4. Hematology/Coagulation/Bone Marrow: In high dose treated males, erythrocyte counts, hemoglobin and packed cell volumes were decreased by 11%, 13% and 13% respectively, when compared to control values.

5. Blood Chemistry/Urinalysis: At the end of treatment period, serum cholesterol levels were increased by 20%, 22% and 34% in low, mid and high dose treated male dogs respectively. In high dose treated females, serum cholesterol levels were decreased by 29% compared to control values. Serum total protein levels were decreased by 11% compared to control in high dose treated dogs (both sexes). These changes were not evident at the end of 2-month recovery period. Treatment related changes were not seen in urinalysis.

6. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related effects were seen.

7. Organ Weights: At high dose, thyroid weights were increased by 43% and 75% in males and females respectively, when compared to the control values. Increase in thyroid weights were still present at the end of 2-month recovery period (28% and 46% respectively).

8. Gross Pathology: Thickening of the stomach wall were seen in most of the treated dogs. Changes in the stomach of treated dogs were still present at the end of recovery period. Additionally, enlarged thyroid was seen in 3/7 high dose treated females and 1/7 high dose treated males.

9. Histopathology: Histopathological changes in the stomach (minimal cryptal, parietal and chief cellular alterations) were seen in most all treated dogs and none in the controls. Additionally, minimal to slight follicular cell hypertrophy in thyroid were seen in mid (males: 2/4 and females: 1/4) and high (males: 4/4 and females: 4/4) dose treated dogs.

10. Morphometric Pathology: Morphometric analysis revealed increases in stomach weight, gastric mucosal and non-mucosal mass and enterochromaffin-like (ECL) cell hypertrophy in all treatment groups. Additionally, ECL cell hyperplasia was seen in mid and high dose treated dogs. Some of these effects were still present at the end of 2 months of recovery period.

Table L-1. **Summary of Stomach Weights, Absolute and Relative to Body Weight, Combined Sexes. Study D00394 [redacted]307640 Sodium.**

				Absolute		Relative to Body Weight	
Study Phase	N	Statistic	Dose (mg/kg)	Mean Stomach Wt. (g)	% Increase	Mean Stomach Wt. g/100 g Body Wt.	% Increase
Treatment	8	Mean	0	99.1	0	0.76	0
	8	Mean p-value	2	168.8* .001	70	1.31* .001	72
	8	Mean p-value	8	178.8* <.001	80	1.58* <.001	108
	8	Mean p-value	25	184.1* <.001	86	1.36* <.001	79
		S.E.M.		13.1		0.10	
Reversibility[a]	6	Mean	0	83.8	0	0.68	0
	6	Mean p-value	8	123.5* .001	47	1.04* .034	53
	6	Mean p-value	25	131.7* <.001	57	1.14* .011	68
		S.E.M.		6.5		0.10	

*Mean is significantly different from control mean based on trend test (Tukey *et al.*, 1985) at the nominal .05 level.
[a]The 2 mg/kg group was not included in the 2-month reversibility phase.
S.E.M. The standard error of the least squares mean.

Table L-2. **Summary of Gastric and ECL Cell Morphometry in Dog Stomachs, Combined Sexes, Study D00394, [redacted]307640 Sodium.**

Dose (mg/kg)	N	Statistics	Mean Mucosal Thickness (mm)	Est. Mean Mucosal Mass (g)	Est. Mean Non-Mucosal Mass (g)	% Mucosal Tissue	Mean Number ECL cells/mm^2[a]	Mean Total Area/ECL cell (μm^2)[a]
					Treatment Phase			
0	8	Mean	0.597	29.3	69.9	29.60	523	47.7
2	8	Mean p (trend)	0.717 NT	64.4* <.001	104.5* .014	38.14** NT	636 .115	59.5* .039
8	8	Mean p (trend)	0.806** NT	67.1* <.001	111.7* .004	37.26 NT	739* .005	62.9* .010
25	8	Mean p (trend)	0.676 .111	53.1* <.011	131.0* <.001	29.13 .819	663* .025	72.1* <.001
		S.E.M.	0.044	6.0	9.2	2.22	49	3.8
					Reversibility Phase[b]			
0	6	Mean	0.590	25.8	58.0	31.10	446	42.9
8	6	Mean p (trend)	0.671 NT	33.5 NT	90.1* .002	26.52 NT	498 NT	45.0 NT
25	6	Mean p (trend)	0.749 .134	38.5 .064	93.1* .001	29.22 .657	542 .237	45.6 .438
		S.E.M.	0.070	4.4	5.9	2.92	54	2.4

