CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202342Orig1s000

PHARMACOLOGY REVIEW(S)

Addendum to Pharmacologist's Review Dated April 8, 2013 of NDA 20-2342 (SDN 37)

Sponsor and Address: Hanmi USA Inc.
200 Park Avenue
Florham Park, NJ 07932

Reviewer: Sruthi Tallapragada King, Ph.D.
Division of Gastroenterology and Inborn Errors Products

Drug: Esomeprazole Strontium

Category: Proton Pump Inhibitor

The following statements in the nonclinical review of NDA 20-2342 (SDN 37) for esomeprazole strontium dated April 8, 2013 are revised as follows. The revised text is highlighted in **bold**.

Review page 7 (of 85), paragraph 1:

There was evidence of a growth abnormality (physeal dysplasia in the femur) in the offspring of dams receiving treatment with EST or EMT at doses ≥ 169 mg esomeprazole/kg/day, as compared to control.

Revised text:

There was evidence of a growth abnormality (physeal dysplasia in the femur) in the offspring of dams receiving treatment with EST or EMT at doses ≥ **138** mg esomeprazole/kg/day, as compared to control.

Review page 7 (of 85), paragraph 4:

Doses at or above 140 mg esomeprazole/kg/day (as either salt) produced treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decrease femur weight and thickness) and affected overall growth and development.

Revised text:

Doses at or above 140 mg esomeprazole/kg/day (as either salt) produced treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decreased femur weight and **length**) and affected overall growth and development.

Review page 11 (of 85), paragraph 1:

In a juvenile rat toxicity study, administration of either esomeprazole strontium or esomeprazole magnesium at oral doses of salt equimolar to 140 to 280 mg esomeprazole/kg/day (34 to 57 times the daily maximum recommended human dose (MHRD) of 40 mg on a body surface area basis) produced deaths at the high dose and treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decrease femur weight and thickness) and affected overall growth and development.

Revised text:

In a juvenile rat toxicity study, administration of either esomeprazole strontium or esomeprazole magnesium at oral doses of salt equimolar to 140 to 280 mg esomeprazole/kg/day (34 to 57 times the daily maximum recommended human dose (**MRHD**) of 40 mg on a body surface area basis) produced deaths at the high dose and treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decrease femur weight and **length**) and affected overall growth and development.

Review page 13 (of 85), paragraph 4:

Doses at or above 140 mg esomeprazole/kg/day (34 times the daily MHRD of 40 mg on a body surface area basis) produced treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decrease femur weight and thickness) and affected overall growth and development.

Revised text:

Doses at or above 140 mg esomeprazole/kg/day (34 times the daily **MRHD** of 40 mg on a body surface area basis) produced treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decrease femur weight and **length**) and affected overall growth and development.

Review page 27 (of 85), paragraph 1:

Physeal dysplasia in the femur was observed at doses ≥ 169 mg esomeprazole/kg/day.

Revised Text:

Physeal dysplasia in the femur was observed at doses ≥ **138** mg esomeprazole/kg/day.

Review page 50 (of 85), paragraph 1:

Physeal dysplasia in the femur was observed at doses ≥ 169 mg esomeprazole/kg/day.

Revised Text:

Physeal dysplasia in the femur was observed at doses ≥ **138** mg esomeprazole/kg/day.

Review page 68 (of 85), paragraph 1:

Femur length and weight were affected in doses ≥ 84.7 mg/kg/day EST and at 280 mg/kg/day EMT.

Revised Text:

Femur length and weight were affected in doses ≥ 84.7 mg/kg/day EST and at **311.1** mg/kg/day EMT.

_____ _____
Sruthi Tallapragada King, PhD Date
Pharmacologist

Comments

_____ _____
Sushanta Chakder, PhD Date
Supervisory Pharmacologist

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

SRUTHI T KING
04/26/2013

SUSHANTA K CHAKDER
04/26/2013

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 20-2342
Supporting document/s:	37
Applicant's letter date:	October 29, 2012
CDER stamp date:	October 31, 2012
Product:	Esomeprazole Strontium
Indication:	Treatment of gastric acid related disorders
Applicant:	Hanmi USA Inc. 200 Park Avenue Florham Park, NJ 07932
Review Division:	Division of Gastroenterology and Inborn Errors Products
Reviewer:	Sruthi Tallapragada King, PhD
Supervisor/Team Leader:	Sushanta Chakder, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Stacy Barley, RN, MSN, MHA

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 20-2342 are owned by Hanmi USA Inc. or are data for which Hanmi USA Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 20-2342 that Hanmi USA Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 20-2342.

Reference ID: 3289165

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1 Executive Summary

1.1 Introduction

Gastric acid secretion is dependent on the H^+/K^+-ATPase pump ("proton pump"), which is located in the gastric parietal cells and is stimulated by histamine, gastrin, or acetylcholine. Excessive gastric acid secretion can manifest itself as a variety of digestive disorders including gastroesophageal reflux disease (GERD), erosive esophagitis, and gastric and duodenal ulcers. Proton pump inhibitors have been used successfully in patients suffering with digestive disorders related to excessive gastric acid secretion. In the current NDA, the applicant is seeking marketing approval of esomeprazole strontium (HM 70231) for the following indications: treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome. The drug product is formulated as 40 mg and 20 mg delayed release capsules for oral administration. This is a 505(b)(2) application, and the reference listed drug (RLD) is esomeprazole magnesium (Nexium®, AstraZeneca)

The current submission is a Class 2 resubmission (response to complete response). The applicant, Hanmi USA Inc., received a complete response (CR) in November 2011 following the first cycle review due to lack of adequate nonclinical safety data to support the use of esomeprazole strontium in pregnant and lactating women and in children under the age of 2. In order to address the deficiencies identified in the CR letter, the applicant conducted reproductive and developmental toxicology studies in rats, comparing esomeprazole strontium tetrahydrate (EST) to esomeprazole magnesium trihydrate (EMT), at equimolar concentrations of esomeprazole. Bone effects of treatment with EST and EMT were assessed in all developmental toxicology studies. Bone parameters were assessed because strontium (Sr), like calcium, is known to affect the skeletal system and published studies with dietary Sr have shown treatment-related adverse effects on bone growth in juvenile rats.

1.2 Brief Discussion of Nonclinical Findings

The applicant conducted a segment II embryo-fetal development study and two segment III pre- and postnatal developmental toxicity studies with an emphasis on bone development, in addition to standard toxicology parameters. The applicant also conducted a Segment III pre- and post-natal developmental toxicity study in animals receiving a calcium and vitamin D deficient diet. This study was conducted to address the Division's concern regarding the potential adverse effects of Sr on bone under nutrient deficient conditions. All studies were conducted in rats, per prior agreement with the Division (Please refer to Complete Response Letter issued to this applicant by the Division on November 15, 2011 and the minutes from the Type A End of Review Conference held on January 5, 2012 and the Type C meeting held on February 7, 2012).

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The results of the newly submitted nonclinical studies are briefly summarized below. The segment II embryofetal development study revealed that neither EST nor EMT was teratogenic in rats. In the pre- and postnatal developmental toxicity studies, EST and EMT shared a similar toxicity profile overall. There was evidence of a growth abnormality (physeal dysplasia in the femur) in the offspring of dams receiving treatment with EST or EMT at doses ≥ 169 mg esomeprazole/kg/day, as compared to control. Growth of offspring was negatively affected by treatment with both EST and EMT in the standard and modified diet studies, as indicated by decreases in body weight, developmental delays (attainment of sexual maturation in both sexes), decrease in femur weight and length, and decrease in thickness of cortical bone. There was also a decrease in mean tibial growth plate thickness in offspring of dams treated with EST and EMT, as compared to vehicle. A decrease in maternal femur weight was observed in animals treated with EST and EMT in both the standard and modified diet studies; however, statistically significant decreases in femur weight were observed at the two highest doses of EMT, as compared to vehicle treatment, in the standard diet study only.

In the Segment II and Segment III studies, the exposure to Sr increased in a greater than dose-proportional manner in EST-treated dams, as compared to vehicle. Fetal exposure to Sr (in plasma, bone, and liver) also increased in a dose-dependent manner, although they were lower than maternal levels. Fetal bone levels of Sr were greater than liver levels. The Sr levels (in plasma, bone, liver) in EMT and vehicle treatment groups were similar Calcium levels in the plasma and bone were not affected by treatment with EST (or EMT), as compared to control.

Exposure to esomeprazole also increased in a greater than dose-proportional manner in EST and EMT-treated dams in the embryofetal development and pre- and postnatal developmental toxicity studies, as compared to vehicle-treated dams. Esomeprazole absorption and kinetics were unaffected by the salt (Sr or Mg) or the by the diet modification in the segment III study. In the embryofetal development study, fetal plasma levels of esomeprazole were similar or higher than maternal plasma levels after dosing, indicating that fetuses were exposed to esomeprazole during gestation. However, exposure to esomeprazole in F1 offspring was lower than maternal levels on postnatal day (PND) 21 in the Segment III pre- and postnatal development studies.

The applicant conducted a 28-day juvenile rat toxicity study with 14-day recovery period with EST and EMT administered at oral doses of salts equimolar to 70, 140, and 280 mg esomeprazole/kg/day from PND 7 through PND 35. There was an increase in the number of deaths in the animals treated with 280 mg esomeprazole/kg/day (as either Sr or Mg salt). Doses at or above 140 mg esomeprazole/kg/day (as either salt) produced treatment-related decreases in body weight and body weight gain, produced changes in bone morphology (decrease femur weight and thickness) and affected overall growth and development. Partial recovery from treatment-related decrease in body weight was observed at the higher doses of EST and EMT, as compared to full recovery at the lower doses and control treatment.

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Overall, the data submitted in the current submission revealed that EST and EMT share a similar toxicity profile in rats when administered during pregnancy and lactation and have similar effects on growth and development in young animals (after in utero, lactational, or direct exposure to EST or EMT). Neither salt of esomeprazole was teratogenic. Femur length, weight, cortical thickness and tibial growth plate thickness were affected in a similar manner in offspring of dams treated with EST and EMT during pregnancy and lactation. Physeal dysplasia in the femur was identified as an adverse effect with the potential for growth abnormalities in pups born to dams treated with EST and EMT where the esomeprazole dose was $\geq$ 138 mg/kg/day.

Taken together, the applicant has adequately addressed the deficiencies in the complete response letter (dated November 15 2011) by conducting the recommended embryo-fetal development and pre- postnatal developmental toxicity studies in rats. The findings of the studies indicate that EST, although not teratogenic, does produce adverse effects to the fetus at high doses when administered throughout pregnancy and lactation. These studies also show that at equimolar doses of esomeprazole, the toxicity profile of EST and EMT are similar.

1.3 Recommendations

1.3.1 Approvability

This product is approvable for marketing for the indications proposed in adults.

1.3.2 Additional Non Clinical Recommendations

The sponsor should make the following recommended changes in labeling prior to approval.

1.3.3 Labeling

(b) (4)

5 Pages Of Draft Labeling Have Been Withheld In Full As b4 (CCI/TS) Immediately Following This Page

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2 Drug Information

2.1 Drug

CAS Registry Number: 934714-36-0

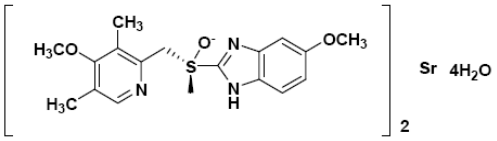
Generic Name: Esomeprazole Strontium Tetrahydrate (EST)

Code Name: HM 70231

Chemical Name: Bis(5-methoxy-2-[(S)-[4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole-1-yl) strontium tetrahydrate

Molecular Formula/Molecular Weight: $(C_{17}H_{18}N_3O_3S)_2 \cdot Sr \cdot 4H_2O$/848.5

Structure or Biochemical Description



Pharmacologic Class: Gastric parietal cell H^+/K^+-ATPase antagonist (proton pump inhibitor)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 078801 (esomeprazole strontium, Hanmi USA Inc)
NDA 21153 (Nexium®, AstraZeneca): Reference listed drug (RLD)
DMF #s [redacted] (b) (4)

2.3 Drug Formulation

The drug product (HM 70231) is formulated as 20 and 40 mg delayed release capsules. The composition of HM 70231 capsules is detailed in the applicant's tables below.

Table P.1-1 Composition of the HM 70231 Capsules 20 mg and 40 mg

Component	Unit Quantity (mg/cap)		Function	Reference to Standards
	20 mg	40 mg		
(b) (4)				
Active ingredient:				
Esomeprazole strontium tetrahydrate (as esomeprazole)	24.65 (20.0 mg)	49.3 (40.0 mg)	Active	In-house
Excipients:				
Sugar sphere (b) (4)	(b) (4)		(b) (4)	USP NF
Hypromellose (b) (4)				USP
Calcium carbonate				USP
Polysorbate 80				USP NF
Talc				USP
	(b) (4)			USP
				USP NF
Total				
	(b) (4)			
				USP
				USP
				USP
				USP NF
				USP NF
				USP NF
				USP NF
				USP NF
				USP
				USP
Total (b) (4)	89.6	179.2		
				(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

The applicant's table below shows the identified impurities.

Table P.5-12 Impurities A, C, D, E1 and E3

Name of impurity/description	Chemical name and structure	Origin of impurity
Impurity A (USP) (b) (4)		(b) (4)
Impurity C		
Impurity D		
Impurity E3		(b) (4)
Impurity E1		

The proposed limit for each identified and unidentified impurity is not more than (b) (4), which satisfies the ICH identification threshold for impurities in drug products with a maximum daily dose of ≤ 2 g/day. Therefore, from a nonclinical standpoint, there are no safety concerns associated with these impurities.

2.6 Proposed Clinical Population and Dosing Regimen

Patients with gastric acid-related diseases: for treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions

including Zollinger-Ellison syndrome. The table below summarizes the proposed clinical doses of esomeprazole strontium for the indications sought:

Indications	Age group	Dose	Frequency
GERD	Adults	20 mg, 40 mg	Once daily for 4-8 weeks
Risk reduction of NSAID-associated gastric ulcer	Adults	20 mg, 40 mg	Once daily for up to 6 months
Pathological hypersecretory conditions	Adults	40 mg	Twice daily
H. pylori eradication (triple therapy)	Adults	40 mg 1000 mg Amoxicillin 500 mg Clarithromycin	Once daily for 10 days Twice daily for 10 days Twice daily for 10 days

2.7 Regulatory Background

This submission is a Class II resubmission to a Complete Response. The applicant was given the following advice to address deficiencies in the first cycle application:

- Submit results of a segment II (embryofetal development) and an enhanced segment III (pre- and post-natal development) reproductive toxicity study in one species to demonstrate the safety of esomeprazole strontium in pregnancy and lactation.
- The studies must include esomeprazole magnesium as an active comparator and a placebo control, in addition to at least 3 dose levels of esomeprazole strontium.
- Both studies should include toxicokinetic evaluations. The dose levels must be associated with sufficient maternal plasma levels of esomeprazole (refer to ICH S5: http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html).
- The enhanced segment III study should include dosing groups fed normal and nutrient deficient (calcium and vitamin D deficient) diets to better understand the impact of nutritional changes on the development of adverse effects from esomeprazole strontium.
- The study should include direct dosing of the pups if there is an insufficient exposure to esomeprazole strontium through milk.
- The enhanced segment III study should be conducted with an emphasis on bone pathology (examination of long bone, growth plates and mineralization patterns), in addition to the standard toxicology parameters.

The applicant received additional guidance and clarification on study design from the Division prior to initiating their proposed embryofetal development study

3 Studies Submitted

3.1 Studies Reviewed

Study #	Study Title	GLP Status
(b) (4) 895003	An Oral (Gavage) Study of the Effects of Esomeprazole Strontium on Embryo/Fetal Development in Rats	GLP

Reference ID: 3289165

(b) (4) 895004	An Oral (Gavage) Study of the Effects of Esomeprazole Strontium on Pre- and Postnatal Development, Including Maternal Function in Rats Fed Standard Rodent Diet	GLP
(b) (4) 895005	An Oral (Gavage) Study of the Effects of Esomeprazole Strontium on Pre- and Postnatal Development, Including Maternal Function in Rats Fed Reduced Levels of Calcium and Vitamin D	GLP
(b) (4) 895007*	A 29-Day Oral (Gavage) Toxicity Study of Esomeprazole Strontium in Juvenile Rats, with 14-Day Recovery	GLP

* This study was conducted under IND 078801 and submitted to the Division for December 27, 2012 (SDN 27).

3.2 Studies Not Reviewed

(b) (4) 895008*	An Oral (Gavage) Range-Finding Study of Esomeprazole Magnesium in Juvenile Rats	GLP

* This study was conducted under IND 078801 and submitted to the Division for December 27, 2012 (SDN 27).

3.3 Previous Reviews Referenced

IND 078801 Esomeprazole Strontium (SDN 6, August 3, 2010)

NDA 20-2342 Nonclinical Review (SDN 1, dated June 30, 2011, November 7, 2011), Juvenile toxicity Literature Review (SDN 21, dated October 21, 2011), Pregnancy and Lactation Literature Review (SDN 24, October 17, 2011)

4 Pharmacology

No new pharmacology studies were submitted with the resubmission.

5 Pharmacokinetics/ADME/Toxicokinetics

No new PK/ADME studies were submitted with the resubmission. Review of toxicokinetics (TK) data is included with the associated toxicity study.

6 General Toxicology

No new single or repeat-dose toxicity studies were submitted in this review cycle. Please refer to the NDA nonclinical review dated June 30, 2011 for a review of previously submitted general toxicology study reports.

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7 Genetic Toxicology

No genetic toxicity studies were submitted in this review cycle.

8 Carcinogenicity

No carcinogenicity studies were conducted in support of this submission.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No Segment I studies were conducted.

9.2 Embryonic Fetal Development

Study title: An oral (gavage) study of effects esomeprazole strontium on embryo/fetal development in rats

Study no.:	(b) (4) 895003
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 17, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Esomeprazole Strontium (EST), Batch # 10SOSHB21008, 99.5% purity Esomeprazole Magnesium (EMT), Batch # EZFP11030, 99.4% purity

Key Study Findings

In utero exposure to EST and EMT was not teratogenic in rats. The no observed adverse effect level (NOAEL) for maternal toxicity was 84.7 mg/kg/day for EST and 15.6 mg/kg/day for EMT, based on decreases in body weight during gestation. The NOAEL dose for embryo/fetal developmental toxicity was the high dose for both EST and EMT (343.8 and 311.1 mg/kg/day, respectively). The equimolar dose of esomeprazole at the NOAEL of embryofetal developmental toxicity was 280 mg/kg/day.

Methods

Doses:	0 (vehicle control) 17.2, 84.7, 343,8 mg/kg/day EST 15.6, 76.7, 311 mg/kg/day EMT
Frequency of dosing:	Once daily

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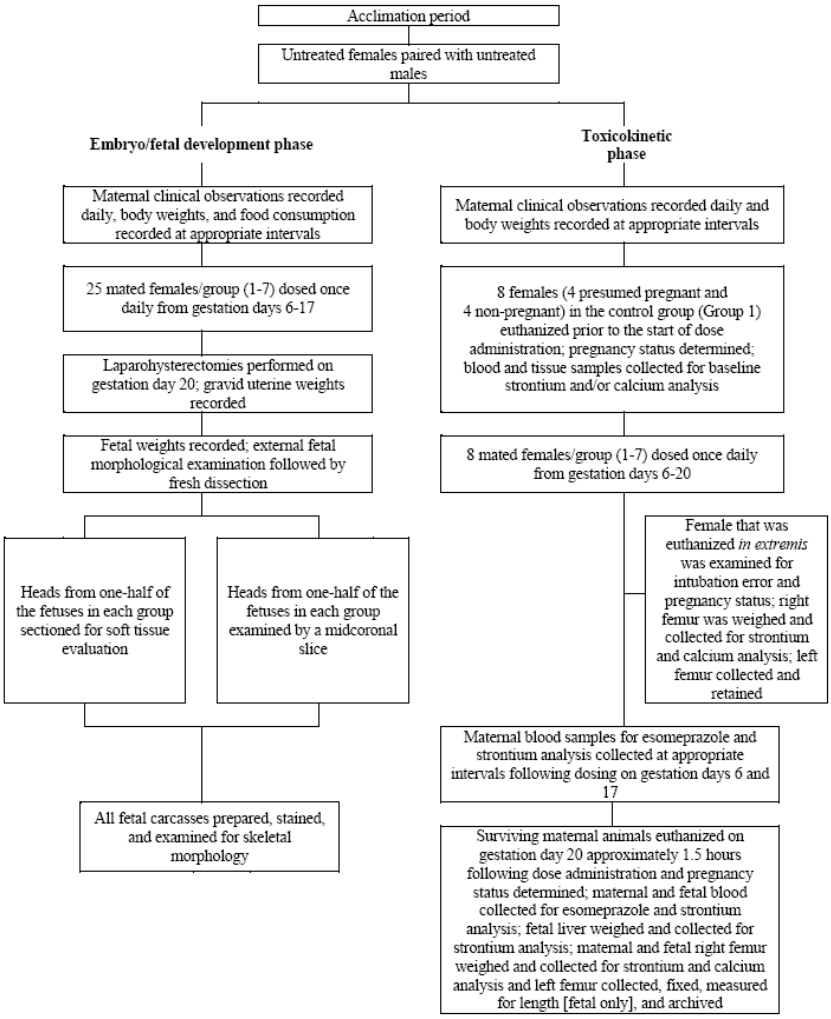
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension/ Methylcellulose, 1500 cps, USP (0.5%)
Species/Strain:	Rats/Sprague-Dawley (Crl:CD(SD)]
Number/Sex/Group:	25 females/dose group
Satellite groups:	Yes, for toxicokinetics (TK): 16 females/vehicle control and 8 females/dose group for all remaining groups
Study design:	See the applicant's flow chart below for study design details
Deviation from study protocol:	None that affected the integrity of the study results



Observations and Results

Mortality

Rats were observed twice daily for mortality and moribundity from gestation day (GD) 0 through 20 (prior to dose administration during the treatment period).

There were no unscheduled deaths (related to treatment) in the main study animals during the dosing period. In the TK group, one control animal was euthanized on GD 14 due to excessive body weight loss (17.8% of GD 6 body weight). All other TK animals survived to GD 20 laparohysterectomy.

Clinical Signs

Clinical observations were recorded daily from GD 0 through 20 (prior to dose administration during the treatment period) for main study and TK animals. Presence of clinical signs at time of dosing was also recorded.

Treatment-related clinical signs included low incidences of clear or red material (wet or dried) around the mouth, particularly in main study animals at the high dose (HD). There were 4 incidences of salivation prior to dosing in 4 main study HD ES animals. Overall, survival and clinical signs were similar at equimolar doses of esomeprazole between the EST and EMT dose groups.

Body Weight

In main study animals, maternal body weight was noted on GD 0, daily on GD 6-18, and GD 20. In the TK animals, maternal body weights were recorded on GD 0 and daily on GD 6-20. Gravid uterine weights were also determined on GD 20.

There was a statistically significant decrease in body weight (up to 5.4%) at the high dose of EST, as compared to control. Body weight decreases of up to 5.2% and 8% were observed at the mid and high doses of EMT, respectively, as compared to control (statistically significant only at the high dose of EMT). Between GD 6-9, dams in the high dose group of EST and EMT had statistically lower mean body weight gains, as compared to control. Lower mean body weight gains were observed in high dose EST dams, as compared to controls, between GD 9-12 and GD 12-18. Similarly, lower mean body weight gains were observed at the mid and high dose in EMT-treated dams between GD9-12 and GD 12-18, as compared to control.

Mean terminal body weight was 4.7% and 6.9% lower in dams treated with EMT at the mid and high doses, as compared to control. Gravid uterine weights (mean values) were lower as well: 12.3% lower in high dose EST-treated dams and 12.9% and 15.2% lower in mid- and high dose-treated EMT dams, respectively, as compared to controls. There was a 4.7% decrease in net body weight in high dose EMT dams and a 17.8% decrease in net body weight change in these animals, as compared to controls.

Feed Consumption

Food consumption was recorded on GD 0, GD 6-18 (daily), and on GD 20.

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Food consumption was comparable between EST and EMT treatment groups, with high dose dams showing greater decreases in mean food consumption, as compared to controls. At the high dose, both EST and EMT dams had statistically significant reduced food consumption between GD 6-9; however, statistically significant decreases in mean food consumption were also recorded in high dose EST females between GD 9-12 and GD 12-18, as compared to controls.

Toxicokinetics

For baseline Sr analysis, blood samples were collected on GD 6 for 4 mated control females (prior to dose initiation) and on the corresponding date for 4 non-pregnant control females. For Sr analysis and TK analysis of exposure to esomeprazole from treated animals, blood samples were collected on GD 6, and 17 at pre-dose, 0.5, 1, 2, 6, and 24 h post dose administration from 4 females/dose group. No rat was bled more than 3 times in a 24-h period. Maternal and fetal blood samples (4/dose group, 4 maternal and 4 corresponding fetal) were also collected on GD 20 for Sr analysis and TK evaluation (esomeprazole) at 1.5 h post dose. Fetal blood was collected via the umbilical vein and pooled by litter.

Sr analysis:
Strontium levels were determined in maternal and fetal plasma, maternal and fetal femur, and fetal liver on GD 20. Strontium levels in the plasma, femur and liver of EMT-treated animals were similar to those in control animals. Likewise, calcium levels in the femur were unaffected by administration of EST or EMT in both mothers and pups and were similar to control. There was a dose-dependent increase in Sr concentration in plasma, femur and liver of fetuses of EST-treated dams. Maternal plasma and femur levels of Sr were higher than fetal levels. These data are summarized in the table below:

Table 1: Sr exposure assessment in maternal and fetal samples in embryofetal development Study (b) (4) 895003

			EST (mg/kg/day)			EMT (mg/kg/day)		
		Control	**17.2**	**84.7**	**343.8**	**15.6**	**76.7**	**311.1**
Dose of Esomeprazole (mg/kg/day)		**0**	**14**	**69**	**280**	**14**	**69**	**280**
Strontium								
Maternal	Plasma (ng/mL)	29.3	495.8	1573.5	4742.5	58.7	31.6	38.5
	Femur (μg/g)	39.6	72.6	250.9	1011.5	36.2	36.9	36.5
Fetal	Plasma (ng/mL)	47.95	396.00	1909.75	5230.00	33.03	35.93	34.73
	Femur (μg/g)	11.02	29.29	98.12	515.17	9.79	6.13	12.65
	Liver (ng/g)	5.33	60.33	235.06	1009.98	9.60	9.85	9.73
Calcium								
Maternal	Femur (mg/g)	149	142	141	143	139	137	132
Fetal	Femur (mg/g)	60.72	51.66	64.77	48.61	53.82	36.99	61.81

Esomeprazole analysis:
There was a dose-dependent increase in exposure to esomeprazole with EST and EMT on both GD 6 and GD 17. Accumulation of esomeprazole in the plasma was observed

on GD 17. T_{max} was similar across all treatment groups (0.5 h) on both GD 6 and GD 17. The plasma half life ($t_{1/2}$) ranged from 1.4-1.9 h for EST and 1.1-4.1 h for EMT on GD 6 and 0.9-1.8 h for EST and 1.1-2.1 h for EMT on GD 17 (as shown in the sponsor's table below).

Table 2.4-3: Esomeprazole pharmacokinetic parameters following oral administration of EST and EMT to pregnant rats during an embryo/fetal development study (GD 6, 17, and 20)

	Esomeprazole Strontium (EST)			Esomeprazole Magnesium (EMT)		
Dosage (mg/kg)	**17.2**	**84.7**	**343.8**	**15.6**	**76.7**	**311.1**
Dosage (mg/kg as esomeprazole base)	**14**	**69**	**280**	**14**	**69**	**280**
	Gestation Day 6					
AUC_{last} (ng·h/ml) ±SE	57.4 ±7.01	891 ±161	25400 ±10500	53.7 ±11.5	1380 ±345	27600 ±6850
C_{max} (ng/ml) ±SD	43.9 ±2.78	822 ±292	16300 ±23100	49.9 ±20.0	859 ±284	9520 ±7170
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5
$T_{1/2}$ (hr)	1.4	1.9	NR	1.1	4.1	NR
EST/EMT Ratio	0.969	0.585	0.833	NA	NA	NA
	Gestation Day 17					
AUC_{last} (ng·h/ml) ±SE	201 ±29.1	3160 ±731	70200 ±20800	185 ±51.6	5860 ±1920	62400 ±26100
C_{max} (ng/ml) ±SD	146 ±42.0	2000 ±1100	27800 ±10500	222 ±177	2490 ±1750	18100 ±15000
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5
$T_{1/2}$ (hr)	0.9	NR	1.8	1.1	1.1	2.1
EST/EMT Ratio	0.985	0.488	1.02	NA	NA	NA
Accumulation Ratio	3.50	3.55	2.76	3.45	4.25	2.26
	Gestation Day 20					
Maternal/Fetal Ratio	0.137	0.227	0.774	0.223	0.213	1.04

AUC_{last} = area under the curve from time of dosing to the last observation that was >LLOQ; EST/EMT Ratio and Accumulation Ratio calculated using AUC_{last}; Maternal/Fetal Ratio calculated using concentrations at 1.5 hours post-dosing. NA = not applicable. NR = not reportable (failed to meet acceptance criteria). LLOQ = lower limit of quantitation. Data are represented by means ±SE (for AUC) or ±SD (for C_{max}).

Fetal plasma levels of esomeprazole were measured on GD 20 (at laparohysterectomy) at 1.5 h post-dose. These data are shown in the table below, along with maternal plasma concentrations at the same time point (Mean ± SD are shown). At 1.5 h post-dose on GD 20, the fetal plasma levels of esomeprazole were generally higher (particularly at the low and mid dose) than the maternal levels. This is reflected in the maternal: fetal concentration ratio.

Table 2: Fetal Esomeprazole concentration in embryofetal development Study (b) (4) 895003

	EST (mg/kg/day)			EMT (mg/kg/day)		
Dose used	17.2	84.7	343.8	15.6	76.7	311.1
Esomeprazole (mg/kg/day)	14	69	280	14	69	280
Fetal	583 ± 269	2960 ± 1850	16300 ± 3460	318 ± 115	3640 ± 2570	16800 ± 5990
Maternal	84.5 ± 53.0	823 ± 909	12800 ± 3720	41.3 ± 20.9	788 ± 590	17900 ± 9540
Maternal/Fetal Ratio	0.137 ± 0.0379	0.227 ± 0.110	0.774 ± 0.0848	0.125 ± 0.0262	0.213 ± 0.0256	1.04 ± 0.266

Dosing Solution Analysis

The concentration determination of EST and EMT in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

Necropsy

Females were euthanized on GD 20 and the contents of the thoracic, abdominal, and pelvic cavities were examined. At euthanasia, the right femur was collected and weighed (after removal of muscle tissue). The left femur, including the proximal tibial epiphysis, was collected, preserved in formalin and the length was recorded. The liver and right femur from 6 fetuses/litter (3 from each horn, if possible) were collected and weighed.

At necropsy, no significant treatment-related findings were observed in either the ES or EM dams.

Organ Weights: The table below summarizes the changes in gravid uterine weights and femur weights of dams on GD 20. There were statistically significant decreases in gravid uterine weight at the high dose in the EST treatment group (12.3%) and at the mid and high dose groups (12.9% and 15.2%, respectively) in the EMT treatment group, as compared to control.

Table 3: Changes in organ weights in maternal animals in Study (b) (4) 895003

		EST (mg/kg/day)			EMT (mg/kg/day)		
Weight (g)	Control	17.2	84.7	343.8	15.6	76.7	311.1
Terminal body	421	416	416	404	421	401	392
Gravid uterus	92.9	89.9	87.1	81.5**	87.8	80.9**	78.8**
Femur	0.8559	0.8570	0.8571	0.8266	0.8393	0.8268	0.8180

** p<0.01 as compared to control

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Laparohysterectomies and macroscopic examinations were performed in a treatment-blinded fashion. The following were recorded: number of corpora lutea on each ovary, weight of trimmed uterus, number and location of all fetuses in the uterus, number of early and late resorptions, and the total number of implantation sites (numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal uterine horn). The placentae were also examined. Group Mean Litter Basis and Proportional Litter Basis were calculated.

Reference ID: 3289165

There were no significant treatment-related differences in the laparohysterectomy parameters evaluated on GD 20 between the EST and EMT dosing groups, as compared to control. Treatment with EST or EMT did not affect number of gravid females, litter size, or sex ratio. The GD 20 laparohysterectomy data are summarized in the applicant's table below (shown as a % of litters affected):

	EST				EMT		
Daily Dose (mg/kg)	0	17.2	84.7	343.8	15.6	76.7	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	280	14	69	280
No. Litters evaluated	25	25	25	25	25	25	24
No. Live Fetuses (%)	95.0	94.3	93.3	96.6	92.5	93.5	94.3
Mean No. Early Resorptions (%)	4.3	5.5	6.7	3.4	6.9	6.5	5.7
Mean No. Late Resorptions (%)	0.7	0.2	0.0	0.0	0.5	0.0	0.0
No. of Litters with Dead Fetuses	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean % Post-Implantation Loss	5.0	5.7	6.7	3.4	7.5	6.5	5.7
Mean Fetal Body Weight (g)- males	3.8	3.7	3.8	3.7	3.8	3.8	3.8
Mean Fetal Body Weight (g)- females	3.6	3.6	3.6	3.5	3.6	3.6	3.5
Sex Distribution							
% Males	49.8	50.8	48.9	53.0	51.5	50.5	48.6
% Females	50.2	49.2	51.1	47.0	48.5	49.5	51.4

* $p \leq 0.05$, as compared to control

Offspring (Malformations, Variations, etc.)

