88/104-Week Oral (gavage) Carcinogenicity Study in Mice
(Report # 92/EIS015/0039)

Testing Laboratories: [redacted]

Study Started: October 23, 1989

Study Completed: April 7, 1993

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

Animals: 33-40 days old CD-1 [redacted] mice (19-30 g)

Drug Batch No.: 89010911, 89120401, 90060702 and 11042522

Methods: In this study dose selection was based on 13-week dose ranging study in mice (for detail see above). Groups of mice (68/sex/group) were given E3810 orally via gavage at daily doses of 2, 20 and 200/100 mg/kg/day for 104 weeks. No information of the pH of the drug solution was given. The high dose level was reduced from 200 to 100 mg/kg/day for both sexes during week 41 of the study due to high incidence of pallor observed in treated males, and during this period a high number of males (exact number not given) were killed in extremis. Two additional groups (68/sex/group) were also included, one group was given vehicle (purified water) in similar fashion while the other group was used as cage control group. After six weeks of treatment the group size was reduced to 60/sex/group. The excess mice i.e. 8/sex/group was initially included to compensate for possible deaths during the first six weeks of study. This is not an acceptable practice. Furthermore, due to increase mortality in vehicle and low dose treated males, male portion of the study was terminated after 88 weeks of treatment. At week 88 survival rate was 25% in vehicle and low dose group treated males. The cause of deaths were not clearly mentioned. The volume of administration was fixed at 10 ml/kg. All mice were observed twice daily for clinical signs and mortality. Physical examinations of all animals were performed weekly. Body weights were recorded weekly during the first 14 weeks then every other week thereafter. Food consumptions were recorded weekly. Just before dosing, blood samples were collected from retro-orbital sinus for hematological test from males during week 87 of treatment and from females during week 103 of treatment. All surviving mice were sacrificed at the end of study period (week 88 for males and week 104 for females) and subjected to complete necropsy. Tissues from control (group 2 = vehicle control) and high dose group and animals killed/dying during the study period were examined microscopically. Stomach slides from all animals which died/killed after week 44 of the study were stained with hemtoxylin and eosin, Alcian blue/Periodic acid-Schiff or Silver-based stain (Grimelius method) and examined microscopically.

Additionally, abnormal gross pathological findings were also examined microscopically. Sponsor used methods of Cox, D.R. (J.R. Stat. Soc. B, 34, 187-220, 1972) and Tarone, R.E. (Biometrika, 62, 679-682, 1975) to analyze the survival data, and the tumor incidence data wre analyzed using Peto method (IARC monograph, Supplement 2, 1980: 311-426).

Results:

1. Observed Effects: Severe pallor, torpor and hypothermia were observed in high dose treated males. The severity of these signs increased and reached maximum during week 37-43 of the study. During this period many high dose treated males (number not specified) were sacrificed in extremis, and top dose level was reduced from 200-100 mg/kg/day from 5th day of week 41. Additionally, 2 high dose treated females had convulsions and subsequently died on day 1 of the study. Convulsions were not observed in rest of the animals during treatment phase. No treatment related clinical signs were seen in animals of low and mid dose groups.

2. Mortality: Originally 68 mice/sex/group were used in the study (see Methods). During the 1st six weeks of treatment, 3 mice (2 males and one female) from vehicle control group and 7 mice from high dose group died. Cause of deaths were unknown. After six weeks of treatment all groups were reduced to 60 mice per group. Excess mice were killed and discarded. In rate of mortality calculation the group size is considered to be 60 animals/sex/group and animals died during the first 6 weeks of treatment were not included. In females, treatmnet had no significant effect on intercurrent mortality rates (see below). However, in males, significant increase in mortality rate was seen in high dose treated group during the period of 7-41 weeks of treatment and most of mortality occured during weeks of 32-43 of the study. At termination, (88 weeks for males and 104 weeks for females) the survival rates were comparable in all groups (22-33% for males and 28-38% for females).

