

(3): Myosis, relaxed nictating membrane, tonic convulsion, coma, liquid stool and tremor. Although no animals died, some severe toxic signs were observed in the 400 and 2000 mg/kg groups. Watery diarrhea and tonic convulsion developed within 60 minutes after dosing and lasted a few hours. Coma developed 6 hours after dosing and persisted for several hours.

(4): Depressed respiration, hypoactivity, lateral position and salivation. Most of these signs disappeared within 24 hours after administration.

(5): Depressed respiration, hypoactivity, lateral position, staggering gait and convulsion. Most of these findings disappeared within 1 hour after injection.

Dogs appeared to be the least susceptible to toxicity by route of oral administration.

Acute I.V. Toxicity Study of E3810 Enantiomers in Rats
(Study # 931214)

Study Started and Completed: August 30, 1993 and
October 18, 1993

Methods: Groups of Sprague-Dawley rats (5/sex/group) were given a single i.v. dose of 0, 50, 100 or 200 mg/kg of S(-) or R(+)E3810. Rats were observed for clinical signs and mortality for 14 days. At the end of observation period all surviving rats were sacrificed and necropsied.

Results: Both enantiomers produced similar clinical signs (bradypnea, salivation and clonic convulsions). Both enantiomers produced death at 200 mg/kg in both sexes [S(-)E3810: males = 5/5 and females = 5/5, R(+)E3810: males = 5/5 and females = 1/5]. The highest non-lethal dose was 100 mg/kg for both enantiomers. The data indicated that there were no significant enantiomeric differences in rats when given via i.v. route.

Addendum: (1) The pH of oral solution for acute toxicity studies in rats was not provided. In the acute toxicity study in dogs, 30 ml of 1% NaHCO₃ solution was given orally before administration of E3810. (2) The acute toxicity studies (921312 and S98613) with degradation products I, II and BCPP and metabolites of E3810 (sulfone-E3810 and thioether-E3810) in rats (5/sex/group) were not previously reviewed. The results of these studies along with the dosing information were summarized in the following table.

| Dose (mg/kg) | Mortality | Clinical Signs of Toxicity |
|--|------------------------------|---|
| Degradation product I, Oral dose 0 500 1500 | 0 0 0 | Diarrhea |
| Degradation product II Oral dose 50 150 500 | 0 3 females died 10/10 | At 150 mg/kg, hypoactivity, lateral position, tachypnea, miosis and lacrimation were observed. At 500 mg/kg, in addition to the above clinical signs of toxicity, hypothermia was also observed. |
| Degradation product BCPP Oral dose 150 500 1500 | 0 0 5/5 females | At 500 mg/kg or higher in females or 1500 mg/kg in males: decreased activity, staggering gait, prone or lateral position, lacrimation were noted. |
| Sulfone-E3810 Oral dose 500 1500 | 0 0 | Tachypnea and hypoactivity were observed at 1500 mg/kg |
| Thioether-E3810 - I.V. dose 0 10 30 100 (male only) | 0 0 0 5/5 | Convulsion was observed at all doses. Irregular respiration and hypoactivity were also observed at 30 mg/kg. All males at 100 mg/kg had convulsion and died during dosing. |

(3) In conclusion, the minimal lethal oral dose of E3810 was identified in mice at 786 mg/kg in males or 983 mg/kg in females. The minimal lethal i.v. dose of E3810 in mice was 205 mg/kg in males or 229 mg/kg in females. The minimal lethal oral dose of E3810 in rats was identified at 1431 mg/kg in males or 1024 mg/kg in females. The minimal lethal i.v. dose of E3810 in rats was 154 mg/kg in males or 123 mg/kg in females. The minimal lethal i.v. dose of E3810 enantiomers [S(-)E3810 and R(+)E3810] in rats was identified at 200 mg/kg in both males and females. The minimal oral lethal dose of degradation product II was identified at 150 mg/kg in female rats (no deaths in males). The minimal oral lethal dose of degradation product BCPP was identified at 1500 mg/kg in female rats (no deaths in males). The minimal i.v. lethal dose of thioether-E3810 in rats was identified at 100 mg/kg in males (females were not included in this study). The highest oral doses tested (1500 mg/kg) of degradation product I and sulfone-E3810 were nonlethal doses in rats. The highest nonlethal oral dose of E3810 in the dog was 2000 mg/kg.

SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY:

RAT:

A 2-Week I.V. Toxicity Study in Rats (952111)

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

Study Start and Completion Dates: February 13, 1995 and
November 2, 1995.

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

Animals: Males (230-280 g, 7 weeks old)
Females (163-218 g, 7 weeks old)
Sprague-Dawley rats

Methods: To evaluate the toxicity of E3810 in rats, rats (10/sex/group) were given E3810 intravenously at 0, 1, 10 and 75 mg/kg/day for 2 weeks. Additional 8 satellite animals (4 males and 4 females) in each group were included for toxicokinetic measurement. Clinical sign of toxicity was observed daily. Food consumption and body weights were determined on days 1, 4, 7, 10 and 14. Ophthalmic examinations were conducted on days -1 and 13. Hematology, clinical chemistry and urinalysis were determined at termination. All animals were necropsied at termination and the organs were weighed. Plasma drug level was determined on days 1 and 14.

Results:

1. Clinical Signs: The treatment related changes were limited in the high dose group and these included hypoactivity, salivation, prone position, bradypnea, flushing of the nose and local reaction at the injection site (swelling and necrosis).
2. Mortality: There were no deaths.
3. Body Weight: There were no clear treatment related changes.
4. Food Consumption: There were no treatment related changes.
5. Ophthalmoscopy: There were no treatment related alterations observed during the study.
7. Hematology: The red blood cell, hematocrit and hemoglobin were slightly but significantly decreased (9.2-9.8%) in the high dose group. Increased platelet count (~25%) and fibrinogen level (25%) were noted in the high dose females.
8. Clinical Chemistry: Some slight changes in the clinical chemistry parameters are not considered treatment related changes.
9. Urinalysis: There were no treatment related changes observed during the study.

10. Organ Weights: Both absolute and relative liver (18%) and thyroid (83%) weights were increased in high dose males. Both absolute and relative thymus weights were decreased in mid (17-18.5%) and high (51%) dose males. The absolute thyroid weight was also increased in the high dose females (45%). Both absolute (39%) and relative (40%) thymus weights were decreased in the high dose females.

11. Gross Pathology: Hypertrophy of the thyroid was noted in one high dose male.

12. Microscopic Pathology: Slight cortical atrophy of thymus and slight or moderate follicular hypertrophy of the thyroid were noted in the high dose group. Histopathological examination also revealed that there were increased subcutaneous connective tissue proliferation, cell infiltration, edema and vasculitis in high dose group.

13. Toxicokinetic Study: There was no obvious difference in the plasma drug level between males and females and between day 1 and day 14, suggesting that the drug was not accumulated over time. The toxicokinetic information was summarized on a table on page 193 in volume 1.16 and this table is attached below.

| Dose (mg/kg) | Study day | Male | | Female | |
|--------------|-----------|---------------------------|-----------------------|---------------------------|-----------------------|
| | | C _{5min} (µg/ml) | AUC (0-2h) (µg·hr/ml) | C _{5min} (µg/ml) | AUC (0-2h) (µg·hr/ml) |
| 1.0 | 1 | 0.901 ± 0.092 | - | 0.842 ± 0.043 | - |
| | 14 | 1.195 ± 0.060 | - | 0.964 ± 0.111 | - |
| 10 | 1 | 8.717 ± 0.503 | 3.241/2.778* | (85.41 ± 6.352)† | (27.30 ± 1.709)† |
| | 14 | 10.47 ± 0.746 | 3.777/3.783* | 10.30 ± 1.568 | 2.423* |
| 75 | 1 | 69.06 ± 7.153 | 23.71 ± 1.335 | 78.51 ± 11.95 | 24.69 ± 3.747 |
| | 14 | 62.28/78.18* | 21.88/27.16* | 84.71 ± 9.786 | 28.70 ± 3.339 |

- not calculated; at 1 mg/kg all rats showed too few quantifiable levels to calculate AUC.