Fetal examinations were conducted in a treatment-blinded fashion. Viable fetuses were examined externally and individually sexed, weighed, euthanized, and tagged for identification. Each fetus underwent external and visceral examination. For late resorptions, the degree of autolysis was determined, a gross external examination was performed (if possible), and the tissues were discarded. Fetuses were then examined for skeletal malformations and developmental variations. External, visceral, and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

There were no significant treatment-related effects on the incidence or number of malformations and variations observed in the EST and EMT dosing groups, as compared to control. The applicant's tables below summarize the malformations observed in the offspring after *in utero* exposure to EST, EMT or vehicle.

DOSE GROUP:	FETUSES 1	2	3	4	5	6	7	LITTERS 1	2	3	4	5	6	7
NUMBER EXAMINED EXTERNALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
POLYDACTYLY	0	0	0	0	0	3	0	0	0	0	0	0	1	0
SYNDACTYLY	0	0	0	0	0	1	0	0	0	0	0	0	1	0
LOCALIZED FETAL EDEMA	0	1	0	0	0	0	0	0	1	0	0	0	0	0
MICROPHTHALMIA AND/OR-ANOPHTHALMIA	1	1	0	0	0	0	0	1	1	0	0	0	0	0
BENT TAIL	1	0	0	0	0	0	0	1	0	0	0	0	0	0
FETAL ANASARCA	1	0	0	0	0	0	0	1	0	0	0	0	0	0
MANDIBULAR MICROGNATHIA	1	0	0	0	0	0	0	1	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
LUNGS- LOBULAR DYSGENESIS	1	0	0	0	0	0	0	1	0	0	0	0	0	0
HYDROCEPHALY	0	0	0	1	0	0	0	0	0	0	1	0	0	0
NUMBER EXAMINED SKELETALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
RIB ANOMALY	1	0	1	0	0	0	0	1	0	1	0	0	0	0
VERTEBRAL AGENESIS	0	0	1	0	0	0	0	0	0	1	0	0	0	0
ILIUM BENT	0	0	0	0	0	1	0	0	0	0	0	0	1	0
BENT LIMB BONE(S)	0	0	0	0	0	1	0	0	0	0	0	0	1	0
STERNEBRA(E) MALALIGNED-(SEVERE)	1	0	0	1	0	0	0	1	0	0	1	0	0	0
VERTEBRAL ANOMALY WITH OR-WITHOUT ASSOCIATED RIB ANOMALY	2	1	0	0	0	0	0	2	1	0	0	0	0	0
TOTAL NUMBER WITH MALFORMATIONS														
EXTERNAL :	2	2	0	0	0	3	0	2	2	0	0	0	1	0
SOFT TISSUE :	1	0	0	1	0	0	0	1	0	0	1	0	0	0
SKELETAL :	3	1	2	1	0	2	0	3	1	1	1	0	1	0
COMBINED :	4	3	2	2	0	4	0	4	3	1	2	0	1	0

1- 0 MKD 2- 17.2 MKD TEST 3- 84.7 MKD TEST 4- 343.8 MKD TEST 5- 15.6 MKD REF 6- 76.7 MKD REF
7- 311.1 MKD REF

The applicant's tables below summarize the variation observed in offspring after *in utero* exposure to EST, EMT or control.

DOSE GROUP:	FETUSES 1	2	3	4	5	6	7	LITTERS 1	2	3	4	5	6	7
NUMBER EXAMINED EXTERNALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
RENAL PAPILLA(E) NOT DEVELOPED-AND/OR DISTENDED URETER(S)	0	1	9	0	1	1	2	0	1	4	0	1	1	2
LIVER- ACCESSORY LOBULE(S)	0	4	0	0	0	1	0	0	2	0	0	0	1	0
MAJOR BLOOD VESSEL VARIATION	1	0	1	0	0	0	0	1	0	1	0	0	0	0
SPLEEN- PALE	0	0	0	0	0	0	1	0	0	0	0	0	0	1
NUMBER EXAMINED SKELETALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
14TH RUDIMENTARY RIB(S)	27	32	14	12	21	31	15	13	12	8	10	9	12	7
STERNEBRA(E) #5 AND/OR-#6 UNOSSIFIED	15	28	17	21	22	16	21	9	12	12	10	9	7	10
7TH CERVICAL RIB(S)	1	3	4	5	1	1	2	1	2	3	5	1	1	2
HYOID UNOSSIFIED	3	7	3	0	8	0	0	2	5	2	0	5	0	0
CERVICAL CENTRUM #1 OSSIFIED	75	60	74	67	60	76	67	19	19	20	20	17	19	17
STERNEBRA(E) MALALIGNED(SLIGHT-OR MODERATE)	4	3	2	3	1	1	1	2	3	2	3	1	1	1
REDUCED OSSIFICATION-OF THE 13TH RIB(S)	5	4	4	3	1	2	0	5	3	3	3	1	1	0
STERNEBRA(E) #1,#2,#3-AND/OR #4 UNOSSIFIED	1	2	1	1	0	0	0	1	2	1	1	0	0	0
REDUCED OSSIFICATION-OF THE VERTEBRAL ARCHES	2	4	1	0	0	1	0	2	4	1	0	0	1	0
PUBIS UNOSSIFIED	0	0	3	1	0	0	0	0	0	2	1	0	0	0
REDUCED OSSIFICATION-OF THE SKULL	0	0	1	1	1	0	0	0	0	1	1	1	0	0
NUMBER EXAMINED SKELETALLY	394	389	375	364	375	353	337	25	25	25	25	25	25	24
BENT RIB(S)	10	1	0	0	0	0	0	3	1	0	0	0	0	0
27 PRESACRAL VERTEBRAE	1	1	0	0	0	0	0	1	1	0	0	0	0	0
25 PRESACRAL VERTEBRAE	1	0	0	0	0	0	0	1	0	0	0	0	0	0
ACCESSORY SKULL BONE(S)	0	0	1	0	0	0	0	0	0	1	0	0	0	0
METACARPAL(S) AND/OR-METATARSAL(S) UNOSSIFIED	0	0	0	3	0	1	0	0	0	0	1	0	1	0
VERTEBRAL CENTRA UNOSSIFIED	1	0	0	0	0	0	0	1	0	0	0	0	0	0

1- 0 MKD 2- 17.2 MKD TEST 3- 84.7 MKD TEST 4- 343.8 MKD TEST 5- 15.6 MKD REF 6- 76.7 MKD REF
7- 311.1 MKD REF

Therefore, *in utero* exposure to EST and EMT did not produce teratogenic effects on the developing fetus when administered to pregnant rats from GD 6 through GD 20.

9.3 Prenatal and Postnatal Development

Study title: An Oral (Gavage) Study of the Effects of Esomeprazole Strontium on Pre- and Postnatal Development, Including Maternal Function in Rats Fed Standard Rodent Diet

Study no.:	(b) (4) 895004
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	• Esomeprazole Strontium (EST), Lot # 10SOSHB21008, 99.5% purity • Esomeprazole Magnesium (EMT), Lot # EZFP11030, 99.4% purity

Key Study Findings

The NOAEL dose for maternal toxicity was 84.7 and 76.7 mg/kg/day for EST and EMT, respectively (69 mg esomeprazole/kg/day), based on a decrease in body weight gain and changes in clinical chemistry and bone morphometry at doses > 69 mg esomeprazole/kg/day. The NOAEL doses for F_1 neonatal/early postnatal toxicity and post-selection toxicity were 17.2 mg/kg/day for EST and 15.6 mg/kg/day for EMT (14 mg esomeprazole/kg/day), based on pup survival. The NOAEL doses for developmental and neurobehavioral toxicity were 84.7 and 76.7 mg/kg/day for EST and EMT, respectively, (69 mg esomeprazole/kg/day), based on delays in sexual maturation and decreased learning and memory behaviors, which may have been secondary to decreased body weights observed at doses > 69 mg esomeprazole/kg/day. The NOAEL dose for bone effects in the F_1 generation was the low dose for both salts (17.2 and 15.6 mg/kg/day for EST and EMT, respectively; equimolar dosage of esomeprazole = 14 mg/kg/day). At doses > 14 mg esomeprazole/kg/day, femur length, cortical thickness and width were affected in both treatment groups. Physeal dysplasia in the femur was observed at doses ≥ 169 mg esomeprazole/kg/day. Adverse effects on maternal bone (decrease in femur weight) were observed in both EST- and EMT-treated dams, with statistically significant decreases at 153.3 and 311.1 mg/kg/day EMT, as compared to control.

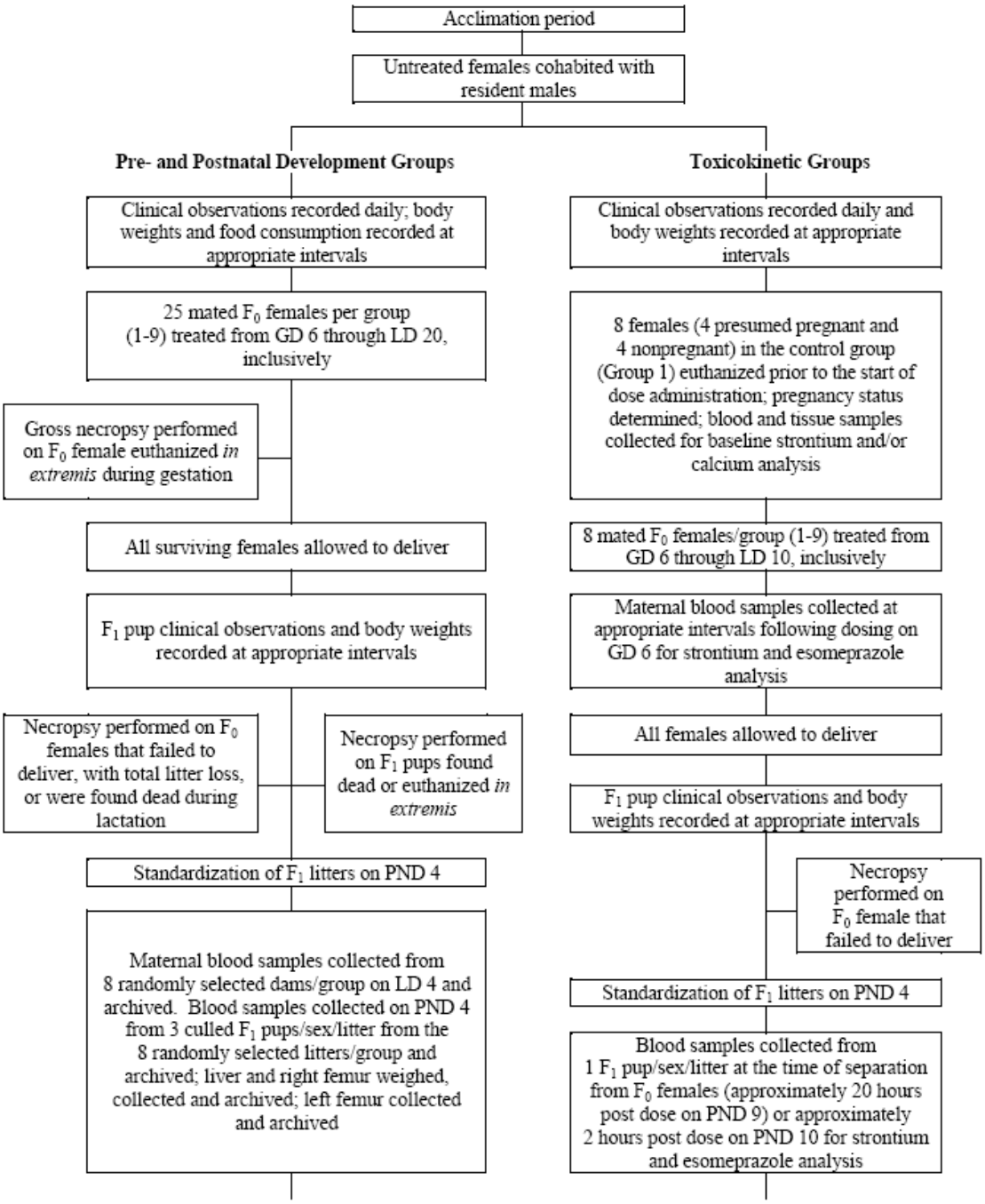
Reference ID: 3289165

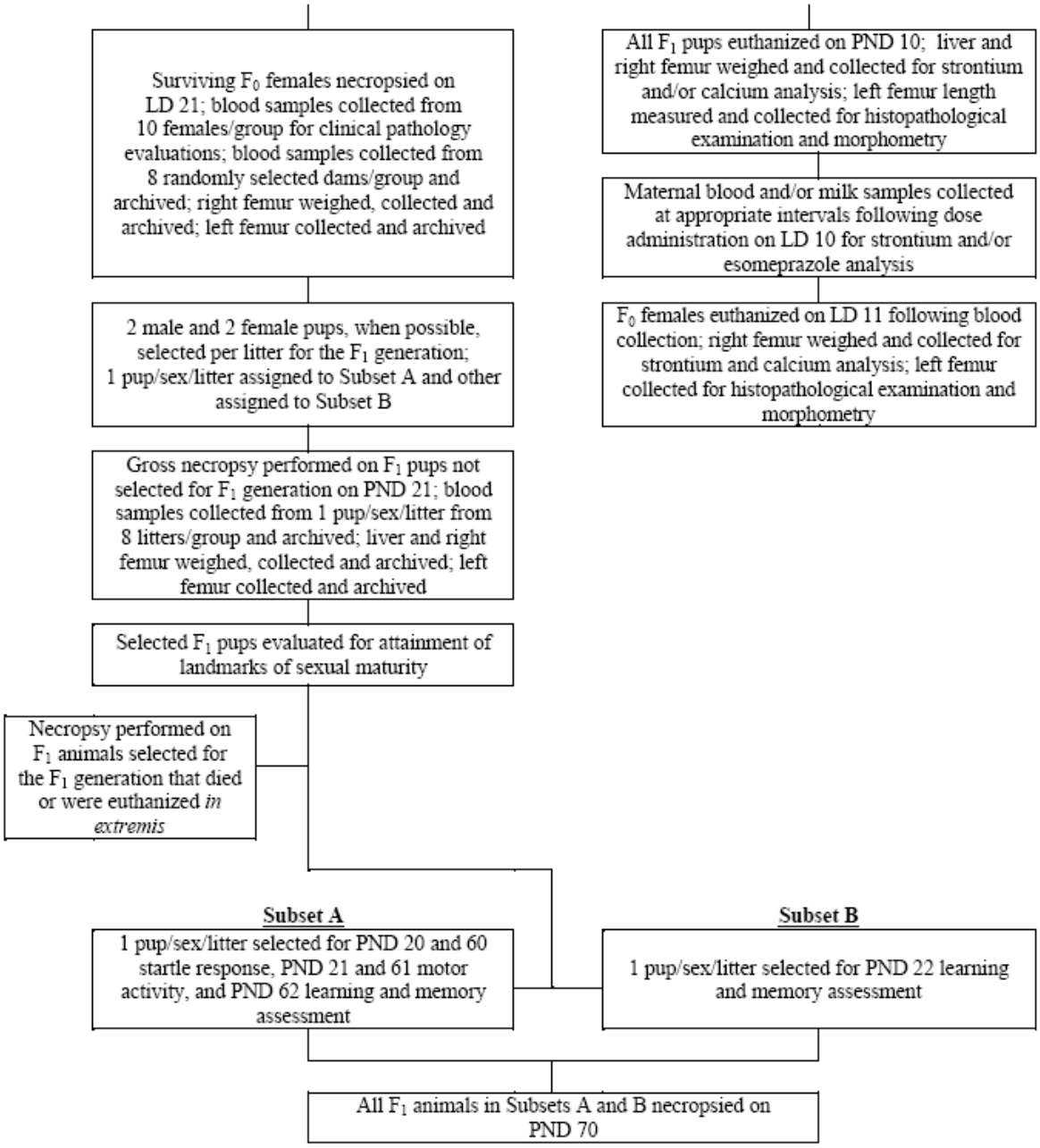
Methods

Doses:	0 (Vehicle Control) ES: 17.2, 84.7, 169.5, 343.8 mg/kg/day EM: 15.6, 76.7, 153.3, 311.1 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension/0.5% methylcellulose (1500 cps)
Species/Strain:	Rat/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	Main Study: 25 females/dose group TK: 16 females (control), 8 females/all other dose group
Satellite groups:	Yes for TK analysis (esomeprazole) and analysis of baseline Sr and Ca
Study design:	See applicant's study design chart below.
Deviation from study protocol:	None which affected study results

Reference ID: 3289165

3. STUDY DESIGN





Observations

F_0 Dams

Survival:	During the treatment period, all rats were observed twice daily for moribundity and mortality prior to dosing and 1 h post-dose during the treatment period.
Clinical signs:	During the treatment period, all rats were observed twice daily prior to dosing and 1 h post-dose during the treatment period.
Body weight:	Individual maternal body weights were measured on gestation days (GD) 0, 6, 9, 12, 15, 18, and 20 and on lactation days (LD) 0 and 1, 4, 7, 10, 14, 17, and 21 for main study animals and on LD 0 (when possible) and 1, 4, 7, and 10 for TK animals.
Feed consumption:	For main study animals, individual maternal food consumption was recorded on GD 0, 6, 9, 12, 15, 18, and 20 and on LD 1, 4, 7, 10, 14, 17, and 21. Food consumption was not recorded for the TK animals.
Uterine content:	Main study pregnant females were allowed to deliver naturally and rear their pups until PND 21 when they were euthanized. TK animals were allowed to deliver naturally and rear their young until PND 11, at which time they were euthanized.
Necropsy observation:	All F0 females (including those which died or were euthanized prior to scheduled sacrifice) underwent a detailed necropsy. The pregnancy status, number and location of corpora lutea and implantation sites were recorded. Uteri were removed and processed for determining implantation loss. Gross lesions were processed for histopathology.
Toxicokinetics:	• Maternal blood samples for Sr and esomeprazole analysis were collected on LD 4 and LD 21 (from 8 randomly selected F0 females) and archived. Blood sampling was done on GD 6, LD10 at predose, and 0.5, 1, 2, 6, and 24 h post-dose from 4 TK females/dose group. Milk collection (for Sr analysis) was done at 1.5 h post-dose on LD 10 from F0 females (which were separated from their pups for 5.5 h prior to milk collection). • For Sr analysis in tissues, right femur from F0 females were collected and weighed on LD 10 The left femur, including the proximal tibial epiphysis, was collected and preserved on LD 21. • Baseline Sr and Ca analysis was conducted on

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blood samples collected on GD 6 from 4 mated control females and on a corresponding date from 4 nonpregnant females. Females that did not deliver were euthanized on GD 25 and necropsied as well.

Dosing Solution Analysis: The concentration determination of EST and EMT in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

Other:

- Blood samples were collected and analyzed for hematology and clinical chemistry.
- Microscopic examination of the left femur was conducted.
- Bone morphometry analysis (tibial growth plate) was performed on all F0 females in control and high dose EST and EMT groups.

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F_1 Generation

Survival:
- Each litter was examined daily. Litter size was noted daily.
- Litters were culled on PND 4 to 2/sex/litter (if possible).
- Selected pups were monitored daily for changes in survival and euthanized on PND 70.

Clinical signs:
- Animals were observed daily for survival and changes in appearance and behavior. A detailed physical examination was performed on PND 1, 4, 7, 10, 14, 17, and 21. Each pup in the TK group received a detailed physical examination on PND 1, 4, 7, and 10. Nursing behavior abnormalities were also recorded.
- TK group pups were individually sexed on PND 0, 4, and 10 and the main study pups were sexed on PND 0, 4, 14, and 21.
- After weaning on PND 21, pups were observed daily and detailed physical examinations were conducted every week until necropsy on PND 70.

Body weight:
- Individual weights of pups were recorded on PND 1, 4, 7, 10, 14, 17, and 21 in the Main Study groups and on PND 1, 4, 7, and 10 for the TK groups.
- After weaning, body weights were recorded twice weekly until necropsy.

Physical development: 1 pup/sex/litter was randomly selected for evaluation of attainment of developmental landmarks. Individual body weights were recorded on the day of attainment of balanopreputial separation (BPS) in males or vaginal patency (VP) in females. Two pups/litter were assessed for each parameter.

Neurological assessment: 1 pup/sex/litter was randomly selected for neurobehavioral assessment. Auditory startle was assessed on PND 20 and 60 (same groups of pups were used for assessment on both days). Locomotors activity was assessed on PND 21 and 61 (same groups of pups were used for assessment on both days). Learning and memory were assessed on PND 22 and PND 62 (different pups were used on each day).

Reproduction: F_1 pups were not mated, per previous agreement with the Division.

Other: Exposure assessment and TK analysis:
- Blood samples were collected on PND 4 and

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PND 21 from 8 litters/group of corresponding dams (which were previously sampled on LD 4 and LD 21) and archived.

- Tissue (liver, right and left femur) were collected from pups on PND 4 and from nonselected pups on PND 21 (4 pups/litter) and processed for further analysis.
- Blood was collected from 1 pup/sex/litter on PND 10 (TK group).
- TK F_1 pups were euthanized on PND 10 and the liver, right and left femur were harvested.

Anatomic Pathology

- Left femur length and right femur weight were determined.
- The tibial growth plate was measured (morphometric analysis), along with microscopic examination of the left femur and bone marrow.

Results

F_0 Dams

1. Survival: One high dose (HD) EST female was found dead on LD 3. Clinical observations included pale body and red material around mouth on the day before its death and necropsy showed clear fluid in the thoracic cavity. Dosing trauma was likely cause of death. One HD EMT female was euthanized *in extremis* on GD 7 after observations of completely shut eyelids, cool and prostrate body, decreased respiration and hypoactivity 1 h post-dose, suggesting a potential treatment-related effect. This female had an entirely resorbed litter (*in utero*). All other females survived until scheduled necropsy. One female each treated with 169.5 and 343.8 mg/kg/day EST had total litter loss on LD 0 or LD 4

2. Clinical Signs: Dried red material around the mouth was observed in a dose-dependent manner in all EST-treated animals and in the 76.7, 153.3 and 311.1 mg/kg/day EMT-treated animals. Observations of wet/dry clear material and/or wet red material around mouth and salivation were comparable between the EST and EMT treatment groups, as compared to control. Lacrimation was also observed in 1-2 animals at the high dose only in both EST and EMT treatment groups. These observations were recorded 1 h post-dose. Overall, observed clinical signs were treatment-related and dose-dependent, particularly in all dose groups of EMT and at the two highest doses of EST.

3. Body Weight: There was a decrease in body weight in high dose EST females beginning on GD 9 and continuing through GD 20 (< 4%), while a slight decrease (< 2%) in body weight was observed in high dose EMT females on GD 15 and

GD 20, when compared to control females. There was a statistically significant decrease (42%, p < 0.01, as compared to control) in body weight gain in high dose EST females between GD 6 to 9. There was a decreasing trend in body weight gain in EMT-treated animals during this interval as well; however, these reductions did not reach statistical significance. During lactation, the overall change in body weight per week was generally similar between EST and EMT-treated animals. When compared over specific study intervals, there was a dose-dependent decrease in body weight gain in EST females, with a statistically significant decrease (53%, p < 0.05 as compared to control) at the high dose during LD 1-LD 4. During the same interval, EMT-treated animals had a 20-33% decrease in body weight gain, although it was not statistically significant compared to control. Decreased body weight gain was also observed during LD 14-17 and LD 17-21. The body weight data are summarized in the applicant's table below.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Gestation Body Weight Gain (g)									
Gestational Days 0-6	24	27	26	26	28	26	26	25	27
Gestational Days 6-9	5	10	8	2	1	7	6	5	3
Gestational Days 9-12	19	18	18	17	11**	15	16	18	13
Gestational Days 12-15	16	19	18	20	15	18	16	17	15
Gestational Days 15-18	35	37	38	35	34	37	36	38	38
Gestational Days 18-20	27	31	28	27	23	28	27	27	25
Gestational Days 6-20	102	114	109	101	85*	105	102	104	94
Lactation Body Weight Gain (g)									
Lactational Days 1-4	15	17	9	8	7*	11	10	12	10
Lactational Days 4-7	9	5	15	12	13	11	12	11	10
Lactational Days 7-10	10	11	9	7	8	10	10	12	9
Lactational Days 10-14	5	8	7	13	7	11	8	4	9
Lactational Days 14-17	8	3	7	3	9	0	-2*	5	4
Lactational Days 17-21	-4	-7	-4	-6	-3	-8	-1	-7	-1
Lactational Days 1-21	43	38	44	38	41	35	37	36	41

* p < 0.05, **p < 0.01, as compared to control

4. Feed Consumption: There was a decrease in food consumption (9%, as compared to control) in high dose EST animals during gestation (GD 6-20). Food consumption in EMT-treated animals was similar to controls during gestation. There was a statistically significant decrease at the high dose in both EST- and EMT-treated animals (17.6% and 9.8%, respectively, as compared to control) between LD 1-21. There was an increase (statistically significant) in food

consumption at the lowest dose of EST and food consumption in all other treatment groups (EST and EMT) was comparable.

5. Gestation Length and Parturition: Gestation lengths were generally longer in EST- and EMT-treated animals, with statistically significant increases in certain dose groups, as compared to control. However, the increase in gestation length was not directly related to dose in either the EST or the EMT treatment group. The increase in gestation length did not affect parturition. The applicant's table below summarizes these data (*p < 0.05, **p < 0.01, as compared to control).

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Mean Duration of Gestation (Days)	21.4	21.8*	21.9**	21.7	22.1**	21.8*	21.8*	21.7	22.0**
Abnormal Parturition	None	None	None	None	None	None	None	None	None

6. Uterine Content: No treatment-related findings were recorded at the scheduled necropsy. There was no difference in the number of implantation sites, number born, or the number of unaccounted sites between EST- and EMT-treated animals, as compared to controls.

7. Necropsy Observation: One high dose EST female was gravid at time of scheduled necropsy and one low dose EST had a depressed area in the kidney. One 153.3 mg/kg/day EMT-treated female had dark red contents in the stomach. One 76.7 mg/kg/day EMT-treated female had a mass in the mammary gland. There were no other macroscopic findings. Femur weights (right femur) were measured and no treatment-related effects were noted.

8. Exposure Assessment:
    a. Baseline Sr and Ca analysis: Sr levels at the implantation sites, uterus and femur were measured in pregnant and nonpregnant control females. Calcium levels were measured in the femur of control pregnant and nonpregnant females. Sr levels were high at the implantation site and uterus in pregnant females. Sr content of the nonpregnant uterus was ~4x lower than that of the pregnant uterus; however, there is high variability (very high standard deviation). The Sr and Ca levels in the femur were similar between pregnant and nonpregnant females. These data are summarized in the table below (mean ± standard deviation, N = 4, NA = Not Applicable).

Reference ID: 3289165

Table 4: Exposure assessment of Sr and Ca in baseline animals in Study (b) (4) **895004**

	Pregnant		Nonpregnant	
Site	**Sr**	**Ca**	**Sr**	**Ca**
Implantation site	178.5 ± 116.44	NA	NA	NA
Uterus	220.3 ± 143.61		54.4 ± 51.41	
Femur	33.3 ± 2.77	151.8 ± 9.78	30.2 ± 3.05	149.3 ± 6.24

b. Plasma Sr and Ca analysis: Maternal plasma Sr and Ca levels are shown in the table below. Sr levels in the plasma increased in a dose-proportional manner on GD 6 and LD10 in EST-treated females. Slight accumulation of Sr was observed (< 2-fold) on LD 10 when compared to trough levels on GD 6. Plasma levels of Sr in EMT-treated animals were similar to controls. Plasma Ca levels, which were measured on LD 21, were similar across all dose groups, including controls. These data are summarized in the applicant's table below.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Strontium Analysis									
Maternal Plasma (ng/mL)									
Gestational Day 6 (Cmax)	41.6	299.0	1341.3	1860.0	3157.5	30.2	32.2	29.2	30.9
Lactational Day 10 (Cmax)	57.9	488.3	2087.5	4265.0	7037.5	112.1	77.6	57.9	51.8
Calcium									
Maternal Plasma (mg/dL)									
Lactational Day 21	10.5	10.6	10.5	10.6	10.2	10.6	10.7	10.6	10.6

c. Milk Sr analysis: The applicant's table below summarizes the concentration of Sr in breast milk on lactation day (LD) 10.

		Esomeprazole Strontium				Esomeprazole Magnesium			
Dosage Level (mg/kg/day)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Strontium (ng/mL)									
Mean	799	2405	9995	19933	52275	701	738	731	758
SD	250.6	678.1	1421.2	2010.8	9138.0	145.3	56.3	75.9	57.3
SE	125.3	339.0	710.6	1160.9	4569.0	72.6	28.2	38.0	28.7
N	4	4	4	3	4	4	4	4	4

SD = Standard deviation of mean; SE = Standard error of mean; N = Number of animals.

Sr levels in the milk of EMT-treated animals are similar to controls. In EST-treated animals, dose-proportional increase in Sr level was measured on LD 10.

d. Femur Sr and Ca analysis: Calcium levels in the maternal femur were not affected by treatment with either EST or EMT and were similar to control treatment. Sr levels in the femur increased in a dose-dependent manner

in EST-treated dams. These data are summarized in the applicant's table below.

Text Table 10. Calcium and Strontium Concentrations in F_0 Female Femurs

		Esomeprazole Strontium				Esomeprazole Magnesium			
Dosage Level (mg/kg/day)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Calcium (mg/g)									
Mean	150.5	144.8	131.4	128.5	133.3	142.6	139.4	135.6	137.9
SD	7.69	12.85	9.38	13.66	23.33	9.53	9.32	10.49	9.30
SE	2.72	4.54	3.13	5.58	8.25	3.37	3.29	3.71	3.29
N	8	8	8	8	8	8	8	8	8
Strontium (µg/g)									
Mean	33.0	79.3	313.0	581.5	1201.9	32.3	36.6	33.0	32.9
SD	2.41	17.56	84.53	83.60	259.99	3.65	12.01	8.61	3.65
SE	0.85	6.21	28.18	34.13	91.92	1.29	4.25	3.04	1.29
N	8	8	9	6	8	8	8	8	8

SD = Standard deviation of mean; SE = Standard error of mean; N = Number of animals.
Values < LLOQ = 70.0 mg/kg Calcium.

9. Esomeprazole toxicokinetics (TK): Esomeprazole TK data are shown in the applicant's table below.

Table 2.4-4: Esomeprazole pharmacokinetic parameters following oral administration of EST and EMT to rats fed a standard diet during a pre- and post-natal development study (GD 6, LD 10, and PND 10)

	Esomeprazole Strontium (EST)				Esomeprazole Magnesium (EMT)			
Dosage (mg/kg)	**17.2**	**84.7**	**169.5**	**343.8**	**15.6**	**76.7**	**153.3**	**311.1**
Dosage (mg/kg as esomeprazole base)	**14**	**69**	**138**	**280**	**14**	**69**	**138**	**280**
	Gestation Day 6							
AUC_{last} (ng·h/ml) ±SE	41.8 ±9.21	776 ±194	7850 ±3750	33500 ±14700	42.8 ±6.6	929 ±155	3830 ±926	30700 ±8190
C_{max} (ng/ml) ±SD	54.4 ±31.5	881 ±568	4300 ±6420	10100 ±10700	32.9 ±2.53	1040 ±407	3230 ±2370	14600 ±5960
T_{max} (hr)	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5
$T_{1/2}$ (hr)	NR	2.1	3.3	NR	1.2	NR	3.6	NR
EST/EMT Ratio	0.977	0.835	2.05	1.09	NA	NA	NA	NA
	Lactation Day 10							
AUC_{last} (ng·h/ml) ±SE	71.2 ±6.63	1640 ±745	6260 ±3570	33200 ±5210	75.0 ±10.7	1220 ±288	4500 ±962	31200 ±6490
C_{max} (ng/ml) ±SD	63.8±7.53	1220 ±1370	5000 ±NA	17800 ±14000	61.3 ±28.0	1170 ±902	3140 ±2870	15000 ±7670
T_{max} (hr)	0.5	0.5	1	0.5	0.5	0.5	0.5	1
$T_{1/2}$ (hr)	1.6	NR	NR	2.0	NR	NR	1.6	2.5
EST/EMT Ratio	0.949	1.34	1.39	1.06	NA	NA	NA	NA
Accumulation Ratio	1.70	2.11	0.797	0.991	1.75	1.31	1.17	1.02
	Postnatal Day 10							
Maternal/Fetal Ratio								
Males	4.17[*1]	187[*1]	166[*3]	875	NC	49.7[*1]	159[*1]	420[*1]
Females	NC	170[*1]	191[*3]	669	NC	34.9[*1]	308[*2]	416[*1]

AUC_{last} = area under the curve from time of dosing to the last observation that was >LLOQ; EST/EMT Ratio and Accumulation Ratio calculated using AUC_{last}; Maternal/Fetal Ratio calculated using concentrations at 2 hours post-dosing. For maternal/fetal ratios, n = 4 except where indicated by [*n]. NA = not applicable. NR = not reportable (failed to meet acceptance criteria). NC = not calculable. LLOQ = lower limit of quantitation. Data are represented by means ±SE (for AUC) or ±SD (for C_{max}). Post-Natal Day 10 and Lactation Day 10 are the same day and refer to pups and dams, respectively.

Analysis of maternal plasma for esomeprazole revealed that EST- and EMT-dosed animals were systemically exposed to esomeprazole in a dose-dependent manner. Exposure was generally similar between EST- and EMT-treated animals and increased in a greater than dose-proportional manner. Exposure to esomeprazole was ≥ 2-fold higher on LD 10 in both EST and EMT treatment groups, as compared to GD 6 values, and minimal accumulation of esomeprazole in the plasma was observed. T_{max} ranged between 0.5 – 1 h.

