

Summary of individual study findings: Fischer 344 rats (20/sex/dose) were given atomoxetine hydrochloride for 1 year in the diet at concentrations of 0, 0.01, 0.03, or 0.1%. These concentrations provided time-weighted average doses of approximately 0, 5, 14, and 46 mg/kg/day to males and 0, 6, 17, and 56 mg/kg/day to females. There was one unscheduled death at LD in males after 330 days with postmortem examination revealing chronic respiratory disease. Matting of the hair in females and chromodacryorrhea in both males and females were the major clinical signs observed. Body wt, body wt gain, and food consumption were reduced in HD and MD groups in males and in all treatment groups in females. Some hematological and clinical chemistry changes were observed. Changes in the liver were evident (increased liver wt relative to body wt, mottling and pallor of the liver were observed grossly at necropsy, and increased incidence and severity of focal vacuolization of hepatocytes in males). Hepatic enzyme induction (as measured by the p-nitroanisole-O-demethylase assay) was slightly elevated in MD and HD females. A slight increase in the incidence of gross and microscopic distention of the uterus.

Study title: a one-year chronic toxicity study of tomoxetine (LY139603) administered orally to beagle dogs

Key study findings: mydriasis and tremors were reported in addition to less frequent sings such as anorexia, and emesis. No effect on body wt or food consumption. Slight increases in the absolute and relative liver wt. Some incidences of cysts in the pituitary in treated animals and not in the control.

Study no: D04382

Volume #, and page #: vol. 41, toxrpt 17, page 1

Conducting laboratory and location: Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Date of study initiation: July 1, 1982

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity: lot#866-83F-248, purity 98.3% according to assay (appendix C) in abstract the sponsor indicated that the purity was 99.97%

Formulation/Vehicle: capsules

Methods (unique aspects): none

Dosing:

Species/strain: beagle dogs

#/sex/group or time point (main study): 4/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: 9-10 months

Weight: M 8.3 ± 0.24 kg

F 7.3 ± 0.16 kg

Doses in administered units: 0, 4, 8, and 16 mg/kg/day

Route, form: oral, capsules

Observations and times:

Clinical signs: physical examinations were performed prior to study initiation, at six months and at termination.

Body weights: before study initiation and weekly during treatment

Food consumption: food consumption was "estimated visually" each day and changes in appetite were noted.

Ophthalmoscopy: ophthalmoscopic examinations were performed prior to study initiation, at three, six, and nine months and at termination.

EKG: not performed

Hematology: blood samples were collected from the jugular vein prior to the start of the study, at one, two, and four weeks, and monthly thereafter. The following parameters were examined: packed cell volume, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte count and morphology, total and differential leukocyte counts, activated partial thromboplastin time, and thrombocyte count. A cytologic evaluation of bone marrow smears including an estimated M:E ratio was performed at termination of the study.

Clinical Chemistry: blood collection was similar to that for hematology. The following parameters were examined: glucose, urea nitrogen, creatinine, total bilirubin, and activities of the enzymes alkaline phosphatase, alanine transaminase, creatinine phosphokinase, lactate dehydrogenase, and aspartate transaminase.

Urinalysis: urine samples were collected prior to treatment initiation, at one, two, and four weeks, and monthly thereafter. The following parameters were examined: specific gravity, glucose content, pH, protein content, occult blood content, color, and clarity.

Gross pathology: gross examination of each animal's general physical condition, body orifices, external and internal organs and tissues at necropsy.

Organs weighed: the weights of the following organs were recorded at necropsy: brain, liver, kidneys, heart, thyroids, adrenals, and testes or ovaries. Organ to body weight (and brain) ratios were calculated.

Histopathology: the following tissues from all animals were collected and examined microscopically by a certified pathologist: kidney, liver, heart, lung, spleen, thymus, lymph node, salivary gland, pancreas, stomach, duodenum, jejunum, ileum, colon, ovary, uterus, adrenal, thyroid (with parathyroid), gallbladder, bone, bone marrow, eye, cerebrum, cerebellum, brain stem, pituitary, gross lesions, trachea, esophagus, and aorta.

Toxicokinetics: not performed.

Results:

Mortality: one male dog from HD group died during the tenth month of the study due to strangulation according to the sponsor.

Clinical signs: mydriasis was seen in all animals at all doses (according to sponsor these signs were observed at 1-h post dosing and continued throughout the day). At LD this was seen mostly during the first week but at the MD and HD the occurrence was seen beyond the first week and sometimes for several weeks. Tremors were

observed in 3 out of 4 M and 1 out of 4 F at the HD. The occurrence of these tremors was seen during several weeks of treatment (up to week 40 of the study). In addition to these two signs, anorexia, emesis, melena, and mucoid stool were observed mainly at MD and HD but not in all animals.

Body weights: no drug effect was seen on body wt. F at HD tended to have lower body wt from control (14% less compared to control) even before treatment.

Food consumption: no data were presented however, according to the sponsor, "food consumption for all dogs was normal during the study".

Ophthalmoscopy: no data were presented but according to the sponsor no treatment-related abnormalities were observed.

Electrocardiography: not performed.

Hematology: some sporadic changes were reported but these were not dose-related and were not seen consistently.

Clinical chemistry: some increases and decreases were seen however, these did not indicate a drug effect since they were sporadic, non dose-related and interchangeable (one week an increase was observed and the next week a decrease, especially in enzyme activities).

Urinalysis: no significant changes were observed.

Organ weights: a slight increase (16%) in the absolute wt of the liver in M at HD, which was also seen in the wt/body wt ratio (15%), however, only the increase in the ratio (16%) was seen in F. These changes were not statistically significant according to the sponsor's statistical analysis. An increase (28%) in the ratio of wt of the heart to body wt was seen in F at HD.

Gross pathology: grossly one M at the LD had a clear cyst in the pituitary.

Histopathology: cysts in the pituitary were observed in the treated groups and not in the control. At the LD, 1 out of 4 M had a large cyst in pars distalis of the pituitary (this was grossly observed) and one animal at HD had multiple large cysts. In F, one of the animals at LD and one at MD had few small cysts in pars distalis of the pituitary. One male at the HD had epithelial hyperplasia with squamous metaplasia in the thymus.

Summary of individual study findings: 4 dogs/sex/group were treated with tomoxetine orally by capsule for one year at doses of 0, 4, 8, and 16 mg/kg/day. One M dog at HD died due to strangulation. The most important physical signs that were observed were mydriasis and tremors. Other less frequent signs included anorexia, emesis, melena, and mucoid stool mainly at MD and HD. No drug effects on body wt or food consumption. No significant changes in hematology or blood chemistry. Slight increase in absolute wt of the liver in M at HD and in the ratio in M and F at HD. An increase in the ratio of the heart wt to body wt in F at HD. There were incidences of cysts in the pituitary in treated animals while none were observed in the control.

Toxicology summary: a variety of studies (both single dose and multiple doses) were submitted. However, only multiple-dose studies (1 month, 3 months and 1 year) in rats and dogs were reviewed here. In the 3-month study in rats, atomoxetine was administered at targeted daily doses of 0, 5, 40, 80, and 160 mg/kg. The most prominent effect of the drug was a decrease in body wt and food consumption. Decreases in

wt/body wt ratio of several organs were observed (spleen, testes, prostate, and uterus). Slight increases in the wt/body wt ratio of the liver was seen in males at HD. Whole tissue alteration in the live and vacuolation were also reported. In a 1-month gavage study, rats were treated with 40, 80, and 160 mg/kg/day. This study confirmed a decrease in food consumption and body wt, in addition to some clinical signs observed at HD (salivation, soiling, and respiratory effects), as a result of treatment with atomoxetine by gavage compared to a similar effect seen when the drug was administered in diet. The finding that a decrease in food consumption was seen when the drug was administered by gavage, similar to what was observed when the drug was administered in diet, might rule out a poor palatability effect of the drug. In the 1-year study, rats were treated with atomoxetine in the diet at concentrations of 0, 0.01, 0.03, or 0.1%. These concentrations provided time-weighted average doses of approximately 0, 5, 14, and 46 mg/kg/day to males and 0, 6, 17, and 56 mg/kg/day to females. Body wt, body wt gain, and food consumption were reduced in HD and MD groups in males and in all treatment groups in females. Changes in the liver were also observed (increased liver wt relative to body wt, mottling and pallor of the liver were observed grossly at necropsy, and increased incidence and severity of focal vacuolization of hepatocytes in males). In a 1-year study, dogs were treated with atomoxetine orally at concentrations of 0, 4, 8, and 16 mg/kg/day. Tremors and mydriasis were observed. No apparent effect on food consumption and body wt even though anorexia was described as part of some of the clinical signs observed. Slight increases in the absolute wt and the wt/body wt ratio of the liver and the wt/body wt ratio of the heart. In a study with IV administration (0, 3, 6, and 12 mg/kg which was dropped to 10 mg/kg due to CNS toxicity) no effect on body wt was observed even though food consumption was decreased in treated animals mostly at HD. Convulsions, head jerking and lateral recumbency were seen at HD, which subsided when the dose was decreased to 10 mg/kg. Tremors and increased activity were observed in animals treated with 10 mg/kg.

