6. Hepatic Drug Metabolizing Enzyme Activities:

Percent Change From Control Values				
Treatment	Sex (M/F)	BND	P-450	TGT
5 mg/kg/day	M	+13	+42	+20
15 mg/kg/day	M	+9	+40	+19
30 mg/kg/day	M	+6	+27	+25
60 mg/kg/day	M	+6	+36	+40**
5 mg/kg/day	F	+6	+41	+9
15 mg/kg/day	F	+1	-2	-5
30 mg/kg/day	F	+7	+60	+6
60 mg/kg/day	F	+11	+49	+39**
120 mg/kg/day	F	+16	+111*	+90**

BND = Benzphetamine N-demethylase
TGT = Thyroxin UDP Glucuronosyltransferase
* = p <0.001
** = p ≤0.005

Treatment had no significant effect on benzphetamine N-demethylase in rats (both sexes). Cytochrome p-450 content was significantly increased in 120 mg/kg/day treated females. Hepatic thyroxine UDP-glucuronosyltransferase activities were also significantly increased in males and females treated with 60 mg/kg/day and females treated with 120 mg/kg/day dose levels.

7. Organ Weights:

In high dose treated females (120 mg/kg/day), liver, kidney and thyroid relative weights were increased by 27.4%, 23.3% and 19.6% respectively while thymus weights were decreased by 27-46% in all treated females. In 30 and 60 mg/kg/day treated males, increased liver (13-14%), kidneys (10-12%) and thyroid (23-25%) and decrease in thymus (47-56%) relative weights were seen. Relative weights of stomach were increased dose-dependently in treated rats (males: 10-43% and females: 7-32%).

8. Gross Pathology: No treatment related effects were seen.

9. Histopathology: Treatment related lesions in kidneys (renal cortical tubular regeneration: at doses of ≥15 mg/kg/day in males and at ≥ 60 mg/kg/day in females), thyroid (slight follicular epithelial hypertrophy: at doses of ≥15 mg/kg/day in males and at ≥60 mg/kg/day in females), thymus (atrophy: at doses of ≥15 mg/kg/day in both sexes) and in the stomach (gastric glandular epithelial eosinophilic changes and hyperplasia of gastric enterochromaffin-like cells: at all doses in both sexes) were seen. The incidences of these findings are as follows.

Number of Rats With Histopathological Findings							
	Sex (M/F)	Control	5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Number Examined		10	10	10	10	10	10
Kidney							
Multifocal Basophilic Cortical Tubular Regeneration (slight-moderate)	M	2	3	5	9	10	---
	F	0	0	1	0	5	10
Thymus							
Atrophy (minimal-moderate)	M	0	0	1	3	7	---
	F	0	0	1	2	6	6
Thyroid							
Follicular Cell Hypertrophy (minimal-slight)	M	3	1	4	7	8	---
	F	0	0	0	0	1	4
Stomach (fundus)							
Eosinophilic Chief Cells (minimal-severe)	M	2	7	9	10	9*	---
	F	4	8	10	10	10	10
ECL Cell Hyperplasia (fundus: slight-severe)	M	0	0	10	10	9*	---
	F	0	6	9	10	9*	10

* = only 9 rats were examined.

10. Morphometric Evaluation of Stomach: Dose related increases in mass and thickness of the gastric mucosa, ECL cell hypertrophy and ECL cell hyperplasia were seen in treated rats. These effects were more prominent in females than in males. Sponsor has not classified what kind of hyperplastic responses (simple or diffuse, linear or chain-forming, micronodular or adenomatoid) were seen in treated rats.

DOG:

A 2-Week I.V. Toxicity Study in Dogs
(952141)

Testing Laboratory: Department of Drug Safety Research, Eisai Co., Ltd., Gifu, Japan

Study Start and Completion Dates: February 7, 1995 and October 24, 1995.