Maternal/Fetal plasma esomeprazole ratio is discussed under "F_1 results Exposure Assessment" below.

10. Dosing Solution Analysis: The concentration determination of EST and EMT in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

11. Other
    a. Hematology: Statistically significant changes in hematology parameters are shown in the applicant's table below (*p < 0.05, **p < 0.001, as compared to vehicle control).

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Hematology (only statistically significant findings are listed)									
Hemoglobin (g/dL)	14.2	14.1	12.9**	12.8**	12.1**	14.4	13.7	13.1*	12.7**
Hematocrit (%)	42.5	42.5	39.7	39.7	37.6**	43.4	42.2	40.7	39.5
Mean Corpuscular Volume (fL)	57.9	57.9	54.0**	52.5**	50.3**	59.9	53.7*	54.1	53.3*
Mean Corpuscular Hemoglobin (pg)	19.3	19.3	17.5**	17.0**	16.3**	19.9	17.4**	17.4**	17.2**
Mean Corpuscular Hemoglobin Concentration (thous/µL)	33.4	33.3	32.4	32.3*	32.3*	33.2	32.4**	32.2**	32.2**
Platelet (thous/µL)	781	756	913	925	1124**	779	818	963	877
Eosinophil Absolute (thous/µL)	0.14	0.13	0.09	0.07	0.06*	0.13	0.11	0.06*	0.07*
Hemoglobin Distribution Width (g/dL)	2.09	2.12	2.51**	2.67**	3.05**	2.08	2.47**	2.59**	2.75**

Overall, effects of treatment with EST and EMT on hematology parameters were comparable. Statistically significant decreases in hemoglobin were noted in EST-treated dams at doses ≥ 84.7 mg/kg/day and in EMT-treated dams at ≥ 153.3 mg/kg/day. There was a statistically significant decrease in hematocrit in high dose EST females only. There was a statistically significant decrease in absolute eosinophil count at the high dose in both EST and EMT groups and at 153.3 mg/kg/day EMT.

    b. Clinical Chemistry: Statistically significant changes in clinical chemistry parameters are shown in the applicant's table below (*p < 0.05, **p < 0.001, as compared to vehicle control).

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Albumin (g/dL)	3.7	3.8	3.8	3.8	3.9	3.8	3.9	3.9	4.0*
Total protein (g/dL)	6.1	6.2	6.1	6.3	6.5	6.2	6.4	6.4	6.5*
ALT (U/L)	123	110	144	118	102	118	158**	137	121
Cholesterol (mg/dL)	79	72	90	90	98**	71	87	92*	103**
Phosphorus (mg/dL)	5.2	6.3	7.0*	6.5	7.0*	5.9	6.9**	6.6*	7.1**

There was an increase in albumin and total protein in dams treated with EMT at the high dose. There was an increase in cholesterol at the high dose in dams treated with high dose EST and 153.3 and 311.1 mg/kg/day EMT. A dose-dependent increase in cholesterol levels was apparent in EMT-treated animals only. Increased phosphorus levels were observed in 84.7 and 343.8 mg/kg/day EST-treated dams and in dams treated with ≥ 76.7 mg/kg/day EMT, as compared to controls. There was also an increase in ALT in 76.7 mg/kg/day EMT animals only, as compared to controls. Overall, it is unclear whether these changes in clinical chemistry are related to treatment with either EST or EMT during pregnancy and lactation.

c. Bone Morphometry, Weight Measurements, and Histopathology: Findings are summarized in the table below.

Table 5: Changes in bone morphometry in maternal animals after treatment with EST or EMT from GD 6 through LD 21 in Study (b) (4) 895004

	Std Control	Esomeprazole Strontium				Esomeprazole Magnesium			
Dose Formula Weight (mg/kg/day)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Dose Esomeprazole (mg/kg/day)	0	14	69	138	280	14	69	138	280
Femur, Hypocellular (N=8)	0/8	1/8	2/8	2/8	4/8	0/8	1/8	2/8	6/8
Minimal	--	1/8	2/8	1/8	3/8	--	1/8	2/8	6/8
Mild	--	0/8	0/8	1/8	1/8	--	0/8	0/8	0/8
Right femur weight (g) (% change vs. control)	0.8764	1.4%	-6.2%	-10.1%	-6.9%	-5.2%	-9.0%	-11.3%*	-13.9%**
Relative femur weight (g/100 g body weight)	0.264	0.272	0.252	0.253	0.253	0.256	0.252	0.247	0.246
Proximal t bial growth plate thickness (mm)	0.165	NA	NA	NA	0.160	NA	NA	NA	0.165

* $p < 0.05$ as compared to control, ** $p < 0.01$ as compared to control

Treatment with EST and EMT, particularly at the higher doses, produced hypocellularity in the bone marrow. However, it was unclear whether there was a direct correlation between bone marrow hypocellularity and hematology findings. Decrease in femur weight was observed in both EST- and EMT-treated animals; however, there was a statistically significant decrease in femur weight at 153.3 and 311.1 mg/kg/day EMT treatment, as compared to control.

F_1 Generation

1. Survival PND 0 through PND 21
   a. The applicant's tables below summarize the postnatal survival (from birth (PND 0) through PND 21) in EST and EMT treatment groups.

	Esomeprazole Strontium					(b) (4)
Dosage Level (mg/kg/day)	0	17.2	84.7	169.5	343.8	Historical Control
Postnatal Survival (% per litter)						
PND 0 (relative to no. born)	100.0	100.0	99.0	94.2	94.1	98.6 (96.1-100.0)
PND 0-1	99.7	99.2	98.3	99.1	99.3	99.0 (96.5-100.0)
PND 1-4 (pre-selection)	99.4	99.2	99.1	94.5	98.9	98.7 (95.7-100.0)
PND 4 (post-selection) – 7	98.2	97.9	99.0	94.3	92.9	98.8 (95.8-100.0)
PND 7-14	99.4	99.3	99.5	98.3	95.4	99.4 (96.2-100.0)
PND 14-21	99.4	99.4	99.5	93.3	88.2	99.4 (95.7-100.0)
Birth to PND 4 (pre-selection)	99.1	98.4	96.5	91.9	92.7	96.4 (91.9-99.3)
PND 4 (post-selection) - 21	97.0	96.9	98.0	86.9	78.8+	97.2 (86.7-100.0)

+ = Significantly different from the control group at 0.05 using Dunn's test.

	Esomeprazole Magnesium				
Dosage Level (mg/kg/day)	0	15.6	76.7	153.3	311.1
Postnatal Survival (% per litter)					
PND 0 (relative to no. born)	100.0	93.9	98.8	98.7	97.0
PND 0-1	99.7	98.7	99.1	99.1	96.9
PND 1-4 (pre-selection)	99.4	99.4	98.4	99.5	92.4
PND 4 (post-selection) - 7	98.2	99.0	98.5	98.1	95.6
PND 7-14	99.4	97.9	100.0	99.5	97.1
PND 14-21	99.4	100.0	100.0	92.2	89.4
Birth to PND 4 (pre-selection)	99.1	92.1	96.4	97.2	88.8
PND 4 (post-selection) - 21	97.0	96.9	98.5	91.1	84.4

There was a statistically significant decrease in postnatal survival (litter proportions) in pups after selection (PND 4 through PND 21) at the high dose of EST (78.8%, as compared to 97% in control). There was a decrease in pup survival (although within historical control of testing laboratory) at the two highest doses of EST and EMT used (169.5 and 343.8 mg/kg/day EST and 153.3 and 311.1 mg/kg/day EMT), particularly after selection (PND 4 through PND 21). Postnatal survival was not affected at the lowest doses tested, 17.2 and 15.6 mg/kg/day EST and EMT, respectively.

   b. General condition of pups: The mean numbers of pups/litter, the mean numbers of liveborn pups/litter, and the percent of male pups/dose were similar across all treatment groups, including vehicle control. The applicant's table below summarizes the general condition of the pups during the early postnatal period (PND 0-PND 4)

	Esomeprazole Strontium				Esomeprazole Magnesium			
Dosage Level (mg/kg/day)	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Found Dead or Euthanized *in Extremis*[a]	6(4)	12(9)	42(14)	41(19)	19(11)	15(9)	23(10)	32(14)
Missing[a]	6(5)	5(5)	7(5)	14(7)	2(1)	3(3)	4(3)	14(9)

[a] = Numbers of pups (litters).

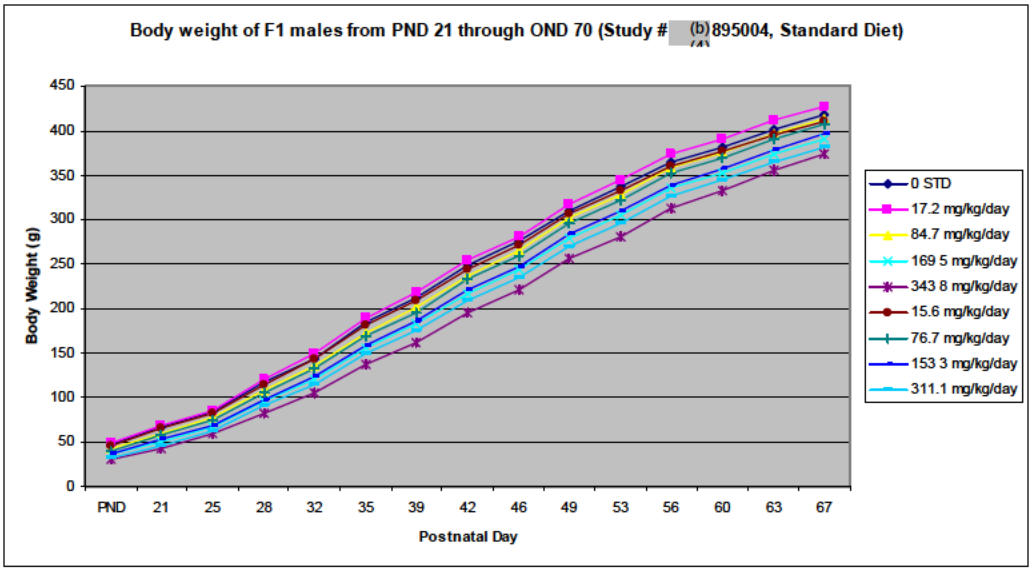
   c. Survival from PND 21 through PND 70: There were a total of 33 and 21 unscheduled deaths in the EST and EMT treatment groups, respectively, after weaning (after PND 21). Of these, 22 and 12 were in the 343.8 and 311.1 mg/kg/day EST and EMT dose groups, respectively.

i. There were 2, 3, and 12 males and 1, 5, and 10 females in the 84.7, 169.5, and 343.8 mg/kg/day EST treatment group which were found dead or euthanized in extremis between PND 22-28. Clinical signs included hypoactivity, cool and/or pale body, decreased respiration, and/or small stature. Because the treatment-related, unscheduled deaths occurred within 7 days of weaning, the applicant attributed them to decreased body weight of the pups (27 of the 33 pups which died had mean body weights ≤ 35.5 g on PND 21, which were 3-5% less than historical controls).
ii. There were 3 and 8 males in the 153.3 and 311.1 mg/kg/day EMT treatment groups and 3, 3, and 4 females in the 76.7, 153.3, 311.1 mg/kg/day EMT dose groups which were found dead or were euthanized in extremis. Clinical signs included small stature, labored respiration and prostrate body, swollen abdominal area, red urine, and impaired use of hind limbs. Because treatment-related, unscheduled deaths occurred within 7 days of weaning, the applicant attributed them to decreased body weight of the pups (15 of the 21 pups which died had mean body weights ≤ 36 g on PND 21, which were ~ 5% less than historical controls). One female in the 15.6 EMT treatment group was euthanized due to findings of swollen dorsal head and red material around the nose and eye. No other deaths were recorded at this dose.

2. Clinical Signs: No significant treatment-related clinical signs were observed in the post-weaning period in both the EST and EMT treatment groups.

3. Body Weight:
   a. Pre-selection: Prior to selection of PND 4, offspring (both sexes) of dams treated with EST ≥ 84.7 mg/kg/day showed a decreasing trend in body weight, with statistically significant decrease of 17.2% ($p < 0.01$, as compared to vehicle treatment) at the highest dose of EST. Decrease in body weight was also observed in offspring of EMT-treated dams, but at ≥ 153.3 mg/kg/day and was ≤ 8.6% as compared to vehicle control.
   b. Post-selection: There were statistically significant decreases in body weight gain observed during PND 10-14 and PND 17-21 in offspring of both EST- and EMT-treated dams at equimolar doses of esomeprazole ≥ 69 mg/kg/day. Decrease in body weight gain during these intervals was similar between EST and EMT treatment.
   c. Post-weaning to PND 70: Body weights of offspring were similar between EST and EMT treatment groups. They are shown in the graphs below.

Figure 1: Body weight of F_1 offspring during the post-weaning period (PND 21 through PND 70) in Study (b) (4) 895004 (A) F_1 Males, (B) F_1 Females



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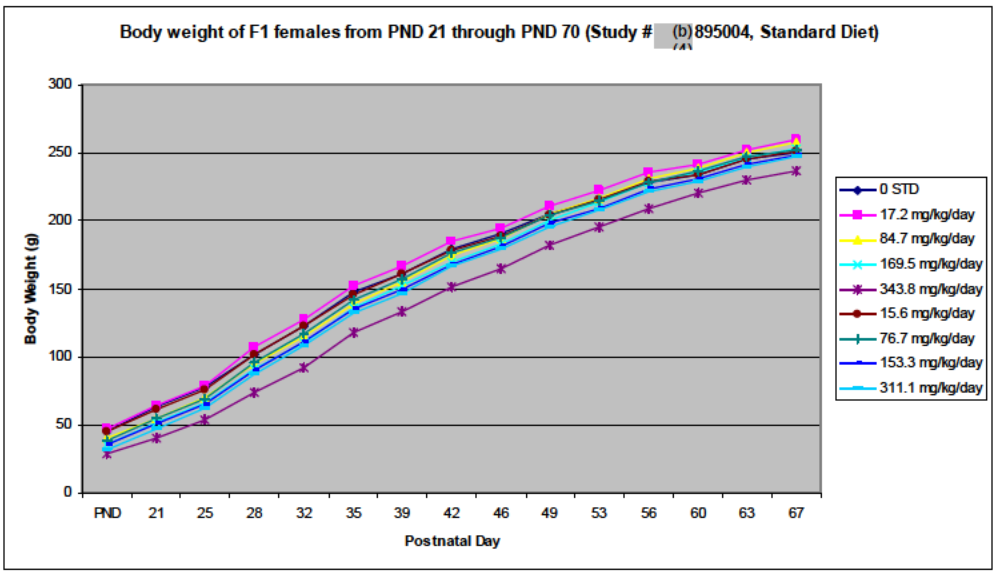

B

4. Necropsy observation
   a. Unscheduled deaths (prior to weaning): Pale liver was observed in 2 pups each in the 169.5 and 343.8 mg/kg/day EST and in 1, 3, and 3 pups in the 76.7, 153.3, and 311.1 mg/kg/day EMT group, respectively. No other significant treatment-related findings were noted.
   b. Scheduled necropsies of unselected pups and pups euthanized due to death of dam: Pale liver was observed in several pups born to dams treated with EST and EMT, particularly at the higher doses. Pale liver was not noted in control pups.

c. Unscheduled deaths (post weaning): Pale liver was noted in several pups born to dams treated with EST and EMT (observed at lower doses in EMT treatment group).
d. Scheduled necropsies on PND 70: No treatment-related findings were observed in either EST or EMT dose groups. Any observed findings were comparable between the treatment groups and the control.

5. Exposure Assessment
   a. Sr and Ca: Sr and Ca assessment was conducted on plasma and tissue samples collected on PND 10 (i.e. LD 10). There was a dose-dependent increase in plasma Sr concentration in EST-treated animals, in a greater than dose-proportional manner, as compared to controls. There was no apparent gender-specific effect on plasma Sr concentration. Exposure to Sr (in plasma, femur, and liver) in EMT-treated animals was similar to controls. Calcium concentration in the femur was unaffected by treatment with either EST or EMT and was similar to control in all animals. The applicant's table below summarizes the Sr and Ca levels in pup plasma, femur and liver.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Strontium Analysis (Postnatal Day 10)									
Pup Plasma (From combined samples collected at approximately 20 hours after the maternal dose on LD 9 or 2 hours after dosing of the dam) (ng/mL)									
Males	26.9	78.7	524.0	1405.7	2532.5	34.8	29.8	29.8	28.4
Females	28.5	80.4	549.1	1322.9	2571.3	27.0	29.6	45.5	32.9
Pup Femur (µg/g)									
Males	2.6	20.3	124.9	318.9	639.6	1.2	2.2	5.6	2.3
Females	3.1	20.2	155.7	319.7	675.1	1.6	2.4	5.3	3.6
Pup Liver (ng/g)									
Males	5.29	25.20	160.33	680.74	899.13	6.29	13.29	8.16	16.06
Females	3.89	25.72	186.54	465.06	1784.88	13.17	11.56	10.80	21.90
Calcium (mg/g)									
Pup Femur (Lactation Day 10)									
Males	27.3	27.9	26.3	27.0	27.4	25.5	26.2	29.4	25.2
Females	28.9	27.9	32.0	27.1	28.2	26.2	26.5	29.2	28.3

b. Esomeprazole: Esomeprazole was measured in fetal plasma on PND 10. The Maternal/neonatal plasma ratio indicated very low exposure to esomeprazole in the pups on PND 10 (i.e. pups are to be getting exposed to esomeprazole through mother's milk). The table below shows the plasma concentration of esomeprazole in PND 10 pups sampled on lactation day10 at 20 h post-dose (post-dose of dams) and on LD 9 at 2 h post-dose. As the data indicate, the pups were being exposed to very low, in some cases, undetectable (below the limit of quantitation) levels of esomeprazole. Esomeprazole levels were not measured in the breast milk.

Table 6: Plasma esomeprazole levels in F1 offspring exposed to EST or EMT via milk in Study (b) (4) 895004

	EST				EMT			
Daily Dose (mg/kg/day)	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Dose as Esomeprazole (mg/kg/day)	14	69	138	280	14	69	138	280
Males								
20 h post dose	BLQ*	BLQ	BLQ	BLQ*	BLQ	BLQ	BLQ	BLQ
2 h post dose	BLQ*	BLQ*	3.32	9.75	BLQ	BLQ*	BLQ*	BLQ*
Maternal/Neonatal Ratio	4.17	187	166	875	NA	49.7	159	420
Females								
20 h post dose	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
2 h post dose	BLQ	BLQ*	3.79	10.3	BLQ	1.87	1.48	BLQ*
Maternal/Neonatal Ratio	NC	170	191	669	NA	34.9	308	416

* BLQ represents the mean concentration.

6. Organ Weights:
   a. PND 4 Culled Pups: There were no statistically significant differences in right femur weight and relative femur weight (relative to body weight) between EST and EMT-treatment groups on PND 4. Femur were statistically significantly lower at doses of esomeprazole > 69 mg/kg/day (as Sr or Mg salt), as compared to control. There were decreases in liver weights, with statistical significance at the highest dose in EST female offspring (14.5% as compared to control) and 13.9% and 18.2% decrease at the highest dose in EMT male offspring, as compared to control. These data are shown in the applicant's table below.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Postnatal Day 4 Culled Pups									
Right Femur[b] (g)									
Males	0.0219	7.3%	-4.6%	-11.0%	-14.6%	14.2%	-3.2%	-6.4%	-10.5%
Females	0.0208	7.7%	-5.3%	-10.6%	-15.4%	14.4%	2.4%	-8.2%	-12.5%
Right Femur (g/100 g Body Weight)									
Males	0.256	0.255	0.250	0.243	0.251	0.270	0.255	0.243	0.263
Females	0.253	0.258	0.246	0.246	0.261	0.274	0.265	0.236	0.260
Liver[b]									
Males	0.3219g	4.8%	-4.3%	-8.8%	-10.8%	6.2%	-13.4%*	-5.5%	-13.9%**
Females	0.3155g	3.9%	-7.1%	-5.9%	-14.5%**	4.2%	-12.5%	-7.1%	-18.2%**
Liver (g/100 g Body Weight)									
Males	3.785	3.700	3.723	3.714	3.869	3.713	3.388	3.660	3.721
Females	3.815	3.812	3.700	3.991	4.015	3.831	3.472	3.650	3.688

   b. PND 10: Femur and liver weights were determined on PND 10 for selected offspring of dams from the TK groups. Statistically significant decreases in pup femur weights were observed in both EST and EMT treatment groups, particularly at the higher doses of EST (169.5 and 343.8 mg/kg/day) and at the mid and high doses of EMT (76.7, 153.3, and 311.1 mg/kg/day). A decrease in femur weight in offspring of EMT-treated dams was observed at a lower dose than was noted in EST. Body weights also

decreased in a similar fashion in the affected dose groups for both EST- and EMT-treated animals. Therefore, the relative femur/body weight ratio was unchanged. The applicant concluded that the decrease in femur weights were likely secondary to decrease in body weight of pups. Liver weights were affected in the same pattern as the femur weight: the offspring of EST-treated dams in the two highest dose groups, and the mid- and two highest dose groups in the pups of EMT-treated dams. Liver/body weight ratio remained unchanged. The applicant's table below summarizes these findings for the pups in each dose group.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Body Weight[b] (g)									
Postnatal Day 10									
Males	21.2	-0.5%	-10.4%	-27.8%**	-29.7%**	-0.9%	-15.6%**	-18.4%**	-34.0%**
Females	19.8	0.0%	-10.1%	-27.8%**	-28.3%**	3.5%	-14.6%*	-22.2%**	-33.3%**
Pup Organ Weights (g) (Postnatal Day 10)									
Right Femur[b]									
Males	0.0683	-6.0%	-14.6%	-32.9%**	-34.1%**	-5.3%	-21.1%*	-23.1%*	-37.9%**
Females	0.0636	-3.6%	-16.0%	-32.7%**	-32.4%**	-2.7%	-19.8%*	-27.4%**	-40.9%**
Right Femur (g/100 g Final Body Weight)									
Males	0.320	0.304	0.306	0.301	0.301	0.308	0.300	0.303	0.304
Females	0.320	0.309	0.300	0.300	0.300	0.300	0.301	0.301	0.287
Liver [b]									
Males	0.6874	0.3%	-11.9%	-28.4%**	-26.4%**	-6.0	-18.3%**	-17.6%**	-32.8%**
Females	0.6625	-0.7%	-13.5%	-28.2%**	-26.6%**	-1.6%	-17.6%**	-21.5%**	-34.2%**
Liver (g/100 g Final Body Weight)									
Males	3.256	3.281	3.202	3.219	3.408	3.086	3.153	3.280	3.325
Females	3.359	3.335	3.234	3.316	3.423	3.183	3.250	3.367	3.317

* $p<0.05$, as compared to control, ** $p<0.01$, as compared to control

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c. On PND 21, there were statistically significant decreases in femur weight at doses of esomeprazole ≥ 138 mg/kg/day (as either the Sr or Mg salt). There was also a decrease in liver weight (statistically significant) at ≥ 169.5 mg/kg/day EST and ≥ 76.7 mg/kg/day EMT. Because of a decrease in body weight during this interval, the relative femur and relative liver weights remained unchanged. These data are shown in the applicant's table below.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Postnatal Day 21									
Right Femur[b] (g)									
Males	0.1899	7.8%	-13.4%	-19.7%*	-44.0%**	4.2%	-13.0%	-13.5%	-32.1%**
Females	0.1757	9.4%	-17.4%*	-15.0%	-46.7%**	-4.8%	-12.6%	-20.4%**	-25.4%**
Right Femur (g/100 g Body Weight)									
Males	0.405	0.408	0.416	0.429	0.389	0.424	0.415	0.433	0.417
Females	0.401	0.406	0.411	0.398	0.388	0.416	0.413	0.408	0.410
Liver[b] (g)									

Males	1.7739	15.5%	-13.3%	-23.4%**	-34.8%**	4.1%	-13.1%	-14.9%	-26.8%**
Females	1.8112	8.7%	-17.0%	-17.3%	-41.0%**	-1.5%	-15.1%*	-21.8%**	-25.5%**
Liver (g/100 g Body Weight)									
Males	3.788	4.062	3.888	3.836	4.159	3.947	3.853	3.958	4.208
Females	4.112	4.132	4.258	4.013	4.416	4.030	4.086	4.123	4.251

7. Developmental Landmarks, Sensory Function, and Behavioral Testing
    a. Developmental landmarks: Balanopreputial separation (BPS) in males was statistically significantly delayed at the high dose of EST (343.8 mg/kg/day), as compared to control treatment group (48.6 days) and historical control values of the testing laboratory (48.2 days). A slight delay in attainment of BPS was also observed in animals treated with EMT at doses > 76.7 mg/kg/day; however, these values were within the historical control values of the testing laboratory. There was also a statistically significant decrease (10%, as compared to control) in body weight on the day of BPS attainment at the high dose in both EST and EMT-treated animals. Similar to the delay in BPS, females treated with high dose EST also showed a delay in attainment of vaginal patency (VP) on PND 37, as compared to control group VP value of 33.7 days and historical control of the testing laboratory VP value of 34 days. There was an 8.9% decrease in mean body weight on the day of VP attainment in high dose EST females, although statistical significance was not reached. Delay in sexual maturity of female offspring of dams treated with EMT was not observed. These results are summarized in the applicant's table below.

		EST				EMT			
Daily Dose (mg/kg)	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0	14	69	138	280	14	69	138	280
Preputial Separation (postnatal Day)	48.6	48.4	49.2	50.1	51.6**	48.3	49.0	49.9	50.2
Body Weight at Time of Preputial Separation[b] (g)	270	1.8%	-2.0%	-6.9%	-10.0%**	-2.6%	-4.8%	-5.8%	-10.0%**
Vaginal Patency (postnatal Day)	33.7	33.6	34.3	34.4	37.0**	34.2	34.3	34.6	35.7
Body Weight at Time of Vaginal Patency[b] (g)	113.1	2.9%	-3.0%	-5.4%	-8.9%	1.1%	-2.7%	-6.5%	-4.1%

    b. Sensory function: Auditory startle response and motor activity were assessed on PND 20 and PND 60 in selected male and female pups. Overall, there were no obvious treatment-related adverse effects on sensory function on offspring of EST- or EMT-treated dams.
        i. On PND 20, there was a decrease in maximum response values (MAX) in offspring in the EST and EMT treatment groups at doses ≥ 69 mg/kg esomeprazole/kg/day, while a decrease in MAX was observed in females at ≥ 138 mg esomeprazole/kg/day (as Sr or Mg), as compared to control. These effects were not observed on PND 60 in either sex. TMAX was only affected in PND 20 females

at the highest dose of EST (280 mg esomeprazole/kg/day). The mean average response values (AVE) were affected only on PND 20 in both sexes at EST doses ≥ 138 mg esomeprazole/kg/day and at EMT doses of 69 mg esomeprazole/kg/day and 280 mg esomeprazole/kg/day.

ii. Locomotor activity increased at the highest dose of EST in PND 61 females only. No treatment-related changes were observed in any other dose groups. Ambulatory counts were also increased on PND 21 and PND 61 in female offspring of dams treated with EST and EMT at the high dose (280 mg esomeprazole/kg/day).

c. Behavioral function: There were no treatment-related changes in either sex on PND 22 or on PND 62 in the straight channel assessment in the Biel Maze-Swimming Trial. On PND 22, male offspring of dams treated with high dose EMT and females in high dose EST had an increase in escape time on Learning Path A. There was a increase in escape time noted in males and females on PND 22 at the high dose of EMT and an increase in errors noted in PND 22 females at the high dose of EMT on Learning Path B. There were no significant treatment-related changes in escape time or errors made in Memory test using Path A. Overall, there were no obvious treatment-related adverse effects on behavioral function on offspring of EST- or EMT-treated dams.

8. Bone Parameters: The following bone parameters were assessed on PND 10 in the offspring of dams in the TK dose groups: femur length, width and cortex thickness, proximal tibial growth plate thickness and bone marrow hypocellularity. Overall, the effects on bone parameters in offspring of dams treated with EST or EMT appeared to be similar at equimolar doses of esomeprazole. These effects were similar in both male and female offspring. There was an increase in incidence in minimal to mild physeal dysplasia in the femur in male and female offspring in both EST and EMT groups where the esomeprazole dose was 138 and 280 mg/kg/day. The physeal dysplasia was considered an adverse effect with a potential for growth abnormalities. The total number of pups (males + females) affected in the EST treatment groups were 1 and 25 (138 and 280 mg esomeprazole/kg/day respectively), while the number affected in the EMT treatment groups were 2 and 18 (138 and 280 mg esomeprazole/kg/day, respectively). Statistically significant decreases in proximal tibial growth plate thickness was observed in offspring of dams treated with doses of EST > 84.7 mg/kg/day (males) and > 169.5 mg/kg/day (females). In EMT treatment groups, growth plate thickness was also decreased, with statistically significant decreases observed at doses of EMT ≥ 15.6 mg/kg/day. These data are summarized in the table below (shown as # animals affected/total number of animals in dose group).

Table 7: Changes in bone morphology in offspring of TK animals treated with EST or EMT in Study (b) (4) 895004

		Control	Esomeprazole Strontium				Esomeprazole Magnesium			
Dose Formula Weight (mg/kg/day)		**0**	**17.2**	**84.7**	**169.5**	**343.8**	**15.6**	**76.7**	**153.3**	**311.1**
Dose Esomeprazole (mg/kg/day)		**0**	**14**	**69**	**138**	**280**	**14**	**69**	**138**	**280**
Males	↓ length	0/24	0/23	0/23	7/21	12/24	0/24	2/23	0/21	14/22
	↓ thickness (cortex)	0/24	0/23	3/23	7/21	9/24	0/24	4/23	4/21	9/22
	↓ width	0/24	0/23	2/23	9/21	10/24	1/24	4/23	7/21	10/22
	Dysplasia, Physis-Minimal	0/24	0/23	0/23	0/21	11/24	0/24	0/23	1/21	8/22
	Dysplasia, Physis-Mild	0/24	0/23	0/23	0/21	5/24	0/24	0/23	0/21	0/22
	Hypocellular, Minimal	0/24	0/23	2/23	4/21	3/24	0/24	5/23	4/21	3/22
	Hypocellular, Mild	0/24	0/23	1/23	0/21	1/24	0/24	0/23	0/21	0/22
	Proximal tibial growth plate thickness (mm)	0.903	0.869	0.792**	0.721**	0.699**	0.835*	0.802*	0.743**	0.649**
Females	↓length	0/24	0/24	0/24	6/21	10/22	0/21	1/24	4/21	17/24
	↓thickness (cortex)	0/24	0/24	0/24	3/21	3/22	0/21	0/24	0/21	1/24
	↓ width	0/24	0/24	0/24	4/21	5/22	0/21	3/24	7/21	8/24
	Dysplasia, Physis-Minimal	0/24	0/24	0/24	1/21	6/22	0/21	0/24	1/21	10/24
	Dysplasia, Physis-Mild	0/24	0/24	0/24	0/21	3/22	0/21	0/24	0/21	0/24
	Hypocellular, Minimal	0/24	0/24	1/24	0/21	2/22	0/21	2/24	4/21	6/24
	Hypocellular, Mild	0/24	0/24	0/24	0/21	0/22	0/21	0/24	0/21	1/24
	Proximal tibial growth plate thickness (mm)	0.818	0.822	0.775	0.717**	0.660**	0.814*	0.759**	0.724**	0.669**

*p<0.05, **p<0.01, as compared to control

9. Macroscopic Examinations:
    a. Unscheduled deaths: Pale liver was noted in 4 EST pups found dead (on PND 22 or 23) and 1 EST pup euthanized *in extremis* (on PND 22) prior to scheduled study termination. One high dose EST female which was found dead (PND 24) had an enlarged spleen. In the EMT group, pale liver was observed in female pups found dead (on PND 22). One male which was found dead on PND 25 had dark red discolored lungs with foamy tracheal contents. It is unclear whether these findings were related to treatment of dams with EST or EMT. All other pups found dead or euthanized *in extremis* did not reveal any significant treatment-related findings at necropsy.
    b. Scheduled deaths: No treatment-related changes were observed at scheduled necropsy of F1 pups on PND 70.

Study title: An Oral (Gavage) Study of the Effects of Esomeprazole Strontium on Pre- and Postnatal Development, Including Maternal Function in Rats Fed Reduced Levels of Calcium and Vitamin D (Modified Diet)

Study no.:	(b) (4) 895005
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	17 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	• Esomeprazole Strontium (EST), Lot # 10SOSHB21008, 99.5% purity • Esomeprazole Magnesium (EMT), Lot # EZFP11030, 99.4% purity

Key Study Findings

The NOAEL doses for maternal toxicity were 169.5 and 153.3 mg/kg/day for EST and EMT, respectively (equimolar dosage of esomeprazole = 138 mg/kg/day), based on a decrease in body weight gain and changes in clinical chemistry and bone morphometry parameters at doses > 138 mg esomeprazole/kg/day. The NOAEL doses for F_1 neonatal/early postnatal toxicity and post-selection toxicity were 17.2 mg/kg/day for EST and 15.6 mg/kg/day for EMT (14 mg esomeprazole/kg/day), based on pup survival. The NOAEL doses for developmental and neurobehavioral toxicity were 343.8 and 311.1 mg/kg/day for EST and EMT, respectively, (280 mg esomeprazole/kg/day). The NOAEL doses for bone effects in the F1 generation were 169.5 mg/kg/day for EST and 76.7 mg/kg/day EMT, based on findings of decreased femur length, width, cortical thickness and tibial growth plate thickness. Physeal dysplasia in the femur was observed at doses ≥ 169 mg esomeprazole/kg/day. The overall toxicity profile of EST and EMT was similar and generally unaffected by diet modification.