[a] Collected from basal region of mucosa only.
[b] The 2-mg/kg group was not included in the 2-month reversibility phase.
* Mean is significantly different from control mean based on trend test (Tukey *et al.*, 1985) at the nominal .05 level.
** Not tested by trend test, mean is significantly different from control mean based on Dunnett's test (Dunnett, 1955) at the nominal .05 level.
NT Not tested due to lack of significance for the trend test at a higher dose.
S.E.M. The standard error of the least squares mean.
Est. Estimated.

11. Serum Gastrin Levels: Serum gastrin levels at 4 hr after drug administration were significantly higher in treated dogs (control: 40-70 pg/ml, low dose: 100-550 pg/ml, mid dose: 100-700 pg/ml and high dose: 80-750 pg/ml), however, the effect was not dose related (data presented graphically). Twenty-four hours after drug administration, serum gastric levels were still somewhat elevated in mid- and high-dose treated dogs. At the end of 2-months of recovery period serum gastrin levels were normal.

12. Toxicokinetics: T_{max} ranged 1-3 hours $AUC_{0-\infty}$ increased with increasing dosages. The plasma $t_{½}$ of [redacted]307640 was estimated to be 0.4 - 0.6 hr (both sexes). Parent drug was the main circulating compound. The 4 metabolites of the drug (thioether-[redacted]307640, sulfone-[redacted]307640, desmethyl-[redacted]307640 and desmethylthioether-[redacted]307640) were also seen in plasma samples.

Pharmacokinetic Parameters of [redacted]307640 in Male and Female Dogs

Dose (mg/kg/day)	Day	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng.hr/ml)	$T_{½}$ (hr)
2	1	2.1 ± 0.3	1267 ± 278	1347 ± 242	0.4 ± 0.1
8		2.6 ± 0.2	3744 ± 778	5309 ± 710	0.5 ± 0.1
25		3.0 ± 0.4	8925 ± 1473	15296 ± 2431	0.4 ± 0.1
2	182	2.7 ± 0.7	1417 ± 427	2000 ± 230	0.5 ± 0.1
8		2.3 ± 0.2	5785 ± 1024	7781 ± 1092	0.5 ± 0.1
25		3.1 ± 0.4	15561 ± 2706	31518 ± 3162	0.5 ± 0.1
2	362	2.1 ± 0.2	2190 ± 495	3053 ± 543	0.5 ± 0.2
8		4.0 ± 0.4	4870 ± 1385	13844 ± 3240	0.6 ± 0.1
25		3.6 ± 0.4	15770 ± 3376	39800 ± 5527	0.5 ± 0.1

Range of Plasma Mean C_{max} Values for 307640 and Metabolites in Male and Female Dogs. Study D00394.

Compound/metabolite	Dose, mg/kg	Males Range of Mean Plasma C_{max} (ng/ml)	Females Range of Mean Plasma C_{max} (ng/ml)
307640	2	1348.0 - 2304.7	1024.9 - 2075.9
307640	8	3593.0 - 4720.6	2907.8 - 7976.9
307640	25	9112.6 -17230.3	8736.6 - 16639.9
Thioether 307640	2	77.2 - 171.2	44.7 - 110.5
Thioether 307640	8	205.5 - 438.1	169.1 - 536.8
Thioether 307640	25	1042.2 - 1481.4	982.3 - 1225.7
Sulfone 307640	2	70.9 - 302.7	73.8 - 235.9
Sulfone 307640	8	305.1 - 525.9	210.9 - 1083.1
Sulfone 307640	25	722.4 - 2331.2	642.9 - 2345.2
Desmethyl 307640	2	71.2 - 79.5	69.4 - 97.2
Desmethyl 307640	8	163.9 - 248.1	196.5 - 362.9
Desmethyl 307640	25	426.9 - 473.6	459.4 - 618.5
Desmethyl Thioether 307640	2	20.8 - 45.8	BQL - 28.6
Desmethyl Thioether 307640	8	40.6 -75.1	44.1 - 91.5
Desmethyl Thioether 307640	25	115.4 - 199.0	120.2 - 170.8

BQL = below quantifiable limits (20 ng/ml).

