Segment II. Teratology Study in Rats
(Study # 884212)

Testing Laboratories: Department Drug Safety Research, Gifu 501-61, Japan.

Study Started: August 30, 1988

Study Completed: March 3, 1992

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Test Species: Slc:SD Sprague-Dawley pregnant rats

No. of Animals: 36 pregnant rats/group

Route of Administration: Oral (gavage)

Dose Levels: 0, 25, 100 and 400 mg/kg/day

Drug Batch No.: 88020601

Methods: The selection of the doses were based on the preliminary teratology study in which oral doses of 50, 100, 200, and 400 mg/kg/day were used. According to sponsor "increased salivation and reduced motility were seen at 400 mg/kg/day". Based on these results, dose levels of 0, 25, 100 and 400 mg/kg were selected for the main study.

In the main study, pregnant rats were given oral doses of 0, 25, 100 and 400 mg/kg from day 7 to 17 day of gestation. Control animals received the vehicle (purified water) throughout the same period. The volume of administration was fixed at 5 ml/kg. During days 7-16 of gestation dams were given food for only 5 hr from 30 min after drug administration. According to sponsor "the lowest dose (25 mg/kg/day) was 62.5 -125 fold higher than the proposed clinical dose (0.2-0.4 mg/kg/day). The absolute biovailability of the intact drug in rats under fasting condition was approximately 6% and the dose was approximately 3.8-7.5 fold higher than the proposed clinical dose when compared by biovability". From day 17 of gestation dams had free access of food. Pregnant dams were observed for mortality and clinical signs on days 7 through autopsy. Body weights were recorded on days 0, 7-20 of gestation and on days 1, 4, 7, 10, 14, 17 and 21 of postpartum. Daily food consumptions were recorded on days 6 to 20 of gestation and days 1-4, 4-7, 7-10, 10-14, 14-17 and 17-21 of postpartum. Two-third pregnant rats were sacrificed on day 20 of gestation, and was examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were

weighed and sexed. Approximately one-half of the fetuses in each litter eviscerated and examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining 1/3 of the dams (12 /group) were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. On day 4 after birth culling was carried out to make 8 offspring (4 male and 4 female) per dam. The offspring were reared by the dams until day 21 of post partum. On day 21 of post partum all dams were sacrificed and necropsied, and examined as mentioned above. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (auditory test, learning ability test, ophthalmological examination, open field test and fertility test). At week 11, males and females of the same group were continuously mated for 14 days. F_1 dams were weighed on day 0, 7, 14 and 20 of gestation. Cesarean section was performed on the F_1 dams on day 20 of gestation and examined as mentioned above. F_2 fetuses were weighed, sexed and examined for external abnormalities.

Results: During the treatment period, body weight gains were reduced by 8-12% along with reduction in food consumptions by 4-7% in high dose group when compared to their respective control values.

Dams Sacrificed at Day 20

No significant effect on body weight gains were seen in treated rats. Food consumptions were significantly decreased in high dose treated dams. The number of corpora lutea, the number of implants, numbers of live/dead fetuses, weights of fetuses and sex ratio did not show any significant difference between the treated groups and the control group. External examinations were normal except one fetus in the control group had hematoma and edema and 2 fetuses of 2 litters in the mid dose group had microphthalmia. Dose related delayed ossification in metatarsus were seen in fetuses of treated dams (control = 10.1%, low dose = 13.4%, mid dose = 18% and high dose = 23.7%). Skeletal anomalies such as fusion of cervical arch (control = 0%, low dose = 0.66% [1/1 litter]. mid dose =1.5% [1/1 litter] and high dose = 3.4% [5/5 litters]) were also seen. However, no treatment related abnormalities were observed on external, skeletal, and visceral examinations in any group.

Effect of E3810 on Maternal and Fetal Parameters in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
# Dam examined	22	23	20	24
# Corpora lutea/Dam	14.6±0.4	14.5±0.3	15.0±0.3	15.0±0.4
# Implants/Dam	13.7±0.7	13.9±0.3	14.2±0.4	13.5±0.6
# Total implant loss(%)	8.0	4.2	5.4	10.4
Resorption/ dam (%)	9.9	10.8	10.1	11.1
# Live fetuses/dam	13.1±0.7	12.4±0.49	12.7±0.5	12.4±0.53
Fetal wt (g)	3.35±0.04	3.44±0.06	3.25±0.05	3.36±0.15
Sex Ratio (M/F)	1.12	0.96	1.0	1.09