† suspected misdosing: administration of 75 mg/kg, rather than 10 mg/kg (on 1st day only).

* individual results: some 10 mg/kg rats had too few quantifiable levels to calculate AUC; at 75 mg/kg on day 14, s.c. dosing was suspected for 1 male and another could not be bled.

In summary, E3810 was tested intravenously at 0, 1, 10 and 75 mg/kg/day for 2 weeks. There were no deaths. The major treatment related changes were mainly in the high dose group and these included clinical signs of toxicity (hypoactivity, salivation, prone position, bradypnea, flushing of the nose), decreased red blood cell, hematocrit and hemoglobin (9.2-9.8%, females only) and histopathological changes associated with altered organ weights. The histopathological changes were slight cortical atrophy of thymus and slight or moderate follicular hypertrophy of the thyroid associated with decreased thymus

weight and increased thyroid weight. No effect dose was identified at 1 mg/kg/day. E3810 was tolerated at doses up to the high dose tested (75 mg/kg/day). The thyroid and thymus were the target organs of toxicity.

4 Week Intravenous Toxicity Study in Rats
(Summary Report of Study No. 882113)

Date of the study: Not specified.

GLP requirement: No statement of compliance with GLP regulations was included.

Animals: Strain, body weight and age of the rat used in the study were not mentioned.

Methods: Four groups of animals each consisting of 10 males and 10 females were given E3810 (batch no. was not identified) prepared in saline at dose levels of 0, 1, 5, 25 and 50 mg/kg/day into the tail vein at a volume of 0.2 ml/kg for 4 weeks. Additional five animals per sex in the control, 25 and 50 mg/kg/day groups were held for an additional four weeks without treatment in order to assess reversibility of any effects.

Results:

Clinical signs: At 50 mg/kg/day, inactivity was seen and necrosis developed at the sites of injection.

Body weight: A five percent reduction in body weight was observed in the 50 mg/kg/day females. It returned to control level after recovery period.

Food consumption: Lower (9%) food consumption was seen in the 50 mg/kg/day females.

Hematology: At 50 mg/kg/day reductions were seen in RBC (8-10%), hematocrit (10-11%), hemoglobin (14-16%), MCV (4-6%) and MCHC (4%) with corresponding increases in reticulocytes (246%), platelets (20-29%) and WBC (29%). At 25 mg/kg/day, it produced slight decrease in hemoglobin (5%).

Blood chemistry and urinalysis: Sponsor stated that data was generally unremarkable. However, no such data were submitted in the IND.

Organ weight: Statistically significant increases in stomach weights were noted in all dose levels (12-17%). At 25 and 50 mg/kg/day, it increased liver weight (10-12%) and decreased thymus weight (26-38%). At 50 mg/kg/day, it increased thyroid (26-95%), spleen (26%), kidneys (7%) and lung (9%). Increased kidney weights were noted at lower dose level (1-25 mg/kg/day). Only the thymus weight decreased and a persistent effect in the female stomach were evident at the end of the recovery period.

Histopathology: Cortical atrophy of the thymus was seen in the 25 and 50 mg/kg/day groups. In the thyroid, hypertrophy of the follicular epithelium and decrease in colloid was seen in the 5 mg/kg/day and above groups. At the end of the recovery period, changes were still evident in the thyroid of the 25 and 50 mg/kg/day groups. One male at 50 mg/kg/day showed slight changes in thymus.

In conclusion, E3810 given intravenously for 4 weeks induced clinical signs of hypoactivity at 50 mg/kg/day dose level. Lesions in the thymus was seen in the 25 and 50 mg/kg/day groups. Changes in thyroid was seen in the 5 mg/kg/day and above groups. Thus, at 1 mg/kg/day, except slight change in stomach and kidney weight, it did not produce any signs of toxicity.

Addendum: (1) The study was initiated on January 19, 1988 and the final report was signed on October 17, 1996 and (2) the testing laboratory is Drug Safety Research Laboratories, Eisai Co., Ltd., Gifu, Japan.

13 week Oral Toxicity Study in Rats
(Study No. 872113)

Date of the study: Sept. 9 1987 to June 14, 1988.

