Results: In the absence of metabolic activation, mutation frequency was increased by 2.9 fold at 100 mcg/ml. Even though the result was significant but it did not satisfy the second criteria of positivity i.e. this response must be seen in at least two successive concentrations. The survival rate was 26% at 100 mcg/ml in the absence of S9 mix. In the presence of S9 mix, mutant frequencies were increased by 2.1, 2.3 and 2.8 fold at 10, 25 and 30 mcg/ml respectively, which meets the criteria of positivity. Furthermore, positive results seen in cultures was not related to toxicity since the survival was greater than 20%. In the presence of S9 mix concentrations of 32.5-40 mcg/ml were cytotoxic. Thus the drug is mutagenic at the tk locus of L5178Y mouse lymphoma cells and it confirms the previous results (study # 930921MLA3736).

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended.

1. Sponsor's Version:

Carcinogenesis, Mutagenesis, Impairment of Fertility:

[redacted]

Evaluation: The 2-year carcinogenicity study in Fischer rats on a restricted feeding regime was not a valid study and thus it should not be included in the labeling. Human body weight of 50 kg is recommended to use to calculate the fold of the animal doses to human dose on a body surface area basis (mg/m^2).

[redacted]

2. Sponsor's Version:

Evaluation: The oral Segment II teratological reproductive toxicity study in rats was not a valid study and thus it should not be included in the labeling. Human body weight of 50 kg is recommended to use to calculate the fold of the animal doses to human dose on a body surface area basis (mg/m^2).

3. Sponsor's Version:

[redacted]

Evaluation: The results of the oral acute toxicity studies were not adequately included.

Suggested Version:

[redacted]

SUMMARY AND EVALUATION:

Aciphex is a substituted benzimidazole proton pump inhibitor. It inhibited the activity of H^+/K^+-ATPase in porcine gastric parietal cells with $IC_{50} = 2.6 \times 10^{-7}$ M. The (±)-E3810 (racemic mixture), R-(+)-E3810 [R-(+) enantiomer], and S-(-)-E3810 [S-(-)enantiomer] are equipotent in inhibiting H^+/K^+-ATPase in porcine gastric parietal cells with IC_{50} of $2.8\text{-}3.0 \times 10^{-7}$ M. By inhibiting the H^+/K^+-ATPase activity on the surface of gastric parietal cells, it blocks the final step of the gastric acid secretion. Its antisecretory activities were demonstrated in a number of animal models.

In the present NDA, sponsor is asking for approval to market aciphex for treatment of gastroesophageal reflux disease, gastric and duodenal ulcers and pathological hypersecretory conditions including Zollinger-Ellison syndrome. The recommended adult oral dose is 20 mg daily for up to 4 weeks (healing of duodenal ulcers), 6 weeks (healing of gastric ulcers) and 8 weeks (healing of erosive or ulcerative GERD). For long-term maintenance of healing of erosive or ulcerative GERD, the recommended adult dose is 20 mg/day. For patients with pathological hypersecretory conditions including Zollinger-Ellison syndrome, the recommended adult oral dose is 60 mg/day. In support of this NDA, the following studies were submitted: pharmacological studies, absorption, distribution, metabolism and excretion (ADME) studies in mice, rats, rabbits and dogs, toxicity studies: **(1)** acute toxicity studies in mice, rats and dogs, **(2)** subacute, subchronic and chronic toxicity studies: 13-week oral toxicity studies in mice and rats, 2-week and 4-week i.v. toxicity study in rats, 3- and 6-month oral toxicity studies in rats, 1-year oral toxicity study in rats, 2-week i.v. toxicity study in dogs, two 13-week