Toxicology conclusions:

As evident from the reviewed studies and from the summaries of the other studies, the major effect of atomoxetine was the decrease in food consumption and body wt that was observed mainly in rodents (dogs did not reflect this in the 1-year study, and only food consumption appeared to be affected when the drug was given IV for two weeks). In view of the decrease in body wt observed in rats, an MTD is considered to be met in rat studies. As for dogs, an MTD can be considered met in view of the physical signs observed (tremors that lasted for several weeks in animals treated with HD). The liver appears to be the organ that is mostly affected by atomoxetine with changes in its wt and other gross and histopathological changes (pallor, mottling, and vacuolation). However, dogs did not reflect hepatotoxicity as a result of treatment with the drug.

Histopathology Inventory for NDA #

Study	3 M	1 Y	1 Y	
Species	Rat	Rat	Dog	

Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow smear	X	X	X
Bone	X	X	X
Brain	*	*	*
Brain (cerebrum, cerebellum, brain stem)	X	X	X
Cecum	X	X	
Cervix	X		
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*		
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder			X
Gross lesions		X	X
Harderian gland	X		
Heart	X*	X*	X*
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland			
Larynx			
Liver	X*	X*	X*
Lungs	X	X	X
Lymph nodes, Not specified	X	X	X
Lymph nodes mandibular			
Lymph nodes, mesenteric			
Mammary Gland	X	X	
Nasal cavity			
Optic nerves			
Ovaries	X*	X*	X*
Pancreas	X	X	X
Parathyroid	X*	X	X
Peripheral nerve			
Pharynx			
Pituitary	X*	X	X
Prostate	X*	X*	
Rectum	X		
Salivary gland	X	X	X
Sciatic nerve	X		
Seminal vesicles	X		

Skeletal muscle	X	X		
Skin	X	X		
Spinal cord	X			
Spleen	X*	X*	X	
Sternum				
Stomach	X	X	X	
Testes	X*	X*	*	
Thymus	X	X	X	
Thyroid	X*	X*	X*	
Tongue	X			
Trachea	X	X	X	
Urinary bladder	X	X		
Uterus	X*	X*	X	
Vagina	X			
Zymbal gland				
Standard List				

X, histopathology performed

*, organ weight obtained

V. GENETIC TOXICOLOGY:

Reviewed by Kathleen Haberny

Study title: THE EFFECT OF LY139603 ON THE INDUCTION OF BACTERIAL MUTATION USING A MODIFICATION OF THE AMES TEST

Key findings:

- Negative in the gradient plate assay, a modified Ames assay, in 8 strains of histidine-dependent *Salmonella typhimurium* and 2 strains of tryptophan-dependent *Escherichia coli* at concentrations estimated to be 0.1-1,000 mcg/ml
- Validity of the assay was not established because the test article stability, basis for dose selection, and the final concentrations achieved were not provided, and the dosing was inadequate due to absence of precipitation and cytotoxicity at the highest concentration tested

Study no: 811012GPA1671

Study type: Mutagenicity *in vitro*: to determine the ability of a compound to revert specific bacterial histidine auxotrophs to the wild type

Volume # 46, and page # 1

Conducting laboratory and location: Toxicology Division, Department GL796, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana, 46140

Date of study initiation: October 12, 1981

GLP compliance: yes (x) no ()

Criteria for positive results: Increase in frequency of appearance of discrete colonies along background lawn along increasing concentration gradient, exceeding solvent controls.

Summary of individual study findings:

Study validity: Appropriate strains were used and the positive control articles STZ and 2-AAF induced a positive mutagenic response. However, this study is not considered valid due to the following:

1. Test article stability and sterility were not reported
2. The basis for concentration selection was not provided
3. There was no precipitation or cytotoxicity to justify the high concentration limit of 1 mg/ml
4. No quantification was provided for concentrations achieved along the gradient

Study outcome :

Results of the Gradient Plate Assay on LY139603

<i>Tester Strain</i>	<i>S9</i>	LY139603		STZ	2-AAF
		MIC*	MCR*	MCR*	MCR*
G46	-	400	-	4.0-100	-
TA535	-	350	-	7.0-100	-
TA100	-	350	-	5.0-100	-
C3076	-	350	-	-	-
TA1537	-	350	-	10.0-100	-
D3052	-	350	-	-	-
TA1538	-	350	-	-	-
TA98	-	300	-	-	-
WP2	-	350	-	30.0-100	-
WP2uvrA-	-	350	-	5.0-100	-
G46	+	450	-	10.0-100	-
TA535	+	40	-	10.0-100	-
TA100	+	300	-	1.0-100	0.5-100
C3076	+	400	-	-	-
TA1537	+	200	-	10.0-100	-
D3052	+	400	-	-	2.0-100
TA1538	+	200	-	-	0.5-100
TA98	+	200	-	-	0.1-100
WP2	+	400	-	20.0-100	-
WP2uvrA-	+	100	-	10.0-100	-

*MIC: minimum inhibitory concentration (mg/ml); MCR: Mutagenic concentration range (mg/ml)

No increase in growth of colonies, indicating mutagenicity, was observed by LY139603 at concentrations from 0.1 – 1000 mcg/ml, either with or without metabolic activation with S9 mix. There was a slight inhibition of bacterial growth at concentrations of 350-400 mg/ml in the absence of metabolic activation, and at 40-450 mg/ml in the presence of metabolic activation. The inhibitory concentration reported may be a typographical error in the submission, because the concentrations are outside the concentrations tested in the mutagenicity assay of up to 1 mg/ml. Therefore, in the absence of observable

precipitation, the high concentration was inadequate according to ICH guidelines. The positive control article STZ increased the mutation frequency in the strains G46, TA1535, TA100, TA1537, WP2 and WP2uvrA- in the absence or metabolic activation. 2-AAF produced dose-related increases in mutation in strains TA100, D3052, TA1537, and TA98 in the presence of metabolic activation.

Study title: THE EFFECT OF COMPOUND LY139603 ON THE INDUCTION OF DNA REPAIR SYNTHESIS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

Key findings:

- LY139603 was negative for mutagenicity measured by induction of UDS in rat hepatocytes at concentrations of 0.5-1000 nmoles/ml

Study no: 820615UDS1671, 820803UDS1671, 820817UDS1671

Study type: Mutagenicity *in vitro*; ability of test article to induce unscheduled DNA synthesis (UDS) in cultured hepatocytes

Volume # 46, and page # - (not paginated)

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and company, Greenfield, Indiana, 46140

Date of study initiation: June 15, 1982

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug LY139603, lot #s 866-83F-248 (Study 820615UDS1671), 866-83F-212 (Studies 820803UDS1671 and 820817UDS1671), radiolabel none, and % purity 98.3%-98.5%

Formulation/vehicle: Test article dissolved in dimethylsulphoxide (DMSO) with serial dilutions in serum-free media

Methods:

Strains/species/cell line: Primary cultures of adult rat hepatocytes (male Fischer 344 rats,)

Dose selection criteria:

Basis of dose selection: Extreme cytotoxicity at the 2 highest concentrations of test article and positive controls, precluding evaluation of UDS

Range finding studies: Cytotoxicity observed at concentrations of 500 and 1000 nmoles/ml MNNG, 100 nmoles/ml 2-AAF, and 500 and 1000 nmoles/ml LY139603 in the main study.

Test agent stability: Not provided

Metabolic activation system: Not applicable

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 0.5-1000 nmoles/ml) and 2-acetylaminofluorene (2-AAF, 0.05-100 nmoles/ml)

Assay 2					
1000	-1.30±1.55	Toxic*	Toxic*	NT*	
500	-2.11±2.09	Toxic*	Toxic*	NT*	
100	-1.67±1.51	-0.31±1.09	54.60±13.40**	Toxic*/**	
50	-1.52±1.38	-0.53±1.19	32.09±5.92**	72.97±10.12**	
10	-1.31±1.26	-1.17±1.44	19.58±5.42**	58.31±7.51**	
5	-1.02±1.29	-0.46±1.17	0.15±2.16	51.82±8.57**	
1	-1.20±1.31	-1.40±1.09	-1.32±1.36	41.89±7.85**	
0.5	-1.45±0.86	-0.90±1.43	-1.21±1.09	40.38±8.15**	
0.1	NT*	NT*	NT*	13.61±5.11**	
0.05	NT*	NT*	NT*	2.39±4.06	
Assay 3					
1000	-0.80±2.00	Toxic*	Toxic*	NT*	
500	-1.18±1.40	Toxic*	Toxic*	NT*	
100	-0.77±0.99	-0.37±0.81	33.11±8.82**	Toxic*/**	
50	-1.05±0.87	-0.92±1.08	17.03±3.90**	77.50±8.20**	
10	-0.86±1.18	-0.46±1.39	2.57±3.06	46.78±8.60**	
5	-0.88±1.69	-0.78±1.68	-0.64±1.57	39.02±7.02**	
1	-1.25±1.50	-0.52±1.17	-0.62±1.34	29.72±6.94**	
0.5	-0.86±1.28	-0.53±1.77	-0.70±1.06	20.61±5.76**	
0.1	NT*	NT*	NT*	4.15±2.94	
0.05	NT*	NT*	NT*	-1.03±1.60	

*NT=Not Tested; Toxic=Cells unevaluable due to extreme cytotoxicity

**Positive Response for UDS

There was no evidence of unscheduled DNA synthesis in rat hepatocytes treated with LY139603 at concentrations of 0.5 – 1000 nmoles/ml, under the conditions of this study. The positive control articles N-methyl-N'-nitro-N-nitrosoguanidine and 2-acetylaminofluorene increased UDS in the hepatocytes, measured by increased net nuclear silver grain counts exceeding the control counts by 3 standard deviations in a dose related manner. The study was conducted appropriately, according to ICH guidelines.

