animals treated from 100 to 500 mmol/kg concentration. The amount of degradants in the animals were _____ mg/kg/day _____ were the major degradants in the solution. A no-effect dose level was not established in the study but the highest tolerable dose of esomeprazole was 35 mg/kg/day and the identified target organs of toxicity were stomach, thyroid and site of injection. The individual degradant present in esomeprazole can not be assessed from the present data.

9. **New 28-Day Continuous Intravenous Infusion Toxicity Study in the Dog:**
(Study Number #500204/0278 AD)

Name of the Laboratory: Astra Zeneca Canada Inc., Mississauga (Canada)

Dates of Initiation and Completion of Study: October 3, 2003 and March 31, 2004

GLP and QAU Requirements: A statement of compliance to GLP and QAU was attached.

Batch #: 117.01

Materials and Methods: Four groups of dogs (3/sex) were administered 0, 100 (35), 250(86) or 490(170) umol/kg (mg/kg/day) esomeprazole (dose concentration = 8.0 mg/ml,

21.6 ml/kg) without degradation products (group 1 to 4) as a slow intravenous continuous infusion via surgically implanted catheters for 28 days (esomeprazole solution concentration 0, 1.6, 4.0, 8.0 mg/ml). One additional group (3/sex/group) animals were administered the doses of 22 mg/kg/day (64 umol/kg/day) esomeprazole (from the commercial freeze dried preparation of esomeprazole injection) as a daily 30 min infusion and the group 5 animals (170 mg/kg/day treatment group) were administered with degradation products at the dose concentration of 8 mg/ml. The commercial batch was stored in accelerated conditions for 6 months to produce degradation compounds. The doses used and # animals used in study are shown in the following table (scanned from sponsor submission).

Text Table 1 Dosage and Dose Groups

Group No.	Dose level		Dose concentration	
	µmol/kg·day	mg/kg·day	µmol/mL	mg/mL
1 ^a	0	0	0	0
2 ^a	100	35	4.6	1.6
3 ^a	250	86	12	4.0
4 ^a	490	170	23	8.0
5 ^a	490 ^c	170	23	8.0
6 ^b	64 ^d	22	23	8.0

^a The dose in these groups was administered as a 24-hour continuous intravenous infusion

^b The dose in this group was administered as a daily 30-minute intravenous infusion

^c Test compound was given with degradation products derived from the drug substance (esomeprazole sodium)

^d Test compound was given with degradation products derived from the drug product (Esomeprazole powder for solution for injection/infusion 40 mg)

- Group 5 (esomeprazole 8.0 mg/mL, with degradation products) - _____
- Group 6 (esomeprazole 8.0 mg/mL, with degradation products) - _____

a = Previously called Ex1

b = Previously called Ax

The object of the study was also to determine the effects of many possible effects of the degradation products in esomeprazole preparation (formed in the proposed commercial preparation of esomeprazole powder used for injection/ infusion 40 mg and not the dried-freeze esomeprazole powder). Two additional groups were given 64 or 490 umol/kg/day "esomeprazole with degradation products" to observe the effect of degradants present in the formulations. The study animals were observed 2, 4 and 6 hours after initial infusion start for 30 min and in group 6, the animals were observed for last 30 min of infusion. The body weights were recorded weekly prior to feeding and the food consumption was recorded daily during the study (the body wts were not recorded on week 4). The

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ophthalmologic examination was performed at the pre- study period and once during week 4. The electrocardiogram (EKG) tracings were recorded from all dogs prior to the start of dosing (pre- study treatment day - 7, - 11, - 12 or - 14), the leads I, II, III, aVR, aVL and aVF from limb electrodes were recorded 2 hours after the daily change of the infusion bag on the second day and towards the end of week 4 of dosing (Day 25, 26 or 27). The gastrin was estimated from 17-hr fasted animals and thyroid hormones were collected at the start and during week 4. Cytochrome P450 enzymes estimations were done on the liver samples collected at the time of necropsy. Blood samples were collected (jugular vein) from all animals were collected during week 4 for blood chemistry and hematology parameters estimation. Urine samples were collected from the animals before treatment and during study week 4/5. All surviving animals were sacrificed, full necropsy performed and the external, cranial, thoracic and abdominal cavities and contents were examined. The femoral-tibial joint cavity from each animal was examined, bone marrow smear was prepared from each animal and stored but not examined. The organ weights of adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, salivary gland, stomach, testes, thyroid glands, thymus and uterus were taken. The following tissues were separated, cleaned and preserved in 10% buffered formalin except the eyes which were fixed in Davidson's' fixative and the testes which were fixed in Bouin's fixative. The organs separated were adrenal glands, aorta (thoracic), bladder (gall), bladder (urinary), brain, cervix, epididymides, eyes, femoral head (bone and marrow), heart (atria, ventricles and papillary muscle), infusion site (jugular vein), intestine - duodenum, intestine - jejunum, intestine - ileum, intestine - caecum, intestine - colon, intestine - rectum, kidneys, lacrimal glands, larynx, liver (right median left lateral and caudate lobes), lungs (caudal lobes), lymph node - axillary, lymph node - mesenteric, muscle (skeletal), nerve (sciatic), skin and mammary gland (abdominal), oesophagus, optic nerves, ovaries, pancreas, parathyroid glands, pituitary gland, prostate gland, salivary gland - parotid, salivary gland - sublingual, salivary gland - submandibular, spinal cord (lumbar and cervical), spleen, sternum (bone and marrow), stomach, testes, thymus, thyroid glands, tongue, trachea, uterus, vagina and any macroscopic abnormalities. The preserved tissues were processed by standard histological techniques, blocked in paraffin wax and sectioned. The tissues from all the control and treatment groups animals were examined by light microscopy.

Results:

1. Clinical Observations: One male of each of mid and high dose groups and, 1 female of high dose treatment group showed decreased activity and sore wet lesions were seen 1 and 2 animals of 250 and 500 umol/kg/day treatment groups. Red colored soft to loose feces were seen in 1, 3, 1, 1 and 3 males and, 0, 0, 1, 2 and 3 females of 0, 100 (35), 250(86) or 490 (170) umol/kg (mg/kg/day) and 490 (bolus dose) esomeprazole treatment groups. The males treated with 490 umol/kg/day esomeprazole had slight reduced activity.

2. Mortality: None of the animals died during the study.

3. Body Weight/Food Consumption Changes:

The body weight loss 12.3, 9.0, 7.0 and 9.3 % in males and, was observed in changes were not affected by the treatment , the mean body weights of animals on day 1 (week 1), were 7.67, 7.87, 8.10, 8.17, 8.30 and 8.53 kg in males and, 6.83, 7.13, 7.77, 7.57, 7.50 and 7.00 kg among females of 0, 100 (35), 250(86) or 490(170) umol/kg (mg/kg/day) treatment groups. On week 5, the body weight on week -1 were 7.90, 6.90, 7.37, 7.60, 7.53 and 8.07 kg in males and, 7.1, 6.7, 7.33, 7.0 and 7.13 and, 6.63 kg in females in 0, 100 (35), 250 (86) or 490 (170) umol/kg (mg/kg/day) and 490 (bolus dose) esomeprazole treatment groups. On study day 28, the food consumption was 177.3, 88.0, 90.7, 82.0 181.0 and, 235.6 g/male and, 276.7, 244.0, 255.3, 351.3 and, 198.7 g/female and, 116, 202, 90 and 192 g/female, respectively in control and 3 treatment groups.

4. Blood Chemistry & Urinalysis Changes:

The hematology parameters were similar and no changes of statistical or clinical importance were seen in animals included in 0, 100 (35), 250(86) or 490 (170) umol/kg (mg/kg/day) and 490 (bolus dose) esomeprazole treatment groups. An increase of gastrin levels were seen in both the pre- feed and the post- feed observation period in animals of all treatment groups (as shown in the table below). Thus esomeprazole induced increase in gastrin levels if given before or after food. The increase was significantly higher during post-feed time than the pre-feed period (as seen in the sponsor's table).

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Table 8 Project no. 500204. Thyroid hormone analyses - Group mean values and S.D.
Males - Pretreatment
Group I - Vehicle Control
Group II - Esomeprazole 35 mg/kg/day
Group III - Esomeprazole 86 mg/kg/day
Group IV - Esomeprazole 170 mg/kg/day
Group V - Esomeprazole 170 mg/kg/day*

Group Sex	Summary information	T3 (ng/dL)	T4 (µg/dL)	TSH (ng/mL)
Im	Mean	98.30	2.203	0.164
	S.D.	14.17	0.534	-
	N	3	3	1
IIIm	Mean	117.87	1.943	@
	S.D.	25.76	0.229	
	N	3	3	
IIIIm	Mean	97.00	1.623	@
	S.D.	2.91	0.410	
	N	3	3	
IVIm	Mean	116.87	1.847	@
	S.D.	29.62	0.453	
	N	3	3	
VIm	Mean	75.27	0.990	@
	S.D.	6.99	0.113	
	N	3	2	

* with degradation products
@ Less than lower limit of quantitation reported for all three animals in each group (T4=0.50 µg/dL), (TSH=0.160 ng/mL)
N Represents the number of animals used in each group to calculate Mean and S.D.

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The blood chemistry parameters were of ALT and AST were not affected by the treatment in both males and females of high dose treatment group. The expression of CYP2E1 and CYP1A1/2 cytochrome P450 activities were increased and these were greater in females than in males.

Urine analysis did not show an abnormality but the amber to brown colored urine was seen in 2 males and 1 female of 100 mmol/ kg/day group and 1/sex animal of 500 umol/kg treatment group.

5. Toxicokinetics: A treatment related non-dose proportional mean plasma concentrations of esomeprazole was seen both in male and female animals between 0 and 4 hours on day 1 and, from day 2 to 29 as shown in the following table (taken from sponsor's submission Table 2). The plasma concentration were reduced in the presence of the degradation products as the concentrations of the active compound were lower in their presence as seen in the table.

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Text Table 2 Summary of median (range) esomeprazole plasma concentrations in dogs following 28 days' continuous intravenous infusion

Daily dose ^a (µmol/kg)	Group	No. and sex	Day	C ^b (µmol/L)	Daily AUC ^d (µmol·h/L)	Total 28 day AUC ^e (µmol·h/L)
100	2	3M+3F	1	9.6 (7.2-11)	-	-
			2-29	7.2 (4.7-12)	170 (110-290)	4800 (3100-8100)
250	3	3M+3F	1	15 (13-19)	-	-
			2-29	11 (8.1-16)	260 (190-380)	7300 (5300-11000)
490	4	3M+3F	1	33 (27-46)	-	-
			2-29	21 (15-26)	500 (360-620)	14000 (10000-17000)
490 ^c	5	3M+3F	1	29 (27-35)	-	-
			2-29	14 (12-25)	340 (290-600)	9500 (8100-17000)

- ^a The dose in these groups was administered as a 24-hour continuous intravenous infusion for 28 days
- ^b The esomeprazole plasma concentration, C, is given as the concentration 4 h after the start of the infusion on Day 1 and as the concentration at steady-state, C_{ss}, between Days 2 and 29
- ^c Test compound was given with degradation products
- ^d The daily AUC over Days 2 to 28 has been calculated using the median plasma concentration at steady state (C_{ss}) multiplied by 24 hours. These values are thus approximate estimates of AUC
- ^e The total AUC over Days 1 to 28 has been calculated using the daily AUC multiplied by 28 days. These values are thus approximate estimates of AUC

In the animals treated with the continuous iv infusion of 490 µmol/kg/day esomeprazole plus degradation products, the plasma concentrations (C_{max}) and (AUC values) were higher in animals treated with intravenous infusion of 490 µmol/kg/day esomeprazole without degradants than the animals included in the group given esomeprazole with degradation products. The plasma concentration of the animals receiving 64 µmol/kg/day (22 mg/kg/day) esomeprazole for 28 days was similar on day 1 and 28 and the t_{1/2} was about 0.5 hr.

Text Table 3 Summary of median (range) esomeprazole plasma concentrations in dogs following 30-minute intravenous infusion for 28 days

Daily dose ^a (µmol/kg)	Group	No. and sex	Day	C _{max} (µmol/L)	AUC (µmol·h/L)
64 ^b	6	3M+3F	1	91 (72-100)	91 (74-110)
			28	90 (81-110)	97 (80-120)

- ^a The dose in this group was administered as a daily 30-minute intravenous infusion for 28 days
- ^b Test compound was given with degradation products

6. Physical Examination & Electrocardiograph Changes: On week 4, the heart rate per minute was decreased only slightly in males and not in females. No statistically significant changes in QT and QT_C intervals were observed in animals included esomeprazole treatment groups.