oral toxicity studies in dogs, two 1-year oral toxicity studies in dogs, **(3)** special toxicity study: four antigenicity studies in mice, rats, guinea pigs and dogs, 2-week pharmacodynamic study in rats and relationship between plasma gastrin level and ECL proliferation, **(4)** carcinogenicity studies: 88/104-week oral (gavage) carcinogenicity study in CD-1 mice, 104-week oral (gavage) carcinogenicity study in F-334 rats and 104-week oral (gavage) carcinogenicity study in Sprague-Dawley rats, **(5)** reproductive toxicity studies: Segment I i.v. fertility and general reproductive performance study in rats, Segment II i.v. teratology studies in rats and rabbits, Segment II oral teratology study in rats and Segment III i.v. pre- and postnatal reproductive toxicity study in rats and **(6)** mutagenicity studies: Ames tests, *in vitro* chromosomal aberration test in CHL/IU cells, *in vivo* mouse micronucleus test, two unscheduled DNA synthesis assays (*in vitro* and *ex vivo*) in rat hepatocytes, CHO/HGPRT forward gene mutation test, two mouse lymphoma cell (L5178Y/$TK^{+/-}$) forward mutation assays at tk locus.

The results of pharmacokinetic studies demonstrated that after oral administration, C_{max} was reached within 5 minutes in mice and rats and ~8-11 minutes in dogs. E3810 was quickly declined following i.v. and oral administrations with half life of ~6-10 minutes in rats, 24 minutes in dogs and 42-90 minutes in humans. Its oral bioavailability was variable since E3810 is unstable in the gastric juice. The oral bioavailability can be improved by pretreatment with sodium bicarbonate buffer or delivering directly into the duodenum or using the enteric coated tablets. For example, in rats, the bioavailability following oral dose of E3810 was ~11-21% while the bioavailability following intraduodenal administration of E3810 was ~37-91%. In humans, the oral bioavailability of enteric coated tablets of E3810 was 52%. E3810 was oxidized at the position of sulfoxide to the sulfone-E3810 (M2) or reduced to the thioether-E3810 (M1). It was demethylated to desmethyl-E3810 (M3). The metabolism of E3810 to its sulfone metabolite (M2) is mediated by CYP3A form of human cytochrome P450 enzyme while the conversion of E3810 to the desmethyl metabolite (M3) is mediated by CYP2C19 form. Thioether-E3810 (M1) is the major metabolite identified in the human plasma. Very low level of M2 was also detected in the human plasma. Both M1 and M3 were detected in the mouse, rat and dog plasmas. The carboxylic acid derivative (M6) was the major metabolite identified in the mouse, rat and dog urine. M6 was also a major metabolite in the dog feces and bile. Mercapturic acid (M5) was also found in the urine of the rat and dog. The desmethyl metabolite (M3) is pharmacologically active with similar potency to the parent compound. The tissue distribution studies indicated that the highest radioactivity was detected in the thyroid followed by the liver, gastric mucosa, bone marrow and pituitary gland in rats. The highest radioactivity was

detected in the bile followed by eye, liver, stomach and kidney in dogs. The volume of distribution was similar in dogs (~0.41/kg) and in humans (0.34 l/kg). In rats the volume of distribution ranged from 0.37 l/kg to 1.17 l/kg. The total clearance was 0.23 l/hr/kg in humans, ~0.6-0.7 l/hr/kg in dogs and ~6-60 l/hr/kg in rats. E3810 was highly bound to plasma protein in humans (96.2-98.6%) and rats and dogs (91.4-93.3%). The major route of excretion was by urine and feces in mice, rats and dogs. The radioactivity was recovered in the feces (42-50% in mice, 46-53% in rats and 53% in dogs) and urine (20-23% in mice, 39-44% in rats and 33% in dogs). In humans, ~90% of the drug was excreted in the urine, mainly as mercapturic acid and carboxylic acid.

In the acute toxicity studies in mice, rats and dogs, 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 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 non-lethal doses in rats. The highest non-lethal oral dose of E3810 in the dog was 2000 mg/kg. The major treatment related clinical signs of toxicity in the acute toxicity studies were hypoactivity, depressed or labored respiration, lateral or prone position and convulsion.

