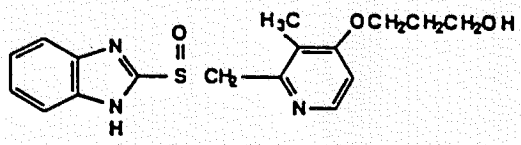
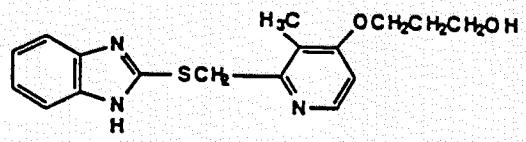
demethyllated-E3810 (lot# T87793-58 and T89497-8)



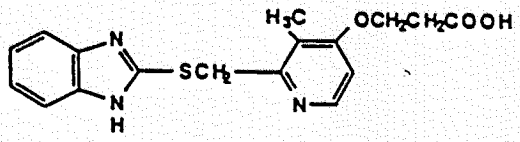
Molecular Formula: $C_{17}H_{18}N_3O_3S$
Molecular Weight: 345.42

demethylated thioether-E3810 (lot# T87793-42-A)



Molecular Formula: $C_{17}H_{19}N_3O_2S$
Molecular Weight: 329.42

carboxylic acid-E3810 (lot# T88090-43-1 and T88090-50)



Molecular Formula: $C_{17}H_{17}N_3O_3S$
Molecular Weight: 343.40

Concentration Employed: 0, 62.5, 125, 250, 500, 1000 and 2000 mcg/plate.

Solvent Control: Dimethylsulfoxide (DMSO).

Positive Control: N-ethyl-N-nitro-N-nitrosoguanidine (2-5 mcg/plate), 2-aminoanthracene (2-20 mcg/plate), 9-aminoacridine (80 mcg/plate), 3,4-benzo[a]pyrene (5 mcg/plate) and 2-nitrofluorene (1 mcg/plate).

Source of Metabolic Activation: Rate liver microsomal enzymes

Criteria of Positivity: More than two fold increase in the number of revertant colonies above the solvent control value are considered positive provided if the effect is also seen in at least three consecutive dose levels. The requirement of positive results in three consecutive dose levels is not proper. In the 4 Amest tests conducted by sponsor, positive results in two consecutive dose levels were sufficient to conclude mutagenic potential of the compound (see above).

Results: Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 mix). Thioether-E3810, sulfone-E3810 and demethylated thioether-E3810 were not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). However, in the absence of S-9 mix, demethylated-E3810 (both lot #s) induced more than 2 fold increase in revertant colonies in strain TA 1535 at 250 and 500 mcg/plate. Additionally it also induced more than 2 fold increase in revertant colonies in strain TA 98 at 500 mcg/plate.

Demethyl-E3810 (lot# T87793-58) in the Absence of S-9 Mix

Dose (mcg/plate)	TA 1535 rev. col/plate	TA 98 rev. col/plate
0	7.0	17.7
62.5	6.7	18.3
125.0	12.3	18.3
250.0	14.7	21.7
500.0	18.3	37.7

Demethyl-E3810 (lot# T89497-8) in the Absence of S-9 Mix

Dose (mcg/plate)	TA 1535 rev. col/plate	TA 98 rev. col/plate
0	6.7	16.3
62.5	7.7	17.3
125.0	9.3	23.0
250.0	14.0	29.7
500.0	25.3	33.7

In the presence of S-9 mix, demethylated-E3810 (lot # T87793-58) induced more than 2 fold increase in revertant colonies in strain TA 98 at 2000 mcg/plate.

Demethyl-E3810 (lot# T87793-58) in the Presence of S-9 Mix

Dose (mcg/plate)	TA 98 rev. col/plate
0	17.7
62.5	17.0
125.0	15.7
250.0	22.3
500.0	25.7
1000.0	26.7
2000.0	39.0 (crystalization)

The metabolite carboxylic acid-E3810 (lot # T88090-50) did not induce more than 2 fold increase in revertant colonies in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix), but carboxylic acid-E3810 from lot # T88090-43-1) did induce more than 2 fold increase in revertant colonies in strain TA 1535 at 1000 and 2000 mcg/plate in the absence of S-9 Mix.