Methods:

Doses:	0 (Vehicle Control, Standard Diet) 0 (Vehicle Control, Modified Diet) EST: 17.2, 84.7, 169.5, 343.8 mg/kg/day EMT: 15.6, 76.7, 153.3, 311.1 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension/0.5% methylcellulose (1500 cps)
Species/Strain:	Rat/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	Main Study: 25 females/dose group TK: 16 females (control), 8 females/all other dose group (See applicant's table below)
Satellite groups:	Yes for TK analysis (esomeprazole) and analysis of baseline Sr and Ca
Deviation from study protocol:	None which affected study results

The Modified Diet Study is identical to the Standard Diet study (Study # (b) (4) 895004), except for the following changes:

1. In addition to a standard diet control, there was a dose group of modified diet control. The modified diet contained approximately 5.7 g calcium and 1500 IU of vitamin D per kg of diet. The modified diet contained about 2/3 the concentrations of calcium and vitamin D in the standard diet (LabDiet® 5002). The selected concentrations of calcium and vitamin D were the lowest doses at which no adverse effects on bone development and normal homeostasis were observed in control animals (based on published data looking at the effects of calcium and vitamin D on growth and development).
2. All EST and EMT dose groups (Groups 1 and 3-10) were initiated on the modified diet beginning on GD 0 and continuing throughout the study (F0 animals in the pre- and postnatal development and toxicokinetic groups and F1 animals in the pre- and postnatal development groups).
3. A standard diet control group was also included (Group 2).
4. The mean Sr concentration in the standard and modified diet was 10.6 mg/kg diet and 10.0 mg/kg diet, respectively). The mean calcium concentration in the standard and modified diet was 8.84 mg/kg diet and 5.7 mg/kg diet, respectively.
5. For statistical comparisons, EST (Groups 3, 4, 5, 6) and EMT (Groups 7, 8, 9, and 10) treatment groups and standard diet control group (Group 2) were each compared to the modified diet control group (Group 1).

Observations

The parameters assessed for F0 Dams and F1 Offspring are identical to those measured in Study (b) (4) 895004. Please refer to the F0 and F1 Observations Section under Study (b) (4) 895004 for detailed descriptions.

Reference ID: 3289165

Results

F_0 Dams

1. Survival: One low dose EST female was found dead on LD 21 with no clinical findings. Total litter loss (on LD 7, 20, or 21) was observed in 2 females treated with 84.7 mg/kg/day EST and 1 high dose EST female.

2. Clinical Signs: Clinical signs in EST- and EMT-treated dams were similar and included findings of dried red material around mouth, nose and/or eye, wet red or clear material around nose and mouth, hypoactivity or lying prostrate 1 h post-dose, pale body, decreased respiration, and/or half-closed eyelids shortly after dosage administration. These observations were similar to those recorded in the standard diet study ([redacted] (b) (4) 895004).

3. Body Weight: Results are shown in the applicant's table below.
    a. Gestation: There was a statistically significant decrease in body weight gain between GD 6-9 in dams treated with ≥ 169.5 mg/kg/day EST and in those treated with ≥ 76.7 mg/kg/day EMT, as compared to control. A statistically significant decrease in body weight gain was again observed between GD 18-20 in the high dose EST group only. Overall, during gestation (GD 6-20), there was a decreasing trend in body weight gain in both EST and EMT treatment groups, with a 21.9% decrease ($p<0.01$, as compared to control) at the high dose of EST and 8.9% and 16.2% decreases ($p<0.05$ and $p<0.01$, as compared to control) at 153.3 and 311.1 mg/kg/day EMT. No other treatment-related changes were observed during the dosing period.
    b. Lactation: There was a decreasing trend in body weight gain in dams treated with EMT at doses ≥ 153.3 mg/kg/day, although a statistically significant decrease was observed only at 153.3 mg/kg/day (p,0.05, as compared to control). During LD 4-11, there was a 69% decrease (p,0.05, as compared to control) in body weight gain at the high dose of EST, while there was an increase in body weight gain observed during this period in the EMT treatment group. Between LD 17-21, there was an increasing trend in body weight gain, with statistically significant increase at the highest dose of EST. Overall, both EST and EMT treatment groups had increased body weight gain during LD 1-21, with a 28% increase ($p<0.05$, as compared to control) at the high dose of EST. These data are shown in the applicant's table below.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Gestation Body Weight Gain (g)										
Gestational Days 0-6	29	32	32	31	31	28	30	29	29	29
Gestational Days 6-9	15	12	11	10	10*	-1**	13	7**	7**	4**
Gestational Days 9-12	15	17	16	17	14	15	16	18	16	15
Gestational Days 12-15	20	18	16	18	20	16	17	17	19	16
Gestational Days 15-18	39	42	41	41	41	40	42	40	40	40
Gestational Days 18-20	34	33	33	34	29	26**	34	31	30	28
Gestational Days 6-20	123	122	118	120	113	96**	121	113	112*	103**
Lactation Body Weight Gain (g)										
Lactational Days 1-4	16	15	14	13	12	16	16	12	8*	9
Lactational Days 4-7	13	11	12	9	11	4*	8	12	14	11
Lactational Days 7-10	10	9	13	13	10	14	11	10	10	13
Lactational Days 10-14	10	8	7	8	7	16	8	9	8	10
Lactational Days 14-17	5	8	9	6	9	3	4	8	6	8
Lactational Days 17-21	-13	-5	-9	-6	-7	0**	-9	-9	-8	-5
Lactational Days 1-21	42	47	46	44	43	54*	39	42	38	46

4. Feed Consumption: There was a statistically significant decrease of 8.3% (p,0.01, as compared to control) in food consumption during gestation (GD 6-20) at the high dose only in both EST and EMT treatment groups. There was a slight decrease in food consumption during this period in the remaining dose groups as well. During lactation (LD 1-21), there was a decreasing trend in food consumption in both EST and EMT treatment groups at doses of ≥ 69 mg esomeprazole/kg/day, with statistically significant decreases at ≥ 138 mg esomeprazole/kg/day, as compared to control. These data are shown in the applicant's table below.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Gestation Food Consumption (g/animal/Day)										
Gestational Days 6-20	24	25	24	25	24	22**	24	24	23	22**
Lactation Food Consumption (g/animal/Day)										
Lactational Days 1-21	53	54	53	50	47**	46**	53	49	45**	46**

5. Gestation Length and Parturition: There were no effects on mean duration of gestation or parturition related to treatment of dams with EST or EMT.

6. Uterine Content: No treatment-related findings were recorded at the scheduled necropsy. There was no difference in the number of implantation sites, number

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born, or the number of unaccounted sites between EST- and EMT-treated animals, as compared to controls.

7. Necropsy Observation:
    a. Unscheduled deaths: One low dose EST female was found dead on LD 21 with red fluid contents in the thoracic cavity. Dosing procedure error was attributed as the likely cause of death. One low dose EMT female was found dead LD 1. Two females in the high dose EMT group were euthanized in extremis on GD 20 due to treatment-related moribundity; necropsy was insignificant.
    b. Scheduled necropsy: No treatment-related effects were recorded at scheduled necropsy. No significant differences between EST and EMT treatment were observed.

8. Exposure Assessment:
    a. Baseline Sr and Ca analysis: There was very high variability in the data. Therefore, it is difficult to compare Sr levels at the implantation site in pregnant animals on the standard and modified diet. The Sr levels in uterus in pregnant dams (both diets) were slightly higher than those in nonpregnant dams. There were no differences in Sr levels in the plasma and femur between pregnant and nonpregnant animals, regardless of the diet they were fed. Calcium levels in the femur were also similar between groups, independent of pregnancy status and diet conditions. These data are summarized in the table below (mean ± standard deviation, N = 4).

Table 8: Baseline strontium and calcium levels in control animals in Study (b) (4) 895005

	Pregnant		**Nonpregnant**	
Site	**Sr**	**Ca (mg/g)**	**Sr**	**Ca**
Standard Diet				
Implantation site (ng/g)	38.5 ± 18.07	NA	NA	NA
Uterus (ng/g)	24.3 ± 2.14		10.9 ± 12.83	
Plasma (ng/mL)	28.3 ± 2.35		32.0 ± 2.71	
Femur (μg/g)	31.9 ± 4.44	132.8 ± 14.89	32.1 ± 6.55	126.8 ± 11.76
Modified Diet				
Implantation site (ng/g)	124.4 ± 134.03	NA	NA	NA
Uterus (ng/g)	36.9 ± 7.82		21.0 ± 2.18	
Plasma (ng/mL)	35.4 ± 2.04		34. 5 ± 4.40	
Femur (μg/g)	30.8 ± 6.30	130.8 ± 13.15	36.2 ± 3.24	139.5 ± 11.24

    b. Plasma Sr and Ca analysis: Maternal Sr and Ca levels in the plasma of EMT-treated animals were similar to controls during gestation and lactation. EST-treated dams showed a dose-dependent increase in plasma Sr concentration. There was slight accumulation of Sr in the plasma (< 3.1-fold than pre-dose levels) on LD 10 at all doses of EST. The mean plasma concentrations of Sr and Ca in EST- and EMT-treated

dams during gestation and lactation are shown in the applicant's table below.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Strontium Analysis										
Maternal Plasma (ng/mL)										
Gestational Day 6 (Cmax)	36.1	34.2	353.8	1081.3	1633.5	2095.0	33.0	28.7	100.4	32.4
Lactational Day 10 (Cmax)	75.7	44.0	498.3	1962.5	3410.0	6047.5	64.3	67.4	57.3	49.7
Calcium										
Maternal Plasma (mg/dL)										
Lactational Day 21	10.3	10.6[c]	10.5	10.4	10.2	10.2	10.5	10.5	10.2	10.6

c. Milk Sr analysis: Concentration of Sr in milk of EST-treated dams increased with dose in a more-than dose-proportional manner. The level of Sr in milk of EMT-treated dams was similar to vehicle-treated dams. The applicant's table below summarizes the concentration of Sr in breast milk on lactation day (LD) 10.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Maternal Milk (ng/mL)										
Lactational Day 10	871	656	3635	13150	23375	45933	1036	939	772	749

d. Femur Sr and Ca analysis: Concentration of Sr in maternal femur increased in a dose-dependent manner with EST treatment. The concentration of Sr in the femur of EMT-treated dams was similar to vehicle-treated dams. Calcium levels in the femur in all animals in all dosing groups were similar, suggesting that calcium levels were unaffected by diet modification or treatment with EST and EMT. These data are summarized in the applicant's table below.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Strontium Analysis										
Maternal Femur (µg/g)										
Lactational Day 10	35.5	37.2	90.6	384.1	704.6	1401.4	35.1	53.6	35.0	37.2
Calcium										
Maternal Femur (mg/g)										
Lactational Day 10	130.7	145.4	132.1	127.9	129.6	124.3	126.1	136.0	126.4	136.1

e. Esomeprazole toxicokinetics (TK): All pregnant animals dosed with either EST or EMT were exposed to esomeprazole systemically. Exposure to esomeprazole was dose-dependent and greater than dose-proportional in

dams from both EST and EMT treatment groups. Tmax and t1/2 were also similar across dosing groups. There was slight accumulation of esomeprazole in the plasma at pre-dose on LD 10 (< 2-fold compared to levels at end of gestation). Maternal plasma concentrations of esomeprazole were consistently higher than those in pups when sampled on LD 10 (2 h post-dose). Maternal/Fetal plasma esomeprazole ratio is discussed under "F_1 Results Exposure Assessment" below. Esomeprazole TK data are shown in the applicant's table below.

Table 2.4-5: Esomeprazole pharmacokinetic parameters following oral administration of EST and EMT to rats fed a diet with reduced calcium and vitamin D during a pre- and post-natal development study (GD 6, LD 10, and PND 10)

	Esomeprazole Strontium (EST)				Esomeprazole Magnesium (EMT)			
Dosage (mg/kg)	**17.2**	**84.7**	**169.5**	**343.8**	**15.6**	**76.7**	**153.3**	**311.1**
Dosage (mg/kg as esomeprazole base)	**14**	**69**	**138**	**280**	**14**	**69**	**138**	**280**
	Gestation Day 6							
AUC_{last} (ng·h/ml) ±SE	38.8 ±4.18	528 ±54.7	7240 ±1380	17000 ±7110	58.8 ±3.73	3440 ±2640	4380 ±1410	23300 ±7020
C_{max} (ng/ml) ±SD	36.2 ±12.5	482 ±137	3520 ±1360	6460 ±6080	52.2 ±13.8	1220 ±1270[*3]	1820 ±948	8630 ±11500[*3]
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$T_{1/2}$ (hr)	0.6	2.2	NR	NR	1.2	NR	3.3	4.4
EST/EMT Ratio	0.660	0.153	1.65	0.730	NA	NA	NA	NA
	Lactation Day 10							
AUC_{last} (ng·h/ml) ±SE	69.2 ±7.04	1010 ±258	8230 ±3670	33800 ±4620	73.0 ±11.0	984 ±193	7410 ±2730	29000 ±9120
C_{max} (ng/ml) ±SD	53.3 ±17.3	671 ±674	6520 ±4010	21700 ±7650	59.6 ±23.6	472 ±211[*3]	3460 ±3800[*3]	12400 ±12600[*3]
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1
$T_{1/2}$ (hr)	1.0	3.6	2.3	2.5	1.5	1.2	1.2	0.7
EST/EMT Ratio	0.948	1.03	1.11	1.17	NA	NA	NA	NA
Accumulation Ratio	1.78	1.91	1.14	1.99	1.24	0.286	1.69	1.24
	Postnatal Day 10							
Maternal/Fetal Ratio								
Males	NC	NC	186[*1]	837[*2]	NC	NC	205[*2]	356[*2]
Females	NC	NC	87.8[*1]	362[*2]	NC	NC	219[*2]	158[*3]

AUC_{last} = area under the curve from time of dosing to the last observation that was >LLOQ; EST/EMT Ratio and Accumulation Ratio calculated using AUC_{last}; Maternal/Fetal Ratio calculated using concentrations at 2 hours post-dosing. For maternal/fetal ratios, n = 4 except where indicated by [*n]. NA = not applicable. NR = not reportable (failed to meet acceptance criteria). NC = not calculable. LLOQ = lower limit of quantitation. Data are represented by means ±SE (for AUC) or ±SD (for C_{max}). Post-Natal Day 10 and Lactation Day 10 are the same day and refer to pups and dams, respectively.

9. Dosing Solution Analysis: The concentration determination of EST and EMT in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

10. Other:
    a. Hematology: Statistically significant changes in hematology parameters are shown in the applicant's table below (*p < 0.05, **p < 0.001, as compared to vehicle control). Red blood cell parameters were affected at doses ≥ 84.7 EST and > 76.7 mg/kg/day EMT (69 mg esomeprazole/kg/day). There were decreases in hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration. A statistically significant decrease in prothrombin time and

absolute lymphocyte counts was observed in EST-treated dams only at 84.7 mg/kg/day and 343.8 mg/kg/day, while EMT-treated dams were not as severely affected. Absolute eosinophil and basophil counts also decreased significantly in both EST and EMT treatment groups at doses > 84.7 mg/kg/day (EST) and > 15.6 mg/kg day EMT (eosinophil only) and ≥ 76.7 mg/kg/day EMT, respectively. These changes in hematology may correlate with the observations of hypocellularity (minimal to mild) observed in maternal femur with EST and EMT, particularly at higher doses.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Clinical Pathology (Lactation Day 21)										
Hematology (only statistically significant findings are listed)										
Red Blood Cells (mil/µl)	8.11	7.56[c]	7.76	7.69	8.04	7.71	7.73	8.06	7.67	8.01
Hemoglobin (g/dL)	15.8	15.4	15.5	13.4**	13.5**	12.8**	15.5	14.2**	13.3**	13.5**
Hematocrit (%)	45.7	44.5	44.5	39.5**	40.4**	38.6**	45.2	42.6*	39.3**	40.8**
Mean Corpuscular Volume (fL)	56.4	59.0[d]	57.3	51.5**	50.3**	50.1**	58.6	53.0	51.3**	51.0**
Mean Corpuscular Hemoglobin (pg)	19.4	20.3[e]	20.0	17.5**	16.8**	16.6**	20.1	17.6**	17.4**	16.9**
Mean Corpuscular Hemoglobin Concentration (thous/µL)	34.5	34.5	34.8	33.8	33.3*	33.1**	34.4	33.2**	33.9	33.2**
Prothrombin Time (sec)	19.6	18.9	18.7	18.2*	18.5	18.3*	19.3	18.6	18.7	18.3
Reticulocyte Absolute (thous/µL)	126.6	133.4	126.5	108.1	139.6	178.0*	116.5	155.2	127.3	130.1
Lymphocyte Absolute (thous/µL)	8.35	7.59	6.88	6.46*	6.81	6.24*	6.74	7.62	7.20	7.64
Eosinophil Absolute (thous/µL)	0.16	0.11	0.09	0.12	0.05**	0.04**	0.10*	0.10*	0.05**	0.08**
Basophil Absolute (thous/µL)	0.04	0.03	0.03	0.02*	0.02**	0.02*	0.03	0.03	0.02**	0.03
Hemoglobin Distribution Width (g/dL)	2.20	2.16	2.24	2.71**	3.02**	3.18**	2.19	2.57**	2.99**	3.04**

b. Clinical Chemistry: Statistically significant changes in clinical chemistry parameters are shown in the applicant's table below (*p < 0.05, **p < 0.001, as compared to vehicle control). There was statistically significant increase in serum cholesterol in dams treated with ≥ 84.7 mg/kg/day EST and with high dose EMT. There was an increase in albumin and total protein level in high dose EMT-treated dams. ALT and AST were also increased in EMT-treated dams at 15.6 and 76.7 mg/kg/day (ALT) and at 76.7 mg/kg/day (AST). The increase in cholesterol, albumin, and total protein was observed in the standard diet study ([(b) (4)]895004) as well.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Serum Chemistry (only statistically significant findings are listed)										
Albumin (g/dL)	3.7	3.8	3.8	3.9	3.9	3.8	3.8	4.0	3.8	4.1*
Total Protein (g/dL)	5.7	6.0	5.9	6.0	6.1	5.9	5.9	6.2*	5.8	6.3*
ALT (U/L)	94	118[c]	105	103	108	97	120*	121*	106	72
AST (U/L)	107	115	106	122	110	117	127	144**	108	98
Phosphorus (mg/dL)	6.3	6.0	6.4	7.0	6.7	6.9	6.7	7.3**	6.4	6.6
Cholesterol (mg/dL)	73	81	90	92*	96**	108**	83	89	89	115**

c. Bone Morphometry, Weight Measurements, and Histopathology: EST and EMT treatment produced changes in maternal bone under modified diet conditions which were similar to those observed in Study (b) (4) 895004. However, maternal bone changes in EST- and EMT-treated animals were not statistically significantly different from modified control. A dose response was not apparent. There was also an increase in incidence of bone marrow hypocellularity (minimal to mild) with increasing dose in both EST and EMT treatment groups. There was also a slight decrease in femur weight (< 5.4% in EST-treated dams and < 10% in EMT-treated dams, as compared to controls). These findings are summarized in the table below.

Table 9: Changes in bone morphology in maternal bone in Study (b) (4) 895005

	Modif Diet Crtl	Std Diet Ctrl	Esomeprazole Strontium				Esomeprazole Magnesium			
Dose Formula Weight (mg/kg/day)	0	0	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Dose Esomeprazole (mg/kg/day)	0	0	14	69	138	280	14	69	138	280
Femur, hypocellular	0/6	0/8	2/8	2/8	2/8	6/7	0/8	0/7	3/7	5/7
Minimal	--	0/8	2/8	2/8	2/8	3/7	0/8	0/7	2/7	4/7
Mild	--	0/8	0/8	0/8	0/8	3/7	0/8	0/7	1/7	1/7
Right femur weight (g) (% change vs. control)	0.8984	0.3%	-3.3%	-2.7%	-5.4%	-3.3%	-3.9%	-9.6%	-5.0%	-5 5%
Relative femur weight (g/100 g body weight)	0.267	0.268	0.263	0.259	0.256	0.271	0.249	0.242	0.254	0.258
Proximal tibial growth plate thickness (mm)	0.150	0.149	NA	NA	NA	0.144	NA	NA	NA	0.154

F_1 Generation

1. Survival, PND 0 through PND 21
   a. The applicant's tables below summarize the postnatal survival (from birth (PND 0) through PND 21) in EST and EMT treatment groups.

	Esomeprazole Strontium					
Dosage Level (mg/kg/day)	0[a]	17.2	84.7	169.5	343.8	Historical Control
Postnatal Survival (% per litter)						
PND 0 (relative to no. born)	99.8	99.2	97.2	98.7	98.8	98.6 (96.1-100.0)
PND 0-1	99.2	98.7	98.2	99.2	97.9	99.0 (96.5-100.0)
PND 1-4 (pre-selection)	98.9	98.0	97.7	97.2	99.2	98.7 (95.7-100.0)
PND 4 (post-selection) – 7	98.5	99.0	93.0	97.4	99.6	98.8 (95.8-100.0)
PND 7-14	100.0	100.0	98.3	97.8	96.5	99.4 (96.2-100.0)
PND 14-21	99.5	100.0	92.6	92.5	89.4	99.4 (95.7-100.0)
Birth to PND 4	97.9	96.0	93.3	95.2	95.9	96.4 (91.9-99.3)
PND 4 (post-selection) - 21	98.0	99.0	85.0	88.3	86.7	97.2 (86.7-100.0)

[a] = Modified diet control group.

	Esomeprazole Magnesium					
Dosage Level (mg/kg/day)	0[a]	15.6	76.7	153.3	311.1	Historical Control
Postnatal Survival (% per litter)						
PND 0 (relative to no. born)	99.8	97.2	97.7	97.8	97.6	98.6 (96.1-100.0)
PND 0-1	99.2	99.2	97.7	97.5	96.0	99.0 (96.5-100.0)
PND 1-4 (pre-selection)	98.9	99.4	97.3	92.9	97.9	98.7 (95.7-100.0)
PND 4 (post-selection) – 7	98.5	97.8	95.2	96.0	98.1	98.8 (95.8-100.0)
PND 7-14	100.0	99.3	98.3	97.4	94.6	99.4 (96.2-100.0)
PND 14-21	99.5	99.0	96.9	90.4	88.0	99.4 (95.7-100.0)
Birth to PND 4	97.9	95.9	92.8	88.4++	91.8+	96.4 (91.9-99.3)
PND 4 (post-selection) - 21	98.0	96.4	90.7	84.5+	81.9	97.2 (86.7-100.0)

[a] = Modified diet control group.
+ = Significantly different from control group at 0.05 using Dunn's test
++ = Significantly different from control group at 0.01 using Dunn's test

Pup survival in the post-selection period was affected at doses ≥ 84.7 mg/kg/day EST, although the decrease in pup survival was within historical range of the testing laboratory. The offspring of EMT-treated dams were more severely affected in the early postnatal period (birth to PND 4) and in the post-selection period (> PND 4) at doses ≥ 153.3 mg/kg/day. The decrease in pup survival dropped below historical control levels for the testing laboratory, suggesting a possible treatment-specific effect secondary to maternal toxicity.

b. General condition of pups in the early postnatal period: The applicant's table below summarizes the general condition of the pups during the early postnatal period (PND 0-PND 4).

	Esomeprazole Strontium				Esomeprazole Magnesium			
Dosage Level (mg/kg/day)	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Found Dead or Euthanized *in extremis*[a]	13(8)	48(18)	32(16)	34(12)	17(12)	38(18)	54(20)	41(14)
Missing	4(3)	7(5)	9(6)	9(7)	6(2)	6(5)	16(12)	13(8)

[a] = Numbers of pups (litters).

The number of pups found dead or euthanized in extremis was slightly higher in the EMT treatment group, as compared to EST and vehicle control. There was an increased incidence of pups with small stature at EST doses of ≥ 84.7 mg/kg/day and at EMT doses of ≥ 153.3 mg/kg/day, as compared to vehicle control (modified diet).

c. Survival, PND 21 through PND 70: There were several unscheduled deaths in the EST and EMT treatment groups. Number of unscheduled deaths was slightly higher in the EST treatment group as compared to EMT.
   i. EST: Between PND 22-28, there were 4, 12, and 5 males and 2, 7, and 6 females in the 84.7, 169.5 and 343.8 mg/kg/day EST dose groups, respectively, which were found dead or were euthanized *in extremis*. One high dose EST group female was found dead on PND 36 and one 169.5 mg/kg/day EST group female was found dead on PND 61. Two high dose EST males were found dead on PND 60 or 65. Clinical signs in animals experiencing unscheduled deaths included hypoactivity, red material around nose or mouth, cool and/or pale body, and impaired use of hindlimb and unkempt appearance. Twenty one of the 40 pups which died ahead of scheduled study termination had mean body weights < 35.5 g, which were lower than the historical control reported by the testing laboratory. The majority of unscheduled EST-related deaths occurred in the post-weaning period (within 7 days after weaning). All other pups survived to scheduled terminal necropsy.
   ii. EMT: There were 3, 6, and 2 males and 6, 6, and 4 females in the 76.7, 153, and 311.1 mg/kg/day EMT treatment groups, respectively, which died prior to scheduled necropsy or were euthanized in extremis between PND 22-28. One male in the 153.3 mg/kg/day was found dead on PND 36 in addition to 2 high dose males on PND 35 or 42 and 1 pup/sex in the 76.7 mg/kg/day EMT group on PND 59 or 68. Clinical signs in these animals prior to death included gasping, decreased respiration, prostrate body, pale/cool body, impaired use of hind limbs, eyelids, red material around nose, yellow material on ventral abdominal, urogenital and thoracic areas, improper mobility (rocking, lurching, swaying while ambulating). Of the 32 animals which died ahead of scheduled necropsy, 13 had mean body weights that were < 35.5 g on PND 21, which were lower than historical control of the testing laboratory. All other pups survived to scheduled terminal necropsy.

2. Clinical Signs: No treatment-related clinical signs were recorded in the post weaning period (PND 21-PND 70).

3. Body Weight: Pup weight was affected in a treatment-related manner.
   a. Pre-selection: On PND 4, pups born to EST-treated dams had statistically significant decreases in body weight (9.9% to 17.2%) at doses ≥ 84.7 mg/kg/day. In offspring of EMT-treated dams, decreases in body weight of 13.5% to 17.6% were observed at doses ≥ 153.3 mg/kg/day.
   b. Post-selection: During PND 10-14, there was a dose-dependent decrease in body weight gain in offspring of dams in both EST and EMT treatment groups, with statistically significant changes at equimolar doses of

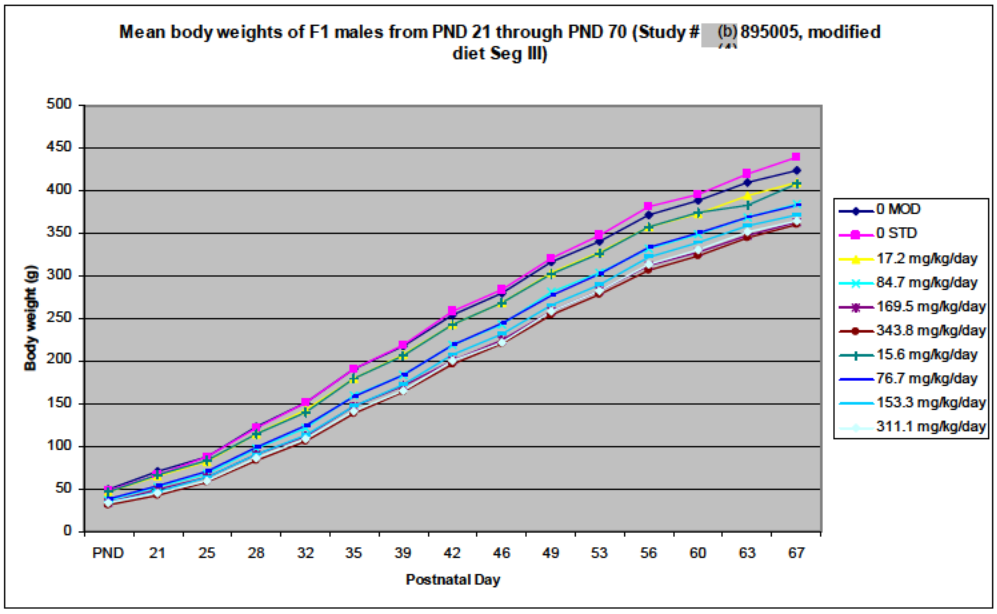
Appears This Way On Original

Reference ID: 3289165

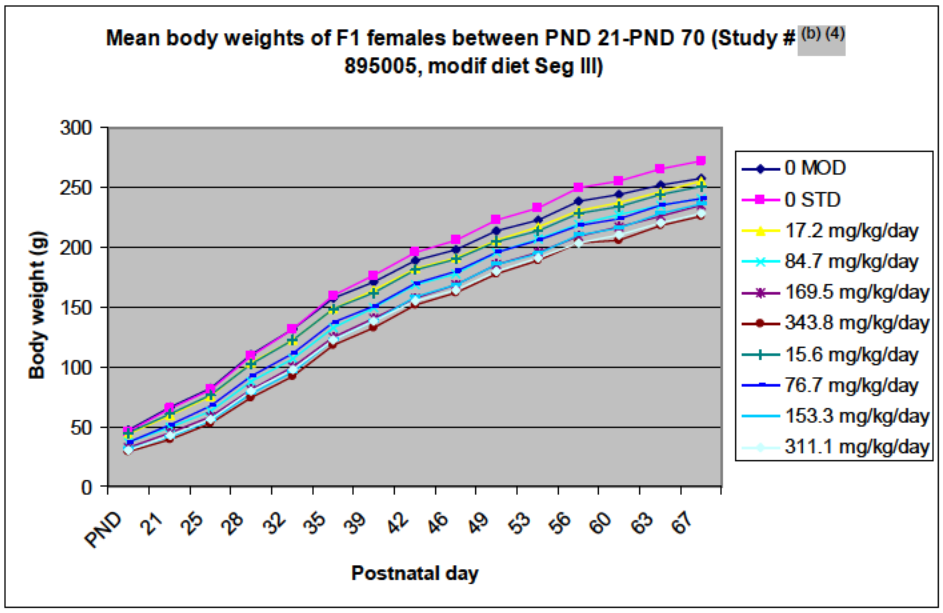
			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Pup Body Weights[a] (g)										
Postnatal Day 4 (Before Selection)										
Males	9.6	-4.2%	-5.2%	-10.4%*	-10.4%*	-16.7%**	-1.0%	-6.3%	-13.5%**	-13.5%*
Females	9.1	-6.6%[c]	-6.6%	-9.9%*	-11.0%*	-17.6%**	-2.2%	-6.6%	-17.6%**	-15.4%**
Postnatal Day 21										
Males	49.7	-1.6%	-6.6%	-23.9%**	-31.0%**	-37.4%**	-5.6%	-22.9%**	-31.4%**	-32.6%**
Females	47.3	-1.7%	-6.3%	-23.3%**	-29.8%**	-37.8%**	-4.4%	-22.2%**	-34.5%**	-36.8%**
Pup Body Weights Gain (g)										
Postnatal Days 10-14										
Males	9.9	10.0	9.7	7.5**	6.8**	6.3**	9.3	7.6**	6.7**	6.5**
Females	9.6	9.9	9.6	7.4**	6.4**	6.2**	9.5	7.4**	6.5**	5.9**
Postnatal Day 17-21										
Males	10.7	10.4	9.5	6.6**	5.3**	4.6**	9.8	6.2**	5.2**	5.4**
Females	9.8	9.8	9.1	6.7**	5.6**	4.3**	9.2	6.0**	4.7**	4.8**

c. Post-weaning to PND 70: Body weights of offspring were similar between EST and EMT treatment groups. They are shown in the graphs below.

Figure 2: Body weights of offspring of dams treated with either EST or EMT during the post-weaning period (PND 21-PND 70), (A) F1 Males, (B) F1 Females)



(A)



(B)

4. Necropsy observation
    a. Unscheduled deaths (prior to weaning): Pale liver was noted in 4, 3, and 3 pups in the 84.7, 169.5, and 343.8 mg/kg/day EST treatment groups, respectively. Pale liver was also noted in 4 and 2 pups in the 153.3 and 311.1 mg/kg/day EMT dose groups. This was considered an adverse finding, related to treatment of dams with EST and EMT at doses ≥ 84.7 mg/kg/day and ≥ 153.3 mg/kg/day, respectively.
    b. Scheduled necropsies of unselected pups and pups euthanized due to death of the dam: Pale liver was recorded in 5, 9, and 15 pups in the 84.7, 169.5, and 343.8 mg/kg/day EST treatment group and in 9, 10, and 8 pups in the 76.7, 153.3 and 311.1 mg/kg/day EMT dose groups, respectively. No other significant treatment-related findings were recorded.
    c. Unscheduled deaths (post weaning): No significant treatment-related findings were recorded.
    d. Scheduled necropsies on PND 70: No other significant treatment-related findings were recorded.