In the 2-week i.v. toxicity study in rats, E3810 was tested intravenously at 0, 1, 10 and 75 mg/kg/day for 2 weeks. In the high dose group, following clinical signs of toxicity were found: hypoactivity, salivation, prone position, bradypnea and flushing of the nose. Thyroid and thymus were the target organs of toxicity evidenced by cortical atrophy of thymus with decreased thymus weight and follicular hypertrophy of the thyroid with increased thyroid weight. Some organ weight changes were also noted in the mid dose group. No effect dose was identified at 1 mg/kg/day.

In the 4-week i.v. toxicity study in rats, E3810 was tested intravenously at 0, 1, 5, 25 and 50 mg/kg/day for 4 weeks. Inactivity and decreased body weight (5%) were seen in the high dose group. Thyroid and thymus were the target organs of toxicity evidenced by cortical atrophy of thymus with decreased thymus weight (20 and 50 mg/kg) and follicular hypertrophy of the thyroid with increased thyroid weight (5 mg/kg or higher). No effect dose was identified at 1 mg/kg/day.

In the 13-week oral toxicity study in rats (Eisai), E3810 was tested orally at 0, 1, 5, 25 and 100 mg/kg/day for 13 weeks. Salivation (100 mg/kg) and increased cholesterol (30-33% at 25 and 100 mg/kg) were noted. Lesions in the thymus (cortical atrophy), thyroid (hypertrophy of follicular epithelium) and stomach (mucosal hypertrophy) were noted at doses of 25 mg/kg or higher. Therefore, the thymus, thyroid and stomach were the target organs of toxicity. No effect dose was identified at 5 mg/kg/day.

In the 1-year oral toxicity study in rats (Eisai), E3810 was tested orally at 0, 1, 5 and 25 mg/kg/day for 1 year. The histopathological examination revealed glandular mucosal hyperplasia, eosinophilic chief cells and periacinar hepatocytic hyperplasia. This study was considered unacceptable due to the feeding restriction (5 hours daily) and unknown pH of the drug solution. Caloric restriction would alter basic biochemical mechanism of toxicity of various drug and expression of carcinogenic property of the drug.

In the 6-month oral toxicity study [redacted] E3810 was tested orally at 0, 5, 15, 30 and 60 mg/kg/day in ad libitum-fed rats for 6 months. The test drug solution was adjusted to pH = 10 with 0.05 M $NaHCO_3$. Salivation was observed at 30 and 60 mg/kg/day. Reduction of body weight gain and increased serum gamma glutamyltransferase activity and cholesterol level were noted at 30 and 60 mg/kg/day. Histopathological examination revealed renal cortical tubular regeneration, follicular epithelial hypertrophy in thyroid, atrophy of the thymus and gastric glandular epithelial eosinophilic changes and hyperplasia of gastric entereochromaffin-like cells in the stomach at doses of 5 mg/kg or higher. Therefore, the stomach, kidney, thyroid and thymus were the target organs of toxicity. No effect dose was not identified.

In 2-week i.v. toxicity study in dogs, E3810 was tested intravenously at 0, 1, 5 and 25 mg/kg/day for 2 weeks. The major treatment related changes were clinical signs of toxicity (vomiting, loose, mucous, muddy or watery stool, salivation, licking of the chops, decreased activity, miosis and conjunctival injection) and histopathological changes including degeneration

of parietal cells and foci of cellular debris in the stomach and follicular hypertrophy in the thyroid in females. No effect dose was identified at 1 mg/kg since the lesions in the stomach and thyroid were also noted in the mid dose group. The stomach and thyroid were the target organs of toxicity.