Carboxylic Acid-E3810 (lot# T89497-8) in the Absence of S-9 Mix

Dose (mcg/plate)	TA 1535 rev. col/plate
0	7.0
62.5	7.3
125.0	5.3
250.0	9.0
500.0	7.0
1000.0	15.0
2000.0	24.0

The data indicated that 2 out of five main metabolits of E 3810, namely demethylated-E3810 and carboxylic acid-E3810 are positive in Ames test.

Addendum: In this Ames test, carboxylic acid-E3810 (lot #T88090-43-1) also induced more than 2 fold increase in revertant colonies in strain TA100 at 2000 µg/ml in the absence of S-9. This information is summarized in the following table.

Dose µg/ml	Revertant Colonies/Plate in TA100
0	80.7
62.5	72.3
125	93.0
250	82.7
500	96.3
1000	130.0
2000	175.0

Ames Test with Carboxylic Acid Metabolite of E3810 (897503)

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

Study Start and Completion Dates: September 11, 1989 and October 2, 1989

GLP and QAU Compliance Statement: Sponsor stated that this study was a non-GLP study.

Methods: To confirm the positive findings with carboxylic acid-E3810 in the previous Ames test (8897105), the potential mutagenic effects of carboxylic acid metabolite of E3810 (lot # T88090-50 and T88090-43-2) were assessed in the reverse mutation assay (Ames test) using the pre-incubation method in 2 strains of salmonella typhimurium (TA1535 and TA100) in the absence of metabolic activation, S-9 mix from rat liver. The following concentrations of carboxylic acid-E3810 were tested: 250, 500, 1000, 1500 and 2000 µg/plate with and without S-9. The positive control (N-ethyl-N'-nitro-N-nitrosoguanidine) was also tested. The result should be considered positive if the test substance induced a dose-dependent increase (more than two folds) in revertant colonies compared to the control.

Results: The results indicated that carboxylic acid-E3810 (lot #T88090-43-2) dose-dependently increased the colonies but this increase was not seen using the lot # T88090-50. The carboxylic acid-E3810 (lot #T88090-50) was purified without reverse-phase column chromatography and carboxylic acid-E3810 (lot #T88090-43-2) with reverse-phase column chromatography. The flushed materials of the column used in the reverse-phase column chromatography also produced a dose-related increase in the colonies in both strains, suggesting that the positive response of carboxylic acid-E3810 (lot #T88090-43-2) may result from contaminants of the purifying column. Sponsor, however, did not identify the possible contaminants from the purifying column. The positive controls also significantly induced increase in the colonies compared to the solvent controls.

In conclusion, the results suggest that the positive response of carboxylic acid-E3810 (lot #T88090-43-2) may result from unidentified contaminants of the purifying column.

Ames Test with E3810 Degradation Product (CEBI)
(S98607)

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

Study Start and Completion Dates: April 6, 1998 and
April 22, 1998

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

Methods: The potential mutagenic effects of E3810 degradation product CEBI were assessed in the reverse mutation assay (Ames test) using the pre-incubation method in 4 strains of salmonella typhimurium (TA98, TA100, TA1535 and TA1537) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations were tested: 313, 625, 1250, 2500 and 5000 μg/plate with and without S-9. The positive controls (sodium azide, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 9-aminoacridine hydrochloride and 2-aminoanthracene) were also tested. The result should be considered positive if the test substance induced a dose-dependent increase (more than two folds) in revertant colonies compared to the control.

Results: The results indicated that E3810 degradation product CEBI did not significantly increase the colonies. The positive controls, however, significantly induced increase in the colonies compared to the solvent controls.

In conclusion, the results suggest that E3810 degradation product CEBI was not mutagenic in this system.