In this study target organs of toxicities were stomach and thyroid. A no effect dose was not established in this study. If one disregards drug effect on stomach (i.e. exaggerated pharmacological effects), then 2 mg/kg/day can be considered as no effect dose. In the earlier submitted 1-year oral toxicity study in dogs, in which dose selection was not appropriate (report # EIS013/0648: 0.2, 1 and 5 mg/kg/day), only stomach and testes were shown to be the target organs of toxicities. By increasing the top dose level to 25 mg/kg/day (i.e. the present study), thyroid also became the target organ of toxicity. Thyroid was also target organ of toxicity in 13-week oral toxicity study in dogs (# 872142).

SPECIAL TOXICITY:

Antigenicity Study in Guinea Pig
(Study No. 886161)

Date of the study: July 4 to Aug. 23, 1988.

Methods: Several groups of animal each consisting of 10 male Harley guinea pigs 4 week of age were used. A group were intramuscularly injected with E3810 dissolved in physiological saline. Another group were injected subcutaneously with a saline solution of E3810 emulsified with Freund's complete adjuvant. Third group were immuninized with E3810-metabolite-conjugated-BSA emulsified with Freund's complete adjuvant.

Results: When the E3810 metabolite conjugated OVA was intravenously injected into the first two groups of animals, anaphylatic reaction occurred. Antibodies were detected in the sera of animals sensitized with E3810 in the passive cutaneous anaphylasxis reaction and the ELISA studies. When E3810 was intravenously injected into third goups, no anaphylatic reactions were evoked.

Additional Antigenicity Study in Guinea Pig
(Study No. 896165)

Date of the study: July 3 to Aug. 22, 1988.

Methods: In this study, antigenicity of omeprazole and E3810 was compared by the appearance of anaphylatic reactions and detection of antibodies to E3810 and omeprazole by PAC and ELISA assays. Groups of guinea pigs were intramuscularly injected with E3810 (5 or 10 mg) or Omeprazole (5 or 10 mg) in physiological saline solution. Other groups were subcutaneously injected with saline solution of E3810 or omeprazole emulsified with Freund's complete adjuvant.

Results: Anaphylatic reactions were observed in both groups when a matabolite of E3810 conjugated OVA or omeprazole conjugated OVA was intravenously injected. Antibodies were dectected in the sera of animal sensitized with Freund's complete adjuvant emulsion of E3810 or omeprazole in the PCA reaction, In ELISA, antibodies were detected in the sera of animals sensitized with Freun's complete adjuvant emulsion of omeprazole.

In conclusion, both had sensitizing antigenicity, but antibodies were detected in the guinea pig sensitized with omeprazole in ELISA assay.

Antigenicity Study in Mice
(Study No. 896122)

Date of the study: July 3 to Aug. 22, 1988.

Methods: Male B6C3F mice 5 weeks of age were used. E3810 or omeprazole were administered intrapertoneally or orally at dose lvel of 200 ug/animal to test its antigenicity. PCA (passive cutaneous anaphylaxis) test and ELISA assays were used. In PCA, antibodies were determined after i.v. injection of E3810, omeprazole, E3810 metabolite conjugated ovalbumin and omeprazole conjugated OVA. In ELISA assay, antibodies were determined by using E3810 metabolite conjugated OVA and omeprazole conjugated OVA as detecting antigens.

Results: The results indicated that E3810 is not antigenic in mice by either the parenteral or oral route of administration.

Anitgenicity Study in Rat[illegible] d Dogs
(Study Numbers 886011 & 886041)

Date of the study: Nov. 9 to Nov. 6, 1988.

Methods: Serum or plasma samples were obtained form the 4 week intravenous toxicity study in rat and the 13 week oral toxicity study in dogs. ELISA assay was performed using E3810-OVA conjugate as detecting antigen.

Results: In rats, antibodies to E3810 were detected in the plasma samples of the 25 and 50 mg/kg/day groups. A positive results was obtained form one dog from the 30 mg/kg/day group but the antibody titer was low and inhibition reactions by E3810 did not occur.

Addendum: The results of antigenic studies were reanalyzed by sponsor and the sponsor's final conclusion was submitted on May 28, 1991 and reviewed on June 13, 1991. The review is attached below.

Antigenic Potential of E3810

Sponsor has conducted five separate studies to assess the antigenic potential of E3810 studies in guinea pigs, and one study each in mice, rats and dogs.