Study title: THE EFFECT OF LY139603 ON THE INDUCTION OF FORWARD MUTATION AT THE THYMIDINE KINASE LOCUS OF L5178Y MOUSE LYMPHOMA CELLS

Key findings:

- LY139603 negative for mutagenicity in L5178Y mouse lymphoma cells at concentrations of 1-17.5 mcg/ml in the absence of metabolic activation with S9, and at 1-35 mcg/ml in the presence of S9

Study no: 820714MLA1671

Study type: *In vitro* test for mammalian gene mutations and clastogenic effects *in vitro*

Volume # 46, and page # (Not paginated)

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140

Date of study initiation: July 14, 1982

Criteria for positive results: Dose-dependent 2X or greater increase in TK-/- frequency compared to frequency in solvent controls, using cultures with >10% total survival only

Summary of individual study findings:

Study validity:

1. Results in the positive control samples demonstrated sensitivity of the cell line to forward mutation at the thymidine kinase locus and efficacy of the metabolic activation system
2. Adequate dosing demonstrated by >50% inhibition of suspension growth at the highest concentrations as described under Study Outcome below

Study outcome: The results of the Mouse Lymphoma Forward Mutation assay are presented in the following table:

Treatment	Concentration (mcg/ml)	Percent Total Survival ^a	Mutation Frequency ^b	Mutation Index ^c
Non-activated Test				
DMSO	(1%)	100	2.1	(1.0)
DMSO	(1%)	100	3.1	(1.0)
EMS	620	12	108.0	41.5
LY139603	17.5	20	3.1	1.2
LY139603	15.0	26	3.3	1.3
LY139603	12.5	56	2.7	1.0
LY139603	10.0	58	4.1	1.6
LY139603	7.5	82	2.5	1.0
LY139603	5.0	74	3.9	1.5
LY139603	2.5	83	3.2	1.2
LY139603	1.0	66	4.3	1.7
Activated Test				
DMSO	(1%)	100	2.5	(1.0)
DMSO	(1%)	100	2.4	(1.0)
3-MC	5	26	24.6	10
LY139603	35	51	2.8	1.1
LY139603	30	67	2.7	1.1
LY139603	25	65	2.8	1.1
LY139603	20	76	1.9	0.7
LY139603	15	69	2.1	0.9
LY139603	10	71	2.8	1.1
LY139603	5	82	2.7	1.1
LY139603	1	108	2.2	0.9

^aPercent Total Survival = %suspension growth X cloning efficiency, where cloning efficiency = Mean # colonies on nonselective plates in treated culture ÷ mean # colonies on nonselective plates of solvent control X 100%

^bMutation Frequency = # TK-/- mutants per 1x10⁵ colony forming cells

^cMutation Index = mutation frequency of treated culture ÷ mutation frequency of solvent control

There was a dose-related increase in cytotoxicity by LY139603. Percent inhibition of suspension growth was 9%-73% (total survival 83%-20%) in the absence of metabolic activation, and 4%-39% (total survival 108%-51%) in the presence of metabolic activation. Survival was decreased 88% by EMS and 74% by 3-MC compared to controls.

The frequency of TK-/- was slightly increased by LY139603 at several concentrations compared to the control frequency, in the absence of metabolic activation, but did not reach the criteria for a positive response (dose-related increase by 2X or greater compared to frequency in solvent controls). In comparison, the positive control articles EMS (without S9) and 3-MC (with S9) produced mutation frequencies of 108 and 24.6, respectively. Therefore, LY139603 was negative for mutagenicity in mouse lymphoma cells under the conditions of this study.

Study title: THE EFFECT OF COMPOUND LY139603 ON THE IN VIVO INDUCTION OF SISTER CHROMATID EXCHANGE IN BONE MARROW OF CHINESE HAMSTERS

Key findings:

- LY139603 was negative for sister chromatid exchanges in the bone marrow of Chinese hamsters under the conditions of this study
- Exposure to LY139603 was inadequate, due to absence of a rationale for dose selection, toxicity in the animals, and/or toxicokinetic data

Study no: 821005SCE1671

Study type: *In vivo* test of direct-acting and activation-dependent genotoxic compounds in mammalian system

Volume # 46, and page # (not paginated)

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140

Date of study initiation: October 5, 1982

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug LY139603, lot # 866-83F-212, radiolabel none, and % purity 98.5%

Formulation/vehicle: LY139603 diluted in 10% aqueous acacia

Methods:

Strains/species/cell line: Inbred adult female Chinese hamsters (Eli Lilly and Company, 31.5-36 g)

Dose selection criteria:

Basis of dose selection: None

Range finding studies: None

LY139603 was negative for clastogenicity in Chinese hamster bone marrow cells under the conditions of this study, the dosing was inadequate due to lack of precipitation and/or toxicity at the highest dose studied.

Study title: THE EFFECT OF TOMOXETINE HYDROCHLORIDE (LY139603) ON THE INDUCTION OF REVERSE MUTATIONS IN *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI* USING THE AMES TEST

Key findings:

- LY139603 negative in the Ames test at concentrations from 400-2000 mcg/plate in the presence and absence of metabolic activation with S9

Study no: 980310AMT1671, 980316AMT1671, and 980407AMS1671

Study type: Mutagenicity *in vitro*: to determine ability of a compound to revert specific bacterial histidine auxotrophs to the wild type

Volume # 46, and page #: Not paginated

Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN 46140

Date of study initiation: March 10, 1998

GLP compliance: yes (x, 980407AMS1671 only) no ()

QA reports: yes (x) no ()

Drug LY139603 (compound 404363 hydrochloride), lot # 399SB7, radiolabel none, and % purity: 98.5%

Formulation/vehicle: Test and positive control articles dissolved and diluted in reagent-grade dimethyl sulfoxide (DMSO)

Methods:

Strains/species/cell line:

Salmonella typhimurium tester strains:

TA1535 (G46 with gal-bio-uvrB deletion and LPS deletion)

TA1537 (C3076 with gal-bio-uvrB deletion and LPS deletion)

TA98 ((TA1538 with addition of R-factor pKM 101)

TA100 (TA1535 with addition of R-factor pKM 101)

Escherichia coli strain:

WP2uvrA (WP2 with uvrA deletion)

Dose selection criteria:

Basis of dose selection: Range-finding assays

Range finding studies: Test article tested at 312.53-5000.5 mcg/plate without and with metabolic activation with S9 mix; Results showed pinpoint colonies and absence of background lawn at 2500-5000 mcg/plate for TA1535, TA1537, TA100, and WP2uvrA, and at 1250, 2500, and 5000.5 mcg/plate for

strain TA98 in the absence of metabolic activation. Pinpoint colonies and absence of background lawn were observed at 2500-5000.5 mcg/plate for strains TA1535, TA1537, TA98, TA100, and WP2uvrA in the presence of metabolic activation. The number of revertants was reduced in strain TA100 at 1250 mcg/plate without metabolic activation, and in strains TA1535 and TA98 at 1250 mcg/plate and TA100 at 312.5, 625, and 1250 mcg/plate with metabolic activation. In a second range-finding assay using five concentrations ranging from 400-2000 mcg/plate, pinpoint colonies and absence of background lawn were observed at 2000 mcg/plate for the strain TA98, and at 1000 and 2000 mcg/plate in strains TA98 and TA100, and the number of revertants was reduced at 1000 and 2000 mcg/plate for strains TA100 and at 1000 mcg/plate for strain TA98 in the absence of metabolic activation. In the presence of metabolic activation, the number of revertants was reduced at 2000 mcg/plate for strain TA100.