7. Pathological Changes: The incidences of inflammation and redness at the infusion sites or the skin areas adjacent to the site were 1, 1, 3 and 4 in males and, 1, 0, 4 and 2 females of 100 (35 mg/kg), 250 (86 mg/kg) and 500 (170 mg/kg) µmol/kg/day treatment groups. Fibrosis of minimal to moderate intensity was seen in 1 and 3 males of control and high

dose treatment groups. No fibrosis was seen in low and mid dose treatment groups animals. Lung mass/nodules were seen in 33.4 and 25% males and females of 250 umol/kg/day (86 mg/kg/day) treatment group and not in low and high dose treatment groups animals.

8. Organ Weight Changes: Treatment related increase in the absolute and relative to brain weight of stomach was observed. The weights were 75.65, 101.23, 102.76, 98.59 and 115.23 and 96.23 g in males and, 74.68, 105.01, 110.62, 101.63, 97.66 and 74.53 g in females of in 100, 250, 490 (without degradation products) and 490 (with degradation products) umol/kg/day treatment groups, respectively. An treatment related increase in the liver relative weight (to brain) was increased by 13.9, 1.4, 10.4 and 31.5% in males and, 7.7, 26.3, 28.0 and 34.2 % in females was seen in 100, 250, 490 (without degradants) and 490 (with degradants) umol/kg/day treatment groups, respectively. The absolute weight of spleen was increased in animals treated with 490 umol/kg/day dose (either by a bolus dose or iv infusion). The changes in the relative weights are shown in sponsor's table below (sponsor's table 11. In addition, a trend of an increase in the thymus weights was seen, i.e., the absolute weight were 109.7, 188.23, 164.7 and 177.9 g in males and, 109.3, 154.8, 123.9 and 110.8 g in females of control and 3 treatment groups, respectively.

Text Table 11 Selected mean organ weights (relative to brain) and percentage change compared to the control group

<i>a) Males</i>						
Dose (umol/kg-day esomeprazole)	0	100	250	490	490*	64*
Liver						
Relative weight (% brain)	335.3	375.1	340.0	370.3	440.5	326.9
% Increase	0	13.9	1.4	10.4	31.4	-2.5
Stomach						
Relative weight (% brain)	101.2	151.9	146.4	139.0	163.7	122.1
% Increase	0	45.7	40.5	33.4	57.1	17.1
Thymus						
Relative weight (% brain)	7.83	6.61	5.64	6.45	3.66	6.37
% Decrease	0	15.7	28.0	17.7	53.3	18.6
* With degradation products						
<i>b) Females</i>						
Dose (umol/kg-day esomeprazole)	0	100	250	490	490*	64*
Liver						
Relative weight (% brain)	274.8	296.0	347.2	354.2	368.8	279.1
% Increase	0	7.7	26.3	28.9	34.2	1.5
Stomach						
Relative weight (% brain)	101.0	146.4	159.0	142.7	140.0	107.2
% Increase	0	40.8	52.9	37.2	34.6	3.0
Thymus						
Relative weight (% brain)	11.30	4.16	5.47	2.08	8.42	5.46
% Decrease	0	63.1	51.6	81.6	25.5	51.6
* With degradation products						

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9. Microscopic Changes: Gastric chief cell atrophy in 0, 3, 3, 3 and 3 males and, 3, 4 and 3 females of control, low, mid, high dose treatment groups and high dose plus degradation

products treatment groups (none in the control group). Gastric foveolar epithelium hyperplasia was noted in 0, 3, 3, 3 and 3 males and, 0, 1, 3, 3, and 3 females of 0, 100, 250, 490 (without degradants) and 490 (with degradants) umol/kg/day treatment groups, respectively. The parietal cell atrophy incidences were in 0, 0, 0, 3 and 3 males and, 0, 0, 1, 2, 3 females of the 5 treatment group, respectively. The crypt necrosis was in 0, 2, 1, 3 and 3 males, and 0, 2, 2, 3 and 3 females of the study groups. There were no incidences in the animals treated at 64 mg/kg/day esomeprazole with degradation products. Thyroid lymphoid atrophy incidences were 1, 2, 2, 1 and 3 males and, 0, 1, 3, 3, 2 and 1 females of study group animals. The incidences of histopathological changes were similar in animals treated with degradation products.

Text Table 12 Treatment-related changes recorded in the stomach of dogs at termination

a) Males

Dose (umol mg/kg-day esomeprazole)	0	100	250	490	490*	64*
<i>Number of dogs with:</i>						
Foveolar epithelial hyperplasia						
Total	0	3	3	3	3	1
Minimal	—	1	2	1	1	1
Slight	—	2	1	2	2	—
Chief cell atrophy						
Total	0	3	3	3	3	1
Minimal	—	—	1	—	—	1
Slight	—	—	2	1	—	—
Moderate	—	2	—	—	—	—
Marked	—	1	—	1	3	—
Severe	—	—	—	1	—	—
Parietal cell atrophy						
Total	0	0	0	3	3	0
Minimal	—	—	—	1	—	—
Slight	—	—	—	2	3	—
Parietal cell vacuolation						
Total	0	0	0	0	1	0
Crypt necrosis						
Total	0	2	1	3	3	0
Minimal	—	1	1	2	—	—
Slight	—	1	—	1	3	—
Mixed cell infiltration						
Total	0	3	3	3	3	0
Minimal	—	2	3	—	—	—
Slight	—	1	—	3	3	—
Mucosal edema						
Total	0	3	3	3	3	0
Minimal	—	3	3	1	1	—
Slight	—	—	—	2	2	—
Total number of dogs examined	3	3	3	3	3	3

* With degradation products

In summary, esomeprazole at 35, 86 and 170 mg/kg/day intravenous doses produced a treatment related non-dose proportional plasma concentrations, treatment related inflammatory changes (thrombus formation) at the infusion site, gastric foveolar epithelium hyperplasia, chief cell and parietal cell atrophy and vacuolation, inflammation and thyroid

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(follicular epithelial hypertrophy), thrombus formation in the lung of animals included in 86 and 170 mg/kg/day treatment groups. The plasma concentrations in the animals treated with degradation products slightly lower on day 2 to 29 but the incidences of histopathological and other changes of animals included in esomeprazole without and with degradation products were similar in these groups. The degradation products did not significantly affect the esomeprazole toxicity. A no-effect dose level was not established in the study and stomach, thyroid and site of injection were the target organs of toxicity. The dose of 100 umol/ kg/ day (35 mg/kg/day) was identified as a 'highest tolerable dose'.

10. Hepatic Cytochrome P450 Induction in 1-Month Continuous Intravenous Infusion Toxicity Study in the Dog: (Study Number 0140AD/GI.000-019-076.2.0)

Name of the Laboratory: AstraZeneca UK Limited, Alderley Park Macclesfield, Cheshire (England)

Dates of Initiation and Completion of Study: 5 September 2002 and 12 May 2003

GLP and QAU Requirements: It was a non-GLP study.

Animal Species: Beagle dogs weighing between 8.8 to 9.9 kg (males) and 6.7 to 8.6 kg (females)

Procedure: The liver sections of the dogs treated with the doses of 0, 100 (35), 250(86) or 500(170) umol/kg (mg/kg/day) esomeprazole (dose concentration = 8.0 mg/ml) as a slow intravenous injection via surgically implanted catheters for 30 min/day for 28 days, were separated and cleaned of extraneous matter and used for the induction of liver enzymes. Liver samples of the study animals of the study killed at the time of study termination and of the animals killed during the study were collected for the estimation of the liver enzymes. The study animals died due to infection at the side of injection were included. The number and study reference numbers of animals of whom the liver samples for liver enzyme investigation were taken are given in sponsor's Table 1 as shown below. The liver samples of all the animals originally included in the study were not taken.

Table 1 Groups and dose levels

Group	Animal number and sex	Animal reference number	Treatment	Nominal daily dose levels	
				µmol/kg	mg/kg
1	2M 3F	1, 4 5, 7, 8	Phys. Saline	0	0
2	3M 3F	9, 10, 11 12, 13, 14	Esomeprazole	100	35
3	2M 3F	15, 16 6, 19, 20	Esomeprazole	250	86
4	3M 2F	3, 21, 23 25, 26	Esomeprazole	500	170

All concentrations and doses of the test compound have been expressed as omeprazole (the neutral form) in this report, although the sodium salt of esomeprazole was used to prepare the test formulations. The formulations of esomeprazole used in this study contained 2-4% esomeprazole degradation products (expressed as a % of the esomeprazole concentration in the formulation, which was 8 mg/mL in all groups)

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P450 isoforms CYP1A1, CYP2B11, CYP3A12 and CYP2C21 were analyzed by an enzyme linked immunoassay (ELISA, manual method) using a Wallac Victor 1420 multilabel counter. The enzyme contents of control and animals of high dose treatment groups were first determined and, if an effect was seen in the high dose group, the other samples were then analyzed.

Results:

Enzyme Estimations: Liver cytochrome P450 isoform CYP1A1 was increased mutifolds in a treatment related manner in dogs treated at 100 (35), 250(86) or 500(170) umol/kg (mg/kg/day) esomeprazole. The increase in liver enzyme in 100 (35), 250(86) or 500(170) umol/kg (mg/kg/day) esomeprazole treated animals was about 10-, 25- and 14-folds in male liver and, about 5, 3 and 7 folds in female liver in comparison to the control animals. The changes in the concentrations of other P450 enzymes were not clinically significant as CYP2B11 enzyme was increased maximum by 2.1, 3.1 and 1.9 times in males and, 0.91, 0.96 and 1.1 times in females included in the study. The amount of other enzymes like CYP2C21 and CYP3A12 was altered in an insignificant manner (see the following sponsor's table).

Table 2 Group mean concentrations of CYP protein in liver microsomes

Group & sex	CYP1A1		CYP2B11		CYP2C21		CYP3A12	
	Conc. pmol/mg	Change ^a						
1M ^b	17	-	25	-	86	-	20	-
2M ^c	170	10	53	2.1	ND	-	ND	-
3M ^b	430	25	77	3.1	ND	-	ND	-
4M ^c	230	14	47	1.9	190	2.2	32	1.6
1F ^c	64	-	98	-	52	-	48	-
2F ^c	320	5.0	89	0.91	ND	-	ND	-
3F ^c	180	2.8	94	0.96	ND	-	ND	-
4F ^b	430	6.7	110	1.1	50	0.96	71	1.5

a Fold change compared to the concentration seen in the relevant controls

b n=2

c n=3

ND = Not determined

In summary, esomeprazole induced CYP1A1 enzyme from 10 to 25 folds in male and, 2.8 to 6.7 folds in female dog liver microsomes in a treatment related manner in animals treated from 100 to 500 mmol/kg concentration. The other cytochromes CYP450 enzymes CYP2B11, CYP2C21 and CYP3A12 were not induced in a significant manner.

2.6.6.4 GENOTOXICITY:

2.6.6.4. BRIEF OVERALL SUMMARY:

The genotoxicity of esomeprazole was evaluated in Ames test with and without of degradation product, in vitro chromosomal aberration test in cultured human peripheral blood lymphocytes with and without degradation products. Esomeprazole was not mutagenic in Ames test but it was clastogenic in a vitro human lymphocytes chromosomal aberration test in the presence and absence of metabolic activation. Omeprazole was also positive in a vitro human lymphocytes chromosomal aberration test in the presence and absence of metabolic activation. It did not induce micronuclei in mouse bone marrow test. The esomeprazole metabolites _____ and its degradants were not clastogenic in chromosomal aberration test. The degradation product _____ when tested for its clastogenic effect in a vitro chromosomal aberration test, it was positive both in the presence and absence of S-9 metabolite.

1. Genetic toxicity evaluation using the Ames Test (Salmonella/E.coli reverse mutation test). A comparison of effects of the test compound with and without degradation products.

(Study No. 02209/SR-0220201-1/D9615-82621)

Date of Initiation & Completion: April 3, 2002 & January 20, 2003

Conducting Laboratory: AstraZeneca R& D,
Möln dal, Sweden

Batch # Esomeprazole & Degradants: a. Esomeprazole sodium 42.5 mg/5 ml vial (a freeze- dried formulation, Batch # 105/01), Formulation Batch # HT 1216-01-01-01 (pure) – Batch #113/ 01 (used for formulations with degradation products); b. Esomeprazole sodium 113/01 (without degradants), Formulation Batch # H 1516- 03- 01- 01 (esomeprazole without degradation products") _____

_____ In addition, the constituted solutions prepared from Batch HT 1216-01-01-01 were subjected to a storage period at room temperature that exceeded the proposed in-use period of clinical use, before they were used in the Ames test (with a possibility of additional degradation products).