In guinea pigs, antibody to E3810 was produced when E3810 was given parenterally with or without adjuvant. Furthermore there were a significant correlation between severity of anaphylactic shock and antibody titers as determined by PCA reaction and ELISA. Thus E3810 was antigenic in guinea pigs. Similar results were observed in the second study in guinea pigs. In mice E3810 was not antigenic whether given parenterally or orally, while in rats it was antigenic when given parenterally. Antibodies to E3810 were detected in the plasma of rats following 4 week i.v. administration of E3810. When E3810 was given orally to dogs for 13-weeks (study # 872142), low level of antibody titer was found in one dog in the 30 mg/kg/day dose group, and inhibition reaction by E3810 did not occur. However, the two dogs from the 30 mg/kg/day dose group which were sacrificed in extremis had abnormal histological findings in multiple organs (see above) and indicated possible immunotoxicity of the drug. Thus there is no clear evidence that E3810 has no antigenic potential. One can only conclude that E3810 has antigenic potential when the drug is given parenterally while oral administration has low antigenic potential.

A Two Week Pharmacokinetic Study of E3810 in Rats: Strain Differences Between SD and F 344 Rats and Effects of Food Restriction on PK Parameters
(Study # T 93009)

Methods: This study was conducted assess the effect of the drug on plasma gastrin levels in two strains of rats (F344 and SD) which were fed ad libitum or fed for only 5 hours ("restricted feeding schedules") after drug administration. In this study plasma levels of E3810 were also monitored. Groups of F 344/Du Crj or Crj: CD (SD) rats (3/sex/group) were given oral (via gavage) dose of vehicle (distilled water), 20 (0.4% W/V) or 50 (1.0% W/V) mg/kg/day of E3810 (pH of the drug solutions were not mentioned) for 14 consecutive days. The volume of administration was fixed at 5 ml/kg. Parallel groups of animals were also included in this study which were fed restricted diet i.e. they were given food only for 5 hours after vehicle or drug administration. Body weights and food intakes were not recorded. Blood samples were collected from abdominal aorta or jugular vein (3/sex/time point) for monitoring plasma gastrin levels at 1, 2, 4, 8 and 24 hours after dosing on days 1 and 14 of the study. Blood samples were also collected at 5, 15, 30 min., 1 and 2 hours after drug administration on days 1 and 14 of the study to measure E3810 levels in plasma. Plasma gastrin levels were measured by radioimmunoassay and plasma E3810 levels were measured by HPLC methods.

Results: The C_{max} and $AUC_{0-2\,hr}$ of plasma E3810 levels in food restricted F 344 rats (both sexes) and SD rats (males only) were much higher than those in ad libitum fed rats on day 1 of the study. Similar results were seen on day 14 of the study. However, this conclusion should be viewed with caution, because the plasma levels of E3810 on day 14 of the study were significantly lower than were seen on day 1 of the study, which is indicative of technical difficulties in measuring E3810 levels in plasma. The above results do indicate that drug absorption/exposure was increased in rats which were maintained on food-restricted regimen.

Table 1. Pharmacokinetic parameters of E3810 levels in plasma on Days 1 and 14 after oral administration

Strain of rat	Sex	Feeding Condition	Dose (mg/kg/day)	Day 1		Day 14	
				C max (μg/ml)	AUC(0-2hr) (μg*hr/ml)	C max (μg/ml)	AUC(0-2hr) (μg*hr/ml)
SD	Male	ad libitum	20	0.053	0.035	0.068	0.019
			50	0.487	0.441	0.185	0.041
		Restricted	20	0.909	0.265	0.086	0.095
			50	5.413	1.512	1.250	0.637
	Female	ad libitum	20	0.623	0.133	0.235	0.069
			50	3.756	3.074	0.268	0.208
		Restricted	20	0.204	0.109	0.196	0.198
			50	3.467	0.966	2.483	2.704
F344	Male	ad libitum	20	0.152	0.032	0.117	0.017
			50	0.405	0.084	0.345	0.139
		Restricted	20	0.524	0.178	0.081	0.107
			50	3.482	0.748	0.264	0.218
	Female	ad libitum	20	0.103	0.015	0.124	0.102
			50	0.339	0.096	0.306	0.127
		Restricted	20	1.063	0.196	0.599	0.131
			50	6.399	1.813	0.772	0.548

N=3 at each sampling point

Table 1, page 150454, Amendment dated 6/10/93

The pre-dose plasma gastrin levels in rats (both sexes) of restricted feeding regimen were generally much lower than seen in rats given food ad libitum (85-250 pg/ml versus 230-499 pg/ml). Sponsor did not measure gastrin levels as a function of time in control animals. The plasma gastrin levels are highly erratic as a function time, therefore no conclusion can be made about the duration and extent of hypergastrinemia induced by the drug.