Test agent stability: Drug Substance Characterization provided, Material submitted to Deep Freeze House Sample Program, however stability not determined

Metabolic activation system: S9 mix prepared from Aroclor 1254-induced rat livers

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2-nitrofluorene (2NF), 9-aminoacridine (9 AmAc), and 2-aminoanthracene (2AA) as presented in the following table:

Metabolic Activation	Strain	Positive Control	(mcg/plate)
-	TA1535	MNNG	1.25
	TA1537	9AmAc	60
	TA98	2NF	0.5
	TA100	MNNG	1.25
	WP2uvrA	MNNG	2.5
+	TA1535	2AA	2.5
	TA1537	2AA	2.5
	TA98	2AA	1.25
	TA100	2AA	1.25
	WP2uvrA	2AA	10

Comments: None

Exposure conditions :

Incubation and sampling times: 48 hours at 37°C

Doses used in definitive study: 400-2000 mcg/plate

Study design: Bacterial test strains were incubated with LY139603, positive control article or vehicle negative control (DMSO), dilution medium containing L-histidine, L-tryptophan, and biotin, dilution salts as appropriate, and S9 (activation plates only) in 2.5 ml top agar distributed evenly over base agar medium, and the revertant colonies were counted

Analysis:

No. of replicates: Assay conducted in triplicate

Counting method: Colony Counter; 86% plate counted; correction factor determined using counted and total area of plate

Criteria for positive results:

1. 2X increase in revertants in strains TA98, TA100, and WP2uvrA and 3X increase in number of revertants in strains TA1535 and TA 1537 compared to controls
2. Significant dose correlation in 2 successive test article concentrations
3. Positive responses reproducible

Summary of individual study findings:

Study validity:

1. Normal range of bacterial colonies for each strain in the vehicle control plates, consistent with historical control range
2. 5% or fewer plates lost through contamination
3. Appropriate strains used
4. Dose selection appropriate
5. Positive response by the positive control articles; although first range-finding study invalid due to absence of positive response with the positive control article 2NF in the strain TA98

Study outcome: The results are presented in the following tables:

Results of Range-Finding/Mutagenicity Study 980310AMT1671*

Treatment	Mcg/plate	TA1535	TA1537	TA98	TA100	WP2uvrA
Colony Count Without Metabolic Activation						
DMSO	0.05 ml	9	14	16	83	28
LY139603	312.53	16 (1.78X)	9	23 (1.44X)	79	48 (1.71X)
	625	13 (1.44X)	14	49 (3.06X)	73	53 (1.89X)
	1250	13 (1.44X)	13	Toxicity	48	37 (1.32X)
	2500	Toxicity	Toxicity	Toxicity	Toxicity	Toxicity
MNNG	5000.5	Toxicity	Toxicity	Toxicity	Toxicity	Toxicity
	See under positive controls above	1314 (146X)	-	-	1355 (16.3X)	127 (4.5X)
9AmAc		-	42 (3X)	-	-	-
2NF		-	-	16	-	-
Colony Count With Metabolic Activation						
DMSO	0.05 ml	12	15	16	153	35
LY139603	312.53	13	10	21 (1.31X)	108	49 (1.4X)

2AA	625	14	9	29 (1.81X)	76	35
	1250	7	17	5	47	31
	2500	Toxicity	Toxicity	Toxicity	Toxicity	Toxicity
	5000.5	Toxicity	Toxicity	Toxicity	Toxicity	Toxicity
	See under positive controls above	153 (12.75X)	171 (11.4X)	780 (48.8X)	826 (5.4X)	345 (9.8X)

*Values in parenthesis represent multiples of the control values

Results of Range-Finding/Mutagenicity Study 980316AMT1671

Treatment	Mcg/plate	<i>TA1535</i>	TA1537	TA98	TA100	WP2uvrA
Colony Count Without Metabolic Activation						
DMSO	0.05 ml	7	8	17	107	49
LY139603	400	12	10	29 (1.71X)	91	55
	600	10	10	22	105	62
	800	9	10	28	101	50
	1000	6	13	8	73	51
	2000	1	5	Toxicity	17	13
MNNG	See under positive controls above	1051 (150X)	-	-	845 (7.9X)	135 (2.75X)
9AmAc		-	193 (24X)	-	-	-
2NF		-	-	141 (8.3X)	-	-
Colony Count With Metabolic Activation						
DMSO	0.05 ml	7	10	24	102	38
LY139603	400	6	14	38	67	56 (1.55X)
	600	10	13	43 (1.79X)	72	30
	800	12	12	35	65	45
	1000	5	20 (2x)	45 (1.88X)	73	41
	2000	3	1	5	7	5
2AA	See under positive controls above	116 (16.6X)	178 (17.8X)	819 (34X)	640 (6.3X)	521 (13.7X)

Results of Definitive Mutagenicity Study 980407AMS1671: Corrected Counts

Treatment	Mcg/plate	<i>TA1535</i>	TA1537	TA98	TA100	WP2uvrA
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Colony Counts Without Metabolic Activation						
DMSO	0.05 ml	12	15	24	95	34
		10	12	22	105	28
		9	12	23	103	34
LY139603	400	9	9	30	10	31
		8	12	34	100	45
		8	8	27	85	45
	600	8	10	20	103	45
		7	16	28	98	50
		8	10	36	90	40
	800	7	9	29	57	35
		7	12	21	64	43
		7	9	24	52	45
	1000	5	10	3	47	34
		7	17	3	52	42
		5	15	10	62	30
	2000	Toxicity	Toxicity	Toxicity	14	13
		Toxicity	Toxicity	Toxicity	9	9
		Toxicity	Toxicity	Toxicity	15	23
MNNG	See under positive controls above	467	-	-	523	190
		393	-	-	466	173
		413	-	-	541	209
9AmAc	See under positive controls above	-	223	-	-	-
		-	163	-	-	-
		-	180	-	-	-
2NF	See under positive controls above	-	-	119	-	-
		-	-	117	-	-
		-	-	112	-	-
Colony Counts With Metabolic Activation						
DMSO	0.05 ml	7	15	36	109	20
		8	15	31	112	29
		9	14	29	108	34
LY139603	400	7	14	38	122	36
		6	16	36	94	42
		8	15	37	119	37
	600	7	12	37	95	42
		8	12	40	98	40
		9	15	40	107	43
	800	8	12	31	86	43
		7	16	33	73	43
		6	10	35	87	49
	1000	2	9	14	29	47
		1	10	16	49	37
		5	15	22	64	51
	2000	1	5	Toxicity	22	8
		1	7	Toxicity	13	13
	See under positive controls above	0	5	Toxicity	23	10
2AA		194	119	659	570	215
		206	147	731	520	231
		162	156	692	542	159

*Toxicity observed as pinpoint colonies and lack of background lawn

Results of Definitive Mutagenicity Study 980407AMS1671: Means \pm SD for Triplicate Plates

Treatment	Mcg/plate	TA1535	TA1537	TA98	TA100	WP2uvrA
Colony Counts Without Metabolic Activation						
DMSO	0.05 ml	10 \pm 2	13 \pm 2	23 \pm 1	101 \pm 5	32 \pm 3
LY139603	400	8 \pm 1	10 \pm 2	30 \pm 4	95 \pm 9	40 \pm 8
	600	8 \pm 1	12 \pm 3	28 \pm 8	97 \pm 7	45 \pm 5
	800	7 \pm 0	10 \pm 2	25 \pm 4	58 \pm 6	41 \pm 5
	1000	6 \pm 1	14 \pm 4	5 \pm 4	54 \pm 8	35 \pm 5

MNNG	2000	Toxicity	Toxicity	Toxicity	13±3	15±7
9AmAc	See under	424±38	-	-	510±39	191±18
2NF	positive	-	189±31	-	-	-
	controls above	-	-	116±4	-	-
Colony Counts With Metabolic Activation						
DMSO	0.05 ml	8±1	15±1	32±4	110±2	31±3
LY139603	400	7±1	15±1	37±1	112±15	38±3
	600	8±1	13±2	39±2	100±6	42±2
	800	7±1	13±3	33±2	82±8	45±3
	1000	3±2	11±3	17±4	47±18	45±7
	2000	1±1	6±1	Toxicity	19±6	10±3
2AA	See under	187±23	141±19	694±36	544±25	202±38
	positive					
	controls					
	above					

LY139603 was negative for mutagenicity in the Ames test under the conditions of this study, at concentrations of 400-2000 mcg/ml in *S. typhimurium* and *E. coli* strains TA1537, TA1535, TA98, TA100, and WP2uvrA, conducted with and without metabolic activation with S9. Although the positive control 2NF was negative in strain TA98 in the first range-finding Toxicity/Mutagenicity study, thus invalidating that preliminary study, further evaluation showed a positive response in a subsequent range finding study and in the definitive mutagenicity study. The study validity was demonstrated by the appropriate use of strains and doses based on toxicity at the highest concentrations.