Positive Controls:

Positive control Chemical	Solvent	Dose per plate		Bacterial Strain	Metabolic activation
		µg	µmol		
sodium azide	water	0.50	0.0077	TA1535 TA100	-
2-nitrofluorene	dimethyl sulfoxide	0.50	0.0024	TA98	-
9-aminocacridine	dimethyl sulfoxide	70	0.30	TA1537	-
potassium dichromate	water	25	0.085	WP2 uvrA	-
2-aminanthracene	dimethyl sulfoxide	2.0	0.010	TA1535 TA100 TA98 WP2 uvrA TA1537	+
		5.0	0.026		

Methods: The mutagenicity potential of the proposed commercial formulation for intravenous esomeprazole preparation with and without degradation products was determined by using genetically modified Salmonella strains TA 1535, TA100, TA98, TA 1537 and Escherichia coli WP2 uvrA as indicator organisms. The fortified rat liver homogenate (S-9) fraction for metabolic activation was obtained from Aroclor 1254 pre-treated rats. Esomeprazole at the concentration of 0.145, 0.483, 1.45, 4.83 or 14.5 umol/plate (58.0, 167, 500, 1670 and 5000 ug) was added in cultures and these were exposed to 4 specific histidine auxotrophs of Salmonella tryphimurium (TA-1535 and TA-100 for base pair substitutions and TA-1537 and TA-98 for the detection of frame shift mutagens) in the presence or absence of S-9 fraction in the toxicity experiment. The E. coli WP2 urva reverse mutation system (for measuring reversion from $trp(-)$ to $trp(+)$) was also used in the test. The esomeprazole from the concentration of 1670 ug/plate was toxic in the presence and absence of S-9 mix. The final concentrations used in the mutagenicity test were:

Results: In a concentration range finding study, esomeprazole with and without degradants was found as toxic at 1.45 and 4.83 umol/plate concentration in cultures with TA1537 and other microbes (TA1535, TA100, TA98 and WP2 uvrA) in the presence and absence of S-9 activation mix. Esomeprazole when used at the reduced concentration of 0.145, 0.483, 1.45, 2.9 and 4.83 umol/plate without metabolic activation showed toxicity in cultures with TA100 strain. The concentrations from 4.83 to 0.483 umol/plate esomeprazole alone or without degradation products showed toxicity. The esomeprazole degradation products used were _____ in the different solutions of esomeprazole " with degradation products" in this study. It was claimed by sponsor that only small or very small amounts of _____ were also present in the solutions of esomeprazole " without degradation products". The sponsor should have employed a suitable low concentration of the esomeprazole and its degradants.

In a complimentary study (Study No. 02209), genetic toxicity of esomeprazole for intravenous infusion and injection, i.e., Esomeprazole powder for solution for injection/ infusion 40 mg (as esomeprazole sodium 42.5 mg/5 ml vial was determined in the Ames Salmonella/E.coli reverse mutation test with and without degradation products. One of the esomeprazole solutions used had been prepared from a " degraded" batch of esomeprazole, and the constituted solution had been subjected to further degradation prior to use. However, formulation analysis showed that a particular degradation product _____ that can be present in the esomeprazole solutions during normal clinical intravenous use was not present in this degraded solution. In the repeat study #02209, the solution was prepared by a different technique by using the drug substance esomeprazole sodium (non- degraded), and not the proposed commercial formulation for intravenous use. This solution was subjected to " degradation storage" during preparation, in order to ensure the presence of _____ in the solution tested. The test is described below:

In the pre-incubation mutagenicity tests, the test compound toxicity was observed at the dose of 0.869 mmol (300 mg)/plate and above with strains TA1535, TA100 and TA1537 without metabolic activation while strains TA98 and WP2 uvrA showed evidence of toxicity at 1.45 umol (500 mg) and at 2.17 umol (750 m g) per plate respectively (Tables 3 and 5). In the presence of metabolic activation

The concentrations used in plate incorporation test were 0.145, 0.483, 1.45 and 14.5 umol (50. 167, 500, 1670 or 5000 ug/plate) in the presence and absence of S-9 mix.

Results: In the cultures without S-9 mix: In the plate incorporation test: The test compound esomeprazole was toxic at 4.83 umol (1670 ug) concentration in the cultures in the absence of S-9 mix with strains TA1535, TA98 and TA1537 as it elicited a reduction of colonies. A concentration of 14.5 mmol (5000 mg)/plate with strains TA100 and WP2 uvrA showed toxicity. In the cultures with metabolic activation, signs of toxicity of reduced revertant colonies was seen at 4.83 mmol (1670 m g) per plate with strains TA1535 and TA1537 and at 14.5 mmol (5000 mg) per plate with strain.

Mutagenicity Test: The concentrations of esomeprazole used were 18.8, 37.5, 75.0, 150 and 300 mg/plate. The highest concentration of 300 ug/plate was toxic to all bacterial cultures. Esomeprazole with or without degradation product did not elicit increase in the number of revertant colonies up to 150 mg/plate; the high concentration of 300 mg/plate was toxic in the study.

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Table 6 Pre-incubation test 2, with and without metabolic activation

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BACTERIAL MUTATION STUDY - WITH and WITHOUT METABOLIC ACTIVATION																					
Study No. 02209		Liquid pre-incubation method test 2																			
Start 021119	End 021122	Positive controls												µg/plate							
Test compound	esomeprazole with degradation products	TA1535 (-S9 mix)	sodium azide	0.50																	
Batch	WT 1311-01-01-03	TA1537 (-S9 mix)	9-aminoacridine	70.00																	
Concentration of stock solution	0.145 ml/L	TA1535 (+S9 mix)	1-aminoanthracene	2.00																	
Solvent	0.9 % sodium chloride	TA1537 (+S9 mix)	2-aminoanthracene	5.00																	
Metabolic activation:	Induced rat liver homogenate																				
Bacterial strain and number of revertant colonies per plate																					
		TA 1535 (-S9 mix)					TA1537 (-S9 mix)					TA1535 (+S9 mix)					TA1537 (+S9 mix)				
		1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD
Solv. control		12	13	16	14	2	8	12	5	8	4	22	23	11	19	7	12	14	11	12	2
Pos. control		257	232	295	261	31	487	628	781	699	77	236	208	214	210	16	74	57	62	75	21
Dose/plate																					
	µmol																				
	µg																				
0.0544	18.8	9	12	10	10	2	7	11	11	10	2	8	19	13	13	6	12	5	7	8	4
0.109	37.5	8	8	13	10	3	5	7	6	6	1	15	15	10	14	3	14	6	12	11	4
0.217	75.0	13	8	16	13	4	5	9	7	7	2	13	7	13	11	3	16	9	11	12	4
0.434	150	10	13	13	12	2	5	9	7	7	2	12	14	9	12	3	7	11	9	9	2
0.869	300	16t	9t	7t	11	5	2t	7t	8t	6	3	8	18	9	9	1	13t	6t	8t	9	4

In a complimentary Study No. 02209, genetic toxicity of esomeprazole compound was re-evaluated. Esomeprazole powder was stored in alkaline medium for favourable conditions for degradation product formation. One of the esomeprazole concentrations, a low concentration of 50 µg/plate showed increase in revertants but not at higher concentrations, therefore not important. Esomeprazole, and the constituted degradants in solution was not mutagenic in the repeat test.

3. Genetic Toxicity Evaluation Using an Ames Salmonella/E.coli test of a degradation product of Esomeprazole: (Study No. 0137BV)

Date of Initiation & Completion: January 30, 2003 & April 22, 2003

Conducting Laboratory: AstraZeneca R& D, Mölndal, Sweden

Batch No. _____ - 709/ 02 (Molecular mass 313.3 g/ mol Purity _____)
Source AstraZeneca R& D Mölndal, Sweden)

Positive Controls:

Positive control Chemical	Solvent	Dose per plate µg	µmol	Bacterial Strain	Metabolic activation
sodium azide	Water	0.50	0.0077	TA1535 TA100	-
2-nitrofluorene	dimethyl sulfoxide	0.50	0.0024	TA98	-
9-aminoacridine	dimethyl sulfoxide	70	0.30	TA1537	-
potassium dichromate	Water	25	0.085	uvrA/ pKM101	-
2-aminoanthracene	dimethyl sulfoxide	2.0	0.010	TA1535 TA100 TA98 uvrA/ pKM101 TA1537	+

Methods: The test compound of the study _____ was obtained from sponsor, Astra Zeneca, Sweden, as an aqueous solution (50 mg/ml). It was tested in 2 parts, the plate incorporation and liquid pre-incubation test. In a concentration ranging study, it was tested from 0.62 to 16.2 uml/plate in the first test and 0.162 to 16.2 umol/plate in the second test in the presence and absence of S-9 mix.

Results: The test compound _____ did not show a toxicity up to the highest concentration of 16.2 umol/plate in the absence and presence of activation by S-9 mix.

Table 3 Bacterial mutation study – without metabolic activation

Study No. 01378V Liquid pre-incubation method
Start 2003-02-10 End 2003-02-21

Test compound	Batch	Concentration of stock solution	Solvent	Positive controls	ug/plate
_____	709702	162 umol/L	50.7 g/L	TA 1535 sodiumazide	0.50
				TA 150 sodiumazide	0.50
				NR2 uvrA potassium dichromate	25.00
				TA98 2-nitrofluorene	0.50
				TA1537 9-aminoacridine	75.50

sterile distilled water

Factorial strain and number of revertant colonies per plate

	TA 1535					TA 150					NR2 uvrA					TA98					TA1537					
	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	
Solv. Control ^a	9	13	14	12	1	85	118	94	99	17	158	95	95	97	16	24	14	28	25	2	13	9	7	9	2	
Solv. Control ^b	11	10	16	12	3	151	184	165	119	27	81	78	94	78	17	22	22	25	23	2	19	11	14	11	2	
Pos. control	315	315	328	319	6	389	373	375	379	8	1049	1049	988	1028	35	109	120	120	108	12	615	544	641	619	68	
Dose/plate																										
umol																										
ug																										
0.162 50.7	19	14	16	16	3	84	114	106	105	19	153	115	97	113	18	19	33	27	27	7	19	7	18	9	2	
0.324 101.4	11	9	18	13	5	95	109	110	104	8	99	134	101	108	23	22	22	32	25	6	5	5	10	7	3	
1.62 507	7	7	17	11	6	112	97	113	107	8	101	109	95	102	7	19	18	31	23	7	7	5	9	7	2	
5.40 1690	11p	14p	23p	16	6	103p	117p	89p	103	13	73	184	195	96	14	29p	28p	30p	29	1	9p	12p	18p	12	2	
16.2 5070	p	17	11	9	12	4	193	115	119	199	3	90	192	97	96	6	22	26	22	23	3	8	11	8	9	2

^a sterile distilled water pH adjusted to pH 11
^b sterile distilled water not pH adjusted
p precipitate formed on the plates

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4. Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test of _____
(Interim Study Report # 0306BV/GI. 000-049-664)

Name of the Laboratory: AstraZeneca S-151 85 Sodertalje, Sweden

Date of Initiation and Completion: February 13, 2004 and March 30, 2004

GLP Compliance: a statement of compliance was attached

Batch #: _____ (Purity _____, a degradation compound of esomeprazole)

(The solutions were prepared in dimethyl sulphoxide at concentration of 50.5 mg/ml for Test 1 and 40.6 mg/ml for Test 2. The test solution used at the concentration of 113, 56.5, 14.1, 5.65 and 1.41 umol/l (or 50.8, 40.8, 20.4, 5.1, 2.04 and 0.508 ug/ml).

Positive Controls & Bacterial Strain Used: Salmonella strain TA1535, TA1537, TA98, TA 100 and Escherichia coli strain uvrA/pKM101.

Methods:

The test was performed by adding to an aliquot of 100 µl of the appropriate bacterial culture with 100 µl of solvent, positive control or 1 of the test formulation dilution and 500 µ L of sodium phosphate buffer or S9 mix. Two ml of molten 0.6% agar maintained (at approx. 45°C) supplemented with low concentrations of biotin and histidine (each at 0.05 mmol/ L) for the Salmonella strains and tryptophan (0.018 mmol/l) for the Escherichia strain was added. The mixture was immediately poured onto minimal glucose agar plates and incubated for 3 days at 37°C. The plates were scored for revertant colony number.

Criteria for Evaluation: 1. The increase in the number of revertant colonies in the presence of one or more doses of the test compound (with or without metabolic activation) in a dose-related manner and in a reproducible manner.

Results:

The numbers of colonies in solvent control were within acceptable ranges for each of the indicator strains as shown in the tables below (). The positive control agents produced an increase in the revertant colonies in the presence and absence of the S9 mix. The test compound was toxic at the highest concentration of 5050 ug/plate in all of the strains in the presence and absence of S-9 mix used in the study and the number of colonies showed a decrease at this concentration. The concentrations used in mutagenicity test was 50.8, 169, 508, 1690 and 5080 ug/plate.