GLP and QAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (8.1-9.7 kg, 6-7 months old)
Females (6.8-8.7 kg, 6-7 months old)
Beagle dogs

Methods: To evaluate the toxicity of E3810 in dogs, dogs (3/sex/group) were given E3810 intravenously at 0, 1, 5 and 25 mg/kg/day for 2 weeks. E3810 was dissolved in physiological saline at 0.1, 0.5 and 2.5% (w/v) and the test solution was given at an injection rate of 10-20 ml/min daily. Clinical sign of toxicity was observed daily. Food consumption and body weights were determined. Hematology, clinical chemistry and urinalysis were determined before study started and at termination. Ophthalmological examination was also conducted before study started and at termination. ECG was conducted before treatment started and on days 1 and 14. All animals were necropsied at termination and the organs were weighed. Histopathological examination was also conducted. Plasma level of test drug was determined on day 1 and at termination.

Results:

1. Clinical Signs: Frequency of vomiting and stool changes (loose, mucous, muddy or watery stool) were increased in the mid and high dose groups as compared to the control. In the high dose group, following clinical signs of toxicity were also noted: salivation, licking of the chops, decreased activity, miosis and conjunctival injection.

2. Mortality: There were no deaths.

3. Body Weight: There were no treatment related changes.

4. Food Consumption: There were no treatment relate changes.

5. Ophthalmoscopy: There were no treatment related alterations observed during the study.

6. ECG: There were no treatment related changes.

7. Hematology: The major treatment related changes were found in the high dose males and these included decreased red blood cell count (17%), hematocrit (12%) and hemoglobin (15%). Similar changes were also seen in the high dose females. Other changes were slight and sporadic.

8. Clinical Chemistry: Changes were slight and sporadic and are not considered significant.

9. Urinalysis: There were no treatment related changes.

10. Organ Weights: The absolute and relative liver weights were increased in the high dose males by 34% and 30%, respectively. The absolute liver weight in females was also increased by 49%. The absolute and relative thyroid weights were increased by 57% and 53% in high dose males.

11. Gross Pathology: There were no treatment related changes.

12. Microscopic Pathology: The followings were the treatment related changes: degeneration of parietal cells in stomach in 1 male and 2 females in mid dose group and all high dose group animals, foci of cellular debris in stomach in 1 high dose female, follicular hypertrophy in the thyroid in 1 mid dose female and 1 high dose female, slight C-cell hyperplasia in 1 control male, 1 low dose male and female, 2 mid dose males and 2 high dose females.

13. Plasma Level of Test Drug: The AUC values of E3810 were 1.1, 5 and 30.2 μg.hr/ml (males) or 1.1, 5.5 and 31 μg.hr/ml (females) on day 1 for the low, mid and high dose groups, respectively. On day 14, AUC values of E3810 were 1.2, 6.2 and 29.2 μg.hr/ml (males) or 1.1, 6.9 and 40.4 μg.hr/ml (females) for the low, mid and high dose groups, respectively. The half life was ~0.28-0.44 hours on both days 1 and 14 for males and females.

In summary, E3810 was tested intravenously at 0, 1, 5 and 25 mg/kg/day for 14 days. The major treatment related changes were found in the high dose group and these included clinical signs of toxicity (vomiting, loose, mucous, muddy or watery stool, salivation, licking of the chops, decreased activity, miosis and conjunctival injection), slight decreased red blood cell count (17%), hematocrit (12%) and hemoglobin (15%), slight increased liver weights (30-49%) and thyroid weights (53-57% in males) and histopathological changes including degeneration of parietal cells and foci of cellular debris in stomach and follicular hypertrophy in the thyroid in females. Some of these changes were also seen in the mid dose group. The high dose was maximum tolerated dose. No effect dose was identified at 1 mg/kg. The stomach and thyroid were the target organs of toxicity.

13 Week Oral toxicity Study in Dogs
(Study No. 872142)

Date of the study: June 16, to Sept. 25, 1987.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Beagle dogs weighing 6.9-9.7 kg and 6-7 months of age were used.

Methods: Six groups of animals each consisiting of three males and three females were given E3810 in the form of enteric coated tablets (Lot no. K751802 and K751803) at dose levels of 0, 1, 3, 10 and 30 mg/kg/day and omeprazole at dose level of 10 mg/kg/day for 13 weeks. Additional one per sex in the control group and two per sex in the 30 mg/kg/day Of E3810 were kept for a five week recovery period after treatment. Tissues were stained with H & E for microscopic examination.

Results:

Plasma level of E3810 and omeprazole: Plasma level of test articles were determined on day 1, 35 and 91 of dosing. Tmax of 2-4 hours were obtained. AUC of omeprazole was larger than E3810.