Intercurrent Mortality Rates

Weeks	CC	%	VC	%	Low Dose	%	Mid Dose	%	High Dose	%
Male Mice										
7-41	4/60	6.7	6/60	10.0	12/60	20.0	8/60	13.3	19/60	31.7
42-88	36/56	64.3	41/54	75.9	34/48	70.8	37/52	71.1	21/41	51.2
Terminal	20	----	13	----	14	----	15	----	20	----
Survival Rate	----	33.3	----	21.7	----	23.3	----	25.0	----	33.3
Female Mice										
7-41	1/60	1.7	2/60	3.3	1/60	1.7	1/60	1.7	3/60	5.0
42-88	17/59	28.8	16/58	27.6	17/59	28.8	22/59	37.3	26/57	45.6
89-104	19/42	45.2	24/42	57.1	20/42	47.6	14/37	37.8	14/31	45.2
Terminal	23	----	18	----	22	----	23	----	17	----
Survival Rate	----	38.3	----	30.0	----	36.7	----	38.3	----	28.3

CC = cage control
VC = vehicle control

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

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

5. Organ Weights: Absolute as well as relative weights of the stomach were increased by 46-48% and 106-108% in high dose treated males and females respectively when compared to the vehicle control values. In females the absolute as well as relative weights of stomach were also increased by 36% in mid dose group compared to vehicle control values.

6. Gross Pathology: Dose related statistically significant increase in the incidence of thickened stomach wall were seen in treated mice (males: cage control = 7/60, vehicle control = 4/60, low dose = 9/60, mid dose = 19/60 and high dose = 41/60; females: cage control = 8/60, vehicle control = 6/60, low dose = 8/60, mid dose = 31/60 and high dose = 47/60). Additionally, at high dose, enlarged livers were seen in high dose females (cage control = 1/60. vehicle control = 0/60, low dose = 0/60, mid dose = 2/60 and high dose = 8/60).

7. Histopathology:

Non-neoplastic Findings: In the fundic region of stomach, there were statistically significant increases in mucosal thickness, hyperplastic gastropathy, amorphous deposits in glands, presence of acidic mucin and neuroendocrine cell (NE cell) hyperplasia in mid and high dose treated mice of both sexes. Sponsor did not provide the description of NE cell hyperplasia. Additionally, statistically increased incidence of focal necrosis with inflammatory infiltrate in the liver were seen in high dose treated male mice. The incidences were as follows.

Number of Mice With Non-neoplastic Findings

	Male					Female				
# Examined	CC 56	VC 53	L 56	M 54	H 56	CC 56	VC 58	L 58	M 58	H 58
Fundic Region:										
Increased Mucosal Thickness	13	17	18	42[3]	52[3]	15	19	17	42[3]	53[3]
Hyperplastic Gastropathy	16	11	16	22[1]	24[1]	8	13	8	21	31[2]
Amorphous Deposits in Glands	29	28	27	45[3]	52[3]	34	28	21	45[2]	54[3]
Presence of Acid Mucin	9	9	11	15	13	10	12	13	29[2]	39[3]
NE Cells Hyperplasia (Grimelius method)	10	8	12	15	37[3]	4	6	9	21[2]	42[3]
# Examined	49	60	50	50	60	37	60	40	37	60
Liver Focal Necrosis	7	3	3	2	11	2	5	5	4	11

CC = cage control, VC = vehicle control, L = low dose, M = mid dose and H = high dose;
1 = $p<0.05$, 2 = $p<0.01$ and 3 = <0.001 (pairwise comparison with VC)

Neoplastic Findings: Compared to vehicle control, significant increase in pulmonary adenoma was seen in high dose treated male mice (vehicle control = 1/60 [1.7%], low dose = 1/49 [2.0%], mid dose = 3/48 [6.2%] and high dose = 7/60 [11.7%]; p = 0.031, pairwise comparison). However, the incidence rate of pulmonary adenoma in cage control was 6/49 (12.2%) which is comparable to that seen in high dose treated males. No other treatment related neoplastic changes were evident in this study.