Table 2 Test 1 - Plate incorporation method - with metabolic activation

(Dose / plate)		Bacterial strain and number of revertant colonies per plate (Mean values ± SD)				
µg	µmol	TA1535	TA1537	TA98	TA100	E.coli uvrA/pKM101
Solvent (DMS)		18±1	12±1	37±5	105±2	142±9
50.8	0.141	12±4	13±2	34±1	97±11	133±11
169	0.470	13±1	14±2	33±1	94±4	137±12
508	1.41	15±5	15±1	41±2	89±2	126±5
1690	4.70	15±4	14±1 t	33±1	92±1 t	125±5
5080	14.1	11±2 t	10±3 t	30±1	67±7 t	132±12
Positive control		2AA	2AA	2AA	2AA	2AA
µg / plate		2.0	5.0	2.0	2.0	5.0
No. of colonies		224±5	237±12	1116±37	684±32	1119±32

Positive control: 2-aminoanthracene (2AA)
t: toxicity

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Table 1 Test 1 - Plate incorporation method - without metabolic activation

(Dose / plate)		Bacterial strain and number of revertant colonies per plate (Mean values ± SD)				
µg	µmol	TA1535	TA1537	TA98	TA100	E.coli uvrA/pKM101
Solvent (DMSO)		13±1	8±4	23±5	92±7	99±14
50.8	0.141	16±2	7±2	23±2	95±4	100±6
169	0.470	13±3	6±2	22±2	90±13	103±8
508	1.41	13±3	8±2	20±3	94±12	89±4
1690	4.70	17±1	9±1	21±2	87±1	93±11
5080	14.1	12±3	9±1 t	21±5	82±2 t	92±9
Positive control		NaN ₃	9AA.HCl	2NF	NaN ₃	K ₂ Cr ₂ O ₇
µg / plate		0.5	70	0.5	0.5	25
No. of colonies		258±7	947±101	143±21	349±15	1027±28

Positive controls: sodium azide (NaN₃); 9-aminoacridine HCl (9AA.HCl); 2-nitrofluorene (2NF); potassium dichromate (K₂Cr₂O₇)
t: toxicity

Using the liquid pre-incubation method, the toxicity was observed in the absence of metabolic activation at the concentration of 5.65 mmol (2040 ug) per plate and above with strains TA1535, TA 1537TA98 and TA 100. The toxicity was not observed in E. coli uvrA/ pKM101 (Table 4). In the presence of metabolic activation, the toxicity was seen at the 5.65 umol (2040 mg) per plate and above. The number of revertants were not increased at any of the selected concentration (Table 4).

Table 4 Test 2 - Liquid pre-incubation method - with metabolic activation

(Dose / plate)		Bacterial strain and number of revertant colonies per plate (Mean values ± SD)				
µg	µmol	TA1535	TA1537	TA98	TA100	E.coli uvrA/pKM101
Solvent (DMSO)		11±4	13±2	35±8	94±4	239±13
51.0	0.141	16±4	14±4	36±2	99±4	254±22
204	0.565	12±5	14±3	39±8	93±10	242±20
510	1.41	12±1	16±6	42±7	84±4	214±11
2040	5.65	12±4 t	13±3 t	35±3	85±3 t	240±16
4080	11.3	11±4 t	6±1 t	29±5 t	61±12 t	203±8
Positive control		2AA	2AA	2AA	2AA	2AA
µg / plate		2.0	5.0	2.0	2.0	5.0
No. of colonies		108±6	103±13	659±19	372±35	605±61

Positive Control: 2-aminoanthracene (2AA)
t: toxicity

_____ did not increase the revertants in a dose or treatment related manner, the compound was not mutagenic in the test either in the presence or absence of S9 mix.

5. Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test of _____
(Interim Study Report # 0307BV/GI. 000-049-701)

Name of the Laboratory: AstraZeneca S-151 85 Sodertalje, Sweden

Date of Initiation and Completion: approved 29 March 2004

Batch #: _____ **Purity:** _____

(The solutions were prepared in dimethyl sulphoxide at concentration of 50.5 mg/ml for Test 1 and 40.6 mg/ml for Test 2.

Positive Controls & Bacterial Strain Used:

Positive control chemical	Solvent	Supplier	Dose per plate		Bacterial strain	Metabolic activation
			µg	µmol		
sodium azide	water		0.50	0.0077	TA1535 TA100	-
2-nitrofluorene	dimethyl sulphoxide		0.50	0.0024	TA98	-
9-aminoacridine	dimethyl sulphoxide		70	0.30	TA1537	-
potassium dichromate	water		25	0.085	E.coli uvrA/ pKM101	-
2-aminoanthracene	dimethyl sulphoxide		2.0	0.010	TA1535 TA100 TA98	+
			5.0	0.026	E.coli uvrA/ pKM101 TA1537	

Methods:

The test was performed by adding to an aliquot of 100 µl of the appropriate bacterial culture with 100 µl of solvent, positive control or test formulation dilution and 500 µ L of sodium phosphate buffer or S9 mix. Two ml of molten 0.6% agar maintained (at approx. 45°C) supplemented with low concentrations of biotin and histidine (each at 0.05 mmol/ L) for the Salmonella strains and tryptophan (0.018 mmol/l) for the Escherichia strain was added. The mixture was immediately poured onto minimal glucose agar plates and incubated for 3 days at 37°C. The plates scored for revertants colony number.

Criteria for Evaluation: 1. The increase in the number of revertant colonies in the presence of one or more doses of the test compound (with or without metabolic activation) in a dose-related manner and in a reproducible manner.

Results:

The number of colonies in solvent control was within acceptable ranges for each of the indicator strains as shown in the tables below. The positive control agents produced an increase in the revertant colonies in the presence and absence of the S9 mix. The 2- fold increase in the liquid pre- incubation with strain E. coli uvrA/ pKM101 in the presence 2- aminoanthracene is not considered to interfere with the interpretation of the test results since the bacteria showed sensitivity to mutagens when exposed to the positive control without metabolic activation, in the same assay. The test compound was toxic at the highest concentration of 5050 ug/plate in all of the strains in the presence and absence of S-9 mix used in the study. The concentrations used in mutagenicity test was 50.8, 203, 508, 2030 and 4060 ug/plate.

Table 3 Test 2 - Liquid pre-incubation method - without metabolic activation

(Dose / plate)		Bacterial strain and number of revertant colonies per plate (Mean values ± SD)				
µg	µmol	TA1535	TA1537	TA98	TA100	E.coli uvrA/pKM101
Solvent (DMSO)		9±3	6±3	19±3	84±9	118±8
50.8	0.161	12±3	8±2	23±4	80±20	128±10
203	0.645	14±1	5±2	22±5	80±6	130±6
508	1.61	13±4	9±4	25±3	80±4	109±12
2030	6.45	9±2 t	7±2 t	24±6	63±5 t	94±5
4060	12.9 t, p	11±2	7±3	17±3	53±21	31±8
Positive control		NaN ₃	9AA.HCl	2NF	NaN ₃	K ₂ Cr ₂ O ₇
µg / plate		0.5	70	0.5	0.5	25
No. of colonies		271±11	305±25	159±4	374±13	1177±98

Positive Control: 2-aminoanthracene (2AA)
p: precipitate
t: toxicity

Using the liquid pre- incubation method toxicity was observed in the absence of metabolic activation at the dose of 6.45 m mol (2030 m g) per plate and above with strains TA1535, TA100 and TA1537, at the dose of 12.9 m mol (4060 m g) per plate with strains TA98 and E. coli uvrA/ pKM101 (Table 3). In the presence of metabolic activation toxicity was seen at the dose of 12.9 m mol (4060 mg) per plate and above with strains TA1535, TA100 and TA98 and at the dose of 6.45 m mol (2030 m g) per plate and above with strain TA1537 (Table 4).

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Table 4 Test 2 - Liquid pre-incubation method - with metabolic activation

(Dose / plate)	Bacterial strain and number of revertant colonies per plate (Mean values ± SD)						
	µg	µmol	TA1535	TA1537	TA98	TA100	E.coli uvrA/pKM101
Solvent (DMSO)			13±3	14±2	34±3	94±8	238±24
508	0.161		10±3	14±2	35±6	105±12	251±18
203	0.645		13±3	12±1	35±4	81±2	210±19
508	1.61		14±2	11±4	33±2	87±14	168±31
2030	6.45		15±3	10±1	36±6	87±8	101±14
4060	12.91		8±4	2±1	25±8	41±7	52±6
Positive control			2AA	2AA	2AA	2AA	2AA
µg / plate			2.0	5.0	2.0	2.0	5.0
No. of colonies			98±14	78±8	532±14	309±35	470±14

Positive Control: 2-aminanthracene (2AA)
t: toxicity

Precipitate was observed in the liquid pre- incubation assay in the absence of metabolic activation at the dose of 12.9 mmol (4060 mg) per plate (Table 3).

_____ did not increase the revertants in a dose or treatment related manner, the compound was not mutagenic in the test either in the presence or absence of S9 mix.

These studies showed that esomeprazole or its metabolites was not mutagenic in the Ames test. The degradation products _____ were not positive in the Ames test. Sponsor in the amendment dated April 9, 2004 had submitted the data for the qualification of the degradation products. The safety margin of the total degradation products administered with the clinical IV dose of 0.8 mg/kg esomeprazole was assessed from 28-day intravenous toxicity study in rats and 1-month continuous toxicity study in dogs with esomeprazole containing degradants. The total degradants were _____ in the 28-day toxicity study in rats and, 1-month continuous IV infusion study in dogs, respectively. The estimated highest tolerable doses dose in 1-month continuous intravenous toxicity study in dogs was 35 mg/kg/day and the computed amount of degradants in this dose was _____. The total amounts of degradants in a clinical dose of 0.8 mg/kg was _____. This provided sufficient safety margin of _____ to the total degradants. The no effect dose in 28-day toxicity study in rats was 4 mg/kg/day and this was computed to contain _____ total degradants. The amount of total degradants injected with a clinical dose of 40 mg/day (0.8 mg/kg) would be _____. The margin provided by this was _____ times. The mutagenicity of the compound was not affected by the degradants as the Ames tests and in vivo chromosome aberration study showed no additional mutagenicity.

6. **Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes:**
_____ Study Number 24840-0-449OECD)

Name of the Laboratory: _____

Dates of Initiation and Completion of Study: February 6, 2003 and 20 May 2003

GLP and QAU Requirements: A statement of compliance was attached.

Batch #: _____
_____, Batch No. 30000304 _____
_____ Batch No. 1401

Negative/Positive Controls: Negative and vehicle controls used in the initial and repeat assays with and without metabolic activation were the cultures containing the cells and culture medium. Vehicle controls - DMSO at 10.0 ul/ml concentration in cultures without S-9 activation. In the activation assays, the S9 mix was mixed in the cultures. In the first preliminary assay with the metabolic activation, three concentrations of methanol at 0.9, 1.8, and 3.6 mM/l were also included to examine the possible effects of methanol content of the test article.

Positive Controls: Mitomycin C (MMC- _____, Lot No. 31K2506) for the nonactivation series and cyclophosphamide (CP- _____ Lot No. 91K1176) in the metabolic activation series in sterile, deionized water. In the chromosomal aberrations assays, concentrations of MMC (0.750, 1.00, and 1.50 ug/ml) and CP (20.0, 25.0, and 40.0 ug/ml) were used to induce chromosomal aberrations.

Procedure:

About 0.6 ml of heparinized human blood from healthy volunteers was cultured in a 15 ml tube and the final volume of the culture was 10 ml with and without metabolic activation. Cultures tubes were incubated at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in a humidified atmosphere of $5\% \pm 1.5\%$ CO_2 in air. The medium was RPMI 1640 supplemented with approximately 20% heat-inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 pg/ml), L-glutamine (2mM) and 2% phytohemagglutinin M (PHA-M). In the preliminary chromosomal aberration assays (two trials), duplicate cultures were used at each test article concentration, for the negative, vehicle, methanol {methanol controls for the first trial only} controls, and for the positive controls. In the aberration assay without and with metabolic activation, 22-hour harvest periods were used (1.5 times a cell cycle time of about 15 hours) after the lymphocytes are induced to divide by the addition of PHA-M (Galloway et al., 1994). The ability of _____ and a reference compound, _____ to cause structural chromosomal aberrations in human lymphocytes with and without an exogenous metabolic activation system was investigated. Two days after culture initiation, the cultures were incubated at $37^{\circ}\text{C} + 2^{\circ}\text{C}$ for -3 hours in the presence of the test article at predetermined concentrations, vehicle control, and positive controls with the S9 reaction mixture. The cultures were washed, incubated for the remaining culture period and

harvested with 0.1 ug/ml Colcemid^o for 2±0.5 hours of incubation. The cultures were then harvested (22-hours after initiation of treatment). The supernatant discarded, and the cells were swollen with 75 mM KCl hypotonic solution, cultures were then fixed with absolute methanol: glacial acetic acid (3:1, v/v) fixative and, dropped on clean, glass slides and air-dried. The slides were stained with 5% Giemsa solution for the analysis of mitotic index and chromosomal aberrations. All slides were then air-dried and mounted permanently.