Dams allowed to deliver: No significant differences in the gestation period between the groups were noted. The number of implantation sites were significantly lower in high dose treated dams (mean/litter: control = 14.6 +/- 0.3, low dose = 14.0 +/- 0.4, mid dose = 14.0 +/- 0.4 and high dose = 13.4 +/- 0.3). Number of pups delivered (mean/litter: control = 14.0 +/- 0.4, low dose = 13.5 +/- 0.4, mid dose = 12.4 +/- 0.7 and high dose = 12.3 +/- 0.4) and number of alive pups (mean/litter: control = 14.0 +/- 0.4, low dose = 13.3 +/- 0.4, mid dose = 12.2 +/- 0.6 and high dose = 11.9 +/- 0.6) were significantly lower in mid and high dose group. Hence the litter size in mid and high dose was significantly reduced compared to control group. No external anomalies were found in any pups in any group. Body weight gains in pups (both sexes) of high dose group was 7-8% less than the control values. There were no significant effects on postnatal development and differentiation (ear unfolding, incisors eruption, eyelid opening, palmar grasp, auditory startle, free-fall righting, open field test and water maze test) except at high dose slight decrease in the frequency of rearing and ambulation were observed in open field test. At necropsy of F_1 generation at weaning time revealed a significant increase of kidneys weight in males (19%). There was no significant effect on fertility test and mating performance test of F_1-generation rats. External examination of F_2 fetuses revealed no abnormalities.

Thus no treatment related abnormalities were observed on external, visceral and skeletal examinations in any group. No teratogenic effects at dosage up to 400 mg/kg/day was observed. The postnatal development and the fertility of the offspring were comparable in all groups except at high dose slight decrease in the frequency of rearing and ambulation were observed in open field test.

Segment II. Teratology Study in Rabbits
(Study # 884232)

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

Dates Study Started and Completed: October 6, 1988 and February 12, 1992

GLP Compliance: A statement of compliance with the GLP regulations and quality assurance unit was included.

Test Species: Eisai:JW-NIBS 8 month old female and 12-25 month old male rabbits (2.63-3.52 kg)

No. of Animals: 17 pregnant females/group

Route of Administration: I.V.

Dose Levels: 1, 6 and 30 mg/kg/day (1 ml/kg)

Drug Batch No.: 88020601

Methods: The selection of the doses were based on the preliminary teratology study, in which doses of 3, 10, 30 and 50 mg/kg/day were given during the period of organogenesis. Decreased body weight (loss ?) and reduced food and water consumptions were seen at 30 and 50 mg/kg/day dose levels. In addition toxic signs such as ptosis and prone position were seen in 50 mg/kg/day dose group. Based on these results, sponsor concluded that 30 mg/kg/day was the maximum tolerated dose, and dose levels of 1, 6 and 30 mg/kg/day were selected for the main study.

Pregnant rabbits were given i.v. doses of 0, 1, 6 and 30 mg/kg/day of E3810 from day 6 through 18 of gestation. Control animals received the vehicle (saline) throughout the same period. The volume of administration was fixed at 1 ml/kg. Pregnant rabbits were observed daily for mortality. Clinical signs, body weights and food intakes were recorded on gestation days 0 (body weight only), 6-20, 22, 24, 26 and 28. All surviving dams were sacrificed at day 28 of gestation, and were examined for the number of corpora lutea, the number of implants, number of early/late resorptions, number of live/dead fetuses. Live fetuses were weighted, sexed and examined for external abnormalities. Their survival rate was observed at 3, 6 and 24 hours after putting them in an incubator. All fetuses were eviscerated and one-half fetuses were examined for skeletal malformations and variations, and remaining fetuses were examined for visceral abnormalities.

Results: In this study no mortality was observed. Eight dams (3 in control group, 2 in low dose group and 3 in high dose group) had abortion. Those dams which had abortion revealed no gross abnormalities at necropsy. Crust at the injection sites was seen in 3/15 dams of the high dose group. During the gestation period body weight gains were reduced by 10%, 40% and 93% in low, mid and high dose group respectively compared to control value. Food intakes were significantly decreased during 22-28 days of gestation in high dose group. The number of corpora lutea, number of implants, number of live fetuses, sex ratio and fetal viability at 24 hr did not show any significant difference between the treated groups and the control group. Fetal body weights were 8% and 19% lower in mid and high dose group than the control group fetal weights. No treatment related abnormalities were observed on external and visceral examinations in any group. Significant increase in fetuses with non-ossified proximal tibial epiphysis were seen in high dose treated group (control = 1/41 fetuses, low dose = 5/49 fetuses, mid dose = 1/52 fetuses and high dose 13/42 fetuses).