5. Exposure Assessment
    a. Sr and Ca: There was a dose-dependent increase in plasma Sr concentration in EST-treated animals, in a greater than dose-proportional manner, as compared to controls. There was no apparent gender-specific effect on plasma Sr concentration. Exposure to Sr (in plasma, femur, and liver) in EMT-treated animals was similar to controls. Calcium concentration in the femur was unaffected by treatment with either EST or EMT and was similar to control in all animals. The applicant's table below summarizes the Sr and Ca levels in pup plasma, femur and liver.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
F_1 Litters										
Strontium Analysis (Postnatal Day 10)										
Pup Plasma (From combined samples collected at approximately 20 hours after the maternal dose on LD 9 or 2 hours after dosing of the dam) (ng/mL)										
Males	40.7	26.1	117.6	575.9	1236.3	2835.7	34.1	31.5	32.2	35.9
Females	35.1	26.3	110.8	560.5	1286.3	2710.0	33.8	33.0	73.5	34.1
Pup Femur (µg/g)										
Males	4.7	0.7	26.2	138.6	312.7	835.6	8.6	8.0	6.7	7.7
Females	5.7	0.4	25.6	144.9	323.5	826.8	7.7	10.2	8.1	6.9
Pup Liver (ng/g)										
Males	12.63	5.83	37.06	195.83	420.63	1450.90	12.52	13.86	20.84	17.23
Females	12.68	13.40	121.20	357.83	386.92	822.56	12.60	14.22	13.15	13.60
Calcium (mg/g)										
Pup Femur										
Males	26.5	28.1	28.9	27.0	30.4	32.2	32.5	33.7	34.6	38.2
Females	28.5	27.8	28.6	27.8	30.9	33.1	32.3	33.5	35.6	34.5

b. Esomeprazole: Esomeprazole was measured in fetal plasma on PND 10. The Maternal/neonatal plasma ratio indicated very low exposure to esomeprazole in the pups on LD 10 (i.e. pups are to be getting exposed to esomeprazole through mother's milk). The table below shows the plasma concentration of esomeprazole in pups sampled on LD 10 at 20 h post-dose (post-dose of dams) and on LD 9 at 2 h post-dose.

Table 10: Plasma concentration of esomeprazole in F1 offspring of dams treated with EST or EMT in Study (b) (4) 895005

	EST				**EMT**			
Daily Dose (mg/kg/day)	**17.2**	**84.7**	**169.5**	**343.8**	**15.6**	**76.7**	**153.3**	**311.1**
Dose as Esomeprazole (mg/kg/day)	**14**	**69**	**138**	**280**	**14**	**69**	**138**	**280**
Males								
20 h post dose, LD 10	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ*
2 h post dose, LD 9	BLQ	BLQ	BLQ*	3.32	BLQ	BLQ	3.97	15.4
Maternal/Neonatal Ratio	NA	NA	186	837	NA	NA	205	356
Females								
20 h post dose, LD 10	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ*
2 h post dose, LD 9	BLQ	BLQ	BLQ*	3.85	BLQ	BLQ	3.59	30.5
Maternal/Neonatal Ratio	NA	NA	87.8	362	NA	NA	219	158

* BLQ represents the mean concentration.

6. Organ Weights: There were statistically significant decreases in right femur weight in pups of dams treated with esomeprazole doses ≥ 69 mg/kg/day (23.9% to 36.3% in EST group pups and 28.0% to 43.3% in EMT group pups, as compared to vehicle control). Because of the decrease in body weight during this period, the relative femur weight (relative to body weight) was unchanged. There were statistically significant decreases in liver weights in pups of dams treated with esomeprazole doses ≥ 69 mg/kg/day (23.8% to 36.5% in EST group pups and 30.0% to 35.7% in EMT group pups, as compared to vehicle treatment). Because of the decrease in body weight during this period, the relative liver weight (relative to body weight) was unchanged. The applicant's

table below summarizes the statistically significant changes in organ weights recorded on PND 21 for the pups in each dose group.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Right Femur[a] (g)										
Males	0.2017	-0.9%	-3.3%	-23.9%**	-23.3%**	-36.3%**	-2.5%	-28.0%**	-40.2%**	-35.7%**
Females	0.1848	2.4%	-2.2%	-30.9%**	-34.5%**	-34.7%**	3.1%	-33.1%**	-43.3%**	-37.1%**
Right Femur (g/100 g Body Weight)										
Males	0.409	0.414	0.417	0.390	0.414	0.415	0.386	0.411	0.393	0.393
Females	0.401	0.414	0.405	0.374	0.408	0.399	0.390	0.385	0.383	0.392
Liver[a] (g)										
Males	2.1139	-4.0%	-5.6%	-25.6%**	-28.4%**	-36.5%**	-1.6%	-30.0%**	-34.0%**	-35.1%**
Females	1.9963	-0.1%	-3.6%	-23.8%**	-32.7%**	-31.0%**	1.8%	-31.4%**	-34.9%**	-35.7%**
Liver (g/100 g Body Weight)										
Males	4.248	4.218	4.278	3.952	4.047	4.368	4.073	4.230	4.586	4.200
Females	4.332	4.326	4.301	4.476	4.575	4.519	4.152	4.331	4.835	4.340

* $p<0.05$, as compared to control, ** $p<0.01$, as compared to control

7. Developmental Landmarks, Sensory Function, and Behavioral Testing
    a. Developmental landmarks:
        i. Balanopreputial separation (BPS) in male offspring was observed EST treatment group pups at doses ≥ 84.7 mg/kg/day, as compared to vehicle-treated control. BPS was attained at 49.0, 49.1, and 49.9 days in offspring born to dams treated with 84.7, 169.5, 343.8 mg/kg/day EST, respectively, as compared to control (46.5 days) and historical control values of the testing laboratory (48.2 days). There was also a statistically significant decrease (up to 11.2%, as compared to control) in body weight on the day of BPS attainment in affected EST animals. Delay in BPS was observed in pups of EMT-treated dams at doses ≥ 153.3 mg/kg/day, as compared to control. Delay in BPS was observed in EMT group pups at doses ≥ 15.6 mg/kg/day; however, these values were within the historical control of the testing laboratory. The delay in BPS correlated with up to an 11.6% decrease in body weight in affected EMT animals.
        ii. Vaginal Patency: Female offspring of dams treated with EST at ≥ 169.5 mg/kg/day showed a delay in attainment of vaginal patency (VP). VP was attained on 36.2 and 36.5 days for offspring in the 169.5 and 343.8 mg/kg/day EST dose groups, as compared to control group VP value of 33.1 days and historical control of the testing laboratory VP value of 34 days. There was up to a 13.8% decrease in mean body weight on the day of VP attainment in affected EST females. Delay in VP attainment was also observed in the EMT dose groups at ≥ 76.7 mg/kg/day. VP was attained at 35.0, 36.1, and 35.9 days in the 76.7, 153.3, and 311.1 mg/kg/day EMT groups, respectively, as compared to vehicle control and historical control. There was up to a 13.0% decrease in mean body

weight on the day of VP attainment in affected EMT females. These results are summarized in the applicant's table below.

			EST[$]				EMT[$]			
Daily Dose (mg/kg)	0[M]	0[$]	17.2	84.7	169.5	343.8	15.6	76.7	153.3	311.1
Daily Dose as Esomeprazole (mg/kg)	0[M]	0[$]	14	69	138	280	14	69	138	280
Preputial Separation (postnatal Day)	46.5	46.8	48.0	49.0**	49.1**	49.9**	47.2	48.2	50.0**	49.4**
Body Weight at Time of Preputial Separation[a] g)	257.1	2.6%	0.5%	-4.7%	-11.2%**	-11.1%**	-2.5%	-8.7%**	-7.4%*	-11.6%**
Vaginal Patency (postnatal Day)	33.1	33.0	33.4	34.5	36.2**	36.5**	33.3	35.0*	36.1**	35.9**
Body Weight at Time of Vaginal Patency[a] (g)	117.4	-0.5%	-4.8%	-10.0%**	-8.9%*	-13.8%**	-5.7%	-6.0%	-13.0%**	-12.4%**

b. Sensory function: Auditory startle response and motor activity were assessed on PND 20 and PND 60 in selected male and female pups. Overall, there were no obvious treatment-related adverse effects on sensory function on offspring of EST- or EMT-treated dams.
   i. On PND 20, there was a decrease in maximum response values (MAX) in EST and EMT female offspring at doses ≥ 69 mg esomeprazole/kg/day, while a decrease in MAX was observed in males only at the high dose in EST and at 153.3 and 311.1 mg/kg/day EMT, as compared to control. These results were not observed on PND 60 in either sex. TMAX was affected in PND 20 males and females at the highest dose of EST and in PND 20 females at EMT ≥ 153.3 mg/kg/day. TMAX was unaffected in either sex on PND 60. The mean average response values (AVE) were affected only on PND 20 in males at high dose EMT and in females at 84.7 mg/kg/day and 343.8 mg/kg/day and in EMT females at ≥ 153.3 mg/kg/day.
   ii. The total count (locomotor activity) was unaffected in pups (both sexes) in the EMT dose groups and in male pups in the EST dose groups on either testing day (PND 21 or PND 61). There was an increase in total count in PND 21 females only at doses ≥ 84.7 mg/kg/day, as compared to vehicle treatment on modified diet. There was also an increase in activity count in vehicle treated pups in the standard diet group, as compared to controls in the modified diet group. Similar observations were made for ambulatory counts. Ambulatory count was unaffected in pups (both sexes) in the EMT dose groups and in male pups in the EST dose groups on testing day on PND 21. There was an increase in ambulatory activity in PND 21 only in females in the EST group at doses ≥ 84.7 mg/kg/day, as compared to vehicle treatment on modified diet. There was also an increase in ambulatory activity in vehicle treated pups in the standard diet group, as compared to controls in the modified diet group. There were increases in ambulatory counts on

PND 61 in males in the EST group at doses ≥ 169.5 mg/kg/day, on PND 61 in females in the high dose EST group, and on PND 61 in males in the high dose EMT group.

c. Behavioral function: There was an increase in the escape time from Path A (Learning trial) in both males and females on PND 22 at doses of esomeprazole ≥ 69 mg/kg/day. Escape time was unaffected on PND 62. On PND 62, there was an increase in errors in pups in the high dose EST group only. On PND 22, escape time from Path B (learning trial) increased in high dose EST male pups, in female pups at doses of EST ≥ 84.7 mg/kg/day, in male pups at doses of EMT ≥ 76.7 mg/kg/day. There were increases in escape times in PND 62 females in the EST dose groups of 169.5 and 343.8 mg/kg/day only. There was an increase in errors in PND 62 females in EST doses ≥ 84.7 mg/kg/day. There was an increase in escape time on Path A (memory trial) in PND 22 females at 169.5 and 343.8 mg/kg/day EST only, as compared to control. There were no increases in numbers of errors in any of the treatment groups.

8. Bone Parameters: The following bone parameters were assessed on PND 10 in the offspring of dams in the TK dose groups: femur length, width and cortex thickness, proximal tibial growth plate thickness and bone marrow hypocellularity. Overall, the effects on bone parameters in offspring of dams treated with EST or EMT appeared to be similar. These effects were similar in both male and female offspring. There was an increase in incidence of minimal to mild physeal dysplasia in the femur of male and female offspring from both EST and EMT groups at esomeprazole doses of 138 and 280 mg/kg/day. Physeal dysplasia in the femur was considered an adverse effect with a potential for growth abnormalities. The total number of pups (males + females) affected were 0 and 4 in the 169.5 and 343.8 mg/kg/day EST groups, respectively, and 1 and 11 in the 153.3 and 311.1 mg/kg/day EMT groups, respectively. Therefore, at equimolar doses of esomeprazole, the incidence of physeal dysplasia in the femur was numerically higher in offspring of EMT-treated dams, as compared to modified diet control. Proximal tibial growth plate thickness was statistically significantly decreased in male offspring in the 84.7 and 343.8 mg/kg/day EST dose groups and in the 153.3 and 311.3 mg/kg/day EMT dose groups, as compared to control. In females, proximal tibial growth plate thickness was affected in both EST and EMT treatment groups at doses of esomeprazole ≥ 69 mg/kg/day, as compared to control.

Table 11: Changes in bone morphology in offspring of TK animals treated with EST or EMT in Study (b) (4) 895005

		Modif. Diet Ctrl	Std. Diet Ctrl	Esomeprazole Strontium				Esomeprazole Magnesium			
Dose Formula Weight (mg/kg/day)		**0**	**0**	**17.2**	**84.7**	**169.5**	**343.8**	**15.6**	**76.7**	**153.3**	**311.1**
Dose Esomeprazole (mg/kg/day)		**0**	**0**	**14**	**69**	**138**	**280**	**14**	**69**	**138**	**280**
Males	↓ length	0/18	0/24	1/23	6/24	7/23	8/21	0/23	2/21	10/22	10/17
	↓ thickness (cortex)	0/18	0/24	0/23	2/24	6/23	7/21	0/23	1/21	6/22	8/17
	↓ width	0/18	0/24	0/23	3/24	4/23	11/21	0/23	4/21	6/22	9/17
	Dysplasia, Physis-Minimal	0/18	0/24	0/23	0/24	0/23	1/21	0/23	0/21	0/22	6/17
	Hypocellular, Minimal	0/18	3/24	1/23	4/24	8/23	5/21	0/23	3/21	11/22	4/17
	Hypocellular, Mild	0/18	0/24	0/23	0/24	0/23	3/21	0/23	0/21	0/22	1/17
	Proximal tibial growth plate thickness (mm)	0.858	0.868	0.844	0.743**	0.805	0.762**	0.851	0.812	0.775**	0.761**
Females	↓length	0/18	0/23	1/23	6/24	6/20	5/16	0/24	3/21	6/20	14/22
	↓thickness (cortex)	0/18	0/23	1/23	2/24	5/20	6/16	0/24	1/21	3/20	5/22
	↓ width	0/18	0/23	1/23	4/24	5/20	4/16	0/24	2/21	5/20	4/22
	Dysplasia, Physis-Minimal	0/18	0/23	0/23	0/24	0/20	3/16	0/24	0/21	0/20	4/22
	Dysplasia, Physis-Mild	0/18	0/23	0/23	0/24	0/20	0/16	0/24	0/21	0/20	1/22
	Hypocellular, Minimal	1/18	1/23	0/23	2/24	2/20	1/16	3/24	5/21	7/20	5/22
	Hypocellular, Mild	0/18	0/23	0/23	0/24	0/20	2/16	0/24	0/21	1/20	2/22
	Proximal tibial growth plate thickness (mm)	0.806	0.764[c]	0.788	0.721**	0.737**	0.677**	0.758	0.732**	0.701**	0.687**

*p<0.05, **p<0.01, as compared to control

9. Macroscopic Examinations:
    a. Unscheduled deaths: Pale liver was noted in 1 male and 2 females in the 169.5 and 343.8 mg/kg/day EST treatment groups. Pale liver was also observed in 1/sex/dose in the 76.7 and 153.3 mg/kg/day EMT treatment groups. These animals were euthanized in extremis or found dead between PND 22 and 25. This finding of pale liver was considered adverse. No other significant treatment-related macroscopic findings were noted in offspring which experienced unscheduled deaths.
    b. Scheduled deaths: No treatment-related changes were observed at scheduled necropsy of F1 pups on PND 70.

10 Special Toxicology Studies

Study title: A 29-Day Oral (Gavage) Toxicity Study of Esomeprazole Strontium in Juvenile Rats, with 14-Day Recovery

Study no.:	(b) (4) 895007
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	25 May 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Esomeprazole Strontium (EST), Lot# 10SOSHB21008, 99.5% purity Esomeprazole Magnesium (EMT), Lot# EZFP11030, 99.4% purity Methylcellulose, 1500 cps USP, Lot# 051M0179V

Key Study Findings

There were 11 and 9 unscheduled deaths at the high dose of EST and EMT, respectively. There were higher numbers of unscheduled deaths in the EST treatment groups. Based on the findings at necropsy, the applicant attributed the cause of death to dosing-related injury. Irreversible changes in the stomach and lungs were observed in both EST- and EMT-treated animals, particularly at the high dose. There was partial recovery from the treatment-related decrease in body weight gain in both EST- and EMT-treated animals. A slight delay in sexual maturation was observed in high dose EMT females. Femur length and weight were affected in doses $\geq$ 84.7 mg/kg/day EST and at 280 mg/kg/day EMT. Dose-dependent, systemic exposure to esomeprazole was demonstrated with administration of EST and EMT. Dose-dependent exposure to Sr was observed in plasma and bone of EST-treated animals only. The NOAEL dose for juvenile toxicity was the low dose of EST and EMT (86 mg/kg/day and 77.8 mg/kg/day, respectively, or 70 mg esomeprazole/kg/day).

Methods

Doses:	0 (vehicle control) 86, 171.9, 343.8 mg/kg/day EST 77.8, 155.2, 311.1 mg/kg/day EMT Equimolar dosage levels of esomeprazole: 70, 140, 280 mg/kg/day Dosing rationale was based on a previous dose-range finding study (Study # (b) (4) 895008, not reviewed here)
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution in vehicle/0.5% methylcellulose
Species/Strain:	Rats/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	Main study: 20/sex/group
Age:	7 days old (at initiation of treatment)
Weight:	Main Study: 9.1 to 19.7 g (Males), 9.4 to 19.1 g (Females) TK Study: 11.6 to 20.3 g (Males), 8.6 to 20.3 g (Females)
Satellite groups:	Yes, TK study: 45/sex/group
Unique study design:	Time-mated, pregnant females (between GD 3-7) were individually housed and allowed to deliver naturally and nurse their pups to weaning on postnatal day (PND) 21 (PND 0 was designated as day of parturition). On PND 4, litters were randomly culled to 8 pups/litter and 4/sex and carried into the dosing study. Culled, unselected pups were euthanized. Dams were euthanized on PND 21 after F1 pups were weaned.
Deviation from study protocol:	None which affected study results

Observations and Results

Mortality

Juvenile animals were examined for signs of mortality and moribundity twice daily prior to dosing and 1 h post-dosing from PND 7 until necropsy.

In the main study animals, there was an increase in the total number of unscheduled deaths in the EST-treatment group: 4, 3, and 11 animals at 86, 171.9, and 343.8 mg/kg/day EST, respectively. In EMT-treated animals, there were 3 and 9 unscheduled deaths at 155.2 and 311.1 mg/kg/day dose groups, respectively. In both treatment groups, subacute interstitial inflammation of the lungs, necrosis and luminal decries with possible colon obstruction were observed. In EST-treated animals, necrosis was also observed in the esophagus, along with acute inflammation of the mandibular lymph

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node and inflammation and erosion of the glandular stomach. Based on these findings, the applicant attributed the unscheduled deaths to dosing-related injuries (aspiration of test material). Although not significant, there were slightly higher numbers of deaths in the EST treatment group. In the control group, there were 1 male and 1 female which were found dead on PND 16 and 11, respectively. The cause of death for these animals was determined to be acute inflammation of the urinary bladder and prostate and subacute inflammation of lung (male) and coagulative necrosis in the liver (female). There were no unscheduled deaths in the TK animals. These data are summarized in the Applicant's tables 11 (EST group) and 12 (EMT group) below.

Text Table 11. Eso-Sr-Treated Animals that were Found Dead or Euthanized *in Extremis* during the Dosing Period

Animal No.	Sex	Dosage Level (mg/kg/day)	Disposition (Date)	Clinical Observations (Date)	Cause of Death
55180-07	M	86.0	FD (PND 36)	NSCO	Undetermined
53979-13	F	86.0	FD (PND 7)	Swollen left forelimb (PND 7)[a]	Intubation error[e]
55180-11	F	86.0	EIE (PND 22)	Body pale[a]; Red material around nose[a] (PND 22)	Necrosis and bacterial colonization of the heart valve (vegetative valvular endocarditis) with subsequent embolic shower to the brain, kidney, and liver[e]
55183-12	F	86.0	FD (PND 25)	NSCO	Subacute interstitial inflammation and necrosis of the alveolar septa in the lung[e]
55173-02	M	171.9	FD (PND 31)	NSCO	Subacute interstitial inflammation in the lungs[e]
55174-01	M	171.9	FD (PND 35)	Gasping[b]; Clear material around the nose[b] (PND 35)	Subacute interstitial inflammation in the lungs and alveolar septa necrosis[e]
55191-14	F	171.9	FD (PND 15)	NSCO	Subacute interstitial inflammation in the lung and luminal debris in the rectum with possible obstruction[d]
53973-06	M	343.8	FD (PND 10)	Body cool[a,b,c]; Body pale[a,b,c] (PND 9)	Undetermined
53967-04	M	343.8	FD (PND 36)	Salivation[c] (PND 29)	Undetermined
53970-03	M	343.8	FD (PND 9)	NSCO	Acute inflammation of mandibular lymph node and inflammation and erosion of the glandular stomach[d]
55173-06	M	343.8	FD (PND 30)	NSCO	Undetermined
55177-05	M	343.8	FD (PND 9)	Body cool[a,c]; Body pale[c] (PND 8)	Undetermined
55179-16	M	343.8	FD (PND 35)	NSCO	Subacute interstitial inflammation in the lung[e]
55185-03	M	343.8	FD (PND 9)	Body cool[a,c]; Body pale[c] (PND 8)	Erosion, necrosis, and luminal debris with possible obstruction of the ileum and rectum[d]
55176-02	M	343.8	FD (PND 29)	NSCO	Subacute interstitial inflammation in the lung[e]
53979-10	F	343.8	FD (PND 8)	NSCO	Necrosis in the esophagus, cecum, and colon[d]
55182-10	F	343.8	FD (PND 9)	Body cool[a,c] (PND 8)	Erosion, necrosis, and luminal debris with possible obstruction in the cecum and rectum[d]
55191-11	F	343.8	FD (PND 14)	NSCO	Subacute interstitial inflammation in the lung[e]

FD = Found Dead; EIE = Euthanized *in extremis*
NSCO = No significant clinical observations
[a] = Finding noted at the detailed physical examinations
[b] = Finding noted at time of dose administration
[c] = Finding noted at approximately 1 hour following of dose administration
[d] = Cause of death was attributed to Eso-Sr administration.
[e] = Cause of death was not associated with Eso-Sr administration.

Text Table 12. Eso-Mg-Treated Animals that were Found Dead or Missing during the Dosing Period

Animal No.	Sex	Dosage Level (mg/kg/day)	Disposition (Date)	Clinical Observations (Date)	Cause of Death
55181-06	M	155.5	FD (PND 33)	Eyelids completely shut[c] (PND 21)	Subacute interstitial inflammation in the lung[e]
55183-06	M	155.5	FD (PND 12)	NSCO	Subacute interstitial inflammation in the lung[e]
53977-12	F	155.5	FD (PND 28)	NSCO	Subacute interstitial inflammation and alveolar septal necrosis in the lung[e]
53979-03	M	311.1	FD (PND 9)	Body pale[a,b] (PND 8)	Erosion, necrosis, and luminal debris with possible obstruction in the colon[d]
55182-06	M	311.1	FD (PND 9)	Body cool[a,c] (PND 8)	Undetermined
55185-04	M	311.1	FD (PND 8)	Body cool[c] (PND 7)	Inflammation and erosion in the glandular stomach and subacute interstitial inflammation in the lung[d]
55187-01	M	311.1	FD (PND 9)	NSCO	Undetermined
53976-13	F	311.1	MISS (PND 10)	Body cool[a,b,c]; Body pale[b] (PND 9)	NA
53968-12	F	311.1	FD (PND 21)	NSCO	Erosion and necrosis of the jejunum and necrosis of the alveolar septa in the lung[d]
55177-07	F	311.1	FD (PND 9)	Body cool[a, b, c]; Body pale[c] (PND 8)	Undetermined
55173-13	F	311.1	FD (PND 24)	Body cool[c] (PND 8)	Subacute interstitial inflammation and alveolar septal necrosis in the lung[e]
55185-14	F	311.1	FD (PND 10)	Body cool[a,b,c] (PND 8,9); Body pale[b,c] (PND 9)	Undetermined

FD = Found Dead; MISS = Missing; NA = Not applicable
NSCO = No significant clinical observations
[a] = Finding noted at the detailed physical examinations
[b] = Finding noted at time of dose administration
[c] = Finding noted at approximately 1 hour following of dose administration
[d] = Cause of death was attributed to Eso-Mg administration.
[e] = Cause of death was not associated with Eso-Mg administration.

Clinical Signs

Juvenile animals were examined for clinical signs twice daily prior to dosing and 1 h post-dosing from PND 7 until necropsy.

Clinical signs were comparable between EST and EMT treatment groups during the dosing and post-dosing periods. These included red/yellow/clear material on body, shut eyelids, body which was pale or cool to the touch. There was no apparent dose-dependence and the incidence of clinical signs was low.

Body Weights

Pretest body weights of individual juvenile animals were recorded on PND 1, 4, and 7. During the dosing period, body weights were recorded on PND 7, 10, 14, 17, 21, 24, 28, 31, 35, and 36 for main study and TK animals. For those animals in the recovery groups, body weights were recorded on PND 36, 39, 43, 46, and 50.

Body weight gain in EST- and EMT-treated males during the dosing period was similar between PND 7-10, PND 10-14, PND 14-17, PND 17-21, and PND 21-24. These intervals were characterized by slight decreases (statistically significant) in body weight; however, they were comparable between EST and EMT treatment, as compared to control. Between PND 24-28, there was a 13.7% and 20.2% decrease and a 9% and 18.1% decrease in body weight gain in males treated with 171.9 and 343.8 mg/kg/day EST, respectively, and 155.5 and 311.1 mg/kg/day EMT, respectively, as compared to

control males. In the PND 28-31 interval, there were decreases of ~14% (low and mid dose) and 24% (high dose) in body weight with EST treatment and 17.4% and 24.7% decrease in body weight at mid and high dose EMT treatment, respectively, as compared to controls. Body weight gain in EST- and EMT-treated females during the dosing period was mostly similar, as compared to controls, between PND 7-10, PND 10-14, PND 14-17, PND 17-21, and PND 21-24.

During the recovery period (PND 36-50), body weight gain was similar between males in both EST and EMT treatment groups, as compared to controls. Overall, partial recovery in body weight was observed in all treated males during the treatment-free period, unlike what was observed in females. These data summarized in the table below.

Table 12: Percent change in body weight (from control) in F1 pups of EST- and EMT-treated dams during the post-weaning period (PND 21-PND 70) in Study (b) (4) 895005

		Esomeprazole Strontium			Esomeprazole Magnesium		
	Dose Salt (mg/kg/day)	**86**	**171.9**	**343.8**	**77.8**	**155.5**	**311.1**
	Dose Esomeprazole (mg/kg/day)	**70**	**140**	**280**	**70**	**140**	**280**
Males							
PND	7	-0.7	-2.6	2.6	0	-1.3	0.7
	10	-5	-5.5	-6.4	-2.3	-4.5	-8.6
	14	-2.8	-5.3	-8.2	-1.3	-4.7	-9.1
	17	-2.3	-5.2	-7.5	0.3	-3.9	-8.5
	21	-5.5	-7.1	-11.3	-4.8	-5.5	-11.8
	24	-5.7	-6.5	-11.3	-3.8	-5.4	-11.4
	28	-5.8	-8.2	-13.5	-4.8	-6.2	-13
	31	-7.7	-9.5	-15.3	-5.8	-8.7	-15.7
	36	-14.2	-9.5	-11.8	-5.9	-10.1	-17.8
	39	-11.2	-7.1	-9.2	-3.6	-7.7	-14.8
	43	-9.4	-4.3	-6.4	-1.7	-6	-12
	46	-9.2	-4.6	-5	-1.9	-5	-11.2
	50	-8	-4.3	-3.3	0	-3.7	-8.7
Females							
PND	7	0.7	2.1	2.8	-3.5	2.1	2.8
	10	2.5	0.5	-3.4	-2.9	1	-6.4
	14	1.4	-1.4	-2.7	-1	0.7	-6.4
	17	-0.5	-4.4	-4.9	-1.1	0	-7.6
	21	-0.6	-4.5	-5.2	-2.5	-0.6	-6.6
	24	-0.2	-4.2	-6.4	-1.9	-1.6	-7
	28	-1.1	-6.1	-8.9	-3.2	-2.3	-8.5
	31	-0.9	-6.9	-10.1	-3.6	-1.3	-11
	36	-0.5	-8.2	-9.5	-4.3	-1.3	-9
	39	0.8	-7.5	-9	-4.5	-8	-11.3
	43	1.3	-5.3	-7.2	-5.9	2	-7.9
	46	0.6	-5.2	-5.8	-5.8	2.9	-4.6
	50	0	-6.3	-6.8	-8.7	0.5	-3.9

Feed Consumption

Individual food consumption was recorded on PND 28, 31, 35, and 36 during the dosing period and on PND 36, 39, 43, 46, and 50 during the recovery period. Food consumption was not recorded for the toxicokinetic group rats.

Overall, food consumption (g/animal/day) was higher in males than in females. There was an increasing trend in mean food consumed (per animal/day) during the course of the study (through the dosing and to the end of the recovery period).

Ophthalmic Examination

Ophthalmic examination was conducted on all TK animals towards the end of the dosing period and on recovery animals towards the end of the recovery period.

There were no significant treatment-related ophthalmic findings in EST or EMT-treated animals during the dosing and recovery periods.

Hematology

Samples for hematology analysis was collected from 10 rats/sex/group each on PND 36 (at the end of the dosing period) and on PND 56 (at the end of the 14-day recovery period). Blood was collected from fasted animals. The following hematology parameters were evaluated:

- Total leukocyte count (WBC)
- Erythrocyte count (RBC)
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLATELET)
- Prothrombin time (PT)
- Activated partial thromboplastin time (APTT)
- Reticulocyte count
  - Percent (RETIC)
  - Absolute (RETIC ABSOLUTE)
- Mean Platelet Volume (MPV)
- Red cell distribution width (RDW)
- Hemoglobin Distribution Width (HDW)
- Differential leukocyte count - Percent and absolute
  - -Neutrophil (NEU)
  - -Lymphocyte (LYMPH)
  - -Monocyte (MONO)
  - -Eosinophil (EOS)
  - -Basophil (BASO)
  - -Large unstained cell (LUC)
- Platelet estimate[a]
- Red cell morphology (RBC Morphology)[a]

During the dosing period, statistically significant, dose-dependent decreases in HGB and HCT were observed in EST- and EMT-treated males; these changes were mostly reversible. EMT-treated animals had slightly greater decreases in HGB and HCT, particularly at the high dose, as compared to control. The statistically significant, dose-dependent decreases in MCV, MCH and MCHC observed in EST and EMT-treated males were comparable, during the dosing period and reversed in the recovery period. There was a decrease in APTT of 17.9% and 26.4% in EST-treated males at 171.9 and 343.8 mg/kg/day, respectively, while there was a 22.6% decrease in APTT at the high dose EMT-treated males only, as compared to control, which normalized at the end of the recovery period. There was an increase in reticulocytes that was observed in both EST- and EMT-treated males at the high dose (10.9% and 13.5%, respectively), as compared to control (statistically significant only at high dose EMT); this increase was observed at the end of the recovery period as well (11.6% and 9.2% in high dose EST

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and EMT-treated animals, as compared to control). The 25.6% increase in MPV observed in high dose EMT-treated males, as compared to control males, was reversed at end of the recovery period. The decreases in leukocytes observed during dosing were normalized at the end of recovery.

In females, there was a dose-dependent decrease in RBC, HGB, and HCT in all EST- and EMT-treated animals, as compared to controls; however, there was a recovery from this dosing-related decrease at the end of the treatment-free period. Likewise, the decreases in MCV, MCH, and MCHC observed with EST and EMT treatment (which were statistically significant, as compared to control) were also normalized at the end of the recovery period. The decreases observed in APTT in all doses of EST and EMT (statistically significant at all doses of EST and at MD and HD of EMT only, as compared to control) were partially normalized at the end of the recovery period. The increase in reticulocyte count observed during dosing in EST and EMT treatment groups partially normalized, while the increase in MPV completely normalized and was similar to control treatment in both EST and EMT animals. There was a decrease in lymphocyte count (11.4% to 21%) observed at all doses of EST and EMT, as compared to control, in the recovery animals, which not observed in the main study animals. There was a dramatic increase in RDW (29.1% to 45.1% in EST animals and 16.5% to 25.6% in EMT animals) in the recovery group only, as compared to control.

Clinical Chemistry

Sampling for serum chemistry analysis was collected from 10 rats/sex/group each on PND 36 (at the end of the dosing period) and on PND 56 (at the end of the 14-day recovery period). Blood was collected from fasted animals. The following serum chemistry parameters were evaluated:

Albumin	Gamma glutamyltransferase (GGT)
Total protein	Glucose
Globulin [by calculation]	Total cholesterol (Cholesterol)
Albumin/globulin ratio (A/G Ratio) [by calculation]	Calcium
	Chloride
Total bilirubin (Total Bili)	Phosphorus
Urea nitrogen	Potassium
Creatinine	Sodium
Alkaline phosphatase (ALP)	Triglycerides (Triglyceride)
Alanine aminotransferase (ALT)	Bile acids (Bile Acid)
Aspartate aminotransferase (AST)	Appearance[a]

There was an increase in A/G ratio at the high dose in EMT-treated males and females, There was a decrease in albumin, total protein and globulin at the high doses of ES and EMT in females at the end of dosing, which were reversible. The increase in urea nitrogen at the high dose in both EST and EMT females (statistically significant at high dose EMT only), as compared to control, was not reversible; a dose-dependence in higher urea nitrogen values was observed in EST treated recovery animals. Males and females in the high dose groups had higher chloride levels at the end of dosing; however this was not observed in the recovery animals.

Urinalysis

Urinalysis was not conducted.