In this study the dose selection was not appropriate. In females, treatmnet had no significant effect on intercurrent mortality rates. However, in males, significant increase in mortality rate was seen in high dose treated group during the period of 7-41 weeks of treatment and most of mortality occured during weeks of 32-43 of the study. As a result top dose level for both sexes was reduced from 200-100 mg/kg/day from 5th day of week 41. Furthermore, due to increase mortality in vehicle and low dose treated males, male portion of the study was terminated after 88 weeks of treatment. At week 88 survival rate was 25% in vehicle and low dose group treated males. At termination, (88 weeks for males and 104 weeks for females) the survival rates were comparable in all groups. In the fundic region of stomach, there were statistically significant increases in mucosal thickness, hyperplastic gastropathy, amorphous deposits in glands, presence of acidic mucin and neuroendocrine cell (NE cell) hyperplasia in mid and high dose treated mice of both sexes. Additionally, statistically increased incidence of focal necrosis with inflammatory infiltrate in the liver were seen in high dose treated male mice. Compared to vehicle control, significant increase in pulmonary adenoma was seen in high dose treated male mice (vehicle control 1.7%, low dose 2.0%, mid dose 6.2% and high dose 11.7%; p = 0.031, pairwise comparison). However, the incidence rate of pulmonary adenoma in cage control was 12.2% which is comparable to that seen in high dose treated males. Thus E3810 did not show any carcinogenic effect in 88/104 weeks carcinogenicity study in mice.

Addendum: Tumor and non-tumor data extracted from sponsor's table 10 on pages 133-180 in volume 1.36 are attached in Appendix I.

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 6, 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.

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

RAT STUDY DURATION (weeks): 104
STUDY STARTING DATE: August 1, 1989
STUDY ENDING DATE: April 6, 1993
RAT STRAIN: F-344 rats
ROUTE: Oral gavage
DOSING COMMENTS:

No. Rat in Control1 (C1): 60m, 60f
Control2 (C2): 60m,60f
Low Dose (LD): 55m, 55f
Middle Dose (MD): 55m,55f
High Dose (HD): 60m, 60f

RAT DOSE LEVELS (mg/kg/day)

Low Dose: 2 **Mid Dose:** 6 **High Dose:** 20

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): The basis of dose selection was not provided.

RAT CARCINOGENICITY (negative, positive, MF, M, F): --------

RAT TUMOR FINDINGS:

RAT STUDY COMMENTS: In this oral carcinogenicity in F-344 rats, E3810 was given to F-344 rats by oral gavage at 0, 2, 6 and 20 mg/kg/day for 104 weeks. The basis of dose selection was not provided. The highest dose tested was below MTD. Rats were given food for only 5 hours on each day. It is known that caloric restriction would alter the basic biochemical mechanism of toxicity of various drugs and expression of carcinogenic property of the drug. Therefore, this study was considered invalid. Sponsor was asked to repeat the 104-week carcinogenicity study in Sprague-Dawley rats. These were conveyed to sponsor in the Division's letter dated September 8, 1993.

104-Week Oral (gavage) Carcinogenicity Study in Rats (Report # 91/EIS011/1215)

Testing Laboratories: [redacted]

Date Started: August 1, 1989

Date Completed: April 6, 1993

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

Testing Species & Strain: F-344 Rats [redacted]

No. of Animals: 55-60/sex/group (41-48 days old)

Route of Administration: Oral (gavage)