Criteria for Positive Response. A test article was considered positive if a significant increase ($p < 0.01$) in the number of cells with chromosomal aberrations was observed at one or more concentrations. The linear trend test was positive for the dose responsiveness and if a significant increase was seen at one or more concentrations, then a dose- response was considered. In the event of no significant increase in the %number of cells with chromosomal aberrations at any of the concentrations, the test was considered as negative. The statistical significance, Cochran- Armitage test for linear trend and Fisher's Exact Test were used to compare the percentage of cells with aberrations (-g) in treated cells vs the vehicle controls.

Results:

Assay without metabolic activation: The inhibition of mitosis of 0, 0, 30 and 37% was seen at a concentration of 1.50, 1.60, 1.7 and 1.8 mM/l of _____ The number and percent of cells with chromosomal aberrations were 2.5, 4.0 and 11.5 (these were 26.7 with 1.0 ug/ml MMC culture). No significant mitotic inhibition at the concentrations of 1.8 uM/l esomeprazole was noted in the cultures without the S-9 system observed. No significant increase in polyploidy or endoreduplication was observed in the cultures analyzed. In cultures containing esomeprazole sodium, there was no reduction in the mitotic indices of the cultures treated up to 1.80 mM/l esomeprazole when compared with the vehicle control cultures. A significant increase ($p < 0.01$) in cells with chromosomal aberrations was observed at 1.60 and 1.80 mM/l as shown below in the table 4.

Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~3 Hour Treatment, ~22 Hour Harvest

Assay No.: 24840		Trial No.: B1		Date: 02/13/03		Lab No.: _____		NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS							JUDGE- MENT (%) ^g
Test Article: _____		% MITOTIC INDEX CELLS SCORED	# ENDO- REDUCED CELLS	# POLY- PLOID CELLS	JUDGE- MENT (%) ^f	Gap	Break	chr	chr	mb	TOTALS ^c				
											g	i	g		
CONTROLS															
NEGATIVE: RPMI 1640		A 100	0	0		1					0		1		
		B 100	0	0		2	1				1		5		
		TOTAL 200				3	1				1		4		
		AVERAGE %	0.0	0.0		1.5	0.5				0.5		2.0		
VEHICLE DMSO		100µL/mL													
		A 100	0	0		1					0		1		
		B 100	0	0		5					0		5		
		TOTAL 200				6					0		6		
		AVERAGE %	0	0.0	0.0	3.0					0.0		3.0		
POSITIVE: MMC		1.00µg/mL													
		A 75	0	0		8	3	6			11		16		
		B 75	0	0		5	9	12			19		24		
		TOTAL 150				13	14	18			30		40		
		AVERAGE %	0	0.0	0.0	8.7	9.3	12.0			20.0		26.7		
TEST ARTICLE		1.60mM/L													
		A 100	0	0		2	2				2		2		
		B 100	0	0		1	1				1		3		
		TOTAL 200				2	3				3		5		
		AVERAGE %	0	0.0	0.0	1.0	1.5				1.5		2.5		
		1.70mM/L													
		A 100	0	0		4					0		4		
		B 100	0	0		1	3				3		4		
		TOTAL 200				5	3				3		8		
		AVERAGE %	30	0.0	0.0	2.5	1.5				1.5		4.0		
		1.80mM/L													
		A 100	0	0		9	6				6		15		
		B 100	0	0		3	3				5		8		
		TOTAL 200				12	11				11		23		
		AVERAGE %	37	0.0	0.0	6.0	5.5				5.5		11.5		

chr: chromatid exchange chr: chromosome exchange mb: multiple aberrations, greater than 4 aberrations
^a% Mitotic index reduction as compared to the vehicle control.
^bSignificantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.
^cg = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
^dSignificantly greater in -g than the vehicle control, $p \leq 0.01$. RPMI 1640 = culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

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**Table 4: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation -
~3 Hour Treatment, ~22 Hour Harvest**

Assay No.: 24840 Trial No.: B1 Date: 02/13/03 Lab No.: _____
Test Article: _____ (reference)

		MITOTIC INDEX REDUCTION*	ENDO-REDUPLICATION†	POLY-PLOID CELLS	JUDGE-MENT (±G)‡	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS					TOTALS*		JUDGE-MENT (±G)‡
						Cells Scored	Simple Breaks	chr	chr	mab	±G	±K	
CONTROLS													
NEGATIVE: RPMI 1640													
	A	100	0	0		1					0	1	
	B	100	0	0		2	1				1	3	
	TOTAL	200				3	1				1	4	
	AVERAGE	%	0.0	0.0		1.5	0.5				0.5	2.0	
VEHICLE: DMSO 10.0µL/mL													
	A	100	0	0		1					0	1	
	B	100	0	0		5					0	5	
	TOTAL	200				6					0	6	
	AVERAGE	%	0	0.0	0.0	3.0					0.0	3.0	
POSITIVE: MMC 1.00µg/mL													
	A	73	0	0		8	3	6			11	16	
	B	73	0	0		5	7	12			19	24	
	TOTAL	150				13	14	18			30	40	
	AVERAGE	%	0	0.0	0.0	8.7	9.3	12.0			20.0	26.7	+
TEST ARTICLE 1.60mM/L													
	A	100	0	0		1	3				3	6	
	B	100	0	0		2	1	3			4	6	
	TOTAL	200				3	6	3			9	12	
	AVERAGE	%	0	0.0	0.0	1.5	3.0	1.5			4.5	6.0	+
1.70mM/L													
	A	100	0	0		3	3				3	6	
	B	100	0	0		3	3	1			4	7	
	TOTAL	200				6	6	1			7	13	
	AVERAGE	%	0	0.0	0.0	3.0	3.0	0.5			3.5	6.5	+
1.80mM/L													
	A	100	0	0		1	6	2	1		3	9	
	B	100	0	0		1	6	3			3	10	
	TOTAL	200				6	12	5	1		16	19	
	AVERAGE	%	0	0.0	0.0	3.0	6.0	2.5	0.5		8.0	9.5	+

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chr: chromatid exchange chx: chromosome exchange mab: multiple aberrations, greater than 4 aberrations
 * % Mitotic index reduction as compared to the vehicle control.
 † Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.
 ‡ ±G = # or % of cells with chromosome aberrations; ±K = # or % of cells with chromosome aberrations + # or % of cells with gaps.
 § Significantly greater in -g than the vehicle control, p ≤ 0.01. RPMI 1640 = culture medium DMSO = Dimethylsulfoxide MMC = Mitomycin C

Assay with metabolic activation. A reductions of 0%, 7%, 0%, and 41% was observed in the mitotic indices of the cultures treated with 1.50, 1.60, 1.70, and 1.80 mM/L of _____, respectively, as compared with the vehicle control cultures. Chromosome aberrations were significantly increased at 1.60 and 1.80 mM/L (Table 6). No significant increase in polyploidy or endoreduplication was observed in the cultures analyzed. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the methanol control cultures analyzed (3.60 mM/L).

With _____, reductions of 0%, 14%, and 34% were observed in the mitotic indices of the cultures treated with 1.60, 1.70, and 1.80 mM/L, respectively, as compared with the vehicle control cultures. Like _____esomeprazole sodium was tested in the cultures treated with 1.60, 1.70, and 1.80 mM/L (Table 8) esomeprazole sodium. A significant increase (p<0.01) of cells with chromosomal aberrations was observed at 1.70 and 1.80 mM/L. The increase in polyploidy or endoreduplication was not observed in the study cultures.

The initial assay was repeated with treatment periods of -3 hours without and with metabolic activation and the cultures were harvested -22 hours from the initiation of treatment. Replicate cultures of human whole blood lymphocytes were incubated with 1.50,

1.60, 1.70, 1.80, 1.90, and 2.00 mM/l _____ without and with metabolic activation. The reduction in the mitotic index in cultures without metabolic activation was 3, 9, 51, 72 and 91% at these concentrations, respectively. _____ at the concentrations of 1.60, 1.70, and 1.80 mM/l in cultures produced a significant increase ($p < 0.01$) of cells with chromosomal aberrations and, these were 8.0, 9.5 and 10.0 at 1.6, 1.7 and 1.8 $\mu\text{mol/l}$ concentrations, respectively.

The repeat-assay with metabolic activation, _____ at the concentrations of 1.60, 1.70, and 1.80 mM/l in cultures produced a significant increase ($p < 0.01$) of cells with chromosomal aberrations and, these were 7.0, 12.5, 14.5 and 19.5 at 1.5, 1.6, 1.7 and 1.8 mM/l concentrations, respectively. Esomeprazole at the concentrations of 1.50, 1.60, and 1.70 mM/l in cultures also caused a significant increase ($p < 0.01$) of cells with chromosomal aberrations and, these were 14.0, 9.0 and 19.4 at 1.5, 1.6 and 1.7 mM/l concentrations, respectively.

In summary, esomeprazole sodium and _____ from the concentrations of 1.60, 1.70, and 1.80 mM/l in cultures produced a significant increase ($p < 0.01$) of cells with chromosomal aberrations in the absence and presence of metabolic activation. Esomeprazole was clastogenic in human peripheral blood lymphocytes in the test and it was confirmed in a confirmatory test during the study.

7. Induction of Chromosome Aberrations in the Bone Marrow of Mice. A comparison of the effects of Esomeprazole with and without Degradation Products. (Report No.: 1889/39-D6172/SR 03005-01)

Testing Laboratory: _____

(Sponsor: Global SHE Operations, AstraZeneca
Sodertalje, Sweden)

Batch # Esomeprazole & Degradants:

Dates of Initiation and Completion of Study: September 17, 2002 and 23 January, 2003

GLP and QAU Requirements: The statements of compliance were attached.

Batch #: 105/01 (enantiomeric purity _____) and 113/01. Batch # of formulation used:

Used in group(s) no.	Batch No. esomeprazole sodium	Batch No. freeze-dried powder	With/without degradation products	Concentration esomeprazole in the constituted solution	
				$\mu\text{mol/mL}$	mg/mL
2, 3, 4	105/01	HT 1216-01-01-01	With	23	8
5	113/01	H 1516-03-01-01	Without	23	8

The commercial Nexium injection Batch No. HT 1216-01-01-01 was prepared and stored under accelerated conditions (40°C/75% relative humidity) for 9 months prior to use followed by a period at 40°C (denoted "degradation storage"). The preparation contained the following mixture of relevant degradation products in the freeze-dried powder, i.e., _____ (used in group 2 to 4). The actual amount of degradant injected in 3 groups of the study were 0.88, 1.76 and 3.32 mg/kg/day of _____ Nexium injection Batch No. HT 1516-03-01-01 (without degradants) was used in group 5 of the study.

Positive/Negative Controls: Negative control groups were administered vehicle (saline) two times/day, 6 hr apart, in a volume = 20 ml/kg. The positive control, cyclophosphamide (CPA) 40 mg/kg was administered in a 2.0 mg/ml freshly prepared solution as a single injection 24 hours prior to necropsy.

Procedure:

Twenty four CD-1 male mice were divided in to 4 groups (6 mice/group) were given infusion doses of 0, 230, 460 and 920 umol/kg (0, 80, 160 or 320 mg/kg) esomeprazole with degradation products at the rate of 0.0125 or 0.025 ml/min, respectively for 20 min (this rate adjusted for the doses). The dose selection of the study was based on the study #CLE 1889/41 in which the group of animals were administered the compound at the IV dose of 160 mg/kg or 460 umol/kg two times a day (6 hr apart) via tail vein. The animals treated at 160 mg/kg dose had reduced activity and this was identified 'the highest tolerable dose' (MTD). Another group of 6 mice was administered 920 umol/kg (320 mg/kg) esomeprazole without degradant products. The groups of animals in 320 mg/kg were administered with two equally divided doses 6 hr apart. One group of six male mice received cyclophosphamide (CPA, 40 mg/kg), by a single bolus intravenous injection at the same dose volume as the highest test article dose (20 ml/kg) and was included in 24 hr sampling period. Three additional groups of male rats (6/group) were administered (control), 460 (with degradation products) or 460 (without degradation products) umol/kg, iv esomeprazole two times each in a volume of 20 ml/kg for 48 hr sampling period. Two different batches of the proposed commercial product for solution for injection/infusion 40 mg (as esomeprazole sodium 42.5 mg) in 5 ml vials (a freeze-dried formulation) were used in this study.