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Gross Pathology

All animals (including those which were found dead or were euthanized *in extremis)* underwent a full necropsy. Scheduled necropsies occurred on PND 36 (end of dosing) and on PND 50 (end of recovery). Necropsy included examination of all external and internal surfaces and cavities and macroscopic findings were recorded. The following tissues and organs were preserved in formalin for further analysis:

- Adrenal glands (2)
- Aorta
- Bone with marrow (sternebrae)
- Bone marrow smear[a]
- Brain
  - Forebrain (cerebrum level 1)
  - Midbrain (cerebrum level 2)
  - Hindbrain (cerebrum with pons and medulla)
- Coagulating gland
- Eyes with optic nerve (2)[b]
- Femur[c] and tibia (left), including the proximal tibial epiphysis
- Femur (right)[d]
- Gastrointestinal tract
  - Esophagus
  - Stomach
  - Duodenum
  - Jejunum
  - Ileum
  - Cecum
  - Colon
  - Rectum
- Heart
- Kidneys (2)
- Liver (sections of 2 lobes)
- Lungs (including bronchi, fixed by inflation with fixative)
- Lymph nodes
  - Axillary
  - Mesenteric
  - Mandibular
- Ovaries with oviducts (2)[g]
- Pancreas
- Peripheral nerve (sciatic)
- Pituitary
- Prostate
- Salivary glands [mandibular (2)]
- Seminal vesicles (2)
- Skeletal muscle (rectus femoris)
- Skin (with mammary gland)[e]
- Spinal cord (cervical)
- Spleen
- Testes with epididymides (2)[f]
- Thymus
- Thyroid [with parathyroids, if present (2)][g]
- Trachea
- Urinary bladder
- Uterus with cervix and vagina
- Gross lesions

Scheduled necropsy of animals which survived until completion of dosing did not reveal significant treatment-related findings in EST and EMT-treated animals.

Organ Weights

The following organs were weighed at necropsy (on PND 36 and PND 50):

- Adrenal glands
- Brain
- Epididymides (total)
- Femur, right
- Kidneys
- Liver
- Lungs
- Ovaries
- Pituitary
- Prostate
- Seminal vesicles with coagulating glands (with accessory fluids)
- Spleen
- Testes
- Thymus
- Thyroids with parathyroids
- Uterus (with oviducts and cervix)

There was a dose-dependent decrease in adrenal gland weight in the mid- and high dose EST males (6.3% and 14.6%, respectively) during the dosing period only. In the recovery period, partial recovery of adrenal weight was observed in some dose groups only; in the low dose EST and high dose EMT group, an 8.9% and 9.1% decrease, respectively, in adrenal weight persisted at the end of the recovery period. Lower thymus weights were observe din all EST and EMT-treated males and females at the end of dosing. In high dose animals (both EST and EMT), lower kidney weights were also observed. Mid-dose EST animals had lower prostate weights and spleen weights at the end of the dosing period. There were no treatment-related changes in histopathology observed in the terminal or recovery necropsies. There was a decrease

(7.9%, statistically significant as compared to control) in brain weight at the high dose of EST and EMT in males at scheduled necropsy, which partially recovered, although a 5.7% decrease remained at the end of recovery in high dose EMT. There was a decrease in epididymide weight at the mid and high dose.

Changes in femur length and weight were observed in both EST- and EMT-treated animals. There was a statistically significant decrease in femur length (5% in high dose EST males as compared to control) at the end of dosing. In females, there was up to a 3.9% decrease in femur length (statistically significant, as compared to control) at the mid and high dose of EST at the end of dosing. Femur length and weight was affected in high dose EMT females as well. There as a 3.14% and 14.2% decrease in femur length and weight, respectively, as compared to control, at the high dose of EMT only. All changes in bone length and weight normalized to control levels at the end of the recovery period (as shown in the table below).

Table 13: Changes in femur length and weight in juvenile animals treated with EST or EMT for 28 days following by a 14-day treatment-free period (shown as mean ± standard deviation)

			EST (mg/kg/day)			EMT (mg/kg/day)		
Dose Group		**Control**	**17.2**	**84.7**	**343.8**	**15.6**	**76.7**	**311.1**
Males								
End of dosing necropsy (PND 35)	Length (mm)	25.38 ± 0.791	25.36 ± 0.551	24.83 ± 0.505	24.08 ± 0.555**	25.40 ± 0.747	25.21 ± 0.704	24.79 ± 0.452
	Weight (g)	0.544 ± 0.07915	0.5370 ± 0.07094	0.5084 ± 0.07321	0.4634 ± 0.06100	0.5061 ± 0.04684	0.5004 ± 0.04649	0.4665 ± 0.5747**
Recovery necropsy (PND 50)	Length (mm)	31.20 ± 0.653	30.75 ± 1.146	31.27 ± 0.767	31.14 ± 0.494	31.44 ± 0.858	30.98 ± 1.230	30.61 ± 0.926
	Weight (g)	0.8352 ± 0.08297	0.7693 ± 0.09447	0.8438 ± 0.06856	0.8144 ± 0.08730	0.8251 ± 0.06513	07751 ± 0.13288	0.755 ± 0.06641
Females								
End of dosing necropsy (PND 35)	Length (mm)	24.83 ± 0.399	24.56 ± 0.658	24.09 ± 0.756*	23.87 ± 0.622**	24.24 ± 0.622	24.42 ± 0.682	24.05 ± 0.540**
	Weight (g)	0.4726 ± 0.03203	0.4600 ± 0.04015	0.4129 ± 0.04015*	0.4129 ± 0.03134	0.4411 ± 0.06833	0.4392 ± 0.06701	0.4242 ± 0.05173
Recovery necropsy (PND 50)	Length (mm)	29.07 ± 0.488	29.17 ± 0.3	28.62 ± 0.338	28.86 ± 0.568	28.86 ± 0.568	29.01 ± 0.566	28.76 ± 0.311
	Weight (g)	35.745 ± 2.8336	36.397 ± 2.4177	35.556 ± 3.2838	36.084 ± 2.0590	35.088 ± 3.4939	37.660 ± 3.2321	35.121 ± 1.0908

* $p < 0.05$ compared to control
** $p < 0.01$ compared to control

Histopathology

Adequate Battery: Yes (see list of tissues/organs listed under **Gross Pathology**). The applicant preserved the left femur and tibia (including the proximal tibial epiphysis) from all juvenile animals (scheduled and unscheduled deaths). The femur was processed for histopathology analysis. The tibia was not.

Peer Review: No

Histological Findings: The selected histopathological findings from animals which underwent scheduled necropsies on PND 36 are shown in the applicant's tables 13 (terminal necropsy) and 14 (recovery necropsy) below. There was an increased incidence of minimal to mild intestinal metaplasia (glandular stomach) in both EST and EMT treatment groups. One high dose EMT male and female and one high dose EST female had moderate intestinal metaplasia. These findings may be related to the pharmacology of the test article. There were also findings of perivascular mononuclear cell infiltrates in the lungs (minimal to mild) in all EST- and EMT-treated animals, which may be secondary to aspiration of test article or dosing-related injury.

Text Table 13. Incidence of Selected Histopathologic Findings, PND 36 Primary Necropsy[a]

Dosage Level		Eso-Sr			Eso-Mg		
(mg/kg/day)	0	86.0	171.9	343.8	77.8	155.5	311.1
Males							
Stomach, glandular [b]	**10**	**9**	**10**	**8**	**10**	**10**	**10**
Metaplasia, intestinal	0	8*	10*	8*	9*	9*	10*
Minimal	-	8	9	4	9	9	6
Mild	-	0	1	4	0	0	3
Moderate	-	0	0	0	0	0	1
Lungs [b]	**10**	**9**	**10**	**8**	**10**	**10**	**10**
Infiltrate, mononuclear, perivascular	3	5	10*	5	4	6	9*
Minimal	3	4	9	5	4	6	9
Mild	0	1	1	0	0	0	0
Females							
Stomach, glandular [b]	**10**	**9**	**10**	**10**	**10**	**10**	**10**
Metaplasia, intestinal	0	9*	9*	10*	9*	9*	10*
Minimal	-	9	5	7	9	6	6
Mild	-	0	4	2	0	3	3
Moderate	-	0	0	1	0	0	1
Lungs [b]	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Infiltrate, mononuclear, perivascular	0	3	8*	8*	4	5*	6*
Minimal	-	3	8	8	4	5	6

[a] = Only includes animals that survived to the scheduled necropsy. Does not include animals that were found dead or euthanized *in extremis*.
[b] = Number of tissues examined from each group.
* = Significantly different from the control group at p<0.05 using two-tailed Fisher's Exact test.

Text Table 14. Incidence of Selected Histopathologic Findings, PND 50 Recovery Necropsy

Dosage Level		Eso-Sr			Eso-Mg		
(mg/kg/day)	0	86.0	171.9	343.8	77.8	155.5	311.1
Males							
Stomach, glandular [a]	**9**	**10**	**8**	**4**	**10**	**8**	**6**
Metaplasia, intestinal	0	4	4*	2	4	3	4*
Minimal	-	4	4	2	4	3	4
Lungs [a]	**9**	**10**	**8**	**4**	**10**	**8**	**6**
Infiltrate, mononuclear, perivascular	3	6	4	3	5	4	5
Minimal	3	6	4	3	5	4	4
Mild	0	0	0	0	0	0	1
Females							
Stomach, glandular [a]	**9**	**7**	**9**	**7**	**10**	**9**	**5**
Metaplasia, intestinal	0	4*	6*	6*	4	7*	3*
Minimal	-	4	6	6	4	7	3
Lungs [a]	**9**	**7**	**9**	**7**	**10**	**9**	**5**
Infiltrate, mononuclear, perivascular	0	2	4	5*	4	5*	5*
Minimal	-	2	4	5	4	5	5

[a] = Number of tissues examined from each group.
* = Significantly different from the control group at p<0.05 using two-tailed Fisher's Exact test.

Special Evaluation

Developmental Landmarks:

1. Eye Opening: Eyelid separation was evaluated for main study and TK animals beginning on PND 13 and continued until both eyelids were fully opened. Eye opening was considered present if both eyelids were fully open.

The mean ages of eye opening for either sex were not significantly affected by ES or EM treatment.

2. Balanopreputial Separation and Vaginal Patency: Males in the TK group were checked for balanopreputial separation (BPS) beginning on PND 35. The day of first observation of BPS was recorded along with the body weight on that day. Females in the TK group were checked for vaginal patency (VP) beginning on PND 25. The age at which the vaginal lumen was first observed to open was recorded for each animal along with the body weight on that day. Females were examined until VP was noted. These data are summarized in the table below.

Table 14: Age at which balanopreputial separation and vaginal patency were recorded in EST- and EMT-treated animals in Study (b) (4) 895007 (shown as # of days and with corresponding body weight on day of observation)

	Dose	**Esomeprazole Strontium (mg/kg/day)**			**Esomeprazole Magnesium (mg/kg/day)**		
Landmark	**Control**	**86**	**171.9**	**343.8**	**77.8**	**155.5**	311.1
BPS	45.2	46.1	44.3	46.3	44.7	45.5	46.0
Body weight (g)	251.8	236.5	232.0	248.7	241.8	240.8	232.4
VP	33.6	33.4	34.7	34.7	34.3	33.0	35.5
Body weight (g)	114.9	116.2	115.8	113.2	114.1	113.4	118.2

The historical controls for attainment of BPS and VP were 44.6 days and 34.0 days, respectively. There was a slight delay in attainment of BPS in both treatment groups, as compared to control treatment, but was within the upper limit of the historical control for the testing laboratory. There was a slight delay in VP in the high dose EM-treated females, which was higher than the historical control.

Toxicokinetics

Blood samples for esomeprazole and strontium analysis in the plasma were collected on PND 36 and PND 50 from TK animals immediately after euthanasia. For strontium and calcium analysis in the bone, the right femur was collected at necropsy on PND 36 and PND 50 and stored at -70 ^{0}C until analyses done.

<u>Sr analysis</u>:

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Plasma: There was a dose-dependent increase in Sr levels in the plasma in EST-treated animals on PND 35. The Sr levels in the plasma of EST-treated animals on PND 50 remained higher than Sr levels in controls, suggesting that Sr remained in the plasma during the treatment-free period. Sr levels in EMT-treated animals were unaffected and were similar to controls. These data are shown in the table below.

Table 15: Plasma strontium levels in EST- and EMT-treated animals in Study (b) (4) 895007

Plasma Sr levels (ng/mL) Mean ± SD			**Esomeprazole Strontium (mg/kg/day)**			**Esomeprazole Magnesium (mg/kg/day)**		
Sex	**PND**	**Ctrl**	**86**	**171.9**	**343.8**	**77.8**	**155.5**	**311.1**
Males	36	43.5 ± 17.80	412.1 ± 184.9	783.7 ± 348.96	1755.9 ± 662.15	50.5 ± 22.18	49.2 ± 22.75	48.8 ± 21.95
	50	53.0 ± 11.15	155.4 ± 30.40	253.4 ± 52.88	385.8 ± 56.77	49.9 ± 3.87	51.0 ± 4.65	57.7 ± 6.31
Females	36	48.4 ± 17.53	439.4 ± 207.24	871.7 ± 357.67	1900.0 ± 694.21	55.2 ± 19.64	57.9 ± 25.49	48.6 ± 17.37
	50	53.4 ± 9.14	162.50 ± 35.39	289.9 ± 88.53	492.2 ± 119.72	48.6 ± 7.43	45.7 ± 2.8	50.7 ± 5.97

Femur: Sr levels in the femur increased in a dose-dependent manner in EST-treated animals. Sr levels in the femur of EMT-treated animals were similar to controls. Calcium levels were unaffected by EST administration and were similar to controls. Calcium levels in EMT-treated animals were also similar to controls. These data are summarized in the table below.

Table 16: Strontium and Calcium levels in the femur of EST- and EMT-treated animals on PND 36 and PND 50 in Study (b) (4) 895007 (shown as mean ± standard deviation)

Sr and Ca levels in Femur (Mean ± SD)			**Esomeprazole Strontium (mg/kg/day)**			**Esomeprazole Magnesium (mg/kg/day)**		
Sex	**Parameter**	**Ctrl**	**86**	**171.9**	**343.8**	**77.8**	**155.5**	**311.1**
PND 36								
Males	Sr (µg/g)	27.2 ± 3.79	386.2 ± 101.42	664.2 ± 77.82	1475.3 ± 236.28	24.4 ± 2.69	25.2 ± 3.26	22.5 ± 4.63
	Ca (mg/g)	65.9 ± 7.21	61.4 ± 10.05	56.8 ± 7.32	55.4 ± 5.17	60.6 ± 6.25	60.6 ± 8.70	55.7 ± 5.99
Females	Sr (µg/g)	34.0 ± 8.21	419.5 ± 52.43	806.3 ± 80.94	1545.0 ± 247.94	30.5 ± 6.01	34.7 ± 14.48	25.7 ± 4.13
	Ca (mg/g)	74.5 ± 7.23	66.6 ± 9.19	63.0 ± 7.67	56.5 ± 7.66	69.3 ± 8.62	64.1 ± 6.25	62.2 ± 9.78
PND 50								
Males	Sr (µg/g)	35.2 ± 4.34	230.1 ± 36.25	434.3 ± 73.74	879. ± 206.39	35.4 ± 3.63	37.1 ± 8.15	37.6 ± 6.42
	Ca (mg/g)	88.6 ± 7.31	90.0 ± 9.5	89.3 ± 15.2	92.1 ± 11.87	90.8 ± 8.64	94.3 ± 16.08	93.5 ± 7.42
Females	Sr (µg/g)	41.2 ± 6.68	271.3 ± 49.03	519.1 ± 60.45	1043.9 ± 109.73	41.7 ± 4.94	39.5 ± 6.7	42.9 ± 3.16
	Ca (mg/g)	105.9 ±	97.8 ±	97.2 ±	105.3 ±	105.2 ±	99.0 ±	109.0 ±

		13.14	8.48	6.21	11.07	8.72	13.13	5.39

Esomeprazole TK: Esomeprazole was systemically absorbed after administration of EST and EMT. Exposure to esomeprazole was dose-dependent and similar in both EST- and EMT-treated animals. Minimal accumulation of esomeprazole was observed on PND 35. These data are summarized in the table below.

Table 17: Exposure assessment of esomeprazole in EST- and EMT-treated animals in Study (b) (4) 895004 (Mean values are shown)

		ES (mg/kg/day)			EM (mg/kg/day)		
Sex	**Parameter**	**86**	**171.9**	**343.8**	**77.8**	**155.5**	**311.1**
PND 7							
Males	AUC_{last} (ng·h/mL)	41300	95900	452000	52300	92100	422000
	C_{max} (ng/mL)	15100	28000	63700	15600	27100	60200
	T_{max} (h)	0.5	0.5	1	0.5	1	1
	$T_{1/2}$ (h)	2.9	2.1	3.2	2.0	2.6	2.1
Females	AUC_{last} (ng·h/mL)	41500	95700	351000	46700	101000	398000
	C_{max} (ng/mL)	14900	26800	49300	16800	25200	64800
	T_{max} (h)	0.5	0.5	1	0.5	1	2
	$T_{1/2}$ (h)	2.9	2.2	2.7	2.5	2.2	2.2
PND 35							
Males	AUC_{last} (ng·h/mL)	994	2480	7930	827	5420	12300
	C_{max} (ng/mL)	525	475	1920	375	1690	6190
	T_{max} (h)	1	1	2	0.5	1	0.5
	$T_{1/2}$ (h)	1.4	11	NR	5.1	2.3	1.3
	Accumulation ratio	0.02	0.03	0.02	0.02	0.06	0.03
Females	AUC_{last} (ng·h/mL)	1730	9640	13500	1860	6970	18800
	C_{max} (ng/mL)	503	11200	7740	709	2620	6060
	T_{max} (h)	2	0.5	0.5	1	0.5	0.5
	$T_{1/2}$ (h)	NR	NR	2.1	1.1	NR	NR
	Accumulation ratio	0.02	0.06	0.03	0.04	0.07	0.05

NR = not reported because data failed to meet applicant's acceptance criteria

Dosing Solution Analysis

The concentration determination of EST and EMT in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

10 Integrated Summary and Safety Evaluation

Excessive gastric acid secretion by the parietal cells of the stomach can manifest itself as a variety of digestive disorders including gastroesophageal reflux disease

Reference ID: 3289165

(GERD), erosive esophagitis, gastric and duodenal ulcers. Acid secretion is dependent on the H^+/K^+-ATPase pump ("proton pump"), which is stimulated by histamine, gastrin, or acetylcholine. Proton pump inhibitors have been used successfully in patients suffering with digestive disorders related to excessive gastric acid secretion. Omeprazole is a potent and irreversible proton pump inhibitor that is used for the prevention and treatment of gastric acid-related disorders. Omeprazole is a racemic mixture of (S)- and (R)- enantiomers, of which the (S)- enantiomer (esomeprazole) has higher bioavailability and is a more potent inhibitor of gastric acid secretion than the (R)-form. Esomeprazole magnesium trihydrate (Nexium® 20 mg and 40 mg, AstraZeneca) is currently approved in the United States for the treatment of gastrointestinal reflux disease (GERD) and healing of erosive esophagitis for short term treatment (4-8 weeks), maintenance of healing of erosive esophagitis for up to 6 months; symptomatic GERD; risk reduction of NSAID-associated gastric ulcer for up to 6 months; and *H. pylori* eradication to reduce the risk of duodenal ulcer recurrence (triple therapy: Nexium® plus amoxicillin and clarithromycin).

The applicant, Hanmi USA Inc., has developed esomeprazole strontium tetrahydrate (EST) in a delayed-release capsule formulation (20 mg and 40 mg) and is seeking approval for the same indications as Nexium®, including use in children, via the 505(b)(2) pathway. The applicant is relying on Agency's previous determination of the safety of the reference listed drug, Nexium® (esomeprazole magnesium). In support of the NDA, the applicant had submitted general toxicology studies in adult rats, comparative pharmacokinetics in dogs, and genetic toxicology studies with EST. After initial review of the submitted nonclinical information, it was concluded that the proposed product could be given all of the adult indications for which Nexium® is approved; however, the Division was concerned about the use of esomeprazole strontium in children and in pregnant and lactating women. Based on review of additional information submitted during the review cycle, which included a literature-based (nonclinical) safety summary for the safety of Sr during pregnancy and lactation, it was determined that the Sr-containing esomeprazole product could be used in pediatric clinical trials in children ages 2 and older (Please refer to review of SD # 21 dated October 21, 2011). However, the concern regarding the safety of EST in pregnant and lactating women was not adequately addressed by the applicant (Please refer to review of SD # 24 dated October 17, 2011). The applicant received a complete response (CR) in November 2011.

In order to address the deficiencies identified in the CR letter, the applicant conducted an embryofetal development study (Segment II) and enhanced pre- and postnatal developmental toxicity (Segment III) studies in rats. All studies were conducted with at least 3 doses of EST using EMT as the active comparator, along with vehicle control. The salts were compared at equimolar doses of esomeprazole. In addition to assessing the standard toxicology endpoints in each of the Segment III studies, the applicant also compared the effects of treatment with EST, EMT or the vehicle on bone growth (length, growth plate thickness, width) and studied the effect of a calcium and vitamin D-deficient diet on potential EST- and EMT-mediated effects on growth.

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In this submission (resubmission, response to CR), the applicant presented the results from the Segment II and two Segment III studies in rats. In addition, the applicant also submitted the results of a 28-day neonatal direct dosing toxicity study in rats.

Embryofetal Development Study:

The embryofetal development study in rats revealed that administration of either salt at oral doses of up to 343.8 mg/kg/day (EST) or 311.1 mg/kg/day (EMT) from GD 6 through GD 20 was not teratogenic. There was no evidence of treatment-related fetal harm or adverse effect on bone abnormalities (skeletal malformations) in either treatment group. Exposure to esomeprazole was dose-dependent and similar between EST and EMT treatment groups. Esomeprazole was detectable in fetal plasma and surpassed maternal plasma levels at 1.5 h post-dose. Exposure to Sr increased in a dose-dependent manner in maternal plasma and bone and fetal plasma, liver and bone when dams were treated with EST. Sr levels in EMT- and vehicle-treated animals were similar. Calcium levels in were comparable across all dose groups and were unaffected by the salt.

The no observed adverse effect level (NOAEL) dose for developmental toxicity is 343.8 for EST and 311.8 for EMT (280 mg esomeprazole/kg/day). The NOAEL for maternal toxicity was 84.7 mg/kg/day for EST and 15.6 mg/kg/day for EMT (69 and 14 mg esomeprazole/kg/day, respectively) based on findings of decreased body weight gain during gestation.

Pre- and Postnatal Studies:

The pre- and postnatal developmental studies revealed that EST and EMT shared a similar toxicity profile when EST and EMT were administered to dams from GD 6 through LD 21 (end of lactation). Diet modification, in most instances, did not affect the incidence of adverse effects in either treatment group. Maternal toxicity was observed in both EST and EMT treatment groups at doses ≥ 138 mg esomeprazole/kg/day. These included decreased body weight, food consumption, changes in clinical pathology, decrease in bone weight and bone marrow hypocellularity. Another interesting finding was that of a statistically significant decrease in maternal femur weight, significant only in EMT-treated animals fed the standard diet. There was a slight decrease in maternal bone weight noted in both EST and EMT treatment groups fed the modified diet.

Numerous treatment-related effects were observed in F1 pups, including early neonatal and post-weaning toxicity at doses ≥ 14 mg esomeprazole (as either the Sr or Mg salt). Overall, EST and EMT toxicity was evidenced by decreases in body weight/body weight gain and neurobehavioral and/or general developmental delays. with accompanying decrease in body weight on day of attainment) was observed in pups of dams treated with either EST or EMT at equimolar doses of > 138 mg esomeprazole/kg/day (standard diet conditions) and at ≥ 14 mg esomeprazole/kg/day (modified diet conditions), with a statistically significant delay in sexual maturation in pups in the high dose EST group, as compared to control. Lastly, there were several changes in growing bone in both EST and EMT treatment groups, which included decrease in femur length, thickness and width at doses ≥ 69 mg esomeprazole/kg/day.

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There was a dose-dependent decrease in femur weight, with statistical significance at doses ≥ 84.7 mg/kg/day EST and > 153.3 mg/kg/day EMT. There was also a decrease in proximal tibial growth plate thickness, with statistical significance, at doses > 84.7 mg/kg/day EST and ≥ 15.6 mg/kg/day EMT and an increase in incidence of bone marrow hypocellularity (minimal-mild) at doses > 84.7 mg/kg/day EST and ≥ 15.6 mg/kg/day EMT, with higher incidences in the EMT treatment group. Of note, there were findings of physeal dysplasia in the femurs of offspring of dams treated with ≥ 138 mg esomeprazole (≥ 169.5 mg/kg/day EST and ≥ 153.3 mg/kg/day EMT). This finding was considered to be a growth-adverse effect with the potential for growth abnormalities. The table below summarizes the NOAEL doses for each parameter in the Segment III standard and modified diet studies. The NOAEL doses reveal that the toxicity profile of EST and EMT were similar in the Segment III studies.

Table 18: Comparison of NOAEL doses for various parameters in the Segment III studies (Study (b) (4) 895004 (Standard Diet) and Study (b) (4) 895005 (Modified Diet))

Study	Parameter	NOAEL dose of salt(mg/kg/day)	Equimolar dose of esomeprazole (mg/kg/day)
Standard Diet	Maternal toxicity	EST: 84.7 EMT: 76.7	69
	F1 neonatal/early postnatal toxicity	EST: 17.2 EMT: 15.6	14
	F1 post-selection toxicity	EST: 17.2 EMT: 15.6	14
	F1 Developmental & Neurobehavioral toxicity	EST: 169.5 EMT: 153.3	138
	Bone Effects	EST: 17.2 EMT: 15.6	14
Modified Diet	Maternal toxicity	EST: 169.5 EMT: 153.3	138
	F1 neonatal/early postnatal toxicity	EST: 17.2 EMT: 15.6	14
	F1 post-selection toxicity	EST: 17.2 EM: 15.6	14
	F1 Developmental & Neurobehavioral toxicity	EST: 343.8 EMT: 311.1	280
	Bone Effects	EST: 169.5 EMT: 76.7	Sr salt: 138 Mg salt: 69

Exposure Assessment:

Sr crossed the placenta and was expressed in high levels in breast milk. There was a dose-dependent increase in Sr exposure in EST-treated animals, as compared to control, and maternal Sr levels were generally greater than fetal levels (in plasma and bone). Fetal Sr levels in the bone were higher than in the liver. Calcium levels in plasma and bone in dams and pups were unaffected across all dosing groups and independent of diet modifications.

Esomeprazole was systemically absorbed by the dams, with a dose-dependent increase in exposure in the plasma in both EST and EMT treatment groups. Accumulation of esomeprazole observed in dams at the start of lactation. Although high

levels of Sr were detected in the milk, very low plasma esomeprazole levels were detected in F_1 pups both Segment III studies. The maternal/fetal ratios revealed inadequate exposure of esomeprazole to the fetus. Therefore, the applicant conducted a separate juvenile toxicity study, a direct dosing study in neonatal rats, to appropriately assess the toxicity of systemic exposure to EST.

Juvenile Toxicity Study

In the 28-day juvenile toxicity study with 14-day recovery period, treatment with EST and EMT produced numerous unscheduled deaths at the high dose. Death was likely due to dosing-related injury. Irreversible histopathology changes were observed in the stomach and lungs in treated animals, particularly at high dose for both EST and EMT, as compared to vehicle control. A decrease in body weight, with partial recovery was observed with both EST and EMT treatment, particularly at the higher doses. A decrease in femur length was recorded in high dose EST animals (both sexes), females treated with 84.7 mg/kg/day EST, and in females treated with high dose EMT. There was a decrease in femur weight in high dose EMT males and high dose EST females. A delay in sexual maturation, although not statistically significant from control, was noted in high dose EMT females only. Based on the minimal to mild severity of histopathological findings (in lungs and stomach) and the reversibility of treatment-related changes in body weights and organ weights, the NOAEL doses were the low dose of EST (86 mg/kg/day) and EMT (77.8 mg/kg/day). The equimolar dose of esomeprazole at the NOAEL was 70 mg/kg/day.

Exposure assessment

Esomeprazole was systemically absorbed in a dose-dependent manner after EST and EMT treatment. There was a dose-dependent increase in Sr levels in the plasma and bone in EST-treated animals, while Sr levels in EMT-treated animals was similar to vehicle treatment. Sr levels in plasma and bone remained high in EST-treated animals, as compared to control, when measured on PND 50, although they did not exceed PND 35 levels.

The NOAEL doses from the embryofetal development study and the pre- and postnatal studies provide more than sufficient margins of safety for the proposed clinical dose of 40 mg in adults. These new data bolster the literature summary provided by the applicant in support of the use of EST in pediatric trials in children ages > 2. These data, along with nonclinical data presented in the first cycle, provide support for the marketing approval of the proposed clinical dose of 40 mg in adults. Although EST was not teratogenic, as was demonstrated in the embryofetal study, there was evidence of adverse effects to the fetus when EST was administered to the pregnant and lactating rat. Adverse effects included decreased body weight, developmental delays, and physeal dysplasia at the highest dose of EST administered.

An unexpected finding from these studies was that the toxicity profile of EMT was generally similar to EST across the three reproductive and developmental toxicology studies and in the juvenile toxicity study. Maternal bone was adversely affected in both EST- and EMT-treated animals in the standard diet study; however, statistically significant differences were only observed at the two highest doses of EMT. These data

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are the first demonstration of pre- and postnatal toxicities associated with EMT, with specific emphasis on bone growth. Such studies were not conducted with Nexium® or with Omeprazole. Therefore, these new findings suggest that the contribution of esomeprazole free base and each salt to pre- and postnatal toxicity needs to be further elucidated.

Recommendation:

The applicant has adequately addressed all of the deficiencies identified in the CR letter dated November 15, 2011. From a nonclinical standpoint, the proposed clinical product is recommended for marketing approval for the proposed indications in adults.

12 Appendix/Attachments

None

Reference ID: 3289165

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/s/

SRUTHI T KING
04/05/2013

SUSHANTA K CHAKDER
04/08/2013

Addendum to Pharmacologist's Review Dated June 30, 2011 of NDA 20-2342

Sponsor and Address: Hanmi USA Inc.
200 Park Avenue
Florham Park, NJ 07932

Reviewer: Sruthi Tallapragada King, Ph.D.
Division of Gastroenterology and Inborn Errors Products

Drug: Esomeprazole Strontium delayed release capsules

Category: Proton Pump Inhibitor

The following changes in the original nonclinical review of NDA 20-2342 for esomeprazole strontium dated June 30, 2011 are recommended based on our concerns following further review of the available information.

1. **Section 8.1 Pregnancy Category:**
In Section 8.1 (Pregnancy Category) of the proposed labeling, the nonclinical review of this NDA dated June 30, 2011 stated that the recommended pregnancy category was B. This recommendation was based on the nonclinical studies conducted with esomeprazole magnesium. Hanmi USA Inc. did not conduct any reproductive toxicology studies with esomeprazole strontium. Due to the unknown risks of esomeprazole strontium in pregnancy and lactation, the pregnancy category should be changed to **Category C** instead of the initially recommended **Category B**. The revised labeling should read:

Section 8.1 Pregnancy

Pregnancy Category C

(b) (4)

2. **Section 2.5 Comments on impurities/degradants of concern**
Three potential genotoxic impurities were detected in the drug substance and/or the drug product. The first two impurities (b) (4) The third impurity (b) (4) is a byproduct

(b) (4) The levels of the first two impurities, which contain structural alerts for genotoxicity, were below the detection limit of 0.07% which corresponds to a daily intake of (b) (4) mcg. The (b) (4) mcg /day limit for these two impurities is acceptable as per internal CDER policy, and no further action is recommended. Since the third genotoxic impurity is a byproduct (b) (4) which is already qualified, no further action is recommended. The other identified impurities (A, B, C, E1, and E3) do not have a structural alert, and the specifications for these impurities are within the ICH threshold for impurities in drug products with a maximum daily dose of (b) (4) /day.

3. **Pediatric Use:**
In the nonclinical review of this NDA dated June 30, 2011, statements regarding adverse effects of strontium on bone in young rats and mice were based on published data. At the time of this review, the sponsor had not provided data on the safety of strontium in young animals. In Amendment 21 (Pharmacologist's review dated October 21, 2011), the sponsor submitted a literature review of the effects of stable strontium on the bones of young animals and the relevance of these findings to humans. With these new data, the sponsor provided sufficient evidence of safety of strontium to initiate clinical trials with esomeprazole strontium in pediatric patients aged 2 years or older.

_____ _____
Sruthi Tallapragada King, Ph.D. Date
Pharmacologist

COMMENTS

_____ _____
Sushanta Chakder, Ph.D. Date
Supervisory Pharmacologist

cc:
NDA
HFD- 180
HFD- 180/RPM
HFD- 180/Dr. Tallapragada King
HFD- 180/Dr. Chakder

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/s/

SRUTHI T KING
11/07/2011

SUSHANTA K CHAKDER
11/07/2011

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION OF AMENDMENTS

Application number:	20-2342
Supporting document:	Amendment # 21
Applicant's letter date:	September 8, 2011
CDER stamp date:	September 9, 2011
Product:	Esomeprazole Strontium delayed release capsules
Indications:	Treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome.
Applicant:	Hanmi USA Inc.
Review Division:	Division of Gastroenterology and Inborn Errors Products
Reviewer:	Sruthi Tallapragada King, Ph.D.
Supervisor/Team Leader:	Sushanta Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Stacy Barley, R.N., M.S.N., M.H.A.

Reference ID: 3032885

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1 Executive Summary

1.1 Introduction

Gastric acid secretion is dependent on the H^+/K^+-ATPase pump ("proton pump"), which is located in the gastric parietal cells and is stimulated by histamine, gastrin, or acetylcholine. Excessive gastric acid secretion can manifest itself as a variety of digestive disorders including gastroesophageal reflux disease (GERD), erosive esophagitis, and gastric and duodenal ulcers. Proton pump inhibitors have been used successfully in patients suffering with digestive disorders related to excessive gastric acid secretion. The sponsor submitted a 505(b)(2) application for marketing approval of esomeprazole strontium (HM 70231) for the following indications: treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome The reference listed drug (RLD) is Nexium® (AstraZeneca). The sponsor is seeking approval of this product in children ages 1-17 for all of the indications above.