Study title: THE EFFECT OF TOMOXETINE HYDROCHLORIDE (LY139603) GIVEN ORALLY BY GAVAGE FOR 2 CONSECUTIVE DAYS ON THE INDUCTION OF MICRONUCLEI IN BONE MARROW OF ICR MICE

Key findings:

- No evidence of clastogenicity by LY139603 at doses ranging from 58-232 mg/kg PO, measured by the induction of micronuclei in mouse bone marrow cells

Study no: 980318MNT1671

Study type: *In vivo* assay for clastogenicity

Volume # 46, and page #: (not paginated)

Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN 46140

Date of study initiation: March 18, 1998

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug LY139603 (compound 404363 hydrochloride, lot # 399SB7, radiolabel none, and % purity: 98.5%

Formulation/vehicle: LY139603 dissolved in purified water

Methods:

Strains/species/cell line: Male and female ICR (Hsd:ICR)] mice _____
_____ ages 8-9 weeks, weights 30.1-33.4 g males and 25.3-31.5 g females,
n=5/sex/group)

Dose selection criteria:

Basis of dose selection: Dose range-finding study (980311MTT1671)

Range finding studies:

Results showed median lethal dose (MLD) of 290 mg/kg PO

The high dose chosen for the definitive study, 232 mg/kg, is 80% of the MLD (combined sexes)

Adequate exposure to oral LY139603 suggested by a separate PK study showing a Cmax of 165.3 ng/ml at 2 hours after dosing with 50 mg/kg PO in rats

Test agent stability: 8 days at room temperature (25°C) or refrigerated (5°C)

Metabolic activation system: Not applicable

Controls:

Vehicle: Purified water

Negative controls: Purified water

Positive controls: Cyclophosphamide (CP, 2.5 mg/ml) in purified water, given by oral gavage at 50 mg/kg (20 ml/kg) on Day 1 only

Comments: None

Exposure conditions:

Incubation and sampling times: 48 hours after the first of 2 test article treatments 24 hours apart (24 hours after the last treatment)

Doses used in definitive study: 0, 58, 116, and 232 mg/kg (0, 2.9, 5.8, and 11.6 mg/ml) by oral gavage (20 ml/kg) on Day 0 and Day 1

Study design: All mice given test article, positive control article, and vehicle negative control were sacrificed at 48 hours after the first of 2 test article treatments. The bone marrow was isolated from the femurs and evaluated for polychromatic erythrocytes (PCE), PCE with and without MN, and normochromatic erythrocytes (NCE).

Analysis:

No. of replicates: 5/sex/dose

Counting method: _____ Micronucleus Scoring System: 2000 anucleate PCE counted/animal, numbers of PCE with and without MN, number of NCE; Number of PCE divided by NCE to obtain PCE/NCE ratio indicating bone marrow toxicity

Criteria for positive results: Statistically significant increase in frequency of MPCEs compared to control values

Summary of individual study findings:

Study validity:

1. Adequate number (2000) cells counted per slide
2. Vehicle and positive control agent responses consistent with historical data
3. Significant increase in frequency of micronucleated PCE in positive control group

4. Adequate number of doses

5. Test article tested to 80% median lethal dose or maximum tolerated dose

Study outcome: The treatment-related clinical signs were tremors (HD) and ataxia (MD and HD), and there were 2 treatment-related deaths (1HDM, 1 HDF).

The results are presented in the following table:

Sex	Treatment	Dose (mg/kg PO)	PCE/NCE Ratio*	MPCE/1000 PCE*
Males	Vehicle	0.0	0.9±0.3	0.4±0.4
	LY139603	58.0	1.1±0.2	0.4±0.2
	LY139603	116.0	0.9±0.2	0.5±0.5
	LY139603	232.0	1.3±0.3	0.4±0.5
	CP	50.0	0.9±0.5	3.6±1.8**
Females	Vehicle	0.0	1.4±0.2	0.5±0.4
	LY139603	58.0	0.9±0.3	0.3±0.3
	LY139603	116.0	1.1±0.3	0.2±0.3
	LY139603	232.0	1.1±0.3	0.2±0.3
	CP	50.0	0.9±0.3	2.0±1.3**

*Mean ± standard deviation; high dose means based on 4 animals/sex due to deaths of 1 mouse in each high dose group

**p<0.05 (one-tailed trend test)

There was no evidence of clastogenicity by LY139603 in mouse bone marrow cells at doses of 58-232 mg/kg, under the conditions of this study. The study used an adequate number of animals, adequate dosing including 3 doses up to 80% of the median lethal dose, appropriate incubation time of 48 hours, and evaluated an appropriate number of PCEs and NCEs (approximately 2000 per slide). The validity of the study was also demonstrated by a negative response to the vehicle control within historical control range, and a statistically significant increase in micronucleated polychromatic erythrocytes by the positive control article, cyclophosphamide.

Study title: THE EFFECT OF TOMOXETINE HYDROCHLORIDE (LY139603) ON THE *IN VITRO* INDUCTION OF CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

Key findings:

- Slight treatment-related increase in the percent cells with diplochromosomes at concentrations of 57.5 and 70 mcg/ml in the absence of metabolic activation in the 4-hour exposure assay (4.5% compared to 0 in the controls) and to a lesser extent in the 19-hour assay (1.5% compared to 0 in the controls) at 35 mcg/ml, suggesting increased endoreduplication
- LY139603 was negative for clastogenicity *in vitro* in Chinese hamster ovary cells

Study no: 980402CAB1671, 980408CAB1671, and 980422CAB1671

Study type: Clastogenicity *in vitro*

Volume # 46, and page #: (not paginated)

Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN 46140

Date of study initiation: April 2, 1998 (980402CAB1671), April 8, 1998 (980408CAB1671), and April 22, 1998 (980422CAB1671)

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug LY139603 (compound 404363 hydrochloride), lot # 399SB7, radiolabel None, and % purity: 98.5%

Formulation/vehicle: Test article dissolved in sterile milli-Q water in the preliminary cytotoxicity study, reagent grade dimethyl sulfoxide (DMSO) in the definitive study

Methods:

Strains/species/cell line: Chinese hamster ovary cells

Dose selection criteria:

Basis of dose selection: Preliminary Toxicity Test (Study 980305CTX1671), no evidence of precipitate at up to 1000 mcg/ml.

Range finding studies: Cell survival at concentrations from 1-1000 mcg/ml without metabolic activation was 119%-67% the control survival at 1-100 mcg/ml and 0% at all concentrations of ≥ 200 mcg/ml. Cell survival with metabolic activation at concentrations of 1-1000 mcg/ml was 140%-169% control, with no survival at concentrations of ≥ 200 mcg/ml. In a second test using extended exposure without metabolic activation, survival was 95%, 116%, 74%, and 39% at 1, 10, 50, and 100 mcg/ml, respectively, and there were no surviving cells at concentrations ≥ 150 mcg/ml. Concurrent toxicity evaluation in the genotoxicity study showed survival at 52%-98% at concentrations up to 75 mcg/ml without metabolic activation for 4 hours, and 37%-97% at concentrations up to 112 mcg/ml with metabolic activation for 4 hours. In the activation study, concentrations of 114-118 mcg/ml resulted in cell survival of 23%-28%. However, extended exposure (19 hours) without metabolic activation resulted in increased survival or 191%, 185% and 271% control value at 50, 60, and 70 mcg/ml, respectively, although survival was 50%-115% at concentrations of 0.5-40 mcg/ml and 31% at 45 mcg/ml.

Test agent stability: Not provided in this submission

Metabolic activation system: S9 mix prepared from livers of Aroclor 1254-induced rats

Controls:

Vehicle: Sterile milli-Q water in the preliminary cytotoxicity study, reagent grade dimethyl sulfoxide (DMSO) in the definitive study

Negative controls: DMSO

Positive controls: Cyclophosphamide (CP, 10 and 15 mcg/ml, activated assay) and mitomycin C (MC, 0.5 and 1 mcg/ml, nonactivated assay)

Comments: None

Exposure conditions :

Incubation and sampling times: 4 hours and 19 hours (extended exposure assay)

including gaps (52% compared to 1.5% in the controls), number of aberrations per cell (1.12 compared to 0.005 in the controls), and % cells with >1 aberration (24 compared to 0 in the controls), due to increased chromatid break and exchanges.

4-hour Exposure in Presence of Metabolic activation: No effects by the negative control article and LY139603 at concentrations up to 112 mcg/ml. The positive control article CP induced an increase in % cells with aberrations excluding gaps (34% compared to 0.5% in the controls) and including gaps (38% compared to 1.5% in the controls), number of aberrations per cell (0.41 compared to 0.005 in the controls), and % cells with >1 aberration, due to an increase in chromatic breaks and chromatic exchanges.

19-hour Exposure in Absence of Metabolic activation: No effects by the negative control and LY139603, evaluated singly or by combining the results of the replicate slides, at concentrations up to 35 mcg/ml, except for an increase in the percent cells with diplochromosomes at the highest concentration of 35 mcg/ml (1.5% compared to 0% in the controls). The positive control article (mitomycin C) produced an increase in % cells with aberrations excluding gaps (80% compared to 0.5% in the controls) and including gaps (80% compared to 1.5% in the controls), number of cells with aberrations (2.16 compared to 0.005 in the controls), and % cells with >1 aberration (48% compared to 0 in the controls), due to an increase in chromatid breaks and exchanges.