Dose Levels: 0, 0, 2, 6 and 20 mg/kg/day

Drug Batch No.: 89010911, 89120401, 90060702 and 11042522

Methods: Groups of rats (55-60/sex/group) were given E3810 orally via gavage at daily doses of 2, 6 and 20 mg/kg/day for 104 weeks. Two additional groups (60/sex/group) were also included, one group was given vehicle (purified water) in similar fashion while the other group was used as cage control group. Furthermore, 5 rats/sex/group were also included for monitoring the plasma levels of the drug at week 13 of the study. The volume of administration was fixed at 5 ml/kg. In the initial submission sponsor indicated that E3810 was given along with 1% NaHCO3 becauce the drug has "poor stability in acid solution". In this study no information of the pH of the drug solution was given. It should be noted that in this study rats were given food one-half hour after the drug administration for only 5 hours every day. Thus animals were starved for about 18-19 hr. each day before administration. All rats were observed twice daily for clinical signs and mortality. Physical examinations of all animals excluding satellite groups were performed weekly. Body weights were recorded weekly during the first 14-weeks than every other week thereafter. Food consumptions were recorded weekly. After 102 weeks of treatment blood samples were collected from retro-orbital sinus for hematological tests (blood chemistry test were not done). Blood samples were also collected at 15 minutes after the drug administration on days 1, 29 and 90 of the study from satellite rats for measuring E3810 and its metabolites. All surviving rats were sacrificed at the end of study period and subjected to complete necropsy. Tissues from control (group 2 = vehicle control), high dose group and animals killed/dying during the study period were examined microscopically. Stomach slides

from all animals were stained with hematoxylin and eosin, silver-based stain (Grimelius method) or immunocytochemical stain (chromogranin) for assessing neuroendocrine cells. Additionally, abnormal gross pathological findings were also examined microscopically. In the statistical analysis group 2 (vehicle control) was used as control and group 1 (cage control) was used to clarify the interpretation. Sponsor used methods of Cox, D.R. (J.R. Stat. Soc. B, 34, 187-220, 1972) and Tarone, R.E. (Biometrika, 62, 679-682, 1975) to analyze the survival data, and the tumor incidence data were analyzed using Peto method (IARC Monograph, Supplement 2, 1980: 311-426).

Results:

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

2. Mortality: Dose related increase in mortality was seen in treated males (cage control = 23%, vehicle control = 17%, low dose = 25%, mid dose = 25% and high dose = 37%) and it reached to statistically significant only at high dose ($p < 0.05$ when compared to vehicle control value). In females, the mortality rates were comparable in all groups (cage control = 8%, vehicle control = 18%, low dose = 20% and high dose = 18%).

3. Body Weight/Food Consumption/Water Consumption: No treatment related effects were evident in body weight gains and food consumptions. It should be noted that 104 weeks old male F-344 rat (weight: about 409 g) normally consumes about 16 g of food and female F-344 rat (weight: about 294 g) normally consumes about 12 g of food. In this study 104 weeks old male (mean weight: 310 g) and female (mean weight: 195 g) rats consumed on the average about 14 g and 10 g of food respectively. Hence due to availability of food for only 5 hours/day animals consumed about 13-17% less food than rats given food ad libitum.

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

5. Plasma Levels of E3810 and its Metabolites: E3810 and/or its metabolite(s) were not detected in the plasma sample of low and mid dose treated rats (detection limit = 25 ng/ml). However, in high dose treated rats E3810 and its metabolites were detected on day 1, 29 and 90 of treatment, and levels in female's plasma were always higher than that found in male plasma samples.

Plasma Levels of E3810 and Its Metabolites in High Dose Treated Rats

	Day 1		Day 29		Day 90	
	Male	Female	Male	Female	Male	Female
E3810 (ng/ml)	38 ± 41	234 ± 53	162 ± 175	542 ± 138	249 ± 155	518 ± 255
Metabolites (ng/ml):						
E3810 Desmethyl	30 ± 41	57 ± 53	192 ± 150	401 ± 92	139 ± 109	191 ± 114
E3810 Sulphone	ND	ND	ND	39 ± 18	ND	ND
E3810 Thioether	ND	ND	71 ± 28	78 ± 20	39 ± 29	122 ± 104

ND = Not detected

6. Organ Weight: Dose related increases in stomach weights (males: low dose 2.8%, mid dose 9.6% and high dose 40.7%; females: low dose 3.5%, low dose 16% and high dose 36%) and lung weights (males: low dose 5%, mid dose 9% and high dose 18%; females: low dose 3%, mid dose 10% and high dose 16%) were seen when compared to their respective control values. Additionally, in high dose treated males, seminal vesicles and thymus weights were reduced by 30% and 16% respectively compared to vehicle control values.