Ames Test with E3810 Degradation Product (BCPP)
(S98611)

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

Study Start and Completion Dates: May 18, 1998 and
July 25, 1998

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

Methods: The potential mutagenic effects of E3810 degradation product BCPP were assessed in the reverse mutation assay (Ames test) using the pre-incubation method in 4 strains of salmonella typhimurium (TA98, TA100, TA1535 and TA1537) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations were tested: 78, 156, 313, 625, 1250, 2500 and 5000 μg/plate with and without S-9. The positive controls (sodium azide, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 9-aminoacridine hydrochloride and 2-aminoanthracene) were also tested. The result should be considered positive if the test substance induced a dose-dependent increase (more than two folds) in revertant colonies compared to the control.

Results: The results indicated that E3810 degradation product BCPP did not significantly increase the colonies. The positive controls, however, significantly induced increase in the colonies compared to the solvent controls.

In conclusion, the results suggest that E3810 degradation product BCPP was not mutagenic in this system.

Chromosome Aberration Study in CHL/IU Cells
(Study No. 897402)

Date of the study: Aug. 1 to Oct. 5, 1989.

Methods: E3810 (Lot no. 89010911) was tested at concentrations of 0.0075-0.05 mg/ml in the direct method and 0.0125-0.07 mg/ml in S-9mix method. CHL/IU cells derived from the lung of a female Chinese hamster were used. The cells were harvested after 48 hour incubation with the test articles. In S-9 mix method, the cell were treated with E3810 and S-9 mix for 6 hours and harvested after 21 hours incubation with fresh cell medium. Two hours before harvesting, demecoline was added to each dish. Physiological saline was used as the negative control. N-methyl-N'-nitro-N-nitrosoguanidine and 9,10-dimethyl-1,2-benzanthracene were used as the positive controls. Each 100 metaphase spread was examined for structural or numerical chromosome aberrations.

Results: E3810 at any dose did not increase the incidence of aberrant cells or polyploid cells in both methods. Positive controls increased the incidence of aberrant cell in this study.

Micronucleus Test in Mice
(Study No. 897223)

Date of the study: Aug. 16 to Oct. 5, 1989.

Methods: Male CD1 mice aged of 4 weeks were used. Three groups of animals each consisting of 10 mice were given E3810 at i. p. dose level of 0, 50 and 100 mg/kg and were sacrificed at 24 and 48 hours later dosing. Fourth group consisting of 25 mice were given single i.p. dose of 200 mg/kg and were sacrificed 18, 24, 30, 48 and 70 hours after dosing for perparation of speciemen. The fifth and sixth groups each consisting of 5 mice were given four daily i.p. dose level of 50 and 100 mg/kg/day and were sacrificed 24 hour later. The positive control group were given mitomycin C (MMC, 3 mg/kg, i.p) and sacrificed 30 hour later for preparation of speciemens.

Results: All animals of 200 mg/kg showed hypoactivity and convulsion after dosing. There was no statistically significant difference between control and E3810 groups for the frequency of polychromatic erythrocytes. MMC decreased the frequency of polychromatic erythrocytes significantly. There was no statistically significant defference between the contro and E3810 groups for the incidnece of micronucleated erythrocytes. MMC caused a typical increase in the incidence of micronucleated erythrocytes.

In conclusion, E3810 following singel or mutiple treatment, caused no statistically increases in the incidence of micronucleated erythrocytes.

4. UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF RAT HEPATOCYTES (studies #930805UDS3736 and #930810UDS3736).

Testing Laboratories: [redacted]

Dates Studies Started and Completed: August 5, 1993 and August 16, 1993 for range finding study (# 930805UDS3736). August 10, 1993 and August 20, 1993 for the main study (#930810UDS3736).

Concentration Employed: 0.5, 1, 5, 10, 50, 100, 500 and 1000 mcg/ml in range finding study and 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 mcg/ml in the main study.