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

Animals: Sprague-Dawley rats weighing 70-90 g and 4 weeks of age were used.

Methods: Seven groups of animals each consisting of 12 males and 12 females were given E3810 (Lot no. 87052701) dissolved in 0.5% methylcellulose at dose of 0 (vehicle), 0, 1, 5, 25, and 100 mg/kg/day or omeprazole (lot 87061401) at dose level of 100 mg/kg/day orally by gavage for 13 weeks to rats deprived of food for 19 hours. Additional 8 males and 8 females in the control, vehicle control, 25, 100 and omeprazole groups were held for a 5-week recovery period after treatment. Stomach tissues were stained with hematoxylin and eosin and with Grimelius stain. Microscopic examinations were performed with all tissue samples from the controls and animals treated with 100 mg/kg/day of E3810 or omeprazole. Microscopic examination of tissues from the other groups and recovery groups was limited to the following tissues: liver, stomach, thymus and thyroid.

Results:

Mortality (daily): Two animals receiving 100 mg/kg/day of E3810 died on days 17 and 76. Both animals died of intubation error during administration.

Clinical signs (daily): Transiently increased salivation after the dosing of 100 mg/kg/day of E3810 or omeprazole was seen.

Body weight and food consumption (weekly): Normal.

Ophthalmology (weeks 6, 12 and 17): Two animals in the control group exhibited corneal opacity and hemorrhage in the fundus oculi.

Hematology (weeks 13 and 18): Decreases in RBC (4%), Hct (4-5%) and hemoglobin (4-7%) were seen in the 25 and 100 mg/kg/day of E3810. Decreases in MCH (4%) and MCHC (2%) were observed in the 100 mg/kg/day E3810 group. In the 100 mg/kg/day omeprazole group, decreases in hemoglobin (4%), MCV (2%) and MCH (4%) and increases in reticulocytes (53%) and platelets (18%) were found.

Blood Chemistry (weeks 13 and 18): Elevations in cholesterol (33%), phospholipid (26%) triiodothyronine (14%) and thyroxine (14%) were seen in males treated with 100 mg/kg/day of E3810. Increases in cholesterol (30-33%) and phospholipid (24-25%) were seen in the females receiving 25 and 100 mg/kg/day of E3810. In the omeprazole treated group, elevation of cholesterol (22-42%) and phospholipid (19-33%) were observed. Elevated thyroxine levels were still evident at the end of the five week recovery period.

Urinalysis (weeks 13 and 18): Normal.

Organ weight: In males, increases in liver (13-26%) and stomach (11-21%) and decrease in thymus (25-38%) were seen in the 25 and 100 mg/kg/day of E3810 groups. Increases in liver (17%), kidney (10%) and stomach (17%) were found in the 100 mg/kg/day omeprazole group. In females, decrease in thymus (24-32%) was reported in the 25 and 100 mg/kg/day of E3810 groups. Stomach weights were increased in both 100 mg/kg/day of E3810 and omeprazole groups (20 and 14%, respectively). Decreased thymus weights at 25 and 100 mg/kg/day were still evident in the females at the end of the five week recovery period.

Histopathology: Centrilobular hypertrophy of the liver cells were observed in males treated with 100 mg/kg/day of E3810 or omeprazole. Hypertrophy of the mucosa in the stomach and eosinophilic changes in the chief cell granules were found in the 100 mg/kg/day of E3810 and omeprazole groups. Erosion in pyloric gland was found in the 100 mg/kg/day of E3810 group. Mucosal hypertrophy was observed in males treated with 25 mg/kg/day of E3810. A dose-dependent increase (18-55%) in the thickening of fundic mucosa was found in the 5 mg/kg/day and above groups. The mucosal thickening effect of 100 mg/kg/day of omeprazole was comparable (41-60%) to E3810 at the same dose. Cortical atrophy of thymus was found in all male drug-treated groups, but the incidence was higher in the 100 mg/kg/day E3810 group. In the females cortical atrophy was observed in the 25 and 100 mg/kg/day of E3810 and 100 mg/kg/day of omeprazole groups. In thyroid, hypertrophy of follicular epithelium and decrease of colloid were found in the males treated with 100 mg/kg/day of E3810 and in one male treated with omeprazole. Lesions in kidneys was found in one female treated with 100 mg/kg/day of omeprazole. At end of the five week recovery period, changes in the stomach, thymus and thyroid were still apparent in the 100 mg/kg/day group.