7. Gross Pathology: Dose related statistically significant increase in the incidence of thickened stomach wall were seen in treated rats (males: vehicle control = 5/60, low dose = 10/55, mid dose = 14/55 and high dose = 35/60; females: vehicle control = 2/60, low dose = 4/55, mid dose = 5/55 and high dose = 26/60).

8. Histopathology:

Non-neoplastic Findings: In the glandular region of the stomach, the increased incidences of amorphous deposits in glands, eosinophilic chief cells and mucosal thickness were seen in mid and high dose treated rats. The incidences were as follows:

Non-neoplastic Findings in Stomach

	Male					Female				
# Examined	CC 60	VC 60	L 55	M 55	H 60	CC 60	VC 60	L 55	M 55	H 60
Glandular Region:										
Amorphous deposits in glands	0	0	1	0	41[3]	0	0	0	0	34[3]
Eosinophilic chief cells	0	0	0	3	40[3]	0	0	0	8	52[3]
Increased mucosal thickness	1	0	1	5	13[3]	0	3	2	3	12[1]
NE Cells:										
Hyperplasia (diffuse)	6	7	9	8	20[2]	3	3	14[2]	14[2]	29[3]
Hyperplasia (linear)	0	0	0	0	8[2]	0	0	0	0	5

CC = cage control, VC = vehicle control, L = low dose, M = mid dose, H = high dose.
1 = $p<0.05$, 2 = $p<0.01$, 3 = $p<0.001$ (pairwise comparison with VC).

No usable information was obtained when stomach slides were stained according to Grimelius method due to poor staining of the slides. However, when slides were stained histochemically (chromogranin immunocytochemical method) then statistically significantly increased incidences of diffuse hyperplasia of neuroendocrine (NE) cells were seen in high dose treated males ($p<0.01$, pairwise comparison) and in all treated female ($p<0.01$ for low and mid dose and $p = <0.001$ for high dose group; pairwise comparison). Additionally, increased incidence of linear hyperplasia of NE cells were also seen in high dose treated males ($p = <0.01$, pairwise comparison).

Neoplastic Findings: Statistically significant increases in the incidence of adrenal medulla pheochromocytoma (p = 0.038, pairwise comparison), testicular interstitial cell tumor (p = 0.02, pairwise comparison) and monocytic leukemia (p = 0.019, pairwise comparison) were seen in high dose treated males when compared to incidences in vehicle control group. Additionally, in high dose females, the incidence of pituitary adenomas was significantly higher than that found in vehicle control group (p = 0.048). Sponsor did not do histopathological examinations of all tissues from all groups. However, the above finding does not indicate a dose relationship. According to sponsor, the findings in high dose treated rats are commonly seen in F-344 rats. Sponsor did not provide historical control data. Hence, the biological significance of these findings can not be ascertained at this time. Sponsor should be asked to provide recent (1988-1991) historical control data for this strain (F-344) of rat from the same laboratory which conducted the above study.

Neoplastic Findings:

Table
Neoplastic Findings

Organ/Findings	Sex	CC	VC	L	M	H	p-Value*
Adrenal Medulla							
Malignant Pheochromocytoma	M	1/14	0/60	1/16	0/15	2/60	
Benign Pheochromocytoma	M	2/14	0/60	1/16	3/15	3/60	
Benign + Malignant	M		0/60			4/60	0.038
Testes							
Benign Interstitial Cell Tumor	M	55/55	55/60	47/54	46/50	59/60	0.020
Hemopoietic Tumors							
Malignant Monocytic Leukemia (MML)	M	16/23	12/60	12/17	16/19	18/60	
	F	4/7	6/60	5/13	5/11	10/60	
Fatal Tumors (MML) (life table tests)	M		6/60			15/60	0.019
Pituitary							
Malignant Carcinoma	M	0/17	0/60	0/15	0/20	0/59	
	F	0/17	0/60	0/20	0/25	1/60	
Benign Adenoma	M	1/17	2/60	2/15	6/20	3/59	
	F	8/17	4/60	11/20	12/25	11/60	0.048
Adenoma + Carcinoma	F		4/60			12/60	0.030