In the 13-week oral toxicity study in dogs, E3810 was tested orally (enteric coated tablets) at 0, 1, 3, 10 and 30 mg/kg/day for 13 weeks. The dose of 30 mg/kg was lethal. Diarrhea, increased serum levels of ALP, cholesterol, triglyceride, urea nitrogen and gastrin were observed. The histopathological examination revealed atrophy of thymus and C-cell proliferation of thyroid at doses of 3 mg/kg and higher. The stomach lesion (vacuolization of mucosal epithelium, parietal cell necrosis and degeneration, edema in submucosal lamina propria, chief cell atrophy, proliferation of pyloric epithelium and dilatation of gastric gland) was observed in all treatment groups. Therefore the stomach, thymus and thyroid were the target organs of toxicity. No effect dose was not identified.

In the supplement 13-week oral toxicity study in dogs, E3810 was tested orally (enteric coated tablets) at 0, 0.1, 0.3 and 1 mg/kg/day for 13 weeks. Mucosal thickening and accentuated fold in the stomach and atrophy in the chief cells and degeneration and/or necrosis in the parietal cells were observed in the mid and high dose groups. The stomach was the target organ of toxicity. No effect dose was identified at 0.1 mg/kg/day.

In the 1-year oral toxicity study in dogs (Eisai), E3810 was tested orally (enteric coated tablets) at 0, 0.2, 1 and 5 mg/kg/day for 1 year. The major treatment related changes were the increased gastrin level and histopathological changes in the stomach (mucosal hyperplasia) and testes (degeneration of the tubular germinal epithelium) in the mid and high dose groups. Therefore, the stomach and testes were the target organs of toxicity. The doses tested were too low and additional study was conducted (see next paragraph).

Sponsor repeated the 1-year oral toxicity study in dogs at higher doses [redacted]. In the repeated 1-year oral toxicity study in dogs, E3810 was tested (enteric coated tablets) orally at 0, 2, 8 and 25 mg/kg/day for 1 year. The increased incidence of emesis and soft/water stools were seen in the mid and high dose groups. The serum level of gastrin was increased in all treatment groups. Thickening of the stomach wall, increases in the stomach weight, gastric mucosal and non-mucosal mass and

enterochromaffin-like (ECL) cell hypertrophy were observed in all treatment groups. The histopathological examination also revealed ECL cell hyperplasia and follicular cell hypertrophy in thyroid in the mid and high dose groups. The stomach and thyroid were the target organs of toxicity.

The results from the special toxicity studies on antigenicity of E3810 indicated that E3810 was antigenic in guinea pigs, but not in mice and dogs and it was weakly antigenic in rats.

In the 13-week oral toxicity study in mice, E3810 was tested orally at 0, 25, 100 and 400 mg/kg/day for 13 weeks. The histopathological examination was conducted only on the stomach. The major treatment related changes were dose-dependently increased stomach weight and histopathological change in the stomach (hyperplastic gastropathy of the glandular mucosal wall) in all treatment groups. Slight increase in the liver weight was also seen in the high dose group. Based on these results sponsor arbitrarily selected 2, 20 and 200 mg/kg/day doses for the carcinogenicity study in CD-1 mice.

In the 88/104-week oral carcinogenicity study in mice, E3810 was given to mice by oral gavage at 0, 2, 20 and 200/100 mg/kg/day for 88 weeks (males) and 104 weeks (females). High dose of both sexes was reduced from 200 to 100 mg/kg/day due to a significant increase in mortality in males during weeks of 7-41. The male portion of the study was terminated after 88 weeks of treatment since the survival rate was 25% in week 88 in the control and low dose male groups. These suggested that MTD was between 100 and 200 mg/kg/day and thus the animals were exposed to sufficiently high dose of E3810. At termination, the survival rates were comparable in all groups. Histopathological examination revealed mucosal thickness, hyperplastic gastropathy, amorphous deposits in glands, presence of acidic mucin and neuroendocrine cell hyperplasia in the fundic region of the stomach in mid and high dose groups. Significant increase in the focal necrosis with inflammatory infiltrate in the liver was observed in the high dose group. E3810 was not carcinogenic in this study. This study was considered acceptable in the Division's letter to sponsor dated January 26, 1994.