E3810: Relationship Between Plasma Gastrin Level and ECL Proliferation (Study # 900415)

Testing Laboratories: Department of Drug Safety Research, Research and Development Division, Eisai Co., Ltd., Gifu, 501-61, Japan

Study Started: November 7, 1990

Study Completed: July 7, 1993

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: 7-8 weeks old (F-344/DuCrj, Charles River, Japan) rats (males: 86-104 g and females: 81-107 g).

Drug Batch No.: 90060702

Methods: Groups of female rats (8/group/time point) were given orally (gavage) E3810 at daily doses of 2 and 20 mg/kg/day for 4, 13 and 26 weeks and followed by recovery periods of 4, 13 and 26 weeks. Additionally, male rats (8/group/time point) were also included in groups treated for 13 and 26 weeks and 26 weeks treatment plus 26 weeks recovery. In this study, E3810 was dissolved in water before administration (pH of the solution was not adjusted). The volume of administration was fixed at 5 ml/kg. Animals were fed only for 5 hours (11:00 - 16:00 hr) each day during experimental periods. Thus, rats were starved each day for about 19 hr each day before drug administration. All animals were observed for clinical signs and mortality daily and body weights were recorded weekly. Animals were fasted from 16:00 hr on the day before sacrifice. Just before sacrifice, blood samples were collected from abdominal aorta to monitor plasma gastrin levels. Gastrin levels were measured by RIA methods. Stomachs from each rats were isolated and histopathological examinations were conducted. Stomach from control and high dose groups were also examined under electron microscope. Additionally, morphometric analysis (thickness of gastric [fundic] mucosa, ECL [enterochromaffin-like] cell density, G [gastrin secreting] cell density and BrdU labeling index) were also conducted.

Results:

1. Observed Effects: None.

2. Mortality: None.

3. Body Weight/Food Consumption/Water Consumption: In males, at the end of 13 weeks of treatment body weight gains were reduced by 15.9% and 18.6% at 2 and 20 mg/kg/day respectively. At the end of 26 weeks of treatment, in males, final body weights were 4.5% and 7.2% lower than the weights of the control males. In females, treatment had no significant effect on body weights.

4. Plasma Gastrin Levels: Treatment had no significant effect on plasma gastrin levels in male rats. In high dose treated females gastrin levels were increased by 2.5 fold and 1.7 fold over the control values at the end of 4 and 13 weeks of treatment respectively. No increase in plasma gastrin levels in females were seen after 26 weeks of treatment (2 or 20 mg/kg/day dose levels).

5. Organ Weights: A 20 mg/kg/day dose level, and at the end of 13 or 26 weeks of treatment, relative weights of stomachs were increased by 20-24% in males and 27-28% in females when compared to control values. At the end of recovery period stomach weights were returned to normal.

6. Gross Pathology: In rats of both sexes, 2 mg/kg/day had no effect on stomach. At 20 mg/kg/day for 26 weeks produced thickened gastric mucosa in 7 out of 8 male rats, and 6 out of 8 female rats which disappeared after 26-week of recovery period.

7. Histopathology: In stomach, diffuse/linear neuroendocrine cell hyperplasia and eosinophilic chief cell were seen in rats treated with 20 mg/kg/day for 13 weeks and longer. These changes tend to reverse at the end of recovery period.

Histopathological Findings in Treated (20 mg/kg/day) Rats				
	Males		Females	
Stomach	13 Week	26 Week	13 Week	26 Week
Eosinophilic chief cells	2/8	7/8	3/8	8/8
Neuroendocrine cell hyperplasia (diffuse)	7/8	8/8	8/8	8/8
Neuroendocrine cell hyperplasia (linear)	0/8	0/8	0/8	2/8

8. Electron Microscopic Examinations of Stomach: No treatment related effects were seen.

9. Morphometry Analysis: In stomach, significant increases in thickness of fundic mucosa, ECL cell density, G cell density and BrdU labeling index were seen when rats were treated with 20 mg/kg/day dose for 4 weeks or longer. Some of these changes were still present at the end of recovery period.