Since E3810 is unstable in normal gastric juice, enteric coated tablets were intended for clinical use. Sponsor stated that it was difficult to conduct the study in rabbits by oral administration with an enteric coated tablet or capsule. Therefore the study was conducted by i.v. administration. Overall study is acceptable. In this study maternal and fetal toxicity were seen at 30 mg/kg/day dose level but there was no evidence of a teratogenic potential.

Addendum: The toxicokinetic data were not included and this information was summarized in Table A-16-1 on page 83 in volume 1.12 and this table is attached below.

Table A-16-1: Pharmacokinetic Parameters of E3810 after a Single or Repeated Intravenous Administration of E3810 to Pregnant Rabbits

Dose E3810 mg/kg/day	Day of Drug Administration	Number of Animals	$AUC_{(0-\infty)}$ (ug•hr/mL)	$t_{1/2}$ (hr)	V_d (L/kg)	Cl_{tot} (L/kg/hr)
1.0	First*	5	0.287±0.026	0.147±0.005	0.772±0.091	3.635±0.413
	13th	5	0.364±0.037	0.148±0.013	0.594±0.028	2.881±0.333
6.0	First	3	1.790±0.279	0.157±0.009	0.793±0.115	3.505±0.494
	13th	3	1.700±0.088	0.129±0.005	0.660±0.036	3.549±0.194
30.0	First	3	7.983±0.833	0.162±0.005	0.901±0.117	3.848±0.431
	13th	2	7.280/7.263**	0.187/0.131**	1.110/0.780**	4.121/4.131**

Data are expressed as mean values of 3-5 animals ± SEM
**The first day of drug administration corresponds to Day 6 of gestation*
***Individual results of 2 rabbits (the data from one animal was excluded due to suspected transposition of samples)*

The $AUC_{0-\infty}$ value in healthy volunteers (trial A001-114) was ~0.88 μg.hr/ml following a daily oral dose of E3810 at 20 mg. The calculated ratio of $AUC_{0-\infty}$ of rabbits (30 mg/kg/day) to human (20 mg/day) is ~9.

I.V. Segment III. Perinatal and Postnatal Study in Rats (Study # 3049)

Testing Laboratories: [redacted]

Study Started: February 16, 1990

Study Completed: July 16, 1991

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

Test Species: Pregnant Sprague Dawley rats (Crl: CD SD BR).

No. of Animals: 22-25 pregnant rats/group

Drug Batch No.: 89120401

Route of Administration: I.V.

Dose Levels: 0, 1, 6 and 30 mg/kg/day (1 ml/kg body weight)

Methods: In this study dose selection was based on 4-week i.v. toxicity study in rats in which doses of 1, 5, 25 and 50 mg/kg/day were used (for detail see Segment I study). Pregnant rats were given i.v. doses of 0 (vehicle: saline), 1, 6 and 30 mg/kg from day 17 of gestation to day 20 after parturition. All dams were observed daily for clinical signs and mortality, body weights were recorded daily during the gestation period and on days 0, 4, 7, 14 and 21 day of post partum. Food consumptions were recorded daily during the gestation period and between days 0-4, 4-7, 12-14, and 19-21 of parturition. All dams were allowed to litter normally and raise their pups to weaning. The number of live/dead pups were recorded, and the live pups were weighed and sexed. On day 4 after birth culling was carried out to make 8 offspring (4 males and 4 females). The offspring were reared by the dams until weaning. On day 21 of post partum all dams were sacrificed and necropsied, and examined externally and internally for abnormalities. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pinnae detachment, upper incisor eruption, eye opening, visual and auditory function, learning ability test, estrous cycle and

testes descent). At day 21 of post partum, 1 male and one female from each litter were selected for F1/F2 generation study. At 12 weeks of age they were continuously mated for 14 days. F_1 dams were weighed on days 0, 7, 14 and 20 of gestation. On day 20 of gestation F_1 dams were sacrificed. F_1 dams and F_2 fetuses were examined for abnormalities.