In this study [104 weeks oral (gavage) carcinogenicity study in rats] the basis of dose selection was not given. Furthermore, the carcinogenicity study was conducted in F-344 rats, while sponsor earlier submitted 13-week oral toxicity study (# 872113) in Sprague-Dawley rats in which oral (gavage) doses of 1, 5, 25 and 100 mg/kg/day were used. This study was reviewed by Dr. Sun (date of review: 1/12/90). The highest tested dose (100 mg/kg/day) had no effect on mortality, body weight gain and food intakes. Upon re-examination of the data it was found that animals treated with 25 mg/kg/day and above had elevated serum cholesterol and phospholipid levels along with increased relative weights of liver (only in males) and decreased absolute/relative weight of thymus (in both sexes). Additionally, at high dose, the relative kidney weights were increased by 10% compared to the control values. At the end of the 5-week recovery period,

decreased thymus weights at 25 and 100 mg/kg/day were still present. Histopathological findings revealed centrilobular hpertrophy of the liver in high dose treated males and cortical atrophy of the thymus in both sexes at 25 and 100 mg/kg/day. Additionally vacuolization of mucosal epethelium, parietal cell necrosis, parietal 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 treated rats. If we disregard the gastric changes then the "no effect dose" in Sprague-Dawley rats was 5 mg/kg/day and maximum tolerated dose would be >25<100 mg/kg/day. In all likely-hood, the highest selected dose in F-344 rat carcinogenicity study is below the maximum tolerated dose. Furthermre, in this study rats were starved (without food) for about 18-19 hours each day before drug administration which is not normal procedure to conduct the study. Upon comparison with data of studies in other submission, in this study animals consumed about 13-17% less food and weighed about 24-34% less than rats given food ad libitum due to restricted access to food. Fasting/restricted food supply might adversly affect the toxic responses of the drug as well as metabolism of the drug. It has been known that caloric restriction alter basic biochemical mechanism of toxicity of various drug and expression of carcinogenic property of the drug (National Center for Toxicological Research: Annual Winter Meeting, February 15-17, 1993). Hence, this study should not be accepted as is. Sponsor should be asked to conduct the following studies: (1) 13-week oral dose-ranging toxicity study in rat with normal feeding schedule and then select the maximum tolerated dose (2) repeat 104-week oral (gavage) carcinogenicity study in rat using MTD as the highest dose and the remaining two dose levels should be one-half MTD and one-fourth MTD (3) in carcinogenicity study animals should be given food ad libitum and (4) pH of the drug solutions should be adjusted to about 9.0 before administration in the above mentioned studies.

Addendum: Tumor and non-tumor data extracted from sponsor's table 10 on pages 112-138 in volume 1.44 are attached in Appendix II.

A Two Week Pharmacodynamic Study of E3810 and Omeprazole in Rats
(Study # T93008)

Methods: Groups of female F 344/Du Crj or Crj: CD (SD) rats (15/group) were given oral (via gavage) dose of vehicle (distilled water), E3810 (20 mg/kg/day) or Omeprazole (suspended in 0.5% methyl cellulose: 20 mg/kg/day) for 14 consecutive days. Sponsor did not report pH of the drug solutions and no reason was provided for using two different solvents i.e. distilled water for E3810 and 0.5% methyl cellulose for Omeprazole. The volume of administration was fixed at 5 ml/kg. In this study rats were given food for only 5 hours every day after drug administration i.e. animals were starved for about 19 hours each day before drug administration. Five rats per group were used for monitoring of intragastric pH and plasma gastrin levels on day 1, 7 and 14 of the study. Intragastric pH was recorded at 1, 2, 4, 8 and 24 hours after drug administration on above mentioned days. Blood samples for measuring plasma gastrin levels were collected from jugular vein at 2 and 24 hours after drug administration on day 1, 7 and 14 of the study. Plasma drug levels were not monitored in this study.