In a communication with the sponsor (dated July 26, 2011), the Division stated that juvenile animal studies with esomeprazole strontium would be necessary to demonstrate safety of strontium prior to initiation of pediatric clinical trials. In a teleconference with the sponsor on September 2, 2011, the Division agreed that the sponsor could submit available animal data to support the safety of strontium in children for review. This information, along with a revised pediatric plan, was submitted in the current submission (Amendment 21.).

1.2 Brief Discussion of Nonclinical Findings

In the current submission, the sponsor provided a literature review of the effects of stable strontium on the bones of young animals and the relevance of these findings to humans. The data submitted provide evidence that Sr has adverse effects on growing bone and bone uptake of Sr decreases with age. Under nutrient deficient conditions (low Ca, vitamin D, and/or phosphorous), the deleterious effects of Sr may be more pronounced. The NOAEL for adverse bone effects of Sr in young rats was 140 mg/kg/day.

1.3 Recommendations

The information submitted by the sponsor provides sufficient evidence of safety of strontium to initiate clinical trials with esomeprazole strontium in pediatric patients.

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2 Drug Information

Drug

CAS Registry Number: 934714-36-0

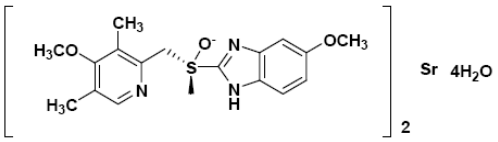
Generic Name: Esomeprazole Strontium Tetrahydrate (EST)

Code Name: HM 70231

Chemical Name: Bis(5-methoxy-2-[(S)-[4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole-1-yl) strontium tetrahydrate

Molecular Formula/Molecular Weight: $(C_{17}H_{18}N_3O_3S)_2 .Sr{\cdot}4H_2O$/848.5

Structure or Biochemical Description



Pharmacologic Class: Gastric parietal cell H^+/K^+-ATPase antagonist (proton pump inhibitor)

Relevant INDs, NDAs, and DMFs

IND 078801 (esomeprazole strontium, Hanmi USA Inc)
NDA 21153 (Nexium, AstraZeneca): Reference listed drug (RLD)
DMF #s (b) (4)

Regulatory Background

In an information request (dated July 26, 2011), in which the Division stated that juvenile animal studies with esomeprazole strontium would be necessary to demonstrate safety of strontium prior to initiation of pediatric clinical trials. The sponsor submitted the current amendment in response to Division's request for available data demonstrating the safety of strontium on developing bone in animals, as discussed in a teleconference with the sponsor on August 2, 2011. The Division agreed to review the submitted data and make a recommendation regarding the need for additional animal studies prior to initiation of pediatric clinical trials.

Also included in this submission was the sponsor's modified pediatric assessment plan. The sponsor is proposing to conduct the following pediatric clinical trials:

Reference ID: 3032885

Indication	Age	Dose	Duration
[redacted]			

(b) (4)

3 Studies Submitted

In the current submission, the sponsor provided a literature review titled “The effects of stable strontium on the skeleton in young animals and a discussion of the relevance of these findings to humans.”

4 Integrated Summary

Esomeprazole strontium is a proton pump inhibitor. The sponsor is seeking approval of esomeprazole strontium capsules for indications identical to the reference drug, esomeprazole magnesium (Nexium®). Nexium® (20 mg and 40 mg, oral) is currently approved in the United States for the treatment of gastrointestinal reflux disease (GERD) and healing of erosive esophagitis for short term treatment (4-8 weeks), maintenance of healing of erosive esophagitis for up to 6 months; symptomatic GERD; risk reduction of NSAID-associated gastric ulcer for up to 6 months; and *H. pylori* eradication to reduce the risk of duodenal ulcer recurrence (triple therapy: Nexium® plus amoxicillin and clarithromycin). Nexium® delayed release capsule is approved in the following populations for GERD: 1-11 years (10 mg or 20 mg), 12-17 years (20 mg or 40 mg), adults (20 mg or 40 mg). Nexium® is not approved for *H.pylori* treatment (triple therapy combination) in ages 17 and under. The sponsor is proposing to conduct 2 pediatric clinical trials. The maximum dose of esomeprazole strontium to be used in the clinical trials will be 40 mg and 10 mg in children weighing > 20 kg and < 20 kg, respectively.

In the current amendment, the sponsor provided a literature review ("Stable strontium effects on the skeleton: Implications of findings in young animals and relevance to the human condition") to support of the safety of strontium in children, specifically focusing on the skeletal effects. Below is a review of the information provided by the sponsor.

In a study with radiolabeled Sr, Forbes et al.[1], demonstrated that uptake of ^{85}Sr was highest in the bone (tibia) of young animals (17 days old) and was inversely proportional to age. Expressed as a percent of administered dose, the concentration of 85Sr was highest in 17 day-old rats (2.2%), followed by 1.3% (22 days old), 0.4% (39 days old), and 0.2% in adult (> 89 days old).

Kroes *et al*.[2], demonstrated in SPF Wistar rats (130-170 g body weight[3]) that continuous dosing of up to 12 weeks with $SrCl_2$ in the diet (under normal Ca, phosphorus, and vitamin D at 0.85%, 0.75%, 1.8 IU/g, respectively) did not produce gross skeletal changes at up to 4800 ppm (4800 mg/kg diet[4]), although there was a dose-dependent increase in accumulation of Sr in the bone. In a separate study[5], when male Wistar rats (21 days old) were fed a diet containing 2% Sr (~300 mg/day[6]) under normal Ca, P, and vitamin D conditions for up to 6 weeks, growth retardation, increased mortality, hemorrhage, paralysis, and widening and increased calcification of the epiphyseal cartilage were noted. In another dietary study[7], female Wistar rats (80-109 g body weight, ~5 weeks of age) were administered up to 0.5% $SrCO_3$ (875 μmol/day or ~ 77 mg/day) for ~4 weeks and did not show any growth effects related to Sr. There was a slight decrease in bone formation, bone resorption and marked decreases in Ca metabolic parameters. Nuefeld *et al.*, demonstrated in male Sprague-Dawley rats (100-125 g body weight) that Sr-induced changes in Ca metabolism alters the mineralization process[8]. The study by Storey[9] clearly demonstrated that the rachitic changes associated with Sr treatment are more pronounced in young animals, as compared to those in adults. Female rats (young and adult animals weighing 40-60 g and 200-250 g, respectively) were placed on a diet (normal Ca and P at 1.9% and 0.9%, respectively) containing 0.19%, 0.38%, 0.75%, 1.0% (young animals only), 1.5%, and 3.0% Sr for 20 days. In young animals, at doses above 0.19%, there was a dose-dependent decrease in calcification as evidenced by an increase in the widening of the epiphyseal cartilage,

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[1] Forbes GB and Reina JC (1969) *J Nutr* **102**: 647-652
[2] Kroes REM *et al.* (1977) *Toxicology* **7**:11-21
[3] SPF Wister rat with a body weight of 130-170 g is likely ~6-8 weeks of age based on historical data (Harlan Laboratories)
[4] Daily food consumption: 15-30 g/day (adult Wistar and Sprague Dawley rat); Sr dose approximated to be ~72 mg/day (based on 15 g/day food intake for 6-8 week old young animal)
[5] Kshirsagar SG (1976) *J Nutr* **106**:1475-1483
[6] Daily food consumption: 15-30 g/day (adult Wistar and Sprague Dawley rat); Sr dose approximated to be ~300 mg/day
[7] Morohashi T *et al.*, (1994) *Jpn J Pharmacol* **64**: 155-162
[8] Nuefeld EB and AL Boskey (1994) *Bone* **15**(4): 425-430
[9] Storey E (1961) *Australas Ann Med* **10**: 213-222

Reference ID: 3032885

an increase in uncalcified bone matrix, and a decrease in bone ash weight, with the greatest changes at the highest doses. In adult animals, widening of the epiphyseal cartilage was noted only in 1.5% and 3.0% Sr dose groups. The NOAEL dose for adverse bone effects of Sr in young animals was ~140 mg/kg/day, while the NOAEL in adult animals was 690 mg/kg/day. There was a dose-dependent increase in Sr content in bone and serum in both young and adult animals. Storey[10] exposed young rats (50-75 g) and adult rats (250-300 g) to strontium carbonate (1.8%) in the diet, which also contained 1.5% calcium and 0.9% phosphorus, for 7 months. Continuous administration of Sr, even under conditions of adequate calcium, phosphorus and vitamin D exposure, first induced rickets in young rats with eventual non-uniform skeletal growth. Histopathological changes in the bones of young animals included areas of uncalcified epiphyseal cartilage, leading to the formation of localized defects, multiple cartilagenous nodules in the epiphysis, metaphysis, and bone shaft. The skull and teeth were also affected in young animals, while changes in adults were much less severe. However, only one dose was used in this study, and NOAEL dose was not established.

Evaluation:

The submitted literature review of the available nonclinical data on strontium in young animals provides sufficient support for the sponsor's proposed clinical trial with esomeprazole strontium in pediatric patients. The minimal risk level (MRL) after oral administration of strontium is 2.0 mg/kg/day[11]. The MRL is derived for oral exposure to stable strontium and its compounds for intermediate duration (15-364 days). The NOAEL dose in young animals was 140 mg/kg/day, based on adverse effects on bone. The sponsor notes that the highest clinical dose in Study 1 (treatment of GERD) will be identical to the approved doses of the RLD Nexium®: 10 mg once daily (patients 1-11 years old, weight < 20 kg), 20-40 mg once daily (patients 12-17 years old, weight > 20 kg). The 40 mg capsule of esomeprazole strontium (ES) contains 5.1 mg of strontium (Sr). The table below shows the Sr content at each proposed dose of ES and each dose in relation to the MRL and NOAEL for adverse bone effects (140 mg/kg/day).

Daily Dose (mg/day)	Sr content (mg)	Body weight (kg)	Daily Sr dose/kg body weight (mg/kg/day)	Fold over MRL	Safety Margin over NOAEL
40	5.1	60	0.085	23.5	~1650x
		20	0.255	7.8	~550x
10	1.3	10	0.13	15.4	~1077x

The maximum exposure to Sr at the highest proposed dose in the 1-11 year age group is 0.13 mg/kd/day, which is 15.4-times the minimum risk level (MRL) and is much lower than the daily exposure to Sr (3.3 mg/day) via drinking water, diet, and inhaled air. The

[10] Storey E (1962) *J Bone Joint Surg* **44-B**: 194-208
[11] " Toxicological profile for Strontium" Agency for Toxic Substance and Disease Registry (ATSDR), 2004

Reference ID: 3032885

NOAEL dose provides ~550-fold margin of safety over the proposed highest clinical dose of 40 mg in patients weighing at least 20 kg.

Recommendations:

From a nonclinical standpoint, the proposed doses in the pediatric clinical trials appear to be safe. However, it should be noted that the effects of chronic use of ES in children are unknown and are beyond the scope of this assessment.

Reference ID: 3032885

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION OF AMENDMENTS

Application number:	20-2342
Supporting document:	Amendment # 24
Applicant's letter date:	October 4, 2011
CDER stamp date:	October 6, 2011
Product:	Esomeprazole Strontium delayed release capsules
Indications:	Treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome.
Applicant:	Hanmi USA Inc.
Review Division:	Division of Gastroenterology and Inborn Errors Products
Reviewer:	Sruthi Tallapragada King, Ph.D.
Supervisor/Team Leader:	Sushanta Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Stacy Barley, R.N., M.S.N., M.H.A.

Reference ID: 3029857

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Reference ID: 3029857

1 Executive Summary

1.1 Introduction

The sponsor submitted a 505(b)(2) application for marketing approval of esomeprazole strontium (HM 70231) for the following indications: treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome. Reproductive toxicity studies in animals have not been conducted with esomeprazole strontium. Thus, the safety of the drug in pregnant and lactating women is not known. As discussed in a teleconference with the sponsor on September 28, 2011, the Agency requested the sponsor to provide evidence of safety of strontium in pregnancy and lactation. In the current submission, the sponsor submitted summaries of available nonclinical information to address the Agency's concerns regarding the safe use of this drug product in pregnant and lactating women.

1.2 Brief Discussion of Nonclinical Findings

In the current submission, the sponsor provided a literature review of the available nonclinical information for strontium in support of the safety of the use of esomeprazole strontium during pregnancy and lactation.

1.3 Recommendations

The information submitted by the sponsor is not sufficient to demonstrate the safety of the use of esomeprazole strontium during pregnancy and lactation. We recommend that the sponsor conduct a standard segment II (embryofetal development) and an enhanced segment III (pre- and post- natal development) study in one species (rat) to demonstrate the safety of esomeprazole strontium on early stages of development. The studies should include esomeprazole magnesium as an active comparator and a placebo control, in addition to at least 3 dose groups of esomeprazole strontium. The segment III study should also include dosing groups on normal and nutrient deficient (Ca and vitamin D deficient) diet to better understand the impact of nutritional changes on the use of this product. Furthermore, the segment III study should be conducted with an emphasis on bone pathology (examination of long bone, growth plates and mineralization patterns) in addition to standard toxicology parameters. We recommend that the sponsor submit protocols for review prior to initiating the studies.

2 Drug Information

2.1 Drug

CAS Registry Number: 934714-36-0

Reference ID: 3029857

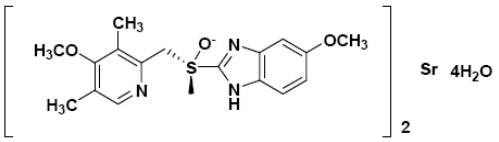
Generic Name: Esomeprazole Strontium Tetrahydrate (EST)

Code Name: HM 70231

Chemical Name: Bis(5-methoxy-2-[(S)-[4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole-1-yl) strontium tetrahydrate

Molecular Formula/Molecular Weight: $(C_{17}H_{18}N_3O_3S)_2 .Sr{\cdot}4H_2O$/848.5

Structure or Biochemical Description



Pharmacologic Class: Gastric parietal cell H^+/K^+-ATPase antagonist (proton pump inhibitor)

2.2 Regulatory Background

The sponsor submitted an amendment in response to a request from the FDA made during a teleconference on September 28, 2011.

3 Studies Submitted

The sponsor submitted a literature review available nonclinical information to support the safe use of strontium during pregnancy and lactation.

4 Integrated Summary

Esomeprazole strontium is a proton pump inhibitor. The sponsor is seeking an approval of esomeprazole strontium capsules for indications identical to the reference drug, esomeprazole magnesium (Nexium®). Nexium® (20 mg and 40 mg, oral) is currently approved in the United States for the treatment of gastrointestinal reflux disease (GERD) and healing of erosive esophagitis for short term treatment (4-8 weeks), maintenance of healing of erosive esophagitis for up to 6 months; symptomatic GERD; risk reduction of NSAID-associated gastric ulcer for up to 6 months; and *H. pylori* eradication to reduce the risk of duodenal ulcer recurrence (triple therapy: Nexium® plus amoxicillin and clarithromycin). Nexium® has a pregnancy category label B, based on reproductive toxicology studies in rats and rabbits. In the current label, the use of Nexium® during lactation is not recommended, unless benefit to the mother outweighs risk to her child, since omeprazole (the racemate of esomeprazole) is excreted in breast milk.

No reproductive toxicology studies have been conducted with esomeprazole strontium. Strontium is a common element in the environment and can act as a calcium

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analog in the body. Strontium can cross the placenta and can be excreted into breast milk. Strontium mainly distributes to the bone, where it can replace calcium. Infants and children absorb more strontium from the gut, as compared to adults, and therefore may be more susceptible to the bone effects of excess strontium, such as rickets.

In the current amendment, the sponsor provided responses to several issues raised during a September 28, 2011 teleconference. The sponsor has submitted a literature review ("Fetal in utero and Neonatal Lactational Exposure to Strontium: Relevance to use of Strontium Containing Products by Women of Reproductive Potential") to address the Agency's concern regarding the safety of strontium in women who are pregnant or lactating and in utero and post-natal exposure to strontium. (b) (4) [redacted] The sponsor also stated that postmarketing data from Korea, where this drug is already approved, indicates that administration of esomeprazole strontium to 2 pregnant women (out of 21,714 total patients) did not reveal any adverse events in these 2 women; no additional details were provided. The post-marketing plan is not reviewed here.

The literature review submitted by the sponsor is summarized below.

In-utero exposure to strontium:

The sponsor cited a study by Lansdown *et al.* [1] to support the claim that strontium has no effect on skeletal development. In this study, pregnant Wistar rats (3 females/dose) were treated with 25, 50, 100, or 200 mg/kg strontium nitrate subcutaneously from gestational day (GD) 9 through GD 19, the period of fetal osteogenesis. Control animals were treated with equal volume of distilled water (1 mL). Animals were sacrificed on GD 19 and fetuses were processed for histology, alizarin and sodium rhodizonate staining, and atomic absorption spectrophotometry. There was no treatment-related difference in the total number of fetuses (30-36 fetuses/dose) or resorption (2-6/dose) sites. Alizarin staining did not reveal any skeletal abnormalities or abnormal zones of calcification. Faint sodium rhodizonate staining in the ossifying regions indicated the presence of strontium. Fetal strontium concentration was independent of dose, while fetal calcium was higher in fetuses from mothers treated with 50 mg/kg/day strontium nitrate. This reviewer agrees with the sponsor's assessment that strontium exposure *in utero* did not affect fetal skeletal development. However, the timing of *in utero* treatment does not meet the criteria for an acceptable segment II teratology study (treatment duration should be GD 6-16 for rats).
Other studies cited by the sponsor include data from Comar *et al.*[2] and Wasserman *et al.*[3] where placental transfer of strontium (radioactive) administered in the food, drinking

[1] Lansdown AB et al. (1972) *Experientia* **28**(5): 558-560.
[2] Comar CL *et al.* (1955) *Proc Soc Exp Biol Med* **92**(4) : 859-863.

Reference ID: 3029857

water, or via maternal subcutaneous injection (administration into fetal sac) was studied. When compared to pups born to mothers maintained on normal diet, pups born to mothers treated with strontium showed a greater preference for Ca incorporation into bones, as compared to strontium. However, mothers on a low calcium diet had pups which had a higher ratio of strontium to calcium. In rats and rabbits, strontium exposure to the fetus was shown to be influenced by the placental barrier, maternal excretion of strontium via the urine, and fetal discrimination for calcium over strontium. In a chronic dietary study (3-3.5 years treatment) with ^{90}Sr in pregnant dogs, Burykina[4] showed that ^{90}Sr was transferred through the palcenta and bone concentration of the pups was 17-46% of the levels in maternal skeletons. Pups born to treated mothers showed a preference for calcium over strontium, similar to what was observed in rats and rabbits. Pups retained 0.4-0.05% of ^{90}Sr measured in the maternal skeleton at 2.5-5 years after stopping treatment. Furthermore, the amount of ^{90}Sr transferred to the fetus via the milk was 10-17 times higher than through the placental barrier. No adverse effects on growth were reported. No other developmental parameters were reported in this study. This reviewer agrees with the observations reported by the sponsor. However, it should be noted that the dog is not a standard species for embryofetal and pregnancy/lactation studies.

The sponsor also provided evidence of strontium use in humans during pregnancy. As observed in animals, Rivera *et al.* [5] reported placental discrimination of calcium over strontium in the human fetus; however, this study is limited by sample size. Studies in women (6 cases of women and their stillborn infants) who were exposed to radioactive strontium due to radiation fallout in the Techa river system[6] revealed that maternal to fetal transfer of strontium was low, with timing (maternal age) of exposure influencing fetal concentrations of strontium only slightly. The coefficient of transfer from mother to fetus was very low (0.012-0.032) when ^{90}Sr was ingested by the mother during the period of intensive compact bone growth. The rate of transfer was much higher (0.21-0.26) when exposure to ^{90}Sr occurred during the reproductive age of the mother.

To support the limited exposure of the fetus to strontium via breast milk, the sponsor cited papers by Comar *et al*[7] and Wasserman *et al*[8] in which calcium was preferentially excreted into breast milk, as compared to strontium, in various species including cows and goats. Similar findings were reported by Jarvis *et al*[9] in humans. The sponsor also mentioned the case of a pregnant woman who was treated with strontium ranelate for

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[3] Wasserman RH *et al*. (1957) *Am J Physiol* **189**(1): 91-97.
[4] Burykina LN (1967) Strontium-90 transfer to progeny via placenta and milk, pp. 237-246. In Lenihan, J.M.A. et al. (eds.) Strontium Metabolism. London and New York: Academic Press.
[5] Rivera J (1963) *Nature* **200**: 269-270.
[6] Tolstykh EI et al., (1998) Radiat Prot Dosimetry 79(1-4) : 307-310.
[7] Comar CL *et al.*, (1957) *Science* **126**:485-496.
[8] Wasserman RH *et al.*, (1958) *J Dairy Sci* **41**(6): 812-821.
[9] Jarvis AA *et al.*, (1963) *Can Med Assoc J* **88**: 136-139.

Reference ID: 3029857

pregnancy and lactation-associated osteoporosis[10]. There is one maternal/fetal pair included in what is likely a post-marketing or off-lable use study, and no adverse effects on the fetus (congenital malformations or neonatal consequences) were reported.

Safety Evaluation

The sponsor submitted published data in support of their claims of the safety of the use of esomeprazole strontium during pregnancy and lactation. Supporting data include administration of stable strontium and radioactive strontium in the diet or drinking water to animals (rats, rabbits and dogs) and studies in humans who were exposed to strontium from radiation fallout and strontium ranelate (Protelos®) for pregnancy/lactation-induced osteoporosis.

Strontium is naturally occurring in the environment and exposure to elemental strontium occurs through the air, water, soil, and food. The estimated daily exposure of strontium in adults is 3.3 mg/day (0.055 mg/kg/day for 60 kg individual). The minimal risk level (MRL) after oral administration of strontium is 2.0 mg/kg/day[11]. The MRL is derived for oral exposure to stable strontium and its compounds for intermediate duration (15-364 days). Prolonged use of Protelos® (approved in Europe for post-menopausal osteoporosis), which contains 2 g strontium (517 mg/day), has not shown clinically significant adverse effects. Safety and efficacy of Sr-renalate were investigated in 2 large multinational trials and showed that adverse effects were similar to placebo and were generally mild and transient, most common being nausea and diarrhea[12]. However, Protelos® is not approved for use during pregnancy and lactation.

The maximum level of strontium in a 40 mg capsule of HM 70231 is 5.09 mg (0.085 mg/kg for a 60 kg individual). Therefore, the total daily intake of Sr from a 40 mg capsule of HM 70231 is much lower (about 24-fold) than the MRL. In animals, high doses of strontium (>500 mg/kg/day) in young rats and mice produced adverse skeletal effects. The safety profile of strontium in adults is favorable at the proposed clinical dose. However, the developmental effects of this dose of strontium (short-term or chronic exposure) remain unknown. Although the submitted data provide some support of the safety of the issue of strontium in pregnant and lactating females, they are not sufficient to make an informed labeling decision for this product.

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[10] Tanriover MD *et al.*, (2009) *Spine J* **9**(40) : e20-24.
[11] Agency for Toxic Substances and Disease Registry (2004). Toxicological profile for strontium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service
[12] Blake GM and I Fogelman (2006) *Clin Interv Aging* **1**(4): 367-75

Reference ID: 3029857

Recommendations:

The information submitted in the current amendment is not adequate to support the safety of esomeprazole strontium in pregnancy and lactation and to make an informed labeling decision.

- The sponsor should conduct a standard segment II (embryofetal development) and an enhanced segment III (pre- and post- natal development) reproductive toxicity study in one species to demonstrate the safety of esomeprazole strontiumin pregnancy and lactation.
- The studies should include esomeprazole magnesium as an active comparator and a placebo control, in addition to at least 3 dose levels of esomeprazole strontium.
- The segment III study should also include dosing groups on normal and nutrient deficient (Ca and vitamin D deficient) diet to better understand the impact of nutritional changes on the use of this product. Furthermore, the segment III study should be conducted with an emphasis on bone pathology (examination of long bone, growth plates and mineralization patterns), in addition to the standard toxicology parameters.
- The sponsor may submit protocols for review prior to initiation of animal studies.

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	20-2342
Supporting document:	1
Applicant's letter date:	October 15, 2010
CDER stamp date:	October 15, 2010
Product:	Esomeprazole Strontium delayed release capsules
Indications:	Treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome.
Applicant:	Hanmi USA Inc.
Review Division:	Division of Gastroenterology Products
Reviewer:	Sruthi Tallapragada King, Ph.D.
Supervisor/Team Leader:	Sushanta Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Stacy Barley, R.N., M.S.N., M.H.A.

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Any information or data necessary for approval of NDA #20-2342 that Hanmi USA Inc., does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved

Reference ID: 2968549

application is for descriptive purposes only and is not relied upon for approval of NDA #20-2342.

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1 Executive Summary

1.1 Introduction

Gastric acid secretion is dependent on the H^+/K^+-ATPase pump ("proton pump"), which is located in the gastric parietal cells and is stimulated by histamine, gastrin, or acetylcholine. Excessive gastric acid secretion can manifest itself as a variety of digestive disorders including gastroesophageal reflux disease (GERD), erosive esophagitis, and gastric and duodenal ulcers. Proton pump inhibitors have been used successfully in patients suffering with digestive disorders related to excessive gastric acid secretion. The sponsor has submitted a 505(b)(2) application for marketing approval of esomeprazole strontium (HM 70231) for the following indications: treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome.

1.2 Brief Discussion of Nonclinical Findings

In the current 505(b)(2) submission, the sponsor is seeking approval of two dosages (20 mg and 40 mg) of a strontium salt of esomeprazole in a delayed release capsule formulation for the treatment of gastric acid related disorders. The sponsor is relying on previous demonstration of safety of Nexium® (esomeprazole magnesium, AstraZeneca), the reference listed drug (NDA # 21-153). Additional nonclinical studies submitted in support of this application include pharmacokinetic/toxicokinetic (PK/TK) studies, single dose, acute toxicity and repeat-dose, chronic toxicity studies, genetic toxicity studies with the drug substance (esomeprazole strontium, EST) and a PK study with the drug product (EST delayed release capsule). When compared to Nexium®, the test article showed a similar PK/TK and toxicity profiles in rats and dogs. EST was negative in the Ames test, the *in vitro* mouse lymphoma assay, and the *in vivo* mouse bone marrow micronucleus assay.

Esomeprazole is the purified S-enantiomer of the racemate omeprazole. The pharmacology and toxicology of esomeprazole and omeprazole in nonclinical species have been extensively characterized. Esomeprazole has a long history of safe use in humans since its approval as a magnesium salt in 2001. In the current NDA, the sponsor proposed a strontium (Sr) salt of esomeprazole. The total daily exposure to Sr in each 40 mg capsule of HM 70231 is 5.1 mg, or 0.085 mg/kg/day for a 60 kg individual. The minimum risk level (MRL) for orally administered Sr is 2 mg/kg/day, which is 23.5 times higher than the total exposure to Sr in a single dose of HM 70231. Therefore, this new Sr salt of esomeprazole is not expected to have any additional adverse effects at the proposed high dose.

1.3 Recommendations

1.3.1 Approvability

Reference ID: 2968549

From a nonclinical standpoint, the proposed doses of (20 mg, 40 mg) esomeprazole strontium appear to be safe and should be approved.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following changes should be made in the sponsor's proposed labeling.

Sponsor's Proposed Version:

(b) (4)

3 Pages Of Draft Labeling Have Been Withheld In Full As b4 (CCI/TS) Immediately Following This Page

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 934714-36-0

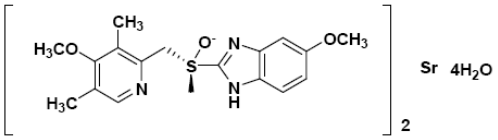
Generic Name: Esomeprazole Strontium Tetrahydrate (EST)

Code Name: HM 70231

Chemical Name: Bis(5-methoxy-2-[(S)-[4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole-1-yl) strontium tetrahydrate

Molecular Formula/Molecular Weight: $(C_{17}H_{18}N_3O_3S)_2 \cdot Sr \cdot 4H_2O$/848.5

Structure or Biochemical Description



Pharmacologic Class: Gastric parietal cell H^+/K^+-ATPase antagonist (proton pump inhibitor)

2.2 Relevant INDs, NDAs, and DMFs

IND 078801 (esomeprazole strontium, Hanmi USA Inc)
NDA 21153 (Nexium, AstraZeneca): Reference listed drug (RLD)
DMF #s (b) (4)

2.3 Drug Formulation

The drug product (HM 70231) is formulated as 20 and 40 mg delayed release capsules. The composition of HM 70231 capsules is detailed in the sponsor's tables below.

Table P.1-1 Composition of the HM 70231 Capsules 20 mg and 40 mg

Component	Unit Quantity (mg/cap)		Function	Reference to Standards
	20 mg	40 mg		
(b) (4)				
Active ingredient:				
Esomeprazole strontium tetrahydrate (as esomeprazole)	24.65 (20.0 mg)	49.3 (40.0 mg)	Active	In-house
Excipients:				
Sugar sphere (b) (4)	(b) (4)		(b) (4)	USP NF
Hypromellose (b) (4)				USP
Calcium carbonate				USP
Polysorbate 80				USP NF
Talc				USP
(b) (4)				USP
				USP NF
				USP
				USP
				USP
				USP NF
				USP NF
				USP NF
				USP NF
				USP NF
				USP
				USP
Total (b) (4)	89.6	179.2		

(b) (4)

Table P.1-2 Composition of the HM70231 Capsules (Hard Gelatin Capsule shells)

Component	Composition (%)	Function	Reference to Standards
Hard Gelatin Capsule shells [1]			
Titanium dioxide	(b) (4)		USP
Gelatin			USP
(b) (4)			

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

The sponsor's table below shows the identified impurities.

Table P.5-12 Impurities A, C, D, E1 and E3

Name of impurity/description	Chemical name and structure	Origin of impurity
Impurity A (USP) (b) (4)	(b) (4)	
Impurity C		
Impurity D		

Impurity E3	(b) (4)
Impurity E1	

The proposed limit for each identified and unidentified impurity is not more than (b) (4), which satisfies the ICH identification threshold for impurities in drug products with a maximum daily dose of ≤ 2 g/day. Therefore, from a nonclinical standpoint, there are no safety concerns associated with these impurities.

2.6 Proposed Clinical Population and Dosing Regimen

Patients with gastric acid-related diseases: for treatment of gastroesophageal reflux disease (GERD), risk reduction of NSAID-associated gastric ulcer, *H. pylori* eradication to reduce risk of duodenal ulcer recurrence, and pathological hypersecretory conditions including Zollinger-Ellison syndrome. The table below summarizes the proposed clinical doses of esomeprazole strontium for the indications sought:

Indications	Age group	Dose	Frequency
GERD	Adults	20 mg, 40 mg	Once daily for 4-8 weeks
	(b) (4)		
Risk reduction of NSAID-associated gastric ulcer	Adults	20 mg, 40 mg	Once daily for up to 6 months
Pathological hypersecretory conditions	Adults	40 mg	Twice daily
H. pylori eradication (triple therapy)	Adults	40 mg 1000 mg Amoxicillin 500 mg Clarithromycin	Once daily for 10 days Twice daily for 10 days Twice daily for 10 days

2.7 Regulatory Background

A Type A, Pre-IND meeting was held on November 28, 2007 to discuss the development plan for esomeprazole strontium. At this time, the Agency agreed that the comparative pharmacokinetic and toxicological studies conducted by Hanmi were sufficient and no additional toxicological studies (chronic toxicity or carcinogenicity) were necessary to support the IND and subsequent NDA application. IND 078801 was submitted on October 14, 2008. On April 27, 2009, a Type A, Pre-NDA meeting was held to discuss the proposed 505(b)(2) NDA application for esomeprazole strontium for the prevention and treatment of gastric acid related diseases. On June 29, 2009, the

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sponsor was granted a Type C meeting to discuss their position that additional trials were not necessary for this product.

3 Studies Submitted

Type of Study	Study#	Test Laboratory	Lot #	GLP
Pharmacokinetics/Toxicokinetics				
Comparative pharmacokinetic study of esomeprazole salts in rats	PT-05-086	Hanmi Pharm. Co., LTD, Gyeonggi-do, Korea	EST: 105093-SEH181 EMT: ES/MG/200506022	Not stated
Comparative pharmacokinetic study of esomeprazole salts in dogs	PT-06-019	Hanmi Pharm. Co., LTD, Gyeonggi-do, Korea	EST: ESPO-12 EMT: FF9705	Not stated
Toxicokinetic study of 13-week repeated oral dose toxicity of esomeprazole strontium tetrahydrate (EST) in SD rats	T06133	(b) (4)	EST: S05043-SFFA191 EMT: Not provided	Yes
Comparative pharmacokinetic study of esomeprazole capsules in beagle dogs	Kvo-07-112	Hanmi Pharm. Co., LTD, Gyeonggi-do, Korea	HM70231: PHIP-0612-004(G) Nexium: F2039	Not stated
Toxicology				
Single dose acute toxicity study of EST in SD rats	B06132	(b) (4)	EST: S05043-SFFA191 EMT: ES/MG/200506022	Yes
Oral maximum tolerated dose study of EST in beagle dogs	B06134	(b) (4)	EST: S05043-SFFA191 EMT: ES/MG/200506022	Yes
13-week oral repeated dose toxicity study and toxicokinetics of EST in SD rats	B06133	(b) (4)	Not provided	Yes
Bacterial reverse mutation test of EST	B06135	(b) (4)	EST: S05043-SFFA191	Yes
In vitro mammalian cell gene mutation test of EST (L5178Y/TK+/- mouse lymphoma assay)	B06136	(b) (4)	EST: S05043-SFFA191	Yes
In vivo micronucleus test of EST	B06137	(b) (4)	EST: S05043-SFFA191	Yes

3.1 Studies Reviewed

Study # Kvo-07-112: Comparative pharmacokinetic study of esomeprazole capsules in beagle dogs

Reference ID: 2968549

3.2 Studies Not Reviewed

The following studies were previously reviewed under IND 078801. Each study is briefly summarized in this review.