In the 104-week oral (gavage) carcinogenicity study in 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 given. The highest dose in this carcinogenicity study was less than MTD. Rats were given food for only 5 hours on each day.

Caloric restriction would alter basic biochemical mechanism of toxicity of various drugs and expression of carcinogenic property of the drug. Therefore, this study was not considered as a valid study. Sponsor was asked to repeat the 104-week carcinogenicity study in Sprague-Dawley rats.

In the 3-month oral dose ranging study in rats (Lilly), E3810 was tested orally at 0, 25, 60, 130 and 300 mg/kg/day for 3 months. The stomach, kidney and thyroid were the target organs of toxicity as evidenced by multifocal basophilic cortical tubular regeneration in the kidney, follicular cell hypertrophy in thyroid, eosinophilic chief cells and ECL cell hyperplasia and hypertrophy in the stomach. These histopathological changes were found in all treatment groups and the incidences were dose dependent. In males, the multifocal basophilic cortical tubular regeneration in the kidney (1/10), follicular cell hypertrophy in thyroid (2/10) and eosinophilic chief cells (4/10) and ECL cell hyperplasia (8/20) at 25 mg/kg (the lowest dose tested) and thus this dose was considered as MTD. At 25 mg/kg/day, the only histopathological change was ECL cell hyperplasia identified in one female and at next higher dose (60 mg/kg), there were follicular cell hypertrophy in thyroid (1/10) and eosinophilic chief cells (7/10) and ECL cell hyperplasia (5/10) in females. Therefore, the dose of 60 mg/kg/day was identified at MTD in females. No effect dose was not identified.

In the repeated 2-year oral carcinogenicity study in rats, Sprague-Dawley rats (70 males/group or 60 females/group) were treated with rabeprazole by oral gavage at 0, 0, 5, 15, 30 and 60 mg/kg/day (males) or 0, 0, 5, 15, 30, 60 and 120 mg/kg/day (females) for 2 years. The dose selection was based on findings from the 3 month oral (gavage) toxicity study in rats (R31193, R31293 and R01994). In this study, MTD was identified at 25 mg/kg/day for males and 60 mg/kg/day for females. Both Division and CAC recommended sponsor to use doses of 7.5, 15 and 30 mg/kg/day in males and 15, 30 and 60 mg/kg/day in females. However, sponsor instead chose the doses of 5, 15, 30 and 60 mg/kg/day in males and 5, 15, 30, 60 and 120 mg/kg/day in females in the 2-year carcinogenicity study. In the current study, the high dose tested (60 mg/kg in males and 120 mg/kg in females) exceeded MTD since there were significantly increased mortality and histopathological changes in the stomach and kidney. Slightly lower (91-92%) in the body weight in the high dose group as compared to control was noted in both males and females. A significant increase in the mean gastrin level was seen in all treatment groups. There were no treatment related changes in the tumor incidence in males but in females malignant and benign carcinoid tumors in the stomach were detected in the treatment

groups. The incidence of the carcinoid tumors (malignant and benign) in the stomach in females was 0, 0, 1, 3, 6, 5 and 7 in the control1, control2, 5, 15, 30, 60 and 120 mg/kg groups, respectively. The combined benign and malignant carcinoid tumors in the stomach were found to have positive linear trends using data from pooled control, 5, 15, 30, 60 and 120 mg/kg/day dose groups ($p<0.001$). Significant positivity is retained even after deleting 120 mg/kg/day group; 60 and 120 mg/kg/day groups; and 30, 60 and 120 mg/kg/day groups from comparisons. There was no significant heterogeneity found between the two control groups. This study is considered acceptable.