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Dose Group ($\mu\text{mol/kg}$)	Dose (mg/kg)	Dose volume (mL/kg)	24 hour sampling (number of animals)	48 hour sampling (number of animals)
Vehicle (x2) (saline)**	-	20	6 males	6 males
230 (with degradation products)	80	10	6 males	-
460 (with degradation products)	160	20	6 males	-
460 (x2) (with degradation products)**	160	20	6 males	6 males
460 (x2) (without degradation products)**	160	20	6 males	6 males
Positive control (CPA)	40	20	6 males	-

** Animals given two administrations received each dose separated by a 6-hour period

The animals of the study were killed after 24 hr or 48 hr (only highest dose and negative control groups) after the administration of the second dose of the esomeprazole in the sequence of treatment and having received 2 mg/kg, ip colchicine to arrest the cells in metaphase. Femurs of each of the animals was separated, cleaned, the bone marrow cells separated, purified, fixed on slide and stained in Giemsa stain (pH 6.8). The slides of control, test solution treated and CPA treated animals were scored for mitotic index (MI) or percentage of cells in mitosis, based on 1000 cells per animal.

Assay Criteria: A test article was considered positive if a significant increase (the difference was considered significant when $p < 0.01$) in the number of cells with chromosomal aberrations was observed at one or more concentrations or the increase showed a positive linear trend test in a dose relation manner. Increased number of cells (%) with gaps or occurring at very high concentrations of the compound was considered as equivocal.

Results:

Clinical Observations: At 24 hr observation period, abnormal breathing was seen in animals treated with 230 or 460 $\mu\text{mol/kg}$ esomeprazole and swollen and sore/blackened tail was seen in mice treated with two doses of 460 $\mu\text{mol/kg}$ with or without degradation products. Additionally, the convulsions were reported in group of animals treated with 460 (x2) $\mu\text{mol/kg}$ esomeprazole with degradants and 1 of 6 mice died following the treatment. No change of the clinical signs was noted at 48 hr period.

Chromosomal Aberration Effect:

The inhibition of mitosis of 2.9, 3.1, 3.7, 3.1, 2.9 and 3.0 % was observed in cell cultures obtained from mice treated at the dose of 0, 230, 460, 920 (460 $\mu\text{mol/kg}$ x2 doses with

degradation products) and 920 (460 umol/kg x2 doses without degradants) esomeprazole and positive control (CPA), respectively.

No increase in the number of cells (%) with aberration was seen in the preparations of negative control or the selected doses of esomeprazole. Esomeprazole at the doses of 0, 230, 460, 920 (460 umol/kg x2 doses with degradants) and 920 (460 umol/kg x2 doses without degradants) did not produce significant dose related increase in the aberrations. An increase in the aberrations was seen in positive control (CPA) as shown below.

Table 1

Group data - 24 hour sample time

Treatment (µmol/kg/day)	Number of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps (% ± SD)	Significance †	Mitotic index % (group mean)
Vehicle (x2*)	6	1200	3	0 (0 ± 0)	-	2.9
230; + Deg	6	1200	4	0 (0 ± 0)	NS	3.1
460; + Deg	6	1200	3	1 (0.08 ± 0.41)	NS	3.7
460 (x 2)*; + Deg	5	1000	3	1 (0.1 ± 0.45)	NS	3.1
460 (x 2)*; - Deg	6	1200	5	1 (0.08 ± 0.41)	NS	2.9
CPA*	6	1200	186	165 (13.8 ± 17.8)	p ≤ 0.001	3.0

† Statistical significance

NS = Not significant

SD = standard deviation

Deg = degradation products

* = Administered as a single dose at 40 mg/kg

* 2 administrations, 6 hours apart on a single day

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Table 2
Group data - 48 hour sample time

Treatment ($\mu\text{mol/kg/day}$)	Number of animals	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps (% \pm SD)	Significance †	Mitotic index % (group mean)
Vehicle (x2)*	6	1200	2	0 (0 \pm 0)	-	3.6
460 (x 2)*; + Deg	6	1200	3	1 (0.08 \pm 0.41)	NS	2.5
460 (x 2)*; - Deg	6	1200	1	0 (0 \pm 0)	NS	3.0

† Statistical significance
NS = Not significant
SD = standard deviation
Deg = degradation products

* 2 administrations, 6 hours apart on a single day

2.6.6.8 SPECIAL TOXICITY STUDIES:

1. Vaso- and Tissue Irritation Study in Dogs after Intravenous and subcutaneous Administration for 5 Days: (Study No. 99073/Project #82621)

Method: The vaso- and tissue irritation potential of esomeprazole (H 199/18; batch #600/93) formulations in saline or polyethylene glycol 400 (PEG400) were tested in dogs. Both of these formulations were stated by sponsor to be used in clinical preparation. The routes of administration of the test formulations of the study were selected because intravenously administered compound may inadvertently cause peri-vascular spill in the clinic and may be likely to cause a tissue reaction. The dose schedules, the sites of injection and the number of animals in during the study are shown in the following table (sponsor's tables 1, 4 on pp 2045, 2052 of the submission).

Table 1 Formulation concentrations and dosage for esomeprazole administered intravenously and subcutaneously to dogs

Group	Formulation	H 199/18 conc.		I.V.				S.C. Vol.	Dose H 199/18 I.V.+S.C	
		$\mu\text{mol}/\text{ml}$	mg/ml	Vol. ml/kg	Time min	Rate ml/kg-h	Rate $\mu\text{mol}/\text{kg-h}$		mg/kg+ mg/dog	$\mu\text{mol}/\text{kg}+\mu\text{mol}/\text{dog}$
1	Phys. saline H 199/18 (saline sol.)	0	0	0.2	3.0	4.0	0	1.0	0+0	0+0
		5.8	2.0	0.2	3.0	4.0	23	1.0	0.4+2.0	1.2+5.8
2	Phys. saline H 199/18 (saline sol.)	0	0	0.2	3.0	4.0	0	1.0	0+0	0+0
		12	4.0	0.2	3.0	4.0	46	1.0	0.8+4.0	2.3+12
3	Phys. saline H 199/18 (saline sol.)	0	0	0.1	3.0	2.0	0	1.0	0+0	0+0
		23	8.0	0.1	3.0	2.0	46	1.0	0.8+8.0	2.3+23
4	Phys. saline H 199/18 (saline sol.)	0	0	0.05	3.0	1.0	0	1.0	0+0	0+0
		46	16	0.05	3.0	1.0	46	1.0	0.8+16	2.3+46
5	40% PEG 400 vehicle H 199/18 (in 40% PEG 400)	0	0	0.05	3.0	1.0	0	1.0	0+0	0+0
		46	16	0.05	3.0	1.0	46	1.0	0.8+16	2.3+46

H 199/18 = esomeprazole

Phys. saline/saline sol. = Physiological saline/saline solution

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Table 4 Intravenous administration

Gr.	No. and sex	Dog ref. Nos.	Formulation type and No.	Conc. H 199/18		I.V administration					Total i.v. dose H 199/18	
				$\mu\text{mol}/\text{ml}$	mg/ml	Vol. ml/kg	Rate ml/kg-h	Rate $\mu\text{mol}/\text{kg-h}$	Rate $\text{mg}/\text{kg-h}$	Inf. vein	$\mu\text{mol}/\text{kg}$	mg/kg
1	1M+1F	1+2	Phys saline (IX)	0	0	0.2	4.0	0	0	LC	0	0
			H 199/18 in sal. (I)	5.8	2.0	0.2	4.0	23	8.0	RC	1.2	0.4
2	1M+1F	3+4	Phys saline (IX)	0	0	0.2	4.0	0	0	LC	0	0
			H 199/18 in sal. (II)	12	4.0	0.2	4.0	46	16	RC	2.3	0.8
3	1M+1F	5+6	Phys saline (IX)	0	0	0.1	2.0	0	0	LC	0	0
			H 199/18 in sal. (III)	23	8.0	0.1	2.0	46	16	RC	2.3	0.8
4	1M+1F	7+8	Phys saline (IX)	0	0	0.05	1.0	0	0	LC	0	0
			H 199/18 in sal. (IV)	46	16	0.05	1.0	46	16	RC	2.3	0.8
5	1M+1F	9+10	PEG 400 vehicle (VI)	0	0	0.05	1.0	0	0	LC	0	0
			H 199/18 in PEG 400 (VIII)	46	16	0.05	1.0	46	16	RC	2.3	0.8

Sal. = saline LC = Left cephalic vein RC = Right cephalic vein

The intravenous infusion time was 3.0 minutes for all formulations.

40% PEG 400 was used in formulations VI and VIII

Table 5 Subcutaneous administration

Group	No. and sex	Dog ref. Nos.	Formulation type and No.	Conc. H 199/18		S.C. administration			Total s.c. dose H 199/18	
				$\mu\text{mol}/\text{ml}$	mg/ml	Vol. ml/dog	Peri-vascular vein	$\mu\text{mol}/\text{dog}$	mg/dog	
1	1M+1F	1+2	Phys saline (IX)	0	0	1.0	L.L. saphenous	0	0	
			H 199/18 in sal. (I)	5.8	2.0		R.L. saphenous	5.8	2.0	
2	1M+1F	3+4	Phys saline (IX)	0	0	1.0	L.L. saphenous	0	0	
			H 199/18 in sal. (II)	12	4.0		R.L. saphenous	12	4.0	
3	1M+1F	5+6	Phys saline (IX)	0	0	1.0	L.L. saphenous	0	0	
			H 199/18 in sal. (III)	23	8.0		R.L. saphenous	23	8.0	
4	1M+1F	7+8	Phys saline (IX)	0	0	1.0	L.L. saphenous	0	0	
			H 199/18 in sal. (IV)	46	16		R.L. saphenous	46	16	
5	1M+1F	9+10	PEG 400 vehicle (VI)	0	0	1.0	L.L. saphenous	0	0	
			H 199/18 in PEG 400 (VIII)	46	16		R.L. saphenous	46	16	

Sal. = saline L.L. = Left lateral R.L. = Right lateral

40% PEG 400 was used in formulations VI and VIII

Five groups of dogs (1/sex/group), were administered intravenous (in the cephalic vein) and subcutaneous (beside the lateral saphenous veins) esomeprazole formulation (pH 9-10) for 5 days. The subcutaneous injection was made adjacent to the right and the control formulations adjacent to the left lateral saphenous vein directly after intravenous administration. Each animal served as its own control and was given intravenous or subcutaneous dose of a relevant control formulation (physiological saline or a 40% PEG 400 vehicle) in the contralateral legs of the animals. The clinical signs, body weight, food

consumption and rectal temperature were recorded. All study animals were killed and necropsied on the study day 3 following the final treatment (i.e. on day 8 or after 2 dose-free days).

Results:

Clinical Signs: The swellings were noted after 1 hr of the sc administration of the compound in a dose related manner and slight swelling was noted just before the next day injection (24 hr of the initial injection). The slight swelling was noted in PEG400 only treated animals.

In group 2 (0.8 mg/ml IV + 4.0 mg/kg, SC), 1 of the 2 animals (female) treated with 4.0 mg/ml had minimal hemorrhagic reaction without necrosis. The grade of inflammatory cell reaction and the number of animals affected were increased with the dose. One (female) and 2 animals (1/sex) receiving 8 and 16 mg/ml had discolored area, inflammatory cell infiltration and grade 2 to 3 necrosis. The animals given esomeprazole solution in PEG400 showed increased inflammatory cell reactions of grade 2 in both the animals. The PEG vehicle alone produced inflammatory reaction in male and female dogs from minimum to slight grade. The tissue necrosis at the site of injection was seen in animals treated with 16 mg/ml solution in physiological solution and, also in PEG alone (1 animals) and both animals treated with esomeprazole solution in PEG 400 as shown below (table 6 taken from sponsor's submission).

Table 6 Histopathological reaction at the subcutaneous injection site

Group	Formulation	H 199/18 concentration		Inflam. cell reaction			Necrosis			Total mean score
		μ mol/ml	mg/ml	M	F	Mean M+F	M	F	Mean M+F	
1	Phys. saline	0	0	0	0	0	0	0	0	0
	H 199/18 (saline sol.)	5.8	2.0	1	1	1.0	0	1	0.5	0.75
2	Phys. saline	0	0	2	0	1.0	0	0	0	0.50
	H 199/18 (saline sol.)	12	4.0	0	1	0.5	0	0	0	0.25
3	Phys. saline	0	0	0	1	0.5	0	0	0	0.25
	H 199/18 (saline sol.)	23	8.0	0	2	1.0	0	0	0	0.50
4	Phys. saline	0	0	0	0	0	0	0	0	0
	H 199/18 (saline sol.)	46	16	1	2	1.5	2	3	2.5	2.0
5	40% PEG 400 vehicle	0	0	1	2	1.5	0	2	1.0	1.25
	H 199/18 (in 40% PEG 400)	46	16	2	2	2.0	3	2	2.5	2.25

Grade 0 = No reaction noted

Grade 1 = Minimal reaction

Grade 2 = Slight reaction

Grade 3 = Moderate reaction

Each observation has been included according to the degree of reaction observed (grades 0, 1, 2 or 3) and the total score has been divided by the number of observations for that particular treatment.