Drug Batch No.: 12070203

Solvent Control: DMSO or sterile water

Positive Control: N-Methyl-N-nitro-N-nitrosoguanidine (MNNG: 1-20 mcg/ml) and 2-acetylaminofluorene (2AAF: 0.05-1 mcg/ml).

Methods: Hepatocyte primary cultures from the liver of adult male F344 rats were used for this study. Net increase in nuclear grains induced by each compound were determined by autoradiographic method. The test compound is considered positive when there is a linear dose response and the results are significant (p< or equal to 0.01).

Results: [redacted]307640 was cytotoxic at dose level of 5 mcg/ml. At non-cytotoxic concentrations, LY307640 did not induced DNA repair synthesis in cultured rat hepatocytes. The positive control was genotoxic. The results suggest that LY307640 was not genotoxic in the rat hepatocyte primary culture/DNA repair test at concentration up to 1 mcg/ml.

5. CHO/HGPRT MAMMALIAN CELL-FORWARD GENE MUTATION ASSAY (study # 930811CHO3736).

Testing Laboratories: [redacted]

Dates Studies Started and Completed: August 11, 1993 and August 31, 1993.

Strain Employed: CHO-K1-BH4 (Chinese hamster ovary cell line)

Concentration Employed: 25-110 mcg/ml in the absence of S-9 Mix and 10-50 mcg/ml in the presence of S-9 Mix.

Solvent Control: Sterile water

Positive Control: 3-Methylcholanthrene (3MC: 3 mcg/ml) and ethyl methane sulfonate (EMS: 310 mcg/ml).

Source of Metabolic Activation: Obtained from commercial source.

Criteria of Genotoxic Effect: Mutation frequency in the exposed culture must be at least 2-fold higher than the mean mutation frequency of the negative control cultures. This response must be

noted at two or more successive concentrations, and cultures must exceed 5% total survival with at least one culture showing > 10% total survival.

Drug Batch No.: 12070203

Results: In the absence of S9 mix significant increases in the mutation frequency were observed at 90, 100 and 110 mcg/ml (mutation frequency per million colony forming cells: negative control = 2.3, 90 mcg/ml = 7.8, 100 mcg/ml = 6.7 and 110 mcg/ml = 6.1) (sponsor's table 7, page 17978 of Amend. # 051). Mutation frequencies in [redacted]307640 treated cultures in the presence of S-9 Mix did not meet the criteria of positivity. Significant increases in the mutation frequencies of the positive test control cultures were observed. Thus [redacted]307640 is mutagenic In CHO/HGPRT forward gene mutation assay .

(study # 930915CHO3736): Sponsor repeated part of the above test (in the absence of S-9 mix). Duplicate experiments were conducted with unspecified "procedural changes". The concentrations of the drug used were 50-130 mcg/ml, and ethyl methane sulfonate (EMS: 230 mcg/ml) was used as positive control. One of the experiment was negative, but in the second experiment increases in the mutation frequency were observed at 50, 70, 100, 120 and 130 mcg/ml (mutation frequency per million colony forming cells: negative control = 2.4, 50 mcg/ml = 7.8, 70 mcg/ml = 5.6, 100 mcg/ml = 5.6, 120 mcg/ml = 9.3 and 130 mcg/ml = 5.6) (sponsor's table 7, page 17979 of Amend. # 051). Significant increases in the mutation frequencies of the positive test control cultures were observed. Thus LY307640 is mutagenic In CHO/HGPRT forward gene mutation assay.

Table 7. A Summary of Results for the Chinese Hamster Ovary Forward Mutation Assay with Compound 314429. Study 930811CH03736.