In the Segment I fertility and general reproductive performance study in rats, rats were given E3810 intravenously at 0, 1, 6 and 30 mg/kg/day. Male rats were treated for 63 days before mating, throughout mating and until they were sacrificed (total 67 days). Females were treated for 14 days before mating and throughout mating. The treatment of the pregnant females for C. section was continued for additional 8 days during gestation. The treatment of the pregnant females for natural delivery was continued until day 20 after delivery. The only treatment related change in dams was dark purplish-reddening, necrosis and sloughing at the injection sites. No adverse effects on fertility and mating performance were found at doses up to the high dose (30 mg/kg/day).

In the Segment II teratology study in rats, rats were treated by oral gavage at 0, 25, 100 and 400 mg/kg/day from gestation days 7 though 17. Two-thirds of pregnant rats were sacrificed on day 20 of gestation and the remaining one-third of the dams were allowed to deliver spontaneously. No significant treatment related changes in the dams were observed. No teratogenic potential was identified in all groups. No adverse effects on postnatal development and fertility of the offspring were seen except at 400 mg/kg slight decrease in the frequency of rearing and ambulating were observed in open field test. E3810 was not teratogenic in this study. However, this study was not considered as a valid study since the dams were given food for only 5 hours during pregnant days 0-16 and sponsor was asked to repeat the Segment II teratological reproductive study in rats.

In the repeated Segment II teratological reproductive toxicity study in rats, E3810 was given to rats intravenously at 0, 5, 25 and 50 mg/kg/day during days 6 through 17. There was no evidence of teratogenic potential of E3810 in this study.

In the Segment II teratology study in rabbits, rabbits were treated intravenously at 0, 1, 6 and 30 mg/kg/day from gestation days 6 through 18. There was no evidence of teratogenic potential of E3810 in this study.

In the Segment III perinatal and postnatal study in rats, rats were treated intravenously at 0, 1, 6 and 30 mg/kg/day from gestation day 17 through day 20 after parturition. At high dose reduction in spontaneous movement, salivation and dark purplish-reddening at the injection sites were observed. The perinatal and postnatal performance were not adversely affected by i.v. administration of E3810 at doses up to 30 mg/ kg/day.

E3810 was positive when tested in the Ames tests, CHO/HGPRT forward gene mutation test and mouse lymphoma cell ($L5178Y/TK^{+/-}$) forward mutation assays at tk locus. The metabolite, demethylated-E3810, was also mutagenic in an Ames test. Rabeprazole was negative when tested in *in vitro* chromosome aberration test in Chinese hamster lung cells, *in vivo* mouse micronucleus test and unscheduled DNA synthesis assays in rat hepatocytes (*in vitro and ex vivo*).

The toxicity profiles of E3810 were studies in rats and dogs. Following target organs of toxicity were identified in rats: stomach (mucosal hypertrophy, hyperplasia of gastric ECL cells and gastric glandular epithelial eosinophilic changes), thyroid (follicular hypertrophy), thymus (cortical atrophy), liver (periacinar hepatocytic hyperplasia), and kidney (multifocal basophilic cortical tubular regeneration). In dogs, stomach (degeneration and necrosis of pariental cells, foci of cellular debris, atrophy of the chief cell, mucosal hyperplasia, ECL cell hyperplasia), thyroid (follicular hypertrophy) and thymus (cortical atrophy) were the target organs of toxicity. The stomach was also the target organ of toxicity in the oral carcinogenicity studies in mice and rats.

In the present NDA, sponsor is seeking approval to market aciphex for treatment of GERD, gastric and duodenal ulcers and pathological hypersecretory conditions including Zollinger-Ellison syndrome. It is also indicated for long-term maintenance use for healing of erosive or ulcerative GERD. Adequate preclinical studies have been conducted and relevant findings of the preclinical studies should be included in the labeling as recommended. Therefore, from a preclinical standpoint, this NDA is approvable. Sponsor should be asked to revise the labeling as recommended.