Morphometric Analysis of Stomach of Rats Treated With 20 mg/kg/day						
	Percent Over Control					
	Males			Females		
Parameters	4-Week	13-Week	26-Week	4-Week	13-Week	26-Week
Fundic mucosal thickness	ND	14.5	24.9	25.2	45.3	48.3
ECL cell density	ND	147	155	51.5	168	324
G cell density	ND	57	43	39	57	63
BrdU labeling index	ND	55	49	82	88	54

ND = Not done

In this study, no information of the pH of the drug solution was given (the drug is known to have poor stability in acid pH). Rats were starved for 19 hr prior to drug administration. Gastrin levels are the C_{min} values. Sponsor's contention that morphological changes in the stomach (increases in the thickness of fundic mucosa, ECL cell density, G cell density and BrdU labeling index of the fundic mucosa) were related to trophic effect of gastrin is not well founded. No increase in gastrin levels were seen in treated males and females treated with high dose for 26 weeks, yet there were significant morphological changes in stomach.

13-Week Oral Toxicity Study in Mice
(Study # EIS014/3810)

Testing Laboratories: [redacted]

Dates Study Started and Completed: June 22, 1989 and December 3, 1992..

GLP Requirements: A Statement of Compliance with GLP regulation was included.

Animals: CD-1 Mice (4-5 weeks old, 16-18 g)

Drug Batch No.: 89010911

Methods: Groups of 12 male and 12 female mice were given orally (gavage) E3810 at daily doses of 25, 100 and 400 mg/kg/day for 13 weeks. The control group animals received the vehicle (distilled water). The volume of administration was fixed at 10 ml/kg. Additionally, 12 mice/sex/group were also included in this study to monitor drug absorption. There was no restriction of diet in this study. All animals were observed for clinical signs and mortality twice daily, body weights and food consumptions were recorded pre-test and weekly during the treatment phase. All surviving animals were sacrificed at the end of the study period and subjected to complete necropsy. Only tissues from glandular region of stomach of all animals were examined histopathologically.

Results:

1. Observed Effects: Ataxia, hypopnea, bradypnea and prostrate position were observed in high dose treated males. Similar signs were seen in high dose treated females but of lesser magnitude.

2. Mortality: One male from low dose group and one female from mid dose group died during study period. The cause of death were not considered to be treatment related.

3. Body Weight/Food Consumption/Water Consumption: No treatment related effects were seen.

4. Organ Weights: Dose related statistically significant increase in stomach weights were seen in treated mice (males: low dose 2.5%, mid dose 23% and high dose 73%; females: low dose 15%, mid dose 55% and high dose 116%) when compared to the control values. Additionally, liver weights were increased by 22-23% in high dose treated mice (both sexes) compared to the control values. In high dose treated males, seminal vesicles weights were increased by 32% compared to control values.

5. Gross Pathology: Thickening of the glandular mucosal wall were seen in treated mice (males: control = 0/12, low dose = 0/11, mid dose = 1/12 and high dose = 4/12; females: control = 0/12, low dose = 2/12, mid dose = 1/11 and high dose = 5/12).

6. Histopathology: In the stomach, "hyperplastic gastropathy" in the glandular region were seen in treated mice (males: control = 0/12, low dose = 0/11, mid dose = 1/12 and high dose = 6/12; females: control = 0/12, low dose = 2/12, mid dose = 5/11 and high dose = 8/12).

7. Plasma Levels of E3810 and the Thioether Metabolite: The levels of E3810 and its metabolite increased with increasing dosage. The standard deviations are very large, hence data is not that robust.