Results: Reduction in spontaneous movement, salivation and dark purplish-reddening at the injection sites were seen in high dose treated dams. Throughout gestation and lactation period, no significant effects were seen on body weight gains and food consumptions of F_0 dams. Length of gestation was comparable in all groups. No abnormalities were observed at autopsy of F_0 dams which would be attributed to treatment. No drug related effects were seen in the F_1 pups during postnatal period except weights of pups from high dose group was about 7-12% lower than that seen in pups of the control group and this effect was evident throughout the lactation period. Development and reproductive performance were comparable in all groups. No external abnormalities were observed in the F_2 fetuses except 1 out of 239 fetuses of high dose group had excencephaly and open eye lid.

The proposed clinical route of administration is oral therefore sponsor should have used oral route of administration and drug should have been given along with 1% NaHCO3 (for detail see the review of Segment I fertility and general reproductive performance study in rats). Overall study is acceptable. No adverse effect were seen in rats following i.v. administration of up to 30 mg/kg/day of E3810 during perinatal and postnatal period.

MUTAGENICITY:

Ames Test
(Study No. 897102)

Date of the study: June 19 to Oct. 4, 1989

Methods: E3810 (Lot no. 630824) dissolved in 0.05N NaOH was tested at concentrations of 62.5-1000 ug/plate in the absence of matabolic activation and 62.5-4000 ug/plate in the presence of metabolic activation. Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100 and E. coli strain WP2/uvrA were used. Assays were performed by the pre-incubation method. The number of revertant colonies on each plate was counted 48 hours later. Following five postivie controls were dissolved in DMSO: N-methyl-N'-nitro-N-nitrosoguanidine, 2-aminoanthracene, 9-aminoacridine hydrochloride, 3,4-benzopyrene and 2-nitrofluorene. The test article was considered mutagenic if the mean number of revertants of three plates per dose were more than twice the solvent control values among at least three consecutive dose levels or at the highest non-toxic dose level.

Results:

In the absence of metabolic activation: It induced a twofold or more increase in the number of revertant colonies at 125-500 ug/plate with TA1535 and at 375 ug/plate with TA 98. The results of regression analysis indicated linearity of the dose-response with both TA 1535 and TA 98.

In the presence of matabolic activation: It induced a two fold increase in the number of revertant colonies at 500 and 625 ug/plate in TA100. Regression analysis showed a dose-dependent increase in number of revertants with TA100. Positive controls increased a typical number of revertant colonies.

In conclusion, E3810 is mutagenic in the Ames test

Addendum: The testing laboratory is Department of Drug Safety Research, Eisai Co., Ltd, Gif-Ken, Japan.

Study # 930818AMS3736

Testing Laboratories:

Dates Studies Started and Completed: August 18, 1993 and August 20, 1993.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and E. coli WP2 UVrA.

Concentration Employed: 0, 46.875, 93.75, 187.5, 375, 750 and 1500 mcg/plate in the presence of S-9 mix and 0.25, 0.5, 1, 2 and 4 mcg/plate in the absence of S-9 mix.

Solvent Control: Dimethylsulfoxide (DMSO).

Positive Control: 2-aminoanthracene (0.625-10 mcg/plate).

Source of Metabolic Activation: Obtained from commercial source

Criteria of Positivity: Two fold increase in the number of revertant colonies above the solvent control value in strains TA 98, TA 100 and WP2 UVrA, or threefold increase in the number of revertant colonies above the solvent control value in strains TA 1535 and TA 1537 are considered positive provided if the effect is seen in at least two consecutive dose levels. When the above criteria are met for only one dose level, then determination of positive response will be made on scientific judgment.

Results: According to present sponsor (Eli Lilly and Co.), this assay was conducted in similar fashion as done by Eisai (see above). However, in this study sponsor used 46.875-1500 mcg/plate of LY307640 (314429) in the presence of S-9 mix. The highest concentration of the drug used in this study is much lower than that used in Eisai study. The rational for using low concentration levels was not provided. Additionally, positive control gave a weak response in TA 1535 and TA 100. The negative finding in this test with respect to strains TA 100 (which gave positive results in study # 897102) could be false negative. Sponsor did not provide any information about the system in which drug concentrations of 0.25-4.0 mcg/plate were used in the absence of S-9 mix.

Addendum: The final report was completed on February 17, 1994.

Study # 930914AMS3736 [redacted]

Testing Laboratories: [redacted]

Dates Studies Started and Completed: September 14, 1993 and September 16, 1993.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and E. coli WP2 UVrA.

Concentration Employed: 0, 62.5, 125, 250, 500, 750, 1000, 2000, 3000, 4000 and 5000 mcg/plate in the absence of S-9 mix.