Thus esomeprazole up to a concentration of 8 mg/ml produced only hemorrhage, minimal to slight skin reactions and no necrosis. But the solution of 16 mg/ml, sc produced both grade 3 necrosis and inflammatory cell reactions.

2. 10-Day Intravenous Vascular and Perivascular Irritation Study in Dogs. A comparison of the effects of test compound with and without Degradation Products:
(Study No. 02096/D9615-82621)

Batch # and Concentrations of the Compounds Used: See following sponsor's table #2 at p 11 (80):

Table 2 Test formulations

Used in group	Batch No. esomeprazole sodium	Batch No. freeze-dried powder	With/without degradation products	Concentration esomeprazole in the constituted solution $\mu\text{mol/mL}^1$ mg/mL^1		Formulation No.
1	105/01	HT 1216-01-01-01	With	1.0	0.36	II
2	113/01	H 1516-03-01-01	Without	1.0	0.36	III
3	105/01	HT 1216-01-01-01	With	23	8	IV
4	113/01	H 1516-03-01-01	Without	23	8	V

¹Analysis of the formulations used for dosing showed that the esomeprazole concentration in the infusion solution was about 90% of the intended 1.2 $\mu\text{mol/mL}$ (0.4 mg/mL). The concentration of esomeprazole in this solution has therefore been adjusted accordingly when describing the concentrations and doses tested.

Two different batches of esomeprazole powder for solution for injection/ infusion 40 mg (as esomeprazole sodium 42.5 mg/5 ml vial (a freeze-dried market formulation), were used in this study. Esomeprazole with relevant degradation products was prepared by storing the substance of 1 of the 2 batches of the compound under accelerated conditions for 9 months before use.

The portion of the solution was stored at room temperature (degradation storage) that exceeded the proposed use period for clinical use which should form the additional degradation products in the constituted solutions. The other esomeprazole batch (esomeprazole without degradation products), used in groups 2 and 4, was stored at ambient temperature, protected from light, from the time of manufacture and up to use. This batch was used in this study about 8 months after manufacture, and therefore it also contained small amounts of degradation products.

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Table 1 Formulation concentrations and dosage for esomeprazole administered intravenously and subcutaneously to dogs

Group	Formulation	Esomeprazole conc.		I.V.		S.C.		Dose esomeprazole		
		µmol/ml	mg/ml	Vol. ml/kg	Time min	Rate		Vol. ml/dog	I.V.+S.C.	
						ml/kg-h	µmol/kg-h		µmol/kg+ µmol/dog	mg/kg+ mg/dog
1	Physiological saline	0	0	16	60	16	0	1.0	0+0	0+0
	Esomeprazole, with	1.0	0.36	16	60	16	17	1.0	17+1.0	5.8+0.36
2	Physiological saline	0	0	16	60	16	0	1.0	0+0	0+0
	Esomeprazole, without	1.0	0.36	16	60	16	17	1.0	17+1.0	5.8+0.36
3	Physiological saline	0	0	0.8	6	8.0	0	1.0	0+0	0+0
	Esomeprazole, with	23	8	0.8	6	8.0	190	1.0	19+23	6.4+8.0
4	Physiological saline	0	0	0.8	6	8.0	0	1.0	0+0	0+0
	Esomeprazole, without	23	8	0.8	6	8.0	190	1.0	19+23	6.4+8.0

With = with degradation products
Without = without degradation products

Method: The aim of the study was to determine and compare the vaso- and tissue irritation potential of esomeprazole. Four groups of dogs (1/sex) were intravenously injected esomeprazole solutions (with and without degradation products) in the right cephalic vein, and subcutaneously (adjacent to the right lateral saphenous vein). Each dog served as its own control and physiological saline solution was intravenously given in left cephalic vein, and subcutaneously, adjacent to the left lateral saphenous vein. The subcutaneous injections were given immediately after the start of the intravenous infusions and the study dogs were necropsied on the third day of the final dosing or after 2 days of dose-free period. The test formulations of the compound use din the study are shown in the following 3.8 tables of sponsor's EDT submission:

3.8 Dose groups and animal numbers

The animals were assigned to the dose groups in order to balance, as far as possible, the distribution of age and body weight. Litter mates were dispersed throughout the groups. The dogs were grouped as shown in the table below.

Group	No. and sex	Animal ref. No.	Formulation	INTRAVENOUS INFUSION					Infusion vein	Form
				Daily dose		Inf. volume ml/kg	Inf. time min			
				µmol/kg	mg/kg					
DAILY 1 Study 1 Observa Animal Number 1 (sexes)	IM	1	Physiological saline control	0	0	16	60	Left cephalic	I	
		1	Esomeprazole sol. (0.4 mg/mL) with degradation products	17	5.8	16	60	Right cephalic	II	
	IF	2	Physiological saline control	0	0	16	60	Left cephalic	I	
		2	Esomeprazole sol. (0.4 mg/mL) with degradation products	17	5.8	16	60	Right cephalic	II	
	IM	3	Physiological saline control	0	0	16	60	Left cephalic	I	
		3	Esomeprazole sol. (0.4 mg/mL) without degradation products	17	5.8	16	60	Right cephalic	III	
	IF	4	Physiological saline control	0	0	16	60	Left cephalic	I	
		4	Esomeprazole sol. (0.4 mg/mL) without degradation products	17	5.8	16	60	Right cephalic	III	
IM	5	Physiological saline control	0	0	0.8	6	Left cephalic	I		
	5	Esomeprazole sol. (8 mg/mL) with degradation products	19	6.4	0.8	6	Right cephalic	IV		
	6	Physiological saline control	0	0	0.8	6	Left cephalic	I		
	6	Esomeprazole sol. (8 mg/mL) with degradation products	19	6.4	0.8	6	Right cephalic	IV		
IM	7	Physiological saline control	0	0	0.8	6	Left cephalic	I		
	7	Esomeprazole sol. (8 mg/mL) without degradation products	19	6.4	0.8	6	Right cephalic	V		
	8	Physiological saline control	0	0	0.8	6	Left cephalic	I		
	8	Esomeprazole sol. (8 mg/mL) without degradation products	19	6.4	0.8	6	Right cephalic	V		

Results: Diarrhea, increased lacrimation, salivation, hyperventilation and serous nasal

Group	No. and sex	Animal ref. No.	Formulation	SUBCUTANEOUS INJECTION			Perivascular vein	Form.
				Daily dose		Injection volume ml/dog		
				µmol/dog	mg/dog			
1	IM	1	Physiological saline control	0	0	1.0	Left lat. saphenous	I
		1	Esomeprazole sol. (0.4 mg/mL) with degradation products	1.0	0.36	1.0	Right lat. saphenous	II
	IF	2	Physiological saline control	0	0	1.0	Left lat. saphenous	I
		2	Esomeprazole sol. (0.4 mg/mL) with degradation products	1.0	0.36	1.0	Right lat. saphenous	II
IM	3	Physiological saline control	0	0	1.0	Left lat. saphenous	I	
	3	Esomeprazole sol. (0.4 mg/mL) without degradation products	1.0	0.36	1.0	Right lat. saphenous	III	
IF	4	Physiological saline control	0	0	1.0	Left lat. saphenous	I	
	4	Esomeprazole sol. (0.4 mg/mL) without degradation products	1.0	0.36	1.0	Right lat. saphenous	III	
3	IM	5	Physiological saline control	0	0	1.0	Left lat. saphenous	I
		5	Esomeprazole sol. (8 mg/mL) with degradation products	23	8	1.0	Right lat. saphenous	IV
	IF	6	Physiological saline control	0	0	1.0	Left lat. saphenous	I
		6	Esomeprazole sol. (8 mg/mL) with degradation products	23	8	1.0	Right lat. saphenous	IV
4	IM	7	Physiological saline control	0	0	1.0	Left lat. saphenous	I
		7	Esomeprazole sol. (8 mg/mL) without degradation products	23	8	1.0	Right lat. saphenous	V
	IF	8	Physiological saline control	0	0	1.0	Left lat. saphenous	I
		8	Esomeprazole sol. (8 mg/mL) without degradation products	23	8	1.0	Right lat. saphenous	V

discharge were observed on 1 or 2 dosing occasions in esomeprazole treated dogs. The incidences of irritation (struggling) and vocalization were greater in subcutaneously treated animals than the control group animals. Perivascular hemorrhage (grade 2) and inflammatory infiltration were noted in 1/sex treated with 8 mg/ml esomeprazole. The male animal treated with esomeprazole with degradation products and a female animal without degradation products showed this reaction. Therefore, the degradants did not affect the irritation/hemorrhagic potential of esomeprazole. The slight inflammatory reactions were seen in control group male and female. Hematoma at the site of injection was seen in 1 dog (#6) administered 23 umol/kg esomeprazole with degradants on study day 3 at the site of injection.

Thus, esomeprazole with degradants caused only mild irritation at the site of injection

3. Blood Hemolysis & Protein Flocculation in Human Blood Study in vitro. A Comparison of the Effects of the Test Compound With and Without Degradation Products (Document #SR02013-01/Study #02013/D9615 (82621)

Date of Initiation and Completion: April 29, 2002 & January 23, 2003

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

Animals: Female, New Zealand White Rabbits, approximately 13 months old, weight not specified.

Drug Batch No.: Esomeprazole sodium (chemical and enantiomeric pure). The test formulations used are shown in sponsor's EDT on pp 9 of 34).

Test formulations

Batch No. esomeprazole sodium	Batch No. freeze-dried powder	With/without degradation products	Concentration esomeprazole in the constituted solution		Formulation No
			$\mu\text{mol/mL}^1$	mg/mL^1	
105/01	HT 1216-01-01-01	With	1.0	0.36	II
113/01	H 1516-03-01-01	Without	1.0	0.36	III
105/01	HT 1216-01-01-01	With	23	8	IV
113/01	H 1516-03-01-01	Without	23	8	V

¹Analysis of the formulations used for dosing in Study No. 02086 showed that the esomeprazole concentration in the infusion solution was about 90% of the intended 1.2 $\mu\text{mol/mL}$ (0.4 mg/mL). The concentration of esomeprazole in this solution has therefore been adjusted accordingly when describing the concentrations tested

Methods: The blood sample from a healthy female volunteer was obtained and plasma separated. In plasma flocculation study, 1 ml of plasma aliquots was mixed with three

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different proportions of esomeprazole with or without degradation products to achieve 1: 100, 3: 100 and 10: 100 (esomeprazole:plasma, v:v). The samples were incubated for 10 minutes at 37 ° C and the turbidity measured (duplicate samples) at _____. The mean value of the change in absorbance obtained in the sample containing esomeprazole was determined.

To another 2 ml blood, physiological saline or the esomeprazole solutions were added in three different proportions to get 1: 100, 3: 100 and 10: 100 (solution: blood, v: v). The samples were mixed and incubated for 15 minutes at 37°C. The red blood cells removed by centrifugation and the absorbance at _____ was determined for each sample (oxyhemoglobin has absorbency maxima at _____ with a minimum absorbency at _____ and the amount of oxyhemoglobin was found as the difference in absorbance at _____). The fraction of blood hemolysed in the samples was determined by using a standard curve obtained by measuring the same difference in absorbance in samples to which varying amounts of blood had been added to distilled water, and subsequently totally hemolysed. The standard curve was obtained by using blood:distilled water ($\times 10^2$) dilutions of 0.013, 0.017, 0.050, 0.100, 0.200 and 0.500 (absorbance determined at _____).

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Table 3 Hemolysis, Formulations II and III

The absorbance at _____ nm and the fraction of blood which was hemolysed after addition of 0.9% NaCl and the solutions of esomeprazole, formulations II and III.