Mortality: Two dogs in the 30 mg/kg/day were killed in extremis on days 39 and 57.

Clinical signs (daily): Diarrhea was observed in the 30 mg/kg/day group.

Body weight (weekly), food consumption (daily) and water consumption (days 0, 29, 85 and 120): There were no treatment related changes except for the two animals killed in extremis.

Hematology (days 0, 30, 86, and 122): Decrease in platelet and lymphocyte counts were seen in the two animals killed in extremis. Activated partial thromboplastin time was reduced in the omeprazole and the 30 mg/kg/day of E3810 groups.

Blood chemistry (days 0, 1, 8, 30, 86 & 122): Occassionally high ALT was seen in some animals in the 3 mg/kg/day and above groups. Urea nitrogen was increased in the 3 and 30 mg/kg/day groups (43 and 36%) at week 4. Increase in triglyceride was seen in the 10 mg/kg/day group at week 4 and 12 by 53 and 46%, respectively.

Plasma drug level (days 1, 35 & 91): AUCs for plasma levels of E3810 increased in a dose related manner.

Serum gastrin levels (days 0, 1, 50, 86 & 99): Serum gastrin level markedly increased but not dose-dependently during fasted (3.12 folds) or fed (4.12 folds) when the animals received repeated dose of E3810 for 50 days.

Urinalysis: Normal.

Ophthalmoscopic examinations (days 0, 31, 87 and 120)and EKG (days 0, 29, 85 & 120): Normal.

Function test: ICG and PSP elimination tests as a measure of liver and kidney functions were not affected by the treatment.

Drug metabolizing enzyme activity: The activity of P450 and 5 other metabolizing enzymes in the liver were not affected.

Lymphocyte proliferation test: Because one animal exhibited inflammatory reactions, immunoreactivity was studied. No reduction in stimulation index was found in lymphocyte proliferation test.

Glutathione content: A remarkable increase in glutathione level in the stomach mucosa was found in a dose-related fasion in groups treated with E3810 and in the single group treated with omeprazole. The level remained elevated at the end of recovery period. This mechanism of increased glutathione in the stomach is not clear.

Organ weight: Decrease in thymus weight was seen in all all drug treated male groups (21-34%) and in the females of the 30 mg/kg/day group (71%). In the Omeprazole group, thymus was reduced by 16% in males.

Gross pathology: Mucosal thickening and accentuated fold in the stomach found in all treated animals.

Histopathology: Vacuolization of mucosal epethelium, parietal cell necrosis, paritel cell degeneration, edema in submucosa, edema in lamina propria, chief cell atrophy, proliferation of pyloric mucosal epithelium and dilatation of gastric gland were observed in all drug-treated group. Goblet cell metaplasia was found in one animal each of the 1-10 mg/Kg/day groups.

Electron Microscopic examination: Dilatation of smooth and rougn endoplasmic reticulum, proliferation of Golgi's apparatus and decrease of glycogen granules in hepatocyte was seen in one animal killed in extremis. The chief cell atrophy was still found at the end of 5 week recovery period.

In conclusion, E3810 given as in enteric coated tablets at dose of 30 mg/kg/day for 13 week produced diarrhea, hematological changes, increases in gastrin levels and lesions in thymus and stomach. Lethal dose was 30 mg/kg/day. Morphological changes in the stomach were observed in all drug treated groups. Thus, a "no effect dose" was not established.

Addendum: (1) The testing laboratory is Department of Drug Safety Research, Eisai Co., Ltd., Gifu, Japan (2) the results of this study was 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.