In conclusion, E3810 given orally in solution induced salivation, decreases in hematological parameters, elevations of cholesterol, phospholipid, triiodothyronine and thyroxine as well as lesions in livers, thymus, thyroid and stomach. Lesions in thymus and thyroid were more severe in the 100 mg/kg/day of E3810 than the 100 mg/kg/day of omeprazole. A "no effect dose" of 1 mg/kg/day was established.

Addendum: (1) The testing laboratory is Department of Drug Safety Research, Eisai Co., Ltd., Gifu, Japan, (2) the final report was signed on October 11, 1989 and (3) 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 # 87113
(13-Week Oral Toxicity Study in Rats)

In 13-week oral toxicity study doses of 0, 1, 5, 25 and 100 mg/kg/day were used. A positive control group was also included which received 100 mg/kg/day of omeprazole. Additional animals (8 sex/group) were included in 25 and 100 mg/kg/day dose groups and positive control dose group for 5 week recovery study. Stomach, liver, thymus and thyroid gland were target organs of toxicity and the "no effect dose" was 1 mg/kg/day.

In the present submitted report sponsor reanalyzed the previously submitted data with reference to the effect of drug on liver, kidney, heart and lung. At 25 mg/kg/day and above, increases in serum cholesterol (Males: 13-34%, Females: 30-33%) and serum phospholipid (Males: 10-26%, Females: 23-24%) were seen. Additionally, at 100 mg/kg/day serum LDH activity was increased by 20-23% (both sexes) compared to their respective control values. Elevated serum cholesterol and phospholipid levels were also seen in omeprazole treated rats. Both E3810 and omeprazole increased the activity of cytochrome b₅ in liver microsomes by 19.6-25.6% compared to the control values. In males increased relative weights of liver (13-31%) and decreased relative weight of thymus (24-36%) were seen at 25 mg/kg/day and above, additionally at high dose, the relative kidney weights were increased by 10% compared to the control values. In females, thymus weights were reduced by 24-33% at 25 mg/kg/day and above when 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. Gross pathological examinations revealed thymus atrophy in treated rats (Males: 1/12 in control, 0/12 in 1 mg/kg/day dose group, 0/12 in 5 mg/kg/day dose group, 2/12 in 25 mg/kg/day dose group and 7/12 in 100 mg/kg/day dose group; Females: 1/12 in 25 mg/kg/day dose group, 1/12 in 100 mg/kg/day, and 1/12 in positive control group). Thymus atrophy was still present at the end of recovery period at high dose (Males: 2/8, Females: 1/8, and positive control males: 1/8). Histopathological examinations revealed centrilobular hypertrophy of the liver in high dose treated males, and cortical atrophy of the thymus (males: 2/12 in control group, 1/12 at 1 mg/kg dose group, 0/12 in 5 mg/kg dose group, 2/12 in 25 mg/kg dose group, and 6/11 in 100 mg/kg dose group; females: 0/12 in control group, 0/12 at 1 mg/kg dose group, 0/12 in 5 mg/kg dose group, 3/12 in 25 mg/kg dose group, and 5/11 in 100 mg/kg dose group).

In summary, elevated serum cholesterol and phospholipid levels along with increased relative weights of liver in males and decreased absolute/relative weight of thymus in both sexes were seen at 25 mg/kg/day and above. 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 hypertrophy of the liver in high dose treated males and cortical atrophy in both sexes at 25 and 100 mg/kg/day. Thus if we disregard the G.I. finding (see Dr. Sun review dated 1/12/90) then the "no effect dose" in rats was 5 mg/kg/day.