Results: In F 344 female rats, plasma gastrin levels at 2 hours after drug (E3810: 20 mg/kg/day) were increased by 368% and 941% on day 7 and 14 of the study respectively. When compared to control group values (no elevation of gastrin levels were seen on day 1 of the study). Omeprazole (comparator: 20 mg/kg/day) was more effective in elevating gastrin levels in F 344 female rats than E3810. Additionally, intragastric pH in E3810 or Omeprazole treated F 344 rats were significantly higher than those seen in control group from 1-4 hours after dosing on days 7 and 14 of the study. The magnitude of pH changes were comparable in both treated groups and returned to normal range at 8 hours after drug administration. In SD female rats, treatment with E3810 (20 mg/kg/day) for 2 weeks had no significant effect on plasma gastrin levels, while Omeprazole gave expected results (increase in gastrin levels: 691%). During the study period E 3810 had no effect on intragastric pH. Intragastric pH in Omeprazole treated rats were significantly higher than those seen in control group and returned to normal range at 8 hours after drug administration. The data indicates that F 344 rats were more sensitive to E3810 than SD rats. Omeprazole produced hypergastrinemic response in both strain of rats.

Table 1. Plasma gastrin levels (pg/ml) after oral administration of E3810 and omeprazole in rats

Strain	Treatment	Dose (mg/kg/day)	Day 1		Day 7		Day 14	
			2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
F344	Vehicle Control	0	125.2±13.0	231.0±23.9	114.0±27.5	203.0±30.0	103.4±4.4	179.4±6.5
	E3810	20	121.4±13.4	176.6±6.9	533.8±141.7 *	208.0±17.0	1076.0±256.7 *	218.4±27.0 *
	Omeprazole	20	204.6±29.7	183.4±6.5	1252.3±166.3 **	189.5±17.5	1310.0±149.4 **	225.0±9.5 **
SD	Vehicle Control	0	159.4±26.0	163.2±10.9	81.4±8.1	148.4±5.5	95.8±7.5	159.0±13.1
	E3810	20	218.6±33.9	186.6±15.7	158.8±62.8 *	163.2±10.8 *	94.8±7.7 **	165.2±7.9
	Omeprazole	20	352.6±117.5	207.2±8.2	518.2±149.2	187.6±12.9	757.6±105.7	188.0±15.4

Values are expressed as Mean ± S.E.
N=5
*p<0.05, **p<0.01, compared with vehicle control group of each strain.

Table 2. Gastric pH values after E3810 and omeprazole administration in rats on Day 1

Strain	Treatment	Dose (mg/kg/day)	Time (hours) after administration					
			1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
F344	Vehicle control	0	2.36±0.34	1.96±0.24	2.02±0.11	1.65±0.03 *	-	2.13±0.20
	E3810	20	2.78±0.34 *	2.51±0.22	1.92±0.08	1.85±0.08	-	1.88±0.04
	Omeprazole	20	3.52±0.36	2.10±0.08	2.43±0.53	2.08±0.51	-	1.89±0.05
SD	Vehicle control	20	2.47±0.54	1.72±0.13 *	1.92±0.21	1.90±0.21	-	2.36±0.27
	E3810	20	2.88±0.44	2.12±0.10	1.80±0.11	1.73±0.11	-	1.73±0.06
	Omeprazole	20	4.15±0.89	2.74±0.54	2.43±0.42	1.73±0.06	-	2.12±0.13

Table 3. Gastric pH values after E3810 and omeprazole administration in rats on Day 7

Strain	Treatment	Dose (mg/kg/day)	Time (hour) after administration					
			1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
F344	Vehicle control	0	2.08±0.10 **	2.39±0.43 **	2.25±0.59	2.30±0.78	-	1.79±0.14
	E3810	20	6.09±0.42 **	5.92±0.81 **	3.99±0.74	1.77±0.09	-	1.90±0.15
	Omeprazole	20	5.68±0.26	6.08±0.36	4.30±0.79	1.93±0.06	-	1.89±0.13
SD	Vehicle control	20	1.86±0.11	2.09±0.17	1.82±0.08	1.97±0.10	-	2.20±0.24
	E3810	20	2.63±0.57 *	2.59±0.56	2.02±0.17	1.92±0.16	-	2.22±0.38
	Omeprazole	20	5.56±0.82	3.98±0.67	2.44±0.34	2.06±0.21	-	1.90±0.12