Summary of the Effects of E3810 on Five Key Organ Systems in Dogs From Drug Safety Study # 872142 (13-Week Oral Toxicity Study in Dogs)

In this study doses of 0, 1, 3, 10 and 30 mg/kg/day were used. Positive control group animals received omeprazole 10 mg/kg/day. One/sex from control group and 2/sex from 30 mg/kg/day group of E3810 were used for 5 week recovery study. Diarrhea was observed in high dose treated animals. Two (one male and one female) out of 6 dogs at high dose were sacrificed in extremis on day 39 and 57 of the study. Before sacrifice, both animals had sudden decrease in body weight (8% and 11%), along with increases in ALP activity (173% & 479%), plasma cholesterol (274% & 202%), plasma lipid (174% & 144%), triglyceride (572% & 652%), BUN (137% & 80%) and creatinine (60% & 73%) and decreases in plasma albumin (38% & 46%). Platelets and lymphocyte counts were significantly decreased in these two dogs. Systemic infiltration of neutrophils, thymus atrophy, myocardial necrosis/degeneration, lung congestion, bronchopneumonia, liver congestion and centrilobular atrophy of hepatocytes, activation of Kupffer's cells (male) and bile duct proliferation (female) and swelling of tubular epithelium of kidneys were seen at histopathological examinations. Thus histological findings in multiple organs of the dogs (# 7032 & # 7536) may be due to the immunotoxicity of the drug. Another male dog (# 7041) had similar toxic signs but also had wound in the right ventral neck. The wound was treated with povidone iodine and subsequently recovered and was able to finish the complete dosing regiment i.e. 13-week of treatment. At 30 mg/kg/day, increases in serum cholesterol levels (Males: 26%, Females: 55%) and phospholipid (Males: 15%, Females 33%) were observed. Serum triglyceride and BUN levels were increased by 19-70% and 18-56% respectively in all treated dogs and these effects were not dose related except female's serum triglyceride levels were increased in dose related manner. Plasma gastrin level were increased in all treated males (females levels were not monitored). Absolute and as well as relative weights of the thymus were reduced in all treated males (17-35%) and in high-dose treated females (67-70%). In omeprazole treated males thymus weights were reduced by 22% compared to the control values. At 5 weeks urinalysis revealed presence of RBC in 1/5 females at high dose. Histopathological examinations revealed thymus atrophy (0/6 in control, 0/6 in 1 mg/kg/day dose group, 1/6 in 3 mg/kg/day dose group, 1/6 in 10 mg/kg/day dose group and 2/6 in 30 mg/kg/day dose group) and C-cell proliferation in thyroid (0/6 in control group, 0/6 in 1 mg/kg/day group, 1/6 in 3 mg/kg/day group, 2/6 in 10 mg/kg/day group and 6/6 in 30 mg/kg/day dose group).

In conclusion the lowest dose tested in this study caused significant increases in serum triglyceride and BUN levels along with decrease in absolute/relative weights of the thymus. Thus no effect dose in this study was not established.

Supplement 13 Week Oral Toxicity Study in Dogs
(Study No. 872145)

Date of the study: Dec. 9, 1987 to June 7, 1988.

GLP requirement: A statement of compliance with GLP regulations was mentioned.

Animals: Beagle dog weighing 7.4-9.9 kg and 6 months of age were used.

Methods: Seven groups of animals each consisting of 1-3 males and 1-3 females were given E3810 (Lot 87042201) in enteric coated tablet at dose levels of 0, 0.1, 0.3 and 1.0 mg/kg/day or omeprazole (Lot Nos. 06111201, 06112602, 87061401) at dose levels of 0.3, 1.0 and 3.0 mg/kg/day for 13 week. One animal per sex in the 0.3 and 1.0 mg/Kg/day groups maintained untreated for 5 or 13 weeks after completion of dosing period. The numbers of animals per sex per dose were as follows:

The numbers of animals per sex per dose were as follows :

		Initial	sacrificed	recovery period	
			13 weeks	5 weeks	13 weeks
Control		3	3	0	0
E3810	0.1 mg/kg	3	3	0	0
E3810	0.3 mg/kg	3	1	1	1
E3810	1.0 mg/kg	2	1	0	1
OME	0.3 mg/kg	3	1	1	1
OME	1.0 mg/kg	3	1	1	1
OME	3.0 mg/kg	1	1	0	0

Only the following tissues were stained with H & E and examined by light microscopy: liver, kidney and stomach.

Results:

Mortality: None.

Clinical signs (daily): None.

Body weight (weekly), food consumption (daily) and water consumption (days 0, 32, 86, 121 & 178): Normal.

Hematology (days 0, 35, 87, 122 and 179): Normal.

Blood chemistry (days 0, 1, 8, 15, 35, 57, 87, 95, 99, 122 & 179): Plasma ALT was sporadically high in one 0.3 mg/kg of E3810 and two 1.0 mg/kg/day omeprazole males only.