1-Year Oral Toxicity Study in Rats
(Report # EIS012/0348)

Testing Laboratories: [REDACTED]

Study Started: August 21, 1989

Study Completed: April 5, 1993

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

Animals: 33-44 days old CD [REDACTED] rats (males: 221-250 g and females: 251-280 g)

Drug Batch No.: 89010911 and 89120401

Methods: Groups of 20 males and 20 females rats were given orally (gavage) E3810 at daily doses of 1, 5 and 25 mg/kg/day for 52 weeks. The control group animals received the vehicle (distilled water). The volume of administration was fixed at 5 ml/kg. Additionally, two groups (n=15/sex/group) were also included in this study, one received the vehicle and the other received high dose of E3810 and used for 26-week recovery study. In the initial submission sponsor indicated that E3810 was given along with 1% NaHCO₃ because the drug has "poor stability in acid solution". In this study no information of the pH of the drug solution was given. The dose selection was based on 13-week oral toxicity study in which 1, 5, 25 and 100 mg/kg/day were used. At 25 mg/kg/day, "Increased liver weight in males and cortical atrophy of the thymus in males and females" were seen. At 100 mg/kg/day, "hypertrophy of centrilobular hepatocytes and thyroid follicular epithelium were seen only in males". It should be noted that in the main 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 drug administration. This feeding schedule was also followed during recovery period. All animals were observed for clinical signs daily, body weights and food consumptions were recorded pre-test and weekly during the treatment phase and recovery phase. Ophthalmoscopic examinations were performed on all animals once

pretest and once during week 24 and 49 of the study. At 12, 24, 38 and 50 weeks of treatment a full neurological examination was performed on all control and high dose groups rats. Just before drug administration blood samples were collected from retro-orbital sinus of rats (10/sex/group) during weeks 24, 50 of the study and during week 25 of the recovery period for hematological and serum chemistry (including T3, T4 and gastrin) tests. Plasma drug levels were not monitored. After 24, 50 weeks of treatment and after 25 weeks of recovery period, over night urine samples were collected from 10 rats/sex/group for urinalysis. All surviving animals were sacrificed at the end of the study period and subjected to complete necropsy. Only control and high dose group animals were examined histopathologically. Stomach and liver from low and mid dose groups were also examined microscopically. 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, electron-microscopic examinations were also performed on tissues of kidneys (cortex and medulla), liver and stomach (mucosa from the glandular region) from 3 rats/sex of control and high dose treated groups.

Results:

1. Observed Effects: One male and 3 females of mid dose group and 4 males of high dose group had convulsions during the study period. Convulsion episodes were observed during handling of the animals and most during the period just prior to drug administration. Even though no convulsion was seen in control and low dose treated rats, the findings in mid and high dose treated rats were not considered to be treatment related.
2. Mortality: During treatment-period, 3 rats (2 females from control group and 1 male from high dose group) died during blood sampling.
3. Body Weight/Food Consumption/Water Consumption: No significant treatment related effects were seen. It should be noted that 1 year old male SD rat (weight: about 685 g) normally consumes about 28 g of food and female SD rat (weight: about 397 g) normally consumes about 22 g of food. In this study 1 year old male (mean weight: 573 g) and female (mean weight: 310 g) rats consumed on the average about 22 g and 16 g of food respectively. Hence due to availability of food for only 5 hours/day animals consumed about 22-27% less food than rats given food ad libitum.
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 25%, 45% and 116% in females in low, mid and high dose respectively, when compared to their respective control values. Additionally, plasma thyroxine levels were increased by 26% in high dose treated males compared to control values and by the end of recovery period it was still 8.7% higher than the control values. At the end of recovery period, serum gastrin levels were decreased by 24% and 17% in males and females of high dose group respectively compared to their respective control values. Urinalysis were normal.
6. Vital Signs/Physical Examination/Ophthalmic Examination/Neurological Examination: No treatment related effects were seen.
7. Organ Weights: At the end of treatment period, stomach weights were increased by 3% (relative wt. = 0.8-9%), 8-11% (relative wt. = 4.8-10%) and 37-41% (relative wt. = 27-37%) in low, mid and high dose treated rats (both sexes) respectively when compared to their respective control values. In high dose treated males, thyroid weights were increased by 42% (relative wt. = 28%) and in high dose treated females, kidney absolute as well as relative weights were increased by 8% when compared to their respective control values. At the end of recovery period, increased stomach weights (relative wt. = 12%) were still present in high dose treated rats of both sexes.
8. Gross Pathology: Thickening of the stomach wall were seen in treated rats (males: control = 0/20, low dose = 2/20, mid dose = 3/20 and high dose = 12/20; females: control = 0/20, low dose = 1/20, mid dose = 1/20 and high dose = 4/20). Thickening of the stomach wall was still present at the end of the recovery period (males: control = 3/11 and high dose = 9/14; females: control = 0/14 and high dose = 2/12).
9. Histopathology: In the stomach, glandular mucosal hyperplasia (males: control = 3/20, low dose = 11/20, mid dose = 13/20 and high dose = 19/20; females: control = 0/20, low dose = 3/18, mid dose = 5/20 and high dose = 11/20) and eosinophilic chief cells (males: control = 0/20, low dose = 0/20, mid dose = 0/20 and high dose = 16/20; females: control = 0/20, low dose = 0/18, mid dose = 1/20 and high dose = 14/20) were seen in treated rats. Additionally, peri-acinar hepatocytic hyperplasia were seen in treated male rats (control = 0/20, low dose = 2/20, mid dose = 3/20 and high dose = 13/20). At the end of the recovery period, glandular mucosal hyperplasia in the stomach was still present in 50% of the male rats of high dose group.
10. Gastric Image Analysis: Dose related increase in fundic mucosal thickness was seen in treated rats of both sexes (low dose 4-7%, mid dose 14-18% and high dose 42-49%). Significant increases in chromogranin positive stained cells were seen in mid and high dose treated rats of both sexes. These findings were still present at the end of recovery period (26-week) in high dose treated rats (only high dose group and control group were used in recovery study).