Table 1: Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated With Tomoxetine Hydrochloride (LY139603) in the Absence of Metabolic Activation, Study 980402CAB1671 (Combined Results From Two Cultures)

Treatment (ug/mL)	Cells Scored	Number and Type of Aberration ^a										Number Aberrations Per Cell	% Cells With Aberrations		% Cells With >1 Aberration	% Cells With Diplochromosomes					
		Gap			Chromatid Exchange				Chromosome Exchange				Other				Excluding Gaps (TA)	Including Gaps (TAG)			
		TG	SG	TB	TR	QR	CR	ID	SB	D	R		CI	DM					PU	GT	
Vehicle Control: Dimethyl sulfoxide (µL)																					
200	2										1						0.005	0.5	1.5	0	0
Positive Control: Mitomycin C																					
0.5	25	1		10	5	2	1	1	2			1				1	1.12	48***	52	24	0
Test Compound: LY139603																					
40	200	1	1	2							1						0.015	1.5	2.5	0	0.5
57.5	200			1													0.005	0.5	0.5	0	4.5***
70	200	2	2	1	1												0.01	1	3	0	4***

^a Abbreviations: TG=chromatid gap; SG=chromosome gap; TB=chromatid break; TR=triradial; QR=quadriradial; CR=complex rearrangement; ID=interstitial deletion; SB=chromosome break (includes acentric fragment); D=dicentric; R=ring chromosome; CI=chromosome intrachange; DM="double minute" fragment; PU=pulverized chromosome; GT=>10 aberrations.

***Significantly greater than vehicle control (p<.001).

In conclusion, LY139603 was negative for clastogenicity in Chinese hamster ovary cells, under the conditions of this study, although there was a treatment-related increase in the percent cells with diplochromosomes at concentrations of 57.5 and 70 mcg/ml in the absence of metabolic activation in the 4-hour exposure

assay (4.5% compared to 0 in the controls) and to a lesser extent in the 19-hour assay (1.5% compared to 0 in the controls). The study was conducted appropriately according to ICH guidelines with respect to study design, including concentration range up to toxic levels and exposure duration. The validity of the study was also demonstrated by a negative response to the vehicle control article and statistically significant increase in aberrations by the positive control articles CP and MC, comparable to the results in the historical data.

Study title: THE EFFECT OF COMPOUND 137877 (NORTOMOXETINE HYDROCHLORIDE) ON THE INDUCTION OF DNA REPAIR SYNTHESIS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

Key findings:

- No evidence of unscheduled DNA synthesis in rat hepatocytes exposed to the LY139603 metabolite nortomoxetine hydrochloride at concentrations of 0.5-10 mcg/ml for 20 hours

Study no: 860708UDS2808 and 860715UDS2808

Study type: Evaluation of the LY139603 metabolite Nortomoxetine hydrochloride (Compound 137877) for mutagenicity *in vitro*, measured by unscheduled DNA synthesis in response to chemical-induced DNA damage

Volume # 46, and page #: (not paginated)

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140

Date of study initiation: July 8, 1986

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug Nortomoxetine hydrochloride (compound 137877), **lot #** 687-JJO-244C, **radiolabel** None, and **% purity:** 99.0%

Formulation/vehicle: Test article dissolved in reagent grade dimethylsulfoxide (DMSO), and diluted in serum-free media

Methods:

Strains/species/cell line: Primary cultures of adult rat hepatocytes from livers of male Fischer 344 rats (weights 180-195 g)

Dose selection criteria:

Basis of dose selection: Laboratory experience

Range finding studies: The cells were unevaluable in both studies due to cytotoxicity at concentrations of 50 mcg/ml and above; thus, dosing was adequate

Test agent stability: Not provided in this submission

Metabolic activation system: Not applicable

Controls:

Vehicle: DMSO

Negative controls: 1% DMSO

Positive controls: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 1-20 mcg/ml) and 2-acetylaminofluorene (2-AAF, 0.05-1 mcg/ml)

Comments: None

Exposure conditions :

Incubation and sampling times: 20 hours

Doses used in definitive study: 0.5, 1, 5, 10, 50, 100, 500, 1000 mcg/ml

Study design: The hepatocytes were incubated for 20 hours at 37°C with test article, vehicle negative control, or positive control article, washed, fixed, stained (1% aceto-orcein), dipped in undiluted NTB-2 liquid photographic emulsion, and then incubated for 7 days at 4°C. The cells were then developed and examined by oil immersion microscopy.

Analysis:

No. of replicates: 2

Counting method: The numbers of silver grains were counted (colony counter) in the cytoplasmic background (3 nuclear-sized areas adjacent to nucleus) and cell nucleus, minimum 20 morphologically unaltered nuclei per treatment counted. Net nuclear grain count (gross nuclear grain count minus mean cytoplasmic background count) was calculated.

Criteria for positive results: Nuclear grain counts exceeding control by three standard deviations of the control value in at least 2 successive concentrations

Summary of individual study findings:

Study validity:

1. Appropriate dose selection: cytotoxicity at the highest test article concentration
2. Negative control response comparable to historical control results
3. Minimum 3x SD increase in net nuclear grain counts in the positive control treated cells in at least 2 successive concentrations
4. Adequate incubation time
5. Reproducibility in the replicate study

Study outcome :

The results of the UDS assay on nortomoxetine hydrochloride in rat hepatocytes are presented in the following table:

E. Results of the UDS Assay on Nortomoxetine Hydrochloride in Rat Hepatocytes*

Compound	Concentration (mcg/ml)	Net Nuclear Silver Grains (Mean ± SD)	
		Study 860708	Study 860715
137877	1000	Toxic	Toxic
	500	Toxic	Toxic
	100	Toxic	Toxic
	50	Toxic	Toxic
	10	-1.27±1.22	-0.96±1.25
	5	-1.93±2.16	-1.06±1.31
	1	-1.88±1.36	-1.93±0.43
	0.5	-0.55±1.66	-1.46±1.41
MNNG	20	Toxic/Positive	Toxic/Positive

	10	42.66± 7.23**	32.50± 7.65**
	5	13.48± 6.91**	19.85± 7.77**
	1	0.03± 2.69	1.26± 3.29
2AAF	1	Toxic/Positive	Toxic/Positive
	0.5	65.95± 14.83**	58.49± 11.84**
	0.1	28.40± 4.72**	30.20± 8.61**
	0.05	12.58± 6.33**	11.91± 5.53**
DMSO	1%	-0.83± 1.23	-0.94± 1.52
	1%	-1.58± 1.16	-0.36± 1.58
	1%	01.75± 0.88	-1.10± 0.84
	1%	-0.66± 0.82	-1.86± 1.27

*Toxic indicates that the cells were unevaluable due to cytotoxicity

**Positive for UDS

There was no evidence of unscheduled DNA synthesis in the hepatocytes exposed to the LY139603 metabolite nortomoxetine hydrochloride at concentrations of 0.5-10 mcg/ml for 20 hours in duplicate assays. Concentrations of 50 mcg/ml and higher were unevaluable due to cytotoxicity. The positive control articles MNNG and 2AAF were positive for mutagenicity, increasing the net nuclear silver grains by more than 3 standard deviations of the control. The study parameters, including dose selection, incubation time, and reproducibility, were appropriate according to ICH guidelines. The validity of the study was also demonstrated by a negative response to the control vehicle.

Study title: THE EFFECT OF NORTOMOXETINE HYDROCHLORIDE (LILLY COMPOUND 137877) ON THE INDUCTION OF FORWARD MUTATION AT THE THYMIDINE KINASE LOCUS OF L5178Y MOUSE LYMPHOMA CELLS

Key findings:

- The LY139603 metabolite nortomoxetine hydrochloride was negative for induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells

Study no: 860610MLA2808 and 860827MLA2808

Study type: Evaluation of the LY139603 metabolite Nortomoxetine hydrochloride (Compound 137877) for mutagenicity *in vitro*, measured by loss of TK activity in formerly TK competent L5178Y cells

Volume # 46, and page #: (not paginated)

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN 46140

Date of study initiation: June 10, 1986

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug, Nortomoxetine hydrochloride (Compound 137877), **lot #** 687-JJO-244C, **radiolabel** None, and **% purity:** 99.0%

Formulation/vehicle: Test article dissolved in sterile Milli-Q water with final dilutions in RoP medium

Study outcome :

The results of the mutagenicity study on nortomoxetine hydrochloride in Mouse lymphoma L5178Y cells are presented in the following table:

Results of the Mouse Lymphoma Assay on Nortomoxetine (Means of Triplicate Plates)