Solvent Control: Dimethylsulfoxide (DMSO).

Positive Control: N-ethyl-N-nitro-N-nitrosoguanidine (1.5-10 mcg/plate), 9-aminoacridine (30-60 mcg/plate), and 2-nitrofluorene (0.25-5 mcg/plate).

Source of Metabolic Activation: No test was done in the presence of metabolic activation.

Criteria of Positivity: Same as above.

Results: The drug inhibited the growth of most of the strains at 1000 mcg/plate and higher dose levels. Drug was not mutagenic in any of the tester strains in the absence of metabolic activation. The negative finding in this test with respect to strains TA 1535 and TA 98 (which gave dose related positive results in study # 897102) could be false negative. Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control.

Addendum: The final report was completed on February 21, 1994.

Study # 930809AMS3736

Testing Laboratories:

Dates Studies Started and Completed: August 9, 1993 and August 11, 1993.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and E. coli WP2 UVrA.

Concentration Employed: 0, 31.25, 62.5, 125, 250 and 500 mcg/plate in the absence of S-9 mix and 281.25, 562.5, 1125, 2250 and 4500 mcg/plate in the presence of S-9 mix.

Solvent Control: Dimethylsulfoxide (DMSO).

Positive Control: N-ethyl-N-nitro-N-nitrosoguanidine (1.5-10 mcg/plate), 9-aminoacridine (30-60 mcg/plate), 2-nitrofluorene (0.25-5 mcg/plate) and 2-aminoanthracene (0.625-10 mcg/plate).

Source of Metabolic Activation: Obtained from commercial source

Criteria of Positivity: Same as above.

Results: In the absence of metabolic activation no significant increase in mutant colonies was noted in any strain. However, in the presence of metabolic activation more than 3-fold increse in revertant colonies was seen in strain TA 1535 at 4500 mcg/plate, and a 2-fold increse in revertant colonies was seen in strain TA 98 at 4500 mcg/plate. Hence LY307640 is mutagenic in Ames test.

Study # 930827AMS3736 [redacted]

Testing Laboratories: [redacted]

Dates Studies Started and Completed: August 27, 1993 and August 29, 1993.

Strain Employed: Salmonella typhimurium strains TA 98 and TA 1535.

Concentration Employed: 0, 2500, 3000, 3500, 4000 and 5000 mcg/plate in the presence of S-9 mix.

Solvent Control: Dimethylsulfoxide (DMSO).

Positive Control: 2-aminoanthracene (0.625-10 mcg/plate).

Source of Metabolic Activation: Obtained from commercial source

Criteria of Positivity: Same as mentioned above.

Results: To confirm the above findings in TA 1535 (point mutation) and in TA 98 (frame shift mutation) sponsor repeated part of the above test. In this test bacterial mutation was evaluated in the presence of metabolic activation in Salmonella typhimurium strains, TA 1535 and TA 98 using standard plate incorporation method. In the presence of metabolic activation increse in revertant colonies was not significant in strain TA 98 at 4500 mcg/plate (colony counts: mean control = 29.5 and test = 43 +/- 5), however, a 2.9-fold increse in revertant colonies was seen in strain TA 1535 at 4500 mcg/plate (colony counts: mean control = 10 and test = 29 +/- 7). This repeat test also indicated that LY307640 is mutagenic in Ames test.

Ames Test
(931214AMS3755)

Testing Laboratories: [redacted]

Dates Studies Started and Completed: December 6, 1993 and May 24, 1994

Drug Batch No.: 93092702

Methods: Ames test was conducted to assess the mutagenic potential of the metabolite of E 3810 (demethylated E 3810) by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium [TA 98 and TA 1537 (frame shift); TA 100 and TA 1535 (base pair substitution)] and E. coli WP2 UVrA- in the presence and absence of S9 activation (Arocol 1254-induced rate liver microsomal enzyme mixture). The method used is pre-incubation method. Vehicle (DMSO: dimethyl sulfoxide), demethylated E 3810 (62.50 - 5000 mcg/plate) and positive controls [9-aminoacridine (30 and 60 mcg/plate), 2-aminoanthracene (0.625-10 mcg/plate), N-ethyl-N-nitro-N-nitrosoguanidine (1.5-10 mcg/plate), 2-nitrofluorene (0.25-5.0 mcg/plate)] were plated in triplicate with tester strains TA 98, TA 100, TA 1535 and TA 1537 and TA 1538, and E. coli WP2 UVrA- in the presence and absence of S9 mix and incubated for 48 hour. Revertant colonies were counted.