Serum gastrin level (days 0, 8, 15, 87 and 98): Fasting serum gastrin levels were increased, although increases in the 0.1 mg/kg/day group were comparable with controls.

Urinalysis (days 0, 32, 86, 121 and 178): Normal.

Organ weight: Increased in stomach weight were seen in all drug treated groups except in the 0.1 mg/kg/day group.

Gross pathology: Mucosal thickening and accentuated fold in the stomach were found in all drug treated groups except in the 0.1 mg/kg/dy group. These changes were not found in any groups after the 13 week recovery period.

Histopathology: No morpahological change was found in the 0.1 mg/kg/day group. Atrophy in the chief cells and degeneration and/or necrosis in the parietal cells was found in all other drug treated groups. The above mentioned morphological changes were still found at the end of the recovery priod.

Electronmicrocospic exmaination: Normal.

In conclusion, E3810 in enteric coated tablets produced irreversible morphological changes in stomach at oral dose of 0.3 mg/kg/day of E3810 and Omeprazole. A no effect dose of 0.1 mg/kg/day was established.

Addendum: (1) The testing laboratory is Department of Drug Safety Research, Eisai Co., Ltd., Gifu, Japan (2) the results of this study was 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.

Summary of the Effects of E3810 on Liver, Kidney, Heart, Thymus and Lung in Study # 872145 (13-Week Oral Toxicity Study in Dogs)

In 13-week oral toxicity study in dogs, doses of 0, 0.1, 0.3 and 1.0 mg/kg/day were used. Parallel groups were also included which were given 0.3, 1.0 or 3.0 mg/kg/day of omeprazole. One dog/sex of 0.3 and 1.0 mg/kg/day dose groups were used for 5 or 13 weeks recovery study. Fasting serum gastrin levels were increased in treated dogs in dose dependent manner, however the increase at 0.1 mg/kg/day dose level was close to the control values. Only stomach weights were increased in 0.3 and 1.0 mg/kg/day E3810 treated dogs and in all omeprazole treated

animals. Upon gross pathological examination, mucosal thickening and accentuated fold in the stomach were evident in mid and high dose E3810 treated dogs and in all omeprazole treated dogs. These changes were not seen at the end of 13-week recovery period. Histological examinations were performed only on liver, kidney and stomach. No abnormal histological finding was found in liver and kidney. Atrophy in the chief cells and degeneration/necrosis in the parietal cells were seen in mid and high dose E3810 treated dogs, and these changes were still present in some treated dogs at the end of the recovery period. If we disregard the G.I. findings and consider those gastric changes as pharmacological action of the drug (E3810) then the "no effect dose" in this study will be 1.0 mg/kg/day.

1-Year Oral Toxicity Study in Dogs
(Report # EIS013/0648)

Testing Laboratories: [redacted]

Study Started: October 25, 1989

Study Completed: March 26, 1993

GLP Requirements: A statement of compliance with GLP regulation was included.

Animals: 20-25 weeks old male and female beagle dogs (4.4-8.2 kg).

Drug Batch No.: K990701/ K041900/ K990702/ K051100

Methods: Groups of 4 male and 4 female beagle dogs were given orally (enteric coated tablets in capsules) E3810 at daily doses of 0.2, 1 and 5 mg/kg/day for 52 weeks. The control group animals received capsules containing placebo. Additionally, two groups (n=2/sex/group) were also included in this study, one received the vehicle and the other received mid dose of E3810 and used for 26-week recovery study. In the initial submission sponsor indicated that E3810 was given along with 1% $NaHCO_3$ by gavage in single dose study in dogs becauce the drug has "poor stability in acid solution". In 13-week oral toxicity study enteric coated tablets of E3810 were given. In this study the formulation of enteric coated tablets was not provided. According to sponsor the dose selection was based on 13-week oral toxicity study in which 1, 3, 10 and 30 mg/kg/day were used. Histological revealed changes in the stomach and thymus atrophy in all treated dogs, C-cell proliferation in thyroid at 3 mg/kg/day and higher dose leves, and 30 mg/kg/day was proved to be lethal. Dogs were dosed approximately 2 hr before feeding each day. Unlike 1-year oral toxicity study in rats, there was no restriction of diet in this study. All animals were observed for clinical signs daily, body weights were recorded weekly and food consumptions were recorded daily. Ophthalmoscopic