Test solution	Proportion solution: blood (v:v)	Absorbance ($\times 10^3$)			Fraction of the blood which had been hemolysed ($\times 10^{-2}$)
		_____ nm	_____ nm	_____ nm	
Formulation I 0.9% NaCl	10:100			M= 9	0.02
	10:100				
	3:100				
	3:100				
	1:100			M= 7	0.02
	1:100				
Formulation II Esomeprazole with degradation products 0.36 mg/mL	10:100			M= 1	0.01
	10:100				
	3:100				
	3:100				
	1:100			M= 10	Missing value* 0.02
	1:100				
Formulation III Esomeprazole without degradation products 0.36 mg/mL	10:100			M= 10	0.02
	10:100				
	3:100				
	3:100				
	1:100			M= 8	0.02
	1:100				
	1:100			M= 7	0.02
	1:100				

M=Mean value
* = The test failed due to technical error

Results: The addition of esomeprazole solutions to the blood increased the flocculation and the average changes in absorbance at _____ nm were 1.5, 7 and 6 in formulations II and III with degradation products and, _____ in solution of formulations IV and V with degradation products. The fraction of hemolysis were not changed as shown in the following table:

Table 6 Hemolysis, Formulations IV and V

The absorbance at _____ nm and the fraction of blood which was hemolysed after addition of 0.9% NaCl and the solutions of Esomeprazole, formulations IV and V.

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Test solution	Proportion solution: blood (v:v)	Absorbance ($\times 10^{-3}$)		Fraction of the blood which had been hemolysed ($\times 10^{-2}$)
			am	
Formulation I 0.9% NaCl	10:100			0.01
	10:100			
	3:100			
	3:100			
	1:100			0.01
	1:100			
Formulation IV Esomeprazole with degradation products 8 mg/mL	10:100			0.01
	10:100			
	3:100			
	3:100			
	1:100			0.02
	1:100			
Formulation V Esomeprazole without degradation products 8 mg/mL	10:100			0.02
	10:100			
	3:100			
	3:100			
	1:100			0.03
	1:100			

M=Mean value

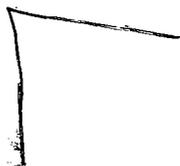
In conclusion, esomeprazole solutions caused slight flocculation of blood proteins but no hemolysis.

LABELING:

The label of Esomeprazole Injection generally conforms to the 21 CFR 42, Subpart B but based on the present review and from the preclinical standpoint, the following changes in the subsection "Overdose" are recommended in the proposed label. The changes are described by first giving the test of the proposed label followed by the reviewer comment and the proposed text of the label:

a. Proposed Text of Label under Category "OVERDOSE":

"OVERDOSAGE



b. REVIEWER'S COMMENTS:

In an acute toxicity study in rats at the intravenous doses of 190 and 310 mg/kg esomeprazole, a doses of 310 mg/ kg was identified as lethal and the dose of 190 mg/kg produced the clinical signs of reduced motor activity, increased or decreased respiratory frequency, abdominal respiration, clonic convulsions in association with tremors, salivation, dyspnea, cyanosis, and ataxia in the animals. Clinical signs in females in general were of greater intensity and duration. Therefore, the sponsor's identified minimum lethal dose of _____, esomeprazole is not acceptable. The first para of the proposed label should be changed as given below.

Sponsor in the second para in the above section of "Overdose" has described the Maximum tolerated dose, pharmacokinetics, etc. This para does not belong to this section and should be removed from the proposed label.

- i. Proposed Text of the Proposed Label: Para 1 of the Overdose Section should read as:
“The minimum lethal dose of esomeprazole in rats after bolus administration was 310 mg/kg (about 62 times the human dose) ~~_____~~ The major signs of acute toxicity were reduced motor activity, changes in respiratory frequency, tremor, ataxia and intermittent clonic convulsions.”
- ii. The second para of the above Overdose section of the proposed label should be deleted.”

2.6.8 OVERALL conclusion and Recommendations

Esomeprazole, an enantiomer of omeprazole is H^+/K^+ -ATPase enzyme inhibitor, acts by accumulating in the acidic compartment of the parietal cell and was similar to its racemic form in the suppression of gastric acid secretion. It has previously been approved as oral capsules under NDA 21-153 (esomeprazole magnesium-AstraZeneca). The sponsor had developed an intravenous injection formulation of esomeprazole for those patients who cannot take oral preparation. Sponsor in the present application did not submit any new preclinical pharmacology studies. A non-clinical bridging program for esomeprazole (H 199/18) consisting of limited number of the pharmacokinetics and toxicology studies was submitted in the present submission. These included the oral/intravenous pharmacokinetic study in the rat, identification of metabolites in vitro by liver microsomal preparations from animals and man, single intravenous dose toxicity study in Sprague-dawley rats with and without degradation products, comparison of acute oral and intravenous toxicity of H199/18 sodium, H199/19 sodium and omeprazole sodium in rats, 28-day IV bolus dose toxicity study in SD rats, 28-day IV bolus dose toxicity study in rats with and without degradants, estimation of maximum tolerated dose in 3-day continuous intravenous infusion toxicity study in dogs followed by a 14 day continuous infusion toxicity studies, 28-day IV bolus toxicity and two 1-month continuous intravenous infusion toxicity studies in dogs, hepatic cytochrome P450 induction in 1-month continuous intravenous infusion study in dogs, mutagenicity evaluation in two Ames test in the presence and absence of degradants, Ames test on degradant products of esomeprazole ~~_____~~ chromosomal aberrations in cultured human peripheral blood lymphocytes, chromosomal aberrations in cultured human peripheral blood lymphocytes, induction of chromosome aberrations in the presence and absence of degradants, in vivo mouse bone marrow chromosomal aberrations test, special toxicity studies on vaso- and tissue irritation study in dogs after intravenous and subcutaneous administration for 5 Days, 10-day intravenous vascular and perivascular irritation study in Dogs (A comparison of the effects of test compound with and without Degradation Products), in vitro hemolysis and protein flocculation in human blood study.

The pharmacokinetic study of esomeprazole in fasted male rats showed that it attained the treatment related plasma concentration with the half-lives of 10 min and the bioavailability (i.d. Vs i.v.) of 2 enantiomers, esomeprazole and omeprazole, varied from 34 to 38% in

rats. The intravenously administered compounds had similar clearance and distribution in the body. The in vitro metabolism in liver microsomal preparation from rat, dog, rabbit and man, esomeprazole was reported to be metabolized into 13 metabolites. These metabolites were the result of sulfonation, hydrolysis and ring modifications and the quantitative amounts of the metabolites varied in animals as M1 and M5 and M6 and M8 were the common metabolites formed in mouse, rat and rabbit microsomes. M8 and M12 were also formed in human microsomes. The metabolic profile of esomeprazole was similar to omeprazole in the presence of degradation products.

A single IV dose of 180 or 310 mg/kg produced reduced motor activity, increased or decreased respiratory frequency, abdominal respiration, clonic convulsions in rats. The dose of 180 mg/kg was not lethal and the minimum lethal dose was 310 mg/kg in the study.

In a 28-day intravenous toxicity study in rats, esomeprazole was tested at intravenous doses of 48, 86 and 160 mg/kg/day. A treatment-related increase in the plasma concentrations were observed with the pharmacological activity of gastric chief cell hypertrophy, chronic nephropathy and inflammatory and other adverse reactions at the site of injection were observed in a treatment-related manner in treatment group animals. The identified target organ of toxicity were CNS, stomach, kidney and site of injection and the 'highest tolerable dose' was 48 and 26 mg/kg/day in males and females, respectively.

In another 28-day intravenous toxicity study, the rats were treated at intravenous doses of 4 and 80 mg/kg/day with degradants synthesized/produced under 3 different conditions (under accelerated conditions, without degradants and the compound used after 9 months of storage and, the solution stored for the extended period – exceeded generously the proposed period of clinical use) to observe the effect of degradation products of esomeprazole. A treatment-related but non-dose proportional esomeprazole concentrations were noted in the animals. The increase in serum iron binding and chief cell eosinophilia incidences in stomach of animals included in 80 mg/kg/day esomeprazole with and without degradants was similar. A dose of 4 mg/kg/day was a 'no effect dose' in the study. The safety margin of 5.2 for the total degradation products administered with the clinical IV dose of 0.8 mg/kg esomeprazole was estimated.

In a 14-day continuous IV toxicity study in dogs (3/sex) esomeprazole was administered in a continuous IV infusion dose of either 120 or 240 mg/kg/day. These doses were lethal and animals showed salivation, CNS depressant effects, noisy breathing and collapse before death. The overt pharmacodynamic effects in stomach were observed and target organ of toxicity was CNS.

In a 28-day iv toxicity study in dogs, esomeprazole at the doses of 0, 4.8, 10 or 22 mg/kg/day esomeprazole (vol = 3 ml/kg) as a slow injection produced CNS related toxicity of reduced activity, tremor and convulsions and the reduced size of parietal cells within the

fundic mucosa, interstitial edema and denser eosinophilic cytoplasm. This suggested the CNS and stomach as the possible target organs of toxicity and a dose of 10 mg/kg/day was 'the identified as the 'highest tolerable dose' for the study.

In 1-month continuous intravenous infusion toxicity study in dogs, 4 groups of animals were administered 0, 35, 86 and 170 mg/kg/day esomeprazole as a continuous infusion in the presence and absence of degradants. Esomeprazole treated animals attained non-dose proportional plasma concentrations, treatment related inflammatory changes (thrombus formation) at the implantation/ infusion site, gastric chief cell and parietal cell atrophy and vacuolation, thyroid (follicular epithelial hypertrophy), thrombus formation in the lung of animals included in 86 and 170 mg/kg/day treatment groups was observed. A treatment related increase in CYP1A1 enzyme from 10 to 25 folds in male and, 2.8 to 6.7 folds in female dogs included 35 to 170 mg/kg/day treatment groups was seen. A no-effect dose level was not established in the study but the identified highest tolerable dose was 35 mg/kg/day and the identified target organs of toxicity were stomach, thyroid and site of injection. The safety of the total degradants administered with the compound in the study in dogs was ———. The estimated total amounts of degradants administered with the 35 mg/kg/day dose was ———. This was estimated to provide the safety margin of ——— to the total degradants administered with the recommended clinical dose 40 mg/day (0.8 mg/kg). This study provided the safety to the total amounts of degradation compounds. The degradation products did not contribute to the toxicity.

In the new 28-day continuous intravenous toxicity study in dogs (submitted in amendment dated April 9, 2004), slow intravenous doses of 0, 35, 86 or 170 mg/kg/day esomeprazole were administered to determine the alteration of the toxicity profile of esomeprazole in the presence of possible degradation products of esomeprazole preparation (formed in the proposed commercial preparation of esomeprazole powder used for injection/infusion 40 mg and not the dried-freeze esomeprazole powder). Two additional groups of animals given bolus iv dose of 22 and 170 mg/kg/day esomeprazole with degradation products were also included for comparison.

A treatment related non-dose proportional plasma concentrations was seen in the animals. Treatment related inflammatory changes (thrombus formation) at the infusion site, gastric foveolar epithelium hyperplasia, chief cell and parietal cell atrophy and vacuolation, inflammation, thyroid (follicular epithelial hypertrophy), thrombus formation in the lung of animals included in 86 mg/kg/day and 170 mg/ kg/day treatment groups. The incidences of histopathological and other changes of animals included in esomeprazole without and with degradation products were similar in these groups. The degradation products did not significantly affect the esomeprazole toxicity. A no-effect dose level was not established in the study and stomach, thyroid and site of injection were the target organs of toxicity. The dose of 100 umol/ kg/ day (35 mg/kg/day) was identified as a 'highest tolerable dose'.

Esomeprazole with and without degradation products was tested for its mutagenicity in Ames test and in vitro chromosomal aberration test in cultured human peripheral blood lymphocytes. It was negative in Ames test and positive in vitro chromosomal aberration test. The esomeprazole degradants (_____) were negative in Ames test.

The special toxicity studies on the vaso and tissue irritation study in dogs indicated that esomeprazole administered by intravenous/subcutaneous route produced minimal to slight bleeding and necrosis in a dose related manner. In the 10-day intravenous vascular and perivascular irritation study, esomeprazole with or without degradation products showed irritation and hemorrhage at the site of injection. Esomeprazole produced only a slight flocculation but no hemolysis in a flocculation test.

The proposed label of the compound should be modified as described in the review.



The proposed label of the compound is acceptable and sponsor may be asked to change the proposed label as suggested in the text of the review. Esomeprazole has been adequately tested in preclinical studies. Therefore, approval of the application is recommended.

**APPEARS THIS WAY
ON ORIGINAL**

RECOMMENDATION:

From preclinical standpoint, approval of the application is recommended.

Yash M. Chopra, M.D., Ph.D.,
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COMMENTS:

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cc:
Original NDA
IND 64,865
HFD-180
HFD-181/CSO
HFD-180/Dr.Chopra
HFD-180/Dr.Choudary
R/D Init.: J. Choudary 6/15/04; 6/21/04
YMC/6/24/04
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/s/

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