Treatment	Conc. (µg/ml)	Percent Cloning Eff.[a]	Percent Total Survival[b]	Mutation Frequency[c]		Mutation Index[d]
Nonactivated Test						
WATER[e]	(1%)	100	100	1.2		
	(1%)	100	100	2.2	2.3[g]	(1.0)
	(1%)	100	100	3.6		
314429	25	83	82	2.5		1.1
	50	82	85	3.8		1.7
	60	94	98	3.4		1.5
	70	92	60	3.4		1.5
	80	100	56	4.2		1.8
	90	67	24	7.8		3.4[i]
	100	94	23	6.7		2.9[i]
	110	86	21	6.1		2.7[i]
EMS[f]	310	66	4	454.0		197.4
Activated Test						
WATER[e]	(1%)	100	100	4.9		
	(1%)	100	100	4.3	4.9[g]	(1.0)
	(1%)	100	100	5.4		
314429	10	112	114	2.2		0.4
	20	118	111	3.1		0.6
	25	96	99	0.0		0.0
	30	120	113	1.0		0.2
	35	101	97	9.5		1.9
	40	84	9	7.1		1.4
	45[h]	-	-	-		-
	50[h]	-	-	-		-
3MC[f]	3	96	106	171.3		35.0

[a]Relative cloning efficiency of treated cultures; solvent RCE = 100%.
[b](% suspension growth) x (% relative cloning efficiency).
[c]HGPRT$^-$ mutants per 1 x 10^6 colony forming cells.
[d]Mutation frequency of treated culture+mutation frequency of vehicle control.
[e]Sterile water served as the vehicle control.
[f]Ethylmethanesulfonate (EMS) and 3-methylcholanthrene (3MC) served as positive controls.
[g]Mean of vehicle controls.
[h]Culture not plated since suspension growth <5%.
[i]Induced mutation is indicated when the mutation index is $\geq$ 2-fold that of the vehicle control at 2 or more successive concentrations.

Table 8. A Summary of Results for the Chinese Hamster Ovary Forward Mutation Assay with Compound 314429. Study 930915CH03736.

Treatment	Conc. (µg/ml)	Percent Cloning Eff.[a]	Percent Total Survival[b]	Mutation Frequency[c]		Mutation Index[d]
Nonactivated Test						
WATER[e]	(1%)	100	100	3.6		
	(1%)	100	100	10.0	6.2[g]	(1.0)
	(1%)	100	100	5.1		
314429	50	86	102	5.4		0.9
	70	102	75	9.1		1.5
	80	136	79	4.3		0.7
	90	117	47	2.0		0.3
	100	80	34	7.2		1.2
	110	123	44	1.9		0.3
	120	127	30	4.6		0.7
	130	130	13	5.4		0.9
EMS[f]	230	77	33	301.5		48.6
Nonactivated Test						
WATER[e]	(1%)	100	100	5.3		
	(1%)	100	100	1.2	2.4[g]	(1.0)
	(1%)	100	100	0.8		
314429	50	103	95	7.8		3.3
	60	80	71	3.8		1.6
	70	91	76	5.6		2.3
	90	79	62	3.8		1.6
	100	90	65	5.6		2.3
	110	86	37	0.0		0.0
	120	87	44	9.3		3.9[h]
	130	73	18	5.6		2.3[h]
EMS[f]	230	105	42	237.5		99.0

[a]Relative cloning efficiency of treated cultures; solvent RCE = 100%.
[b](% suspension growth) x (% relative cloning efficiency).
[c]HGPRT$^-$ mutants per 1 x 10^6 colony forming cells.
[d]Mutation frequency of treated culture+mutation frequency of vehicle control.
[e]Sterile water served as the vehicle control.
[f]Ethylmethanesulfonate (EMS) served as the positive control.
[g]Mean of vehicle controls.
[h]Induced mutation is indicated when the mutation index is $\geq$ 2-fold that of the vehicle control at 2 or more successive concentrations.

6. IN VITRO GENE FORWARD MUTATION ASSAY AT TK LOCUS IN L5178Y MOUSE LYMPHOMA CELLS (study # 930921MLA3736)

Testing Laboratories: [redacted]

Dates Studies Started and Completed: September 21, 1993 and October 4, 1993.

Strain Employed: L5178Y $TK^{+/-}$ mouse lymphoma cells.