examinations were performed on all animals once pretest and once during week 23 and 49 of the study and once during week 23 of the recovery. ECG tracings were recorded on all animals once pretest and once during week 24 (2 and 24 hr after drug administration) and 50 (2 and 24 hr after drug administration) of the study and once during week 24 of the recovery. Blood samples were collected from jugular vein of all dogs once pretest and just before drug administration during weeks 4, 12, 24, 38 and 50 of the study and during weeks 4, 12 and 24 of the recovery period for hematological and serum chemistry (including gastrin) tests. The levels of gastrin will represent Cmin because blood sampling was done at 24 hr after drug administration. Before start of the study and after 3, 11, 23, 37, and 49 weeks of treatment and after 3, 11 and 23 weeks of recovery period, over night urine samples were collected for urinalysis. Over 5 consecutive days in week 49 of the study fecal samples were also collected for examining occult blood. At the end of treatment period and at the end of recovery period all surviving animals were sacrificed and subjected to complete necropsy histopathological examinations. Stomach slides were stained with hematoxylin and eosin, silver-based stain (Grimelius Method) or immunocytochemical stain (chromogranin) for assessing neuroendocrine cells. At the end of treatment period and at the end of recovery period, tissues samples of kidneys (cortex and medulla), liver and stomach (fundus and pylorus) from all animals were also processed and saved for "possible future" electron-microscopic examinations.

Results:

1. Observed Effects: No treatment related effects were seen.

2. Mortality: None.

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

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: At the end of 50 weeks of treatment, serum gastrin levels were increased by 402% and 440% in males and by 85% and 669% in females in mid and high dose respectively, when compared to their respective control values. Additionally, dose related increases in serum triglycerides (low dose 26%, mid dose 37% and high dose 48%), phospholipids (low dose 14%, mid dose 15% and high dose 19%) and cholesterol (low dose 22%, mid dose 27% and high dose 33%) were seen in treated males. In high dose treated females serum phospholipids and cholesterol levels were also increased by 12% and 19%

respectively and in high dose treated males alanine amino-transferase activities were increased by 49% when compared to their respective control values. Only control and mid dose treated dogs were used for recovery study. At the end of recovery period, all values returned to normal. Urinalysis were normal.

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

7. Organ Weights: At the end of treatment period, stomach weights were increased by 6-11% (relative wt. = -8-4%), 67-94% (relative wt. = 56-85%) and 90-119% (relative wt. = 62-110%) in low, mid and high dose treated rats (both sexes) respectively when compared to their respective control values. At the end of recovery period, increased stomach weights were still present in mid dose treated rats of both sexes (relative wt.: males = 16% and females = 25%).

8. Gross Pathology: Thickening of the stomach wall were seen in all mid and high dose treated dogs. These findings were not present at the end of the recovery period (only control and mid dose treated dogs were used for recovery study).

9. Histopathology: Mucosal hyperplasia, increased rugosity and "chief cell cytoplasmic atrophy" in the fundic region of the stomach in all most all mid and high dose treated dogs. Increased incidences of mucosal hyperplasia were also seen in pyloric region of stomach of mid dose treated males (2/4) and high dose treated males (1/4) and female (2/4) dogs. Chief cell cytoplasmic atrophy in the fundic region of the stomach was also seen 1/4 low dose treated male dogs. Additionally, degeneration of the tubular germinal epithelium of testes were seen in 1/4 and 2/4 dogs of mid and high dose groups respectively. At the end of the recovery period, findings at the fundic region of the stomach were still present in mid dose treated dogs.

10. Gastric Image Analysis: Significant increase in fundic mucosal thickness was seen in treated dogs of both sexes (males: low dose 8%, mid dose 70% and high dose 59%; females: low dose -4%, mid dose 46% and high dose 64%). Significant increases in chromogranin positive stained cells were seen in mid and high dose treated male dogs and high dose treated female dogs. Some of these findings were still present at the end of recovery period (26-week) in mid dose treated male dogs (only mid dose group and control group were used in recovery study).