Type of Study	Study #
Pharmacokinetics/Toxicokinetics	
Comparative pharmacokinetic study of esomeprazole salts in rats	PT-05-086
Comparative pharmacokinetic study of esomeprazole salts in dogs	PT-06-019
Toxicokinetic study of 13-week repeated oral dose toxicity of esomeprazole strontium tetrahydrate in SD rats	T06133
Toxicology	
Single dose acute toxicity study of esomeprazole strontium tetrahydrate in SD rats	B06132
Oral maximum tolerated dose study of esomeprazole strontium tetrahydrate in beagle dogs	B06134
13-week oral repeated dose toxicity study and toxicokinetics of esomeprazole strontium tetrahydrate in SD rats	B06133
Bacterial reverse mutation test of esomeprazole strontium tetrahydrate	B06135
In vitro mammalian cell gene mutation test of esomeprazole strontium tetrahydrate (L5178Y/TK+/- mouse lymphoma assay)	B06136
In vivo micronucleus test of esomeprazole strontium tetrahydrate	B06137

3.3 Previous Reviews Referenced

NDA 21-153 (Nexium®, Astra Zeneca)
IND 078801 (Esomeprazole strontium tetrahydrate, Hanmi Inc.)

4 Pharmacology

The sponsor did not conduct any pharmacology studies in support of this NDA. Esomeprazole is the (S)-enantiomer of omeprazole, a potent inhibitor of the H+/K+-ATPase (proton pump inhibitor) in the gastric parietal cell. Proton pump inhibitors significantly decrease gastric acid secretion. Omeprazole was approved for the treatment of symptoms associated with GERD in 1996. Nexium® (magnesium salt of esomeprazole) was approved in 2001. In the current submission, the sponsor is seeking approval to market a strontium salt of esomeprazole (HM70231). Since the pharmacology of esomeprazole and omeprazole (the racemate) has been characterized extensively, the sponsor did not conduct any new pharmacology studies in support of this NDA.

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5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: Comparative Pharmacokinetic Study of Esomeprazole Capsules in Beagle Dogs

Study no.:	Kvo-07-112
Study report location:	Hanmi Research Center, Gyeonggi-do, Korea
Conducting laboratory and location:	Hanmi Research Center, Gyeonggi-do, Korea
Date of study initiation:	September 4, 2007
GLP compliance:	Not stated
QA statement:	Not provided
Drug, lot #:	HM70231 Capsule 40 mg, Lot # PHIP-0612-004(G) Nexium® delayed-release capsule 40 mg, Lot # F2039

Methods

Doses:	40 mg capsule each of HM70231 and Nexium®
Frequency of dosing:	Single oral dose
Route of administration:	Oral capsule
Dose volume:	Single oral capsule followed by 4 mL/kg of water
Formulation/Vehicle:	Capsule
Species/Strain:	Dog/Beagle
Number/Sex/Group:	12 Males
Age:	7.5 months
Weight:	10 ± 1 kg
Satellite groups:	None
Unique study design:	This was a crossover study, with a 1-week crossover between doses of HM70231 and Nexium®. Animals were fasted overnight prior to administration of drug.

Observations and Results

Mortality, Clinical Signs, Body weight

Clinical signs were observed pre- and post-dosing. Body weight was measured the day before each dose. No treatment-related deaths or changes in clinical signs or body weight were reported.

PK sampling and analysis

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Blood for PK analysis was collected from the cephalic vein at 0.25, 0.5, 1, 1.5, 2, 4, 6, and 12 hours post-dose. The concentration of esomeprazole (parent drug) and esomeprazole sulfone (main metabolite) were determined in addition to the following parameters: (peak plasma concentration) C_{max}, time to peak concentration (T_{max}), AUC, half life ($t_{1/2}$), volume of distribution (V_z), and clearance (Cl).

Key Findings:

Overall, the PK properties of orally administered HM70231 delayed release capsule were similar to those of Nexium® capsule in dogs, with no statistical significance. The ratio of test formulation to reference formulation (T/R) for $AUC_{0\text{-}12h}$, C_{max}, and T_{max} were 1.142, 1.053, and 1.024, respectively. The 90% confidence intervals for the ratios of log-transformed $AUC_{0\text{-}12h}$ values (107.1-121.7) for esomeprazole were within the acceptance range for bioequivalence (80-125%). Therefore, the test formulation (HM70231) was shown to be bioequivalent to the reference product (Nexium®) in dogs. The PK parameters of esomeprazole determined from the study are summarized in the table below:

Parameter	HM70231 40 mg	Nexium® 40 mg	(T/R)	(%)
$AUC_{0\text{-}12h}$ (n·gh/mL)	13580.15 ± 1318.73	12042.17 ± 1287.4	1.142	114.2
C_{max} (ng/mL)	6144.17 ± 675.98	6210.00 ± 814.56	1.053	105.3
T_{max} (h)	1.17 ± 0.11	1.17 ±0.13	1.024	102.4
$t_{1/2}$ (h)	0.94 ± 0.08	1.14 ± 0.26		
V_z (L)	4.40 ± 0.57	6.78 ± 2.28		
Cl (L/h)	3.28 ± 0.47	3.75 ± 0.47		

The sponsor also conducted three comparative PK studies under IND 078801 to demonstrate bioequivalence between EST (esomeprazole strontium tetrahydrate) and EMT (esomeprazole magnesium trihydrate). Pharmacokinetic profiles of esomeprazole and esomeprazole sulfone, the major metabolite, were evaluated in rats and dogs after single or repeat dosing via intravenous (i.v.), intraduodenal (i.d.), and oral routes of administration. Detailed reviews can be found under IND 078801. They are briefly summarized below.

The PK profiles of esomeprazole strontium tetrahydrate (EST) and esomeprazole magnesium trihydrate (EMT) after a single dose were characterized in 8-week old Sprague-Dawley (SD) rats (5 males/group) after i.v. and i.d. administration (Study # PT-05-086). Overall, PK parameters (AUC, C_{max}, T_{max}, $t_{1/2}$) of esomeprazole (parent compound) and esomeprazole sulfone (major metabolite) were similar after single administration of EST and EMT, regardless of route of administration. Systemic exposure increased dose proportionately with i.d. administration of EST and EMT. The bioavailability of esomeprazole after i.d. administration of 5 and 10 mg/kg EST was 25.9% and 31.4%, respectively, and 21.4% and 27.7% after 5 and 10 mg/kg EMT, respectively. The $t_{1/2}$ of esomeprazole was similar between EST and EMT (0.18-0.24 h for EST and 0.20-0.22 h for EMT).

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Study # PT-06-019 was a pharmacokinetic study in beagle dogs (6 males/group) after oral administration of a single 40 mg dose of esomeprazole. Gelatin encapsulated enteric-coated pellets of EST and commercial Nexium® tablet were used as the test and reference formulations, respectively. A crossover study was conducted after a 2-week washout period between doses. The mean $AUC_{0\text{-}12h}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max}, and $T_{1/2}$ for esomeprazole after EST administration were 8854.6 ± 1135.6 ng·h/mL, 8938.2 ± 1132.2 ng·h/mL, 4456.6 ± 1748.9 ng/mL, 1.27 ± 0.10 h, and 0.82 ± 0.08 h, respectively. After EMT administration, the $AUC_{0\text{-}12h}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max}, and $T_{1/2}$ for esomeprazole were 7629 ± 605.8 ng·h/mL, 7661 ± 611.0 ng·h/mL, 3971.7 ± 306.9 ng/mL, 1.35 ± 0.18 h, and 0.69 ± 0.09 h, respectively. The ratio of $AUC_{0\text{-}12h}$, C_{max}, and T_{max} values of esomeprazole between test and reference formulations (T/R) were 1.133, 1.102, and 0.923, respectively. The 90% confidence intervals for the ratios of log-transformed $AUC_{0\text{-}12h}$ (104.2-123.1) and C± (96.5-125.8) for esomeprazole were within with acceptance range for bioequivalence (80-125%). Therefore, orally administered EST and EMT were shown to be bioequivalent in terms of systemic exposure and rate of absorption.

5.2 Toxicokinetics

The toxicokinetic profile of esomeprazole and esomeprazole sulfone after repeated oral administration of EST and EMT was studied in rats (Study # T06133). SD rats (3/sex/dose) were administered EST (14, 69, 280 mg/kg/day) or EMT (280 mg/kg/day) for 13 weeks, and plasma concentrations of esomeprazole and esomeprazole sulfone were measured on Day 1, Week 4 and Week 13. C_{max} of esomeprazole was reached within 0.17-0.61 hours after EST administration and 0.17 h after EMT administration. The $T_{1/2}$ of esomeprazole was approximately 2-19 hours. The increase in systemic exposure to esomeprazole was higher than dose-proportional. In males, systemic exposure to esomeprazole after administration of EMT was approximately 2-fold higher, as compared to EST. Both EST and EMT at 280 mg/kg showed slight accumulation in both sexes, based on C_{max} and AUC values from Day 1 to Week 13. Overall, females showed 2-fold higher systemic exposure to drug, as compared to males.

6 General Toxicology

Single- and repeat-dose toxicity studies in rats and dogs submitted under IND 078801 were reviewed previously. A brief summary is provided below.

6.1 Single-Dose Toxicity

In an acute, single-dose toxicity study (Study # B06132) in SD rats (5/sex/dose, 7 weeks old), both EST (1000 and 2000 mg/kg) and EMT (500, 1000, and 2000 mg/kg) produced treatment-related deaths. Treatment with EMT produced more central nervous system (CNS) related clinical signs (convulsions, inanimation, lacrimation, salivation) and death, as compared to EST. The maximum tolerated dose (MTD) of

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EST in rats was ≤ 1000 mg/kg in males and 2000 mg/kg in females. In contrast the MTD of EMT was ≤ 1000 mg/kg in males and 500 mg/kg in females.

In a dose escalation study (Study # B06134), beagle dogs (2/sex, 6 months old) were administered a single oral dose of either EST or EMT (10, 25, 50, 250, 1000 mg/kg) with a 7-day washout period between dosing intervals. Overall, EST showed a toxicity profile similar to EMT. One high dose EMT female died within 1 day of dosing. There was a dose-dependent increase in CNS-related clinical signs (salivation, vocalization, head nodding, tonic extension of limbs and convulsions) at ≥ 25 mg/kg EST or EMT in both sexes, within the first half hour and lasted up to 2 days post-dose in some animals. GI-related clinical signs included dyspepsia, nausea, vomiting and diarrhea in both treatment groups. There were no treatment-related changes in body weight or food consumption. Based on these data, the No Observed Adverse Effect Level (NOAEL) dose for EST in dogs was 10 mg/kg. The MTD for EST in males and females was ≥ 1000 mg/kg.

6.2 Repeat-Dose Toxicity

In a 13-week repeat-dose oral toxicity study (Study # B06133), SD rats (10/sex/dose, 6 weeks old) received 14, 69, and 280 mg/kg/day EST, 280 mg/kg EMT, or vehicle (0.05% carboxymethylcellulose). Except for the dosing-related death of one high dose female on Day 46, there were no other unscheduled deaths during the dosing period. Salivation was observed consistently at 69 and 280 mg/kg EST and 280 mg/kg EMT from Day 3 onwards, throughout the dosing period. Females at all doses of EST showed a decreasing trend in body weight gain, with the greatest decrease in the high dose females (~30%, similar to 280 mg/kg/day EMT-treated females). Males treated with EST or EMT at the highest dose showed a decrease in body weight gain of ≤ 16.6%, as compared to controls, during the dosing period. There was a decrease in hemoglobin, hematocrit, mean corpuscular volume, mean cell hemoglobin (MHC), MHC concentration (MCHC), and eosinophil concentration in the high dose treatment groups (280 mg/kg/day EST or EMT) in males and females, as compared to controls. There were increases in absolute and relative (relative to body weight) liver and kidney weight in the high dose groups (280 mg/kg) for both EST and EMT, as compared to vehicle. The increase in kidney weight correlated with histopathological findings of tubular regeneration in both males and females treated with 69 and 280 mg/kg/day EST and 280 mg/kg/day EMT. Histopathological analysis also revealed lesions and areas of degeneration in the stomach (eosinophilic granular degeneration) in the same treatment groups in both sexes. No other significant, treatment-related changes in histopathology were noted.

Both EST and EMT showed dose-dependent increases in systemic exposure, with potential drug accumulation after repeated administration, which correlated with the consistent observation of clinical signs after Day 3 of the dosing period. The C_{max} was reached at 0.17 to 0.61 h post dose and plasma $t_{1/2}$ was estimated to be between 2 and 19 h. Overall, females had higher systemic exposure to drug than males. Based on these data, the NOAEL dose of EST for males and females was determined to be 14 mg/kg.

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7 Genetic Toxicology

The ability of EST to promote reverse mutations was investigated *in vitro* using the bacterial reverse mutation assay (Study # B06135). *Salmonella typhimurium* TA98, TA100, TA1535, and TA 1537 and *Escherichia coli* WP2*uvrA*(pKM101) cultures were treated with EST at 0, 312.5, 625, 1250, 2500, and 5000 µg/plate (± S9 mix) and compared to concurrent positive and negative controls. EST precipitated at 2500 and 5000 µg/plate in all strains and produced cytotoxicity at 5000 µg/plate in TA98, TA100, TA1535, and TA1537. Negative and positive control values were within historical values provided by the sponsor. Under the conditions tested, EST was not mutagenic.

In Study # B06136, the mutagenic potential of EST was tested in vitro using mouse lymphoma cells (L5178Y/TK$^{+/-}$). EST was used at 68.75, 137.5, 275, 550, and 1100 µg/mL (short incubation) and at 30, 60, 120, 240, and 480 µg/mL (continuous treatment), regardless of metabolic activation. The mutant frequency (MF) did not increase with EST treatment, as compared to negative control, and was independent of metabolic activation or duration of treatment. The relative total growth (RTG) decreased after EST treatment, as compared to negative control. The positive control showed a statistically significant increase in MF. Therefore, under the conditions tested, EST did not show mutagenic ability in the mouse lymphoma assay at doses up to 1100 µg/mL.

An *in-vivo* mouse micronucleus test was conducted to test the ability of EST to induce the chromosomal damage (Study # B06137). Male mice (5/dose) were administered EST by oral gavage at 500, 1000, and 2000 mg/kg and bone marrow was harvested 24 h later. Mitomycin-C- and methylcellulose 1500 cP-treatments were used as positive and negative controls, respectively. The number of polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), and micronucleated polychromatic erythrocytes (MNPCE) were quantitated from bone marrow smears. Positive and negative control treatment produced values within the range of historical controls. Treatment with EST did not alter the total number of erythrocytes (PCE+NCE), the number of PCE, or the proportion of MNPCE. Therefore, under the conditions tested, EST did not induce chromosomal damage in mouse bone marrow erythrocytes.

8 Carcinogenicity

No carcinogenicity studies were submitted in support of this NDA.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology studies were submitted in support of this NDA.

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10 Special Toxicology Studies

No special toxicology studies were submitted in support of this NDA. The sponsor provided a summary of safety information on strontium from published literature and regulatory guidelines. A brief review is presented below.

Safety of Strontium:

The proposed drug substance, esomeprazole strontium, is a new salt of the approved drug Nexium® (esomeprazole magnesium). The strontium salt is a tetrahydrate while the magnesium salt of esomeprazole, is a trihydrate. Strontium (Sr) is an alkaline earth metal in the same family as magnesium and calcium. Under normal physiological conditions, Sr plays a complementary role to calcium and distributes primarily to bone, where it exchanges with calcium in hydroxyapetite. The concentration of strontium in the bone increases from ~0.3 mg Sr/Ca at birth to 0.5 mg Sr/Ca in adulthood. In addition to bone, Sr can also be found in teeth. The pharmacokinetic profile of strontium is similar to calcium. Strontium is absorbed in the gastrointestinal tract and is primarily excreted in the urine and feces. The half-life of elimination is 28 years and 16 years in males and females, respectively, with a dramatic increase in elimination in females after age 50.

Strontium can act as an agonist at calcium receptors and promote bone formation and decrease bone resorption. This effect of Sr has been exploited in pharmaceuticals for preventing bone loss in osteoporosis. Protelos® (strontium renalate), which is marketed in Europe for the treatment of osteoporosis, contains 2 g strontium, which corresponds to 517 mg/day intake. No clinically significant adverse effects in humans have been reported at this daily dose of strontium. Safety and efficacy of strontium renalate were investigated in 2 large multinational trials and showed that adverse effects were similar to placebo and were generally mild and transient, most common being nausea and diarrhea (Blake GM and I Fogelman *Clin Interv Aging* (2006) **1**(4): 367-75). Other clinical applications of Sr, including toothpaste, are listed in the sponsor's table below:

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Table 18 Examples of strontium products approved for use in humans

Product	Ingredient	Maker	Dosage (as Sr)	Indication	Countries launched
Protelos [1)]	Sr ranelate	Servier	2,000 mg susp. (684 mg)	postmenopausal osteoporosis	30 countries worldwide
Metastron [2)]	$^{89}SrCl_2$ (radioactive)		10.9-22.6 mg injection	Painful skeletal metastases	US
Sensodyne [3)] original	$SrCl_2$	GSK	100 g tube	Tooth paste	US, UK, KR
Sensodyne mint	$Sr(OAc)_2$	GSK	100 g tube	Tooth paste	US, UK
Nutrition supplements to prevent osteoporosis	Sr citrate [4)]	227 mg, 1-3 capsule/day: Pure Incapsulation [5)] 315 mg, powder, tablet : Nutricology 500 mg : Ortho Molecular 340 mg, 2 capsule/day : Doctor's Best Inc. [6)]			US
	Sr carbonate	340 mg : Vitamin Research Products			

Source:
Product Information (EMEA 2004, Protelos: Strontium ranelate, Annex I)
Prescribing Information (Amersham Healthcare, Medi-Physics, Inc.)
Product Information (GlaxoSmithKline, GSK)
Mini-report about Strontium & Healthcare (Metagenics, Inc.)
Production Information of Manufacturer
Production Information of Manufacturer

HM 70231 (40 mg esomeprazole strontium) was approved in Korea in 2008. However, the sponsor did not provide any postmarketing safety data on this product.

The estimated daily exposure to Sr is 3.3 mg/day (0.055 mg/kg/day for a 60 kg human) from the drinking water, air and food. The primary toxic effect of high oral doses of strontium in laboratory animals is abnormal skeletal development (rickets). The oral LD_{50} doses in mice were 2350 mg/kg for Sr-nitrate (Llobet JM, et al, (1991) *Res Commun Chem Pathol Pharmacol* **71**(2): 243-246) and $\geq$ 2700 mg/kg for Sr-chloride (Ghosh S et al., (1990) *Biol Trace Elem Res* **25**:51-56). Toxicity studies with Sr-carbonate in weanling rats have demonstrated that the lowest observed adverse effect level (LOAEL) for bone mineralization defects was 550 mg $SrCO_3$/kg/day when administered in the diet. The NOAEL doses in young and adult rats were 140 and 690 mg/kg/day, respectively (Storey E (1961) *Austral Ann Med* **10**:213-222). In a follow-up study, Storey exposed young rats (50-75 g) and adult rats (250-300 g) to strontium carbonate (1.8%) in the diet, which also contained 1.5% calcium and 0.9% phosphorus, for 7 months (Storey E (1962) *J Bone Jt Surg Am* **44B**(1): 194-208). Continuous administration of Sr, even under conditions of adequate calcium, phosphorus and vitamin D exposure, first induced rickets in young rats with eventual non-uniform skeletal growth. Histopathological changes in the bones of young animals included areas of uncalcified epiphysial cartilage, leading to the formation of localized defects, multiple cartilagenous nodules in the epiphysis, metaphysis, and bone shaft. The skull and teeth were also affected in young animals, while changes in adults were much less severe.

Sr toxicity was also assessed in acute and chronic studies with Sr-ranelate. These include a 7-d monkey study (2 g Sr-ranelate/day, from Fisch C. *et al.,* (2006) *Basic and Clin Pharmacol Toxicol* 98: 442-446) and a 52-week study with doses of up to 1250 mg/kg/day (Farlay D. *et al.*, (2005) *J Bone Miner Res* **20**(9): 1569-78), also in monkeys. In a 104-week mouse study with Sr-renalate, no effect on bone

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mineralization was observed at doses up to 1800 mg/kg/day when administered in the diet (Delannoy P et al., *Metabolism* (2002) **51**(7): 906-11).

The sponsor's table below shows *in vitro* genotoxicity studies conducted with Sr.

Table 2.6.6-14 Genotoxicity of Stable Strontium *in vitro*

Species (test system)	End point	Results	Reference
In vitro DNA synthesis reaction	Lack of fidelity in DNA synthesis	No enhanced misincorporation	Loeb et al. 1977
Bacillus subtilis Rec- strain	Growth inhibition	No growth inhibition	Kanematsu et al. 1980
Chinese hamster ovary cells	Reduced cloning efficiency	Non-toxic	Tan et al. 1984

Infants and children absorb more strontium from the gut than adults. Therefore, children are more susceptible to the bone effects of strontium than adults who have mature bone. In a study by Ozgur *et al*., Turkish children whose diet was based on grains grown in soil containing high strontium had impaired bone growth and rickets, especially when calcium, phosphorus, and vitamin D intake were low (Ozgur S *et al*., *Arch Dis Child* (1996) **75**(6): 524-6). Oral administration of strontium salts (carbonate, chloride, or nitrate) to young mice and rats have demonstrated adverse effects on bone (Llobet JM, *et al*, (1991) *Res Commun Chem Pathol Pharmacol* **71**(2): 243-246; Ghosh S *et al*., (1990) *Biol Trace Elem Res* **25**:51-56; Storey E (1961) *Austral Ann Med* **10**:213-222; Storey E (1962) *J Bone Jt Surg Am* **44B**(1): 194-208).

Overall, strontium has a favorable safety profile in adults. However, the short-term and chronic effects of orally administered stable strontium in pediatric patients are not known.

11 Integrated Summary and Safety Evaluation

Excessive gastric acid secretion by the parietal cells of the stomach can manifest itself as a variety of digestive disorders including gastroesophageal reflux disease (GERD), erosive esophagitis, gastric and duodenal ulcers. Acid secretion is dependent on the H^+/K^+-ATPase pump ("proton pump"), which is stimulated by histamine, gastrin, or acetylcholine. Proton pump inhibitors have been used successfully in patients suffering with digestive disorders related to excessive gastric acid secretion. Omeprazole is a potent and irreversible proton pump inhibitor that is used for the prevention and treatment of gastric acid-related disorders. Omeprazole is a racemic mixture of (S)- and (R)- enantiomers, of which the (S)- enantiomer (esomeprazole) has higher bioavailability and is a more potent inhibitor of gastric acid secretion than the (R)-form. Omeprazole undergoes stereoselective metabolism in the liver by CYP450 2C19 and 3A4. The (S)- enantiomer is less sensitive to CYP2C19 metabolism and is therefore maintained for longer periods of time in the blood and shows higher potency against gastric acid secretion. Esomeprazole magnesium trihydrate (Nexium®,

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AstraZeneca) was developed because esomeprazole alone is thermally unstable and lacks crystallinity. Nexium® (20 mg and 40 mg, oral) is currently approved in the United States for the treatment of gastrointestinal reflux disease (GERD) and healing of erosive esophagitis for short term treatment (4-8 weeks), maintenance of healing of erosive esophagitis for up to 6 months; symptomatic GERD; risk reduction of NSAID-associated gastric ulcer for up to 6 months; and *H. pylori* eradication to reduce the risk of duodenal ulcer recurrence (triple therapy: Nexium® plus amoxicillin and clarithromycin).

In the current NDA, the sponsor has developed esomeprazole strontium tetrahydrate in a delayed-release capsule formulation (20 mg and 40 mg) as an alternative to Nexium® and is seeking approval for the same indications as Nexium®. The rationale for developing the strontium salt was based on its thermal stability and improved solubility, as compared to the magnesium salt. The total dose of strontium in one 40 mg capsule of HM 70231 is 5.09 mg (0.085 mg/kg for a 60 kg individual).

Nonclinical studies conducted with esomeprazole strontium in support of this NDA include comparative PK studies in rats and dogs, single- and repeat-dose oral toxicity studies of up to 13 weeks in duration in rats, and *in vitro* and *in vivo* genetic toxicology studies. The sponsor is relying on Agency's previous determination of the safety of the reference listed drug, Nexium®.

Three single dose, comparative PK studies were conducted in rats (i.v. and i.d. administration) and dogs (oral administration) to demonstrate bioequivalence of the drug substance (EST) and drug product (HM 70231) with EMT and Nexium®, respectively. In rats, PK parameters (AUC, C_{max}, T_{max}, $t_{1/2}$) of esomeprazole and esomeprazole sulfone (major metabolite) were similar after single administration of EST and EMT. The bioavailability of esomeprazole after i.d. administration of 5 and 10 mg/kg EST was 25.9% and 31.4%, respectively, and 21.4% and 27.7% after 5 and 10 mg/kg EMT, respectively. The $t_{1/2}$ of esomeprazole was similar between EST and EMT (0.18-0.24 h for EST and 0.20-0.22 h for EMT). Therefore, bioequivalence was demonstrated after i.d. administration. In dogs, oral administration of a single 40 mg dose of either encapsulated EST pellets or Nexium® tablets showed similar PK properties. The ratio of $AUC_{0\text{-}12h}$, C_{max}, and T_{max} values of esomeprazole between test and reference formulations (T/R) were 1.133, 1.102, and 0.923, respectively. The 90% confidence intervals for the ratios of log-transformed $AUC_{0\text{-}12h}$ (104.2-123.1) and C± (96.5-125.8) for esomeprazole were within with acceptance range for bioequivalence (80-125%). In a separate study, PK properties of a single dose (40 mg) of orally administered HM 70231 delayed release capsule were similar to those of Nexium® capsule, with no statistical significance. The T/R ratio for $AUC_{0\text{-}12h}$, C_{max}, and T_{max} were 1.142, 1.053, and 1.024, respectively. The 90% confidence intervals for the ratios of log-transformed $AUC_{0\text{-}12h}$ values (107.1-121.7) for esomeprazole were within with acceptance range for bioequivalence (80-125%). Therefore, the sponsor successfully demonstrated bioequivalence of the proposed clinical product with the currently marketed formulation of esomeprazole in rats and dogs. Because the active ingredient is the same (esomeprazole), the results of PK studies were as expected.

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The sponsor's table below shows a comparison of systemic exposure after single doses of esomeprazole strontium in rats, dogs, and humans.

Table 6. Comparison of Single-Dose Maximum Plasma Esomeprazole Concentrations (C_{max}) and Systemic Exposures (AUC) Between Animals and Humans

Species, Esomeprazole Dose	N (M/F)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng·h/mL)	$AUC_{0\text{-inf}}$ (ng·h/mL)	Study
Rat		Mean ± Standard Error of the Mean			
2.5 mg/kg IV	5/0	3478 ± 300	1043 ± 99.2	1049 ± 98.1	PT-05-086
5 mg/kg ID	5/0	1468 ± 146	541 ± 43.8	543 ± 44.0	PT-05-086
10 mg/kg ID	5/0	3270 ± 208	1312 ± 74.9	1318 ± 75.3	PT-05-086
Dog		Mean ± Standard Error of the Mean			
40 mg PO	12/0	4457 ± 505	8855 ± 1135	8938 ± 1132	PT-06-019
40 mg PO	12/0	6144 ± 676	13580 ± 1319	ND	KVO-07-112
Human		Mean ± Standard Deviation			
40 mg PO	21/8	1079 ± 425	2148 ± 1103	2175 ± 1111	109145
40 mg PO	23/13	999 ± 513	1801 ± 1230	1824 ± 1241	109148
40 mg PO (fasted)	16/9	1083 ± 427	2167 ± 1272	2187 ± 1278	109146
40 mg PO (fed)	16/9	418 ± 342	1020 ± 989	1210 ± 1035	109146

Source: PK data from Studies PT-05-086 (Appendices 1, 3, and 5), PT-06-019 (Appendix 3), KVO-07-112 (Appendix 2); and Clinical Studies 109145 (Table 11-2), 109148 (Table 11-4), and 109146 (Table 11-2).

Esomeprazole was administered as the strontium tetrahydrate salt in all studies. Blood samples were collected from 0 to 2 h in rat, and 0 to 12 h in dog and human.

Abbreviations: ID = intraduodenal, IV = intravenous, ND= not determined, PO = oral.

Single and repeat-dose toxicity and *in vitro* and *in vivo* genotoxicity studies were conducted to establish the safety of esomeprazole strontium. The MTD of EST in rats was ≤ 1000 mg/kg in males and 2000 mg/kg in females. In contrast the MTD of EMT was ≤ 1000 mg/kg in males and 500 mg/kg in females. The NOAEL dose for EST in dogs was 10 mg/kg and the MTD was ≥ 1000 mg/kg in a dose escalation study. In a 13-week subchronic oral toxicity study in rats, the NOAEL of EST was determined to be 14 mg/kg. Similar to treatment with EMT, administration of EST produced salivation, lesions in the stomach and kidney in both sexes, and decrease in body weight gain from Week 4 through to the end of the dosing period in females, as compared to control animals. EST and EMT-treatment did not affect body weight gain as much in males. Treatment with EST produced changes in hematological or clinical parameters that were similar to EMT. Both EST and EMT showed dose-dependent increases in systemic exposure, with potential drug accumulation after repeated administration, which correlated with the consistent observation of clinical signs after Day 3 of the dosing period. Females had higher systemic exposure to drug than males, a finding that was observed in a 13-week repeat-dose toxicity study for EMT.

Genotoxicity studies showed that EST was non-genotoxic in the bacterial reverse mutation assay (up to 500 µg/plate) and the mouse lymphoma assay. In an *in vivo* mouse micronucleus assay, EST did not show clastogenic effects at doses up to 2000 mg/kg. In comparison, esomeprazole magnesium (NDA 21,153) was not mutagenic in the Ames test at doses ranging from 119-11900 µg/plate. In the chromosomal aberration test in rats, esomeprazole magnesium did not increase the frequency of chromosomal aberration, as compared to control, at doses ranging from 487-1950 mg/kg (males) and 430-1750 mg/kg (females). In the mouse micronucleus test,

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esomeprazole magnesium did not significantly increase the frequency of micronucleated polychromatic erythrocytes, as compared to control, at doses ranging from 120-1200 mg/kg. Esomeprazole magnesium was, however, positive in the *in vitro* human lymphocyte chromosome aberration test

Overall, EST had a toxicity profile similar to EMT. The NOAEL dose of 14 mg/kg/day from the 13-week rat study provides more than the required margin of safety for the proposed clinical dose (40 mg/day or 0.67 mg/kg/day for a 60 kg individual).

The proposed drug product is a new salt of an already approved product. To support the safety of strontium in this new salt of esomeprazole, the sponsor provided a review of the available safety information on strontium in animals and humans. Strontium is naturally occurring in the environment and exposure to elemental strontium occurs through the air, water, soil, and food. The estimated daily exposure of strontium in adults is 3.3 mg/day (0.055 mg/kg/day for 60 kg individual). The minimal risk level (MRL) after oral administration of strontium is 2.0 mg/kg/day (ATSDR, 2004). The MRL is derived for oral exposure to stable strontium and its compounds for intermediate duration (15-364 days). Prolonged use of Protelos® (Sr-renalate, approved in Europe), which contains 2 g strontium (517 mg/day), has not shown clinically significant adverse effects. Safety and efficacy of Sr-renalate were investigated in 2 large multinational trials and showed that adverse effects were similar to placebo and were generally mild and transient, most common being nausea and diarrhea (Blake GM and I Fogelman *Clin Interv Aging* (2006) **1**(4): 367-75).The maximum level of strontium in a 40 mg capsule of HM 70231 is 5.09 mg (0.085 mg/kg for a 60 kg individual). Therefore, the total daily dose of Sr in a 40 mg capsule of HM 70231 is much lower (about 24-fold) than the MRL. In animals, high doses of strontium (>500 mg/kg/day) in young rats and mice produced adverse skeletal effects. Thus, the safety profile of strontium in adults is favorable at the proposed clinical dose. However, the developmental effects of this dose of strontium (short-term or chronic exposure) in the pediatric population are unknown.

In conclusion, the sponsor has adequately demonstrated that the proposed clinical formulation is bioequivalent to the reference listed drug and has a favorable safety profile. Therefore, from a nonclinical standpoint, the safety data as presented support the approval for the 20 and 40 mg delayed-release capsule formulations of esomeprazole strontium for the proposed indications in adults.

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/s/

SRUTHI T KING
06/30/2011

SUSHANTA K CHAKDER
06/30/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 20-2342 **Applicant: Hanmi USA Inc** **Stamp Date:**

Drug Name: Esomeprazole Strontium **NDA/BLA Type: NDA, 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

Reference ID: 2876422

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____Yes____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Sruthi Tallapragada King — November 29, 2010
Reviewing Pharmacologist — Date

Sushanta Chakder — November 29, 2010
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

Reference ID: 2876422

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/s/

SRUTHI T KING
12/13/2010

SUSHANTA K CHAKDER
12/13/2010