Table 4. Gastric pH values after E3810 and omeprazole administration in rats on Day 14

Strain	Treatment	Dose (mg/kg/day)	Time (hour) after administration					
			1 hr	2 hr	4 hr	8 hr	12 hr	24 hr
F344	Vehicle control	0	2.09±0.16 **	2.78±0.35 **	1.71±0.06	1.33±0.06	1.74±0.18	1.79±0.04
	E3810	20	6.67±0.28 **	6.59±0.23 **	3.50±0.80 *	1.78±0.22	1.68±0.05	1.77±0.08
	Omeprazole	20	6.00±0.28	6.32±0.55	5.61±0.67	2.81±0.47	1.81±0.17	1.73±0.03
SD	Vehicle control	20	1.69±0.15	1.83±0.07	1.62±0.06	1.70±0.11	1.75±0.18	2.10±0.13
	E3810	20	1.96±0.09 *	1.99±0.17 *	1.53±0.15 *	1.95±0.27	1.63±0.13	1.98±0.10
	Omeprazole	20	4.77±0.69	5.13±0.87	3.39±0.35	1.98±0.11	2.12±0.21	2.20±0.19

Values are expressed as Mean ± S.E.
N=4-5
*p<0.05, **p<0.01, compared with vehicle control group of each strain.

There was a supervisory pharmacologist's addendum dated August 17, 1993 to the pharmacology review of amendments # 023, 029, 036, 038 and 041. This addendum is also attached below.

Supervisory Pharmacologist's Addendum to Dr. Ahmad's Pharmacology Review of Amendments # 023 (April 26, 1993), # 029 (May 28, 1993), # 036 (June 10, 1993), # 038 (June 17, 1993) and # 041 (July 30, 1993)

1. Concur

2. The two week pharmacodynamic (Study # T93008) and pharmacokinetic (Study # T93009) studies in rats were flawed as the pH of the administered dosing solutions were not adjusted upward to counteract the adverse influence of gastric acidity on the drug disposition and the duration of feeding was restricted to 5 hours per day and that too only during the day time.

3. The results of the present pharmacodynamic study (T93008) are at variance with the results of earlier pharmacodynamic studies of E 3810 in Sprague-Dawley (SD) rats and the results of one year chronic toxicity study in SD rats. In the present study, E 3810 did not exert any effects on gastric pH and serum gastrin levels in SD rats. In contrast, treatment with E 3810 in the one year chronic toxicity study in SD rats (Report # EIS012/0348) produced increases (25-116%) in 24-hour sampling gastrin levels ($C_{minimum}$ values as indicated on pages 2 and 3 of Dr. Ahmad's review). Earlier single dose pharmacodynamic studies of E 3810 in SD rats (E 3810 Research Reports W-890384 and W-890378 dated October 10, 1989, pages 0575 to 0607 of Volume 1.3 of initial submission) clearly demonstrated the pharmacological efficacy of the compound both in healing of "ulcers in ulcerogenic rat models" and in acid secretion inhibition in "Pylorus-Ligated Rats". E 3810 was administered in a 0.2% $NaHCO_3$ solution by oral gavage in "Murakami model" and by intraduodenal route in "Shay ulcer model" or "Shay gastric acid secretion inhibition model".

4. The relative plasma levels of E 3810 on days 1 and 14 were erratic (pharmacokinetic Study # T93009). This is compounded by the inappropriate methodological practice. Even though the lowest standard of the calibration curve was 0.01 µg/ml with a coefficient of variation of 31.4 to 45.9%, 0.01 µg/ml was set as the limit of quantitation. The concentration of E 3810 in 175 of 480 samples was determined at 0.01 µg/ml point of the calibration curve. This rendered the estimates of concentrations of a large proportion of these samples questionable and unreliable.