11. Electron Microscopic Examinations: not done.

12. Evaluation of ECL Cells in Fundic Mucosa of Dogs (Report # EIS/013/0648/4931): ECL cells in the fundic mucosa were evaluated using Grimelius staining technique. All intact Grimelius-stained cells containing visible nuclei were counted. No drug induced increase in ECL cell counts were evident in any treated group (combined male and female dogs: control = 18.9 ± 4.9, low dose = 16.0 ± 5.8, mid dose = 15.3 ± 5.7 and high dose = 16.5 ± 6.5). Only in one of the two slides of one high dose treated male (# 3670) had linear and micronodular hyperplasia of ECL cells. No ECL hyperplasia was seen in any of the remaining slides.

The data indicated that stomach and testes were target organs of toxicities. In this study the dose selection was not proper. In the earliar submitted 13-week oral toxicity study (# 872142), a dose level of 10 mg/kg/day produced histological changes in the stomach, thymus atrophy and C-cell proliferation in thyroid and 30 mg/kg/day was proved to be lethal (for detail see my review of Amendment dated 5/28/91: date of review 6/14/91). Sponsor should have atleast used 10 mg/kg/day as the highest dose level in this 1-year oral toxicity study in dogs. The no effect dose in this study was 0.2 mg/kg/day (4 mg/sq. m./day) which on the basis of mg/sq. m/day is about 3.7 times less than the proposed highest dose in DU patient (protocol E-3810-J091-011: 20 mg/day = 0.4 mg/kg/day [50 kg body weight assumed] = 14.8 mg/sq. m./day).

1-Year Oral Toxicity Study in Dogs
(Study # D00394)

Testing Laboratories: [redacted]

Study Started: January 14, 1994

Study Completed: January 18, 1996

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

Animals: 11 - 15 months old male and female beagle dogs (males: 10.92 ± 0.24 kg and females: 10.05 ± 0.26 kg).

Drug Batch No.: CT02521, CT02522, CT02871 and CT02867.

Methods: Sponsor earlier submitted the results of 1-year oral toxicity study in dogs in which dose of 0.2, 1 and 5 mg/kg/day were used. In this study dose selection was not appropriate (for detail see review dated 8/17/93), therefore, sponsor was asked to repeat the study with higher dose levels. Hence, in the present study, groups of dogs (4/sex) were given orally (enteric-coated tablets in capsules) E3810 at daily doses of 2, 8 and 25 mg/kg/day for 1 year. The control group dogs received the capsules containing placebo. Additionally, 3 dogs/sex each were also included in control, mid and high dose groups for 2-month recovery study. All animals were observed for clinical signs and mortality daily, body weights were recorded weekly and food consumptions were recorded daily. Ophthalmoscopic examinations were performed on all animals once pretest and at the end of treatment period. Blood samples were collected from jugular vein from all dogs twice pretest and just before drug administration during days 6, 13, 28, 91, 133, 182, 224, 272, 314 and 361 of the study and blood samples were also collected on days 393 and 4420 from the recovery animals for hematology and serum chemistry tests. Additionally, serum gastrin levels were monitored at 4 and 24 hr after drug administration on days 0, 1, 28, 29, 91, 92, 182, 183, 272, 273, 361 and 362 (treatment phase) and on days 393, 393, 420 and 420 (recovery phase). To monitor toxicokinetic parameters blood samples were also collected at 0.5, 1, 1.5, 2, 3, 4 and 6 hr after daily oral doses of 2, 8 or 25 mg/kg dose on days 1, 182 and 362 of the study. Plasma levels of drug and its metabolites were measured by HPLC/UV method. Overnight urine samples were collected from each dog during pretest, days 92, 183, 362 and 421 of the study for urinalysis. At the end of treatment/recovery period all dogs were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: Dose related increases in the incidences of emesis and soft/water stools were seen in mid and high dose treated dogs.

2. **Mortality**: None.

3. **Body Weight/Food Consumption/Water Consumption**: Inappetence was seen in treated dogs. Several high dose dogs, one mid dose dog from each sex and one female dog were given supplemental diet on various occasions. Purpose of providing supplemental diet was to maintain their weights at reasonable level. Hence, the effect of treatment on body weights were confounded due to availability of supplemental food to treated dogs, and this is in variation of the earlier submitted protocol.