11. Electron Microscopic Examinations: Sponsor has only conducted the electron microscopic examination of samples of glandular mucosa of the stomach and not of samples from liver and kidneys as mentioned in the method section. No ultra-structural differences were observed between four cell types (parietal cells, chief cells, mucous neck cell and neuroendocrine cells) from rats which were given 25 mg/kg/day of E3810 for 52 weeks and those from control rats.

12. Evaluation of ECL Cells in Fundic Mucosa of Rats (Report # EIS 012/0348/4931): In fundic mucosa of rats, about 75% of endocrine cells are enterochromaffin-like (ECL) cells (Hakanson et al., Digestion, 35 (Suppl. 1): 23-41, 1986). Grimelius staining technique stains the argyrophilic ECL cells of the fundic mucosa. The mean ECL cell counts in both sexes increased with increasing dose (control = 65.5 ± 13.6 , low dose = 75.6 ± 18.3 , mid dose = 117.5 ± 55.5 and high dose = 195.1 ± 49.6). At the end of 26 weeks of recovery period, increased ECL cells count was still significantly greater in high dose treated group compared to the control group (control = 74.8 ± 26.5 and high dose = 114.6 ± 61.2). Increase in ECL cell counts in treated groups were also associated with dose dependent linear and micronodular ECL cell hyperplasia. ECL cell hyperplasia was assessed according to Solcia et al (Digestion: 41, 185-200, 1988) criteria. Sponsor further classified degree of hyperplasia as mild, moderate, severe and very severe. This kind of subclassification was not mentioned by Solicia et al. I have combined severe and very severe classifications.

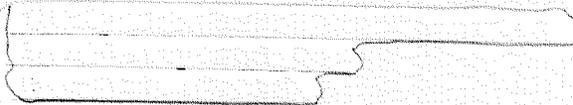
| <u>Males & Females (Combined)</u> | <u>Incidence of ECL Cell Hyperplasia</u> | |
|---------------------------------------|--|---------------------|
| | <u>Linear</u> | <u>Micronodular</u> |
| Control | | |
| Low Dose | 0/40 | 0/40 |
| Mid Dose | 0/40 | 0/40 |
| High Dose | 1/40 | 1/40 |
| | 9/40 | 10/40 |
| Control (recovery) | 0/30 | 0/30 |
| High Dose (recovery) | 0/30 | 4/30 |