Concentration Employed: 10-100 mcg/ml in the absence of S9-mix and 5-40 mcg/ml in the presence of S9-mix.

Solvent Control: Sterile water

Positive Control: 3-Methylcholanthrene (3MC: 2 mcg/ml) and ethyl methane sulfonate (EMS: 620 mcg/ml).

Source of Metabolic Activation: Obtained from commercial source.

Criteria of Genotoxic Effect: Mutation frequency in the exposed culture must be at least 2-fold higher than the mean mutation frequency of the negative control cultures. This response must be noted at two or more successive concentrations, and only cultures showing 20% total survival will be evaluated.

Drug Batch No.: 12070203

Methods: Cells were incubated with appropriate test article, vehicle control or positive control for 4 hours, then cells were washed, resuspended in media and incubated for additional 48 hours. At the end of 48 hr of incubation viability rates and mutation frequency were determined.

Results: In the absence of metabolic activation, mutation frequency was increased by 2.1 fold at 80 mcg/ml. Even though the result was significant but it did not satisfy the second criteria of positivity i.e. this response must be noted at two or more successive concentrations. The higher dose levels (90 and 100 mcg/ml) was cytotoxic in the absence of S-9 mix.

In the presence of metabolic activation (S-9 mix), dose related increase in mutant colonies was seen. In the presence of S-9 mix, mutation frequencies were increased by 2.1 and 3.1 fold at 25 and 30 mcg/ml respectively, and meets the criteria of

positivity (sponsor's table 9, page 17980 of Amend. # 051). In the presence of S-9 mix concentrations 32.5-40 mcg/ml were cytotoxic. Thus the drug is mutagenic at the tk locus of L5178Y mouse lymphoma cells. Sponsor is repeating this assay and results will be submitted at future date.

Table 9. A Summary of Results for the Mouse Lymphoma Forward Mutation Assay with Compound 314429. Study 930921HLA3736.

Treatment	Conc. (ug/ml)	Percent Cloning Eff.[a]	Percent Total Survival[b]	Mutation Frequency[c]		Mutation Index[d]
Nonactivated Test						
WATER[e]	(1%)	100	100	2.1		
	(1%)	100	100	2.1	2.1[g]	(1.0)
	(1%)	100	100	2.1		
314429	10	104	79	2.4		1.1
	20	94	86	2.1		1.0
	30	125	78	2.2		1.0
	40	112	73	2.8		1.3
	50	93	47	3.6		1.7
	60	113	49	2.6		1.2
	70	111	31	3.3		1.6
	80	96	29	4.5		2.1
	90[h]	-	-	-		-
	100[h]	-	-	-		-
EMS[f]	620	58	32	65.6		31.2
Activated Test						
WATER[e]	(1%)	100	100	2.4		
	(1%)	100	100	2.0	2.5[g]	(1.0)
	(1%)	100	100	3.0		
314429	5	96	65	2.8		1.1
	10	87	61	2.0		0.8
	15	94	43	2.4		1.0
	20	87	36	2.9		1.2
	25	69	26	5.2		2.1[i]
	30	80	22	7.7		3.1[i]
	32.5[h]	-	-	-		-
	35[h]	-	-	-		-
	37.5[h]	-	-	-		-
	40[h]	-	-	-		-
3MC[f]	2	97	55	18.2		7.3

[a] Relative cloning efficiency of treated cultures; solvent RCE = 100%.
[b] (% Suspension growth) x (% relative cloning efficiency).
[c] $TK^{-/-}$ mutants per 1 x 10^6 colony forming cells.
[d] Mutation frequency of treated culture-mutation frequency of solvent control.
[e] Sterile water served as the vehicle control.
[f] Ethylmethanesulfonate (EMS) and 3-methylcholanthrene (3MC) served as positive controls.
[g] Mean of vehicle controls.
[h] Culture not plated since suspension growth <20%.
[i] Induced mutation is indicated when the mutation index is ≥ 2-fold that of the vehicle control at 2 or more successive concentrations.