Criteria of Positivity: A two fold increase in the number of revertant colonies above the solvent control value in strains TA 98 and TA 100, and E. coli WP2 UVrA- or threefold increase in the number of revertant colonies above the solvent control value in strains TA 1535 and TA 1537 are considered positive provided if the effect is seen in at least two consecutive dose levels.

Results: The demethylated LY307640 (demethylated E 3810 = compound 317237) was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

Ames Test
(931221AMS3755 and 940111AMS3755)

Testing Laboratories: [redacted]

Dates Studies Started and Completed: December 15, 1993 and May 24, 1994

Drug Batch No.: 93092702

Methods: Ames test was conducted to assess the mutagenic potential of the metabolite of E 3810 (demethylated E 3810) by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium [TA 98 and TA 1537 (frame shift); TA 100 and TA 1535 (base pair

substitution)] and E. coli WP2 UVrA- in the presence and absence of S9 activation (Arocol 1254-induced rate liver microsomal enzyme mixture). The method used is plate incorporation method. Vehicle (DMSO: dimethyl sulfoxide), demethylated E 3810 (28.125-450 mcg/plate in the absence of S-9 mix and 187.5-3000 mcg/plate in the presence of S-9 mix) and positive controls [9-aminoacridine (30 and 60 mcg/plate), 2-aminoanthracene (0.625-10 mcg/plate), N-ethyl-N-nitro-N-nitrosoguanidine (1.5-10 mcg/plate), 2-nitrofluorene (0.25-5.0 mcg/plate)] were plated in triplicate with tester strains TA 98, TA 100, TA 1535 and TA 1537 and TA 1538, and E. coli WP2 UVrA- in the presence and absence of S9 mix and incubated for 48 hour. Revertant colonies were counted.

Criteria of Positivity: A two fold increase in the number of revertant colonies above the solvent control value in strains TA 98 and TA 100, and E. coli WP2 UVrA- or threefold increase in the number of revertant colonies above the solvent control value in strains TA 1535 and TA 1537 are considered positive provided if the effect is seen in at least two consecutive dose levels.

Results: The demethylated [redacted]307640 (demethylated E 3810 = compound 317237) was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

In the above test, the spontaneous revertant colonies for the tester strains TA 98 (in the absence of S-9 mix) and TA 100 (in the presence and in the absence of S-9 mix) in DMSO treated plates were outside the normal historical values. Therefore, sponsor repeated the above test (study # 9340111AMS3755) using 28.125-3600 mcg/plate of demethylated E 3810 in the absence of S-9 mix. The repeat test in the presence of S-9 mix was conducted only in strain TA 100 and the concentrations of demethylated E 3810 used were 187.5-3000 mcg/plate. The results were negative, i.e. demethylated [redacted]3076401 (demethylated E 3810 = compound 317237) was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix).

Ames Test on Main Metabolites of E3810
(Study # 897105)

In this test, mutagenic potential of 5 main metabolites (thioether-E3810, sulfone-E3810, demethyllated-E3810, demethylated thioether-E3810 and carboxylic acid-E3810) were assessed using preincubation method.

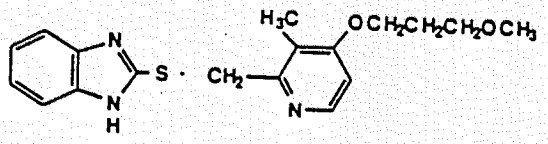
Testing Laboratories: Department of Drug Safety Research, Eisai Co., Ltd., Gifu, Japan.

Dates Studies Started and Completed: August 17, 1989 and March 3, 1993.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and E. coli WP2 UVrA.

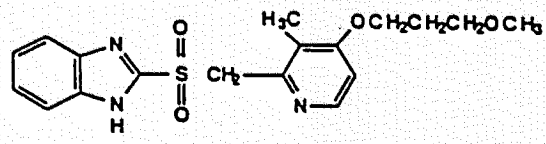
Metabolites Tested and Lot No.:

thioether-E3810 (lot # T87793-43)



Molecular Formula: $C_{18}H_{21}N_3O_2S$
Molecular Weight: 343.45

sulfone-E3810 (lot# T87793-37-A)



Molecular Formula: $C_{18}H_{21}N_3O_4S$
Molecular Weight: 375.45