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). Animals were given food one-half hour after the drug administration for only 5 hours every day. The effect of the drug on gastrin levels were minimised due to lack of food stimulus and sponsor measured Cmin of gastrin i.e. gastrin levels were monitored at 24 hr after drug administration. Only marginal toxicities such as slight increase in T4 levels and slight peri-acinar hepatocytic hyperplasia were seen. In 13-week oral toxicity study, in addition to the drug effect on stomach, thyroid and liver, cortical atrophy of thymus was also observed

at 25 mg/kg/day and higher dose levels. In 13-week oral toxicity study, at the highest tested dose (100 mg/kg/day) hypertrophy of follicular epithelium of thyroid gland was also seen. Sponsor indicated that 5 mg/kg/day was the no effect dose since it only produced gastric changes which was expected pharmacological effects. In this study animals were starved for about 18-19 hr each day before drug administration. Fasting/restricted food supply might adversely 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 a 6-month oral (gavage) toxicity study in rats. In this study animals should be given food ad libitum and pH of the drug solutions should be adjusted to about 9.0 before administration.

6-Month Oral Toxicity Study in Rats
(Study # R00895)

Testing Laboratories:



Study Started: January 17, 1995

Study Completed: April 30, 1996

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

Animals: 6-7 weeks old Sprague-Dawley (SD/tac) male (170.7 ± 12.5 g) and female (146.9 ± 8.0 g) rats.

Drug Batch No.: 13051304

Methods: Sponsor earlier submitted the results of 1-year chronic toxicity study in rats in which rats were maintained on restricted feeding regimen (see review dated 8/17/93). On September 8, 1993 sponsor was asked to conduct 13-week dose ranging study and 6-month oral toxicity study in ad libitum-fed rats. Subsequently, sponsor submitted the results of 3-month chronic toxicity study in rats (see review dated 11/6/94). In the present submission, sponsor submitted the results of 6-month chronic toxicity study in ad libitum-fed rats. Groups of rats (10/sex/group) were given orally (gavage) E3810 (adjusted to pH 10 with 0.05 M NaHCO₃) at daily doses of 5, 15, 30 and 60 mg/kg/day for 6 months. Additionally, one group of 10 females were also included which received 120 mg/kg/day of E3810 for same duration of period. The control group animals received the vehicle (0.05 M NaHCO₃, pH 10) in similar fashion. The volume of administration was fixed at 5 ml/kg. Rats were fed ad libitum during the study period. All animals were observed daily for clinical signs and mortality. Body weights and food intakes were

recorded pretest and weekly during the treatment phase. Just before sacrifice, blood samples were collected from abdominal aorta for hematology and serum chemistry tests. Sixteen hours urine samples were also collected from 5 rats/sex/group for urinalysis. At the end of treatment period, all animals were sacrificed and subjected to complete necropsy and histopathological examinations. Additionally, liver samples from 5 rats/sex/group were used to determine hepatic enzyme induction parameters.

Results:

1. **Observed Effects:** Dose related increase in salivation was seen in ≥ 30 mg/kg/day treated rats.
2. **Mortality:** One male and one female from 60 mg/kg/day dose group died during study period. The cause of death in male was not determined while the female death was accidental.
3. **Body Weight/Food Consumption/Water Consumption:** Treatment had no effect on body weight in males. However, in females, body weight gains were reduced by 4.6% and 11.5% in 60 and 120 mg/kg/day dose groups respectively (at termination, body weights were 3% and 5% lower than the control values respectively). Food intakes were not affected by the treatment in both sexes (control: mean daily food consumptions were 24.12 g/rat/day for males and 17.6 g/rats/day for females).
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.
5. **Blood Chemistry/Urinalysis:** Serum Gamma glutamyltransferase (GGT) activities were increased by 47% and 58% in females treated with 60 and 120 mg/kg/day dose levels. In females treated with 120 mg/kg/day, serum cholesterol and triglycerides levels were increased by 31% and 26% respectively when compared to control values. Urine volumes were increased by 56-59% in 30 and 60 mg/kg/day treated males, while in females urine volumes were increased by 184% in 120 mg/kg/day dose group.

APPEARS THIS WAY
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