Plasma Levels of E3810 and its Metabolites

	Day 1		Day 29		Day 90	
	Male	Female	Male	Female	Male	Female
Low Dose						
E3810 (ng/ml)	ND	74*	76 ± 60	ND	ND	112 ± 52
Metabolites:						
E3810 Desmethyl (ng/ml)	ND	529*	708 ± 318	620 ± 175	458 ± 316	1228 ± 897
E3810 Sulphone (ng/ml)	ND	ND	ND	ND	ND	ND
E3810 Thioether (ng/ml)	70*	143 ± 45	74 ± 25	110 ± 44	197 ± 168	187 ± 35
Mid Dose						
E3810 (ng/ml)	88 ± 71	335 ± 150	289 ± 176	95 ± 46	343 ± 104	527 ± 371
Metabolites:						
E3810 Desmethyl (ng/ml)	ND	641*	2351 ± 1098	2585 ± 255	3501 ± 1220	4156 ± 1415
E3810 Sulphone (ng/ml)	ND	ND	ND	ND	ND	92 ± 44
E3810 Thioether (ng/ml)	217 ± 210	339 ± 198	450 ± 108	231 ± 43	435 ± 232	679 ± 220
High Dose						
E3810 (ng/ml)	1567 ± 1686	3694 ± 5356	8785 ± 10544	391 ± 300	12413 ± 9031	1903 ± 39
Metabolites:						
E3810 Demethyl (ng/ml)	ND	1562 ± 1965	9974 ± 1938	5044 ± 2412	30448 ± 6588	6201 ± 1905
E3810 Sulphone (ng/ml)	97 ± 117	501 ± 672	974 ± 1549	ND	1508 ± 1128	ND
E3810 Theoither (ng/ml)	944 ± 521	784 ± 585	2258 ± 1526	411 ± 54	4293 ± 2203	1504 ± 145

ND = not detected
* = single determination

The data indicate that stomach was the target organ of toxicity. At highest tested dose (400 mg/kg/day), clinical signs such as ataxia, hypopnea, bradypnea and prostrate position were observed in mice of both sexes. Based on this result sponsor arbitrarily selected 2, 20 and 200 mg/kg/day dose levels for the carcinogenicity study in CD-1 mice. The The study is flawed by lack of histopathological examinations of all the tissues other than stomach. Therefore an MTD has not been established.

Addendum: The pH of the drug solution was adjusted to 10-12.

CARCINOGENICITY:

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC)
RODENT CARCIOGENICITY FACTSHEET

NDA: 20,973
CAS #:
DIVISION(s): HFD-180
DRUG NAME(s): Rabeprazole / Aciphex / E3810
SPONSOR: Eisai Inc.
LABORATORY:
P/T REVIEWER(s): Tanveer Ahmad, Ph.D. / Ke Zhang, Ph.D.
P/T REVIEW DATE: August 17, 1993 (Tanveer Ahmad, Ph.D.) and December 15, 1998 (Ke Zhang, Ph.D.)
CARCINOGENICITY STUDY REPORT DATE: April 7, 1993
THERAPEUTIC CATEGORY: Gastric antisecretory agent
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Gastric parietal cell H^+/K^+-ATPase (proton pump) inhibitor
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No
MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): Positive in Ames tests, CHO/HGPRT forward gene mutation assay and gene forward mutation assays at TK locus in L5178Y mouse lymphoma cells.

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1, Std2 etc):

MOUSE STUDY DURATION (weeks): 88 (males) / 104 (females)
STUDY STARTING DATE: October 23, 1989
STUDY ENDING DATE: April 7, 1993
MOUSE STRAIN: CD-1 mice
ROUTE: Oral gavage
DOSING COMMENTS:

No. Mice in Control1 (C!): 60m,60f **Control2 (C2):** 60m,60f
Low Dose (LD): 60m,60f **Middle Dose (MD):** 60m,60f
High Dose (HD): 60m,60f

MOUSE DOSE LEVELS (mg/kg/day)

Low Dose: 2 **Mid Dose:** 20 **High Dose:** 100/200

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): MTD

MOUSE CARCINOGENICITY (negative, positive, MF, M, F): Negative (MF)

MOUSE TUMOR FINDINGS:

MOUSE STUDY COMMENTS: In this oral carcinogenicity study in mice, E3810 was given to mice by oral gavage at 0, 2, 20 and 200/100 mg/kg/day for 88 weeks (males) and 104 weeks (females). The high dose of 200 mg/kg was reduced to 100 mg/kg during week 41 due to a significant increase in mortality in male mice. The male portion of the study was terminated after 88 weeks of treatment since the survival rate was 25% in week 88 in the control and low dose groups. The increased mortality in male suggested that MTD was between 100 and 200 mg/kg/day and the animals were exposed to sufficiently high dose of E3810. This study was considered acceptable in the Division's letter to sponsor on January 26, 1994.