Unscheduled DNA Synthesis in Rat Liver Using an In Ex-Vivo Procedure (Study # 16278-0-494)

Testing Laboratories: [redacted]

Date of Study Started: June 13, 1994

Date of Study Completed: November 14, 1994

Animals: Adult Fisher 344 male rats (199.9-238.9 g)

Doses Used: 250, 500, 750 and 1000 mg/kg

Drug Batch No.: 13051304

Solvent Control: Sodium bicarbonate buffer (0.05 M, pH 10.0).

Positive Control: Dimethylnitrosamine (DMN: 10 mg/kg, 15 mg/kg)

Methods: In the preliminary study, groups of 3 male rats were given a single dose of LY307640 (E 3810) via gavage at 0 (vehicle: 0.05 M sodium bicarbonate buffer, pH 10.0), 500, 1000, 1250, 1500 and 1750 mg/kg (5 ml/kg). All animals were observed for mortality for 3 consecutive days. One out of 3 rats each from 1250 and 1750 mg/kg dose groups died within 8 hr after drug administration. All treated rats, except from low dose group, showed signs of ataxia for up to 8 hr after drug administration. Based on these findings a dose of 1000 mg/kg was chosen as the highest dose for the main study and the remaining doses were 750, 500 and 250 mg/kg. In the main study, groups of 3-4 male rats were given a single dose of LY307640 (E 3810) via gavage at 0 (vehicle: 0.05 M sodium bicarbonate buffer, pH 10.0), 250, 500, 750, and 1000 mg/kg (5 ml/kg). The positive control group animals received DMN (i.p.: 10 mg/kg or 15 mg/kg). Groups of animals were sacrificed at 2-4 hours after drug administration, and another groups were sacrificed at 15-16 hours after the drug administration. Hepatocyte primary cultures from the liver of the rats were obtained, and [^{3}H] thymidine incorporation was measured. Incorporation was followed by autoradiography of the hepatocytes and grains were counted in 150 nuclei other than S-phase. Net increases in nuclear grains induced by each compound were determined. The test compound is considered positive when the mean nuclear grain count is 5 grains per nucleus above the concurrent negative control average leading to a positive number and/or the percent of nuclei with 5 or more net grains to increase at least 10% above the average of the concurrent negative control animals.

Results: No net increase in nuclear grain counts was observed in hepatocytes, when rats were treated with LY307640 at oral (gavage) doses up to 1000 mg/kg at 2-4 hr time point or up to 750 mg/kg (1000 mg/kg was cytotoxic) at 15-16 hr time point. The positive control was genotoxic, while no repair was induced by vehicle (negative control). Thus, results suggest that LY307640 was not genotoxic in this test.

In Vitro Gene Forward Mutation Assay at tk Locus in L5178Y Mouse Lymphoma Cells
(Study # 931012MLA3736)

Testing Laboratories: [redacted]

Dates Study Started and Completed: October 12, 1993 and October 25, 1993.

Strain Employed: L5178Y $TK^{+/-}$ mouse lymphoma cells.

Concentration Employed: 10-100 mcg/ml in the absence of S9-mix and 5-40 mcg/ml in the presence of S9-mix.

Solvent Control: Sterile water

Positive Control: 3-Methylcholanthrene (3MC: 2 mcg/ml and ethyl methane sulfonate (EMS: 620 mcg/ml).

Source of Metabolic Activation: Obtained from commercial source.

Drug Batch No.: 12070203

Criteria of Genotoxic Effect: Mutation frequency in the exposed culture must be at least 2-fold higher than the mean mutation frequency of the negative control. This response must be noted in at two or more successive concentrations, and only cultures showing at least 20% total survival will be evaluated.

Methods: Cells were incubated with various concentrations of test article, vehicle control or positive control for 4 hr, then cells were washed, resuspended in media and incubated for additional 48 hr. At the end of 48 hr of incubation viability rates and mutation frequencies were determined.