Treatment	Concentration (mcg/ml)	% Total Survival	Mutation Frequency*	Mutation Index*
Non-activated Test				
Nortomoxetine	70	NE	-	-
	60	NE	-	-
	50	NE	-	-
	40	NE	-	-
	30	65	2.1	0.9
	29	86	2.1	0.9
	10	98	2.4	1.0
	1	112	2.2	1.0
Solvent Control	0	100	2.7	
	0	100	1.5	
	0	100	2.8	(1.0)
mean			2.3	
EMS	620	19	73.4	31.9
Activated Test				
Nortomoxetine	70	NE	-	-
	60	NE	-	-
	50	NE	-	-
	40	34	2.1	1.0
	30	75	2.8	1.3
	29	71	2.7	1.3
	10	101	2.3	1.1
	1	88	2.5	1.2
Solvent Control	0	100	2.6	
	0	100	1.7	(1.0)
	0	100	2.1	
mean			2.1	
3MC	3	28	32	15.2

* Mutation Frequency = TK-/- mutants per 1x10⁵ colony forming cells

Mutation Index = Mutation frequency of treated culture / control mutation frequency

NE = Not evaluated due to severe toxicity

There were no treatment-related effects on daily cell counts, percent suspension growth, mean colony counts, and percent cloning efficiency. There was no evidence of mutagenicity by nortomoxetine at concentrations up to 30 mcg/ml in the absence of metabolic activation and 40 mcg/ml in the presence of metabolic activation with S9 in L5178Y mouse lymphoma cells in the TK+/- forward mutation assay. Severe cytotoxicity precluded evaluation in the cells exposed to concentrations of 40-70 mcg/ml without metabolic activation and 50-70 mcg/ml with metabolic activation. The vehicle control results were comparable to the historical controls. In comparison, EMS and 3MC were positive in this assay, and increased the mutation frequency and mutation index by >2x compared to the negative control values. The study was conducted appropriately according to ICH guidelines, including dose selection and use of controls.

Study title: THE EFFECT OF NORTOMOXETINE HYDROCHLORIDE (LILLY COMPOUND 137877) ON THE INDUCTION OF REVERSE MUTATIONS IN SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI USING THE AMES TEST

Key findings:

- The LY139603 metabolite, nortomoxetine, was negative in the Ames test

Study no: 860616AMS2808, 860623AMT2808, and 860825AMS2808

Study type: Mutagenicity *in vitro*: to determine ability of the LY139603 metabolite nortomoxetine hydrochloride to revert specific bacterial histidine auxotrophs to the wild type

Volume # 46, and page #: Not paginated

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140

Date of study initiation: June 16, 1986

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug Nortomoxetine hydrochloride (Compound 137877), **lot #** 687-JJO-244C, **radiolabel** None, and **% purity:** 99.0%

Formulation/vehicle: Nortomoxetine hydrochloride in reagent grade dimethylsulphoxide (DMSO)

Methods:

Strains/species/cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100; *Escherichia coli* strain WP2uvrA-

Dose selection criteria:

Basis of dose selection: Toxicity Test

Range finding studies: Concentrations tested: 50, 100, 500, 1000, 2000, 3000, 4000, 5000 mcg/plate; The results showed 0% survival at concentrations of 2000-5000 mcg/plate, and 3%, 49%, 114%, and 111% survival at 1000, 500, 100, and 50 mcg/plate, respectively in the test without metabolic activation. In the toxicity test with metabolic activation, there was 0%-2% survival at 3000-5000 mcg/plate, and 7%, 94%, 107%, 112%, and 106% at concentrations of 2000, 1000, 500, 100, and 50 mcg/plate, respectively.

No precipitation was observed at any concentration

Test agent stability: Not provided in this submission

Metabolic activation system: S9 mix prepared from Aroclor 1254 induced microsomal activation enzymes from male Fischer 344 rat livers

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: MNNG (2.5 and 5 mcg/plate) for strains TA1535, TA100, and WPuvrA- without metabolic activation, 9AmAc (50 and 100 mcg/plate) for strain TA1537 without metabolic activation, 2NF (0.5 and 5 mcg/plate) for strain TA98 without metabolic activation, 2AA (1.25, 2.5 mcg/plate) for the *S. typhimurium* strains in the presence of metabolic activation, and 2AA (5 and 10 mcg/plate) for the *E. coli* strain in the presence of metabolic activation with S9.

Comments: Controls were appropriate

Exposure conditions :

Incubation and sampling times: 48 hours

Doses used in definitive study: 62.5, 125, 250, 500, and 750 mcg/plate without metabolic activation; 125, 250, 500, 1000, and 1500 mcg/plate with metabolic activation

Study design: After incubation with the test, negative control and positive control articles in the presence and absence of S9 mix, at 37°C, the revertant colonies were counted 3 times per plate, and mean count/plate recorded.

Analysis:

No. of replicates: 3

Counting method: Colony Counter, colony size discriminator set at lowers limit, 3x/plate; counts corrected for measurement limit of 90% plate

Criteria for positive results: Dose-related increase in revertants exceeding controls by 2-fold, in at least 2 consecutive concentration levels

Summary of individual study findings:

Study validity:

1. Positive control articles induced significant increases in revertants allowing discrimination between strains and demonstrating sensitivity of the system
2. 5% or fewer plates lost through contamination
3. Appropriate strains used
4. Dose selection adequate

Study outcome :

The results of the evaluation of nortomoxetine hydrochloride for the induction of bacterial mutation in the Ames test are presented in the following table:

Treatment	Concentration (mcg/plate)	Revertant Colony Counts (Mean ± S.D.)*				
		TA1535	TA1537	TA98	TA100	WP2uvrA-
Test Without Metabolic Activation						
Nortomoxetine	750	13±5	9±3	9±3	71±7	18±3
	500	20±2	10±2	28±3	82±11	21±3
	250	23±3	9±2	27±5	90±2	23±1
	125	25±4	11±1	22±2	95±6	26±1
	62.5	20±5	9±1	21±2	100±10	26±5
DMSO	0.05 ml	19±6	12±1	21±3	96±1	26±6
	0.05 ml	25±2	9±2	21±1	95±4	24±1
MNNG	5	3201±171	-	-	3072±92	678±32
	2.5	2378±280	-	-	2310±341	322±26

9AmAc	100	-	1335±161	-	-	-
	50	-	107±26	-	-	-
2NF	5	-	-	937±27	-	-
	0.5	-	-	122±26	-	-
Test with Metabolic Activation						
Nortomoxetine	1500	8±3	9±3	7±1	44±12	10±1
	1000	10±2	12±3	37±8	68±6	15±2
	500	14±1	12±3	45±10	105±5	26±1
	250	12±1	12±2	37±4	110±11	25±3
	125	13±1	14±4	42±10	106±5	31±2
DMSO	0.05 ml	15±1	11±2	42±7	108±6	29±3
	0.05 ml	15±2	13±2	34±3	113±15	26±5
2AA	2.5	193±10	322±3	2221±220	2375±144	-
	1.25	105±8	123±2	1174±36	1243±54	-
2AA	10	-	-	-	-	626±11
	5	-	-	-	-	169±25

*Means are for triplicate plates, corrected for 100% plate area

There were no increases in revertant colonies in the nortomoxetine treated plates compared to the control colony counts. In comparison, the positive control articles MNNG, 9AmAc, 2NF, and 2AA increased the revertant colony counts by greater than 2-fold, in a dose related manner. The study was conducted appropriately according to ICH guidelines, including dose and strain selection, and other methods.

Study title: THE EFFECT OF NORTOMOXETINE HYDROCHLORIDE (LILLY COMPOUND 137877) ON THE IN VIVO INDUCTION OF SISTER CHROMATID EXCHANGE IN BONE MARROW OF CHINESE HAMSTERS

Key findings:

- The LY139603 metabolite nortomoxetine hydrochloride (Compound 137877) was negative for sister chromatid exchanges in the bone marrow of Chinese hamsters under the conditions of this study
- However, exposure to Compound 137877 was inadequate, due to absence of a rationale for dose selection in the study referenced for dose selection (study 821005SCE1671), and absence of toxicity, toxicokinetic data, or precipitate

Study no: 870223SCE2808

Study type: *In vivo* mammalian assay for clastogenicity

Volume # 46, and page #: (not paginated)

Conducting laboratory and location: Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana 46140

Date of study initiation: February 23, 1987

GLP compliance: yes (x) no ()

QA reports: yes (x) no ()

Drug Nortomoxetine hydrochloride (Lilly Compound 137877), **lot #:** 687-JJO-244C, **radiolabel:** not applicable, and **% purity:** 98.0%

Formulation/vehicle: Nortomoxetine hydrochloride in 10% (w/v) aqueous acacia

Methods:

Strains/species/cell line: Adult female Chinese hamsters (weights 32-30 g)

Dose selection criteria:

Basis of dose selection: Reference study 821005SCE1671

Range finding studies: In the reference study, LY139603 (parent compound) was administered at doses of 1.63, 3.25, 6.5, and 13 mg/kg PO; no toxicity was observed by the test article

Test agent stability: Not provided in this submission

Metabolic activation system: Not applicable

Controls:

Vehicle: 10% (w/v) aqueous acacia

Negative controls: 10% (w/v) aqueous acacia

Positive controls: Cyclophosphamide

Comments: Appropriate controls were used

Exposure conditions:

Incubation and sampling times: 19 hours

Doses used in definitive study: 3.25, 6.5, 13 mg/kg PO

Analysis:

No. of replicates: 3 animals/treatment group

Counting method: Sister chromatid exchanges (SCE) and first, second and third divisions of metaphase figures were scored using light microscopy

Criteria for positive results: Dose-related increase in SCE frequency with statistical significance ($p \leq 0.01$, compared to controls) in at least 2 successive doses

Summary of individual study findings:

Study validity:

1. Appropriate controls were used
2. Dosing was inadequate due to absence of a rationale for dose selection in the study referenced for dose selection (study 821005SCE1671), and absence of toxicity, toxicokinetic data, or precipitate

Study outcome: There were no increases in mean number of sister chromatid exchanges per metaphase by nortomoxetine at concentrations from 3.25-13 mg/kg PO (means 2.9-3.6, range 2.5-4.0) compared to the control means (mean 3.1, range 2.8-3.6). In comparison, the positive control article cyclophosphamide increased the mean number of SCEs per metaphase (mean 19.6, range 17.1-21.0). Further, there was no shift in the distribution of metaphase figures to the 1st division by nortomoxetine (11%-16% cells in M1) nor by the negative control article 10% aqueous acacia (14% in M1), whereas the positive control cyclophosphamide increased the metaphase figures in the 1st division (mean % cells in M1 34%). Although nortomoxetine was negative for clastogenicity in

typographical error in the submission, because the concentrations are outside the concentrations tested in the mutagenicity assay of up to 1 mg/ml. The study is not deemed valid due to absence of test article stability data, a basis for dose selection, demonstration of final concentrations along the gradient, absence of precipitation and cytotoxicity at the highest concentration tested. The second Ames test, using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA 100, and *Escherichia coli* strain WP2uvrA was conducted appropriately according to ICH guidelines, and showed no potential for genotoxicity by tomoxetine HCl at concentrations of 400-2000 mcg/ml with and without metabolic activation with S9.

Tomoxetine HCl was also negative in three *in vitro* assays for chromosomal damage in mammalian cells. There was no evidence of unscheduled DNA synthesis at concentrations of 0.5-1000 nmoles/ml, measured by increased net nuclear silver grain counts, in the study on induction of DNA repair synthesis in primary cultures of adult rat hepatocytes. No forward mutations were observed at the TK+/- locus of cultured L5178Y mouse lymphoma cells at concentrations of 1-17.5 mcg/ml in the absence of metabolic activation, and at 1-35 mcg/ml in the presence of metabolic activation with S9. In the Chromosome Aberrations study in Chinese hamster ovary cells, a slight increase in percent cells with diplochromosomes was observed at concentrations of 57.5 and 70 mcg/ml in the absence of metabolic activation in the 4-hour exposure and at 35 mcg/ml in the 19-hour exposure, suggesting increased endoreduplication. However, tomoxetine was negative for clastogenicity in that study at concentrations from 40-75 mcg/ml without S9 and 100-118 mcg/ml with S9 for the 4-hour exposure, and at 15-70 mcg/ml without S9 for the 19-hour exposure. The *in vitro* assays for chromosomal damage were conducted appropriately according to ICH guidelines.

The sponsor conducted two *in vivo* assays for chromosomal damage. Tomoxetine HCl was negative for clastogenicity in the sister chromatid exchange study in Chinese hamster bone marrow at oral doses of 1.63-13 mg/kg. No increase in proportion of first division metaphase figures was observed to indicate disruption of the cell cycle, although exposure to the test article did not achieve currently accepted criteria due to absence of a rationale for dose selection, observable toxicity in the animals and toxicokinetic data. Tomoxetine HCl was negative in the Mouse micronucleus test at doses of 58-232 mg/kg PO, measured by the induction of micronuclei in bone marrow cells.

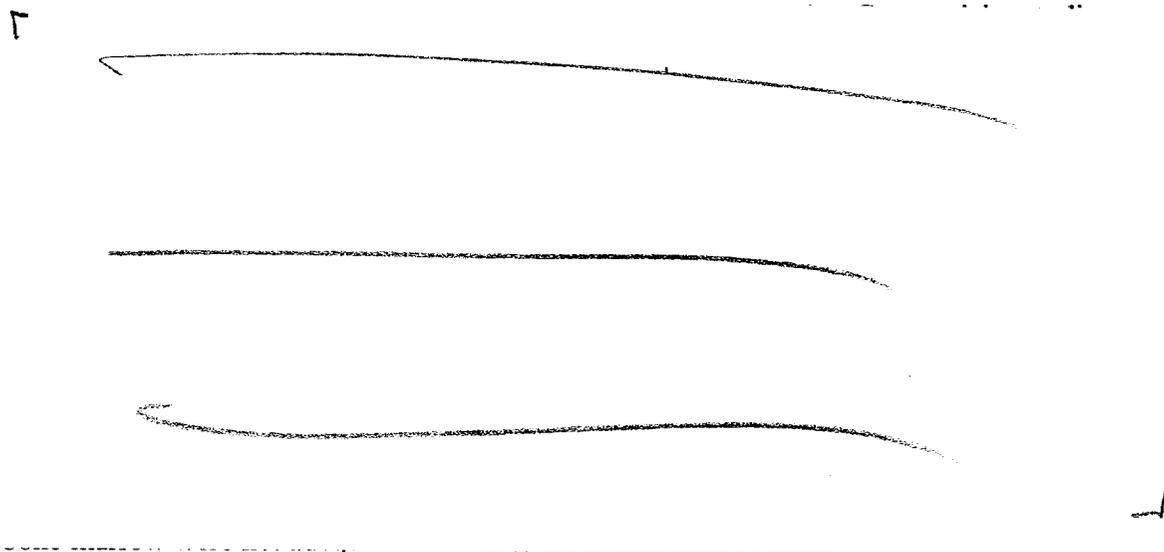
The genotoxic potential of the metabolite nortomoxetine HCl (Compound 137877) was studied in a standard battery that included the Ames test for gene mutation in bacteria, *in vitro* assays for clastogenicity measured by induction of DNA repair synthesis (UDS) and forward mutation at the TK locus of mouse lymphoma cells, and an *in vivo* assay on induction of sister chromatic exchange in Chinese hamster bone marrow. Nortomoxetine HCl was negative for induction of reverse mutations in the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and in the *Escherichia coli* strain WP2uvrA- at concentrations of 62.5-750 mcg/plate without metabolic activation and 125-1500 mcg/plate with metabolic activation with S9. The concentration ranges were appropriately determined based on the results of preliminary toxicity tests. Nortomoxetine was negative in the *in vitro* UDS assay at 0.5-10 mcg/ml and forward

mutation assay in mouse lymphoma cells at 1-70 mcg/ml, with and without metabolic activation. In vivo, nortomoxetine HCl was negative for clastogenicity measured by sister chromatic exchanges in the bone marrow of Chinese hamsters at doses of 3.25-13 mg/kg PO. Although appropriate controls were used in the *in vivo* test, the doses were not supported by data indicating toxicity in the study referenced (Study 821005SCE1671), and therefore, the study is deemed inadequate.

Genetic toxicology conclusions: A standard genotoxicity test battery was conducted for tomoxetine HCl. Tomoxetine was negative for mutagenicity in the standard Ames test and in a modification of the assay using a gradient plate technique. The results of the modified test support those of the standard assay in bacteria, although the concentrations used may be inadequate according to currently accepted criteria. Tomoxetine was also negative for clastogenicity in the *in vitro* assays for unscheduled DNA synthesis in adult rat hepatocytes (UDS assay), forward mutations at the TK locus in L5178Y mouse lymphoma cells, and chromosome aberrations in Chinese hamster ovary cells, and in the *in vivo* assays for sister chromatid exchange in Chinese hamster bone marrow and induction of micronuclei in mouse bone marrow cells.

The metabolite nortomoxetine HCl (Compound 137877) was negative in a standard battery that included the Ames test for gene mutation in bacteria, *in vitro* assays for clastogenicity measured by induction of DNA repair synthesis (UDS) and forward mutation at the TK locus of mouse lymphoma cells, and an *in vivo* assay on induction of sister chromatic exchange in Chinese hamster bone marrow. However, the doses in the *in vivo* study were not supported by data indicating toxicity in the study referenced (Study 821005SCE1671) for dose selection, and therefore, the study is deemed inadequate.

Labeling recommendations: It is recommended that the results of the genotoxicity studies be included in the product label as described under Genetic toxicology conclusions above.



VI. CARCINOGENICITY:

Study title: a chronic toxicity/oncogenicity study in B6C3F1 mice maintained for two years on diets containing tomoxetine (LY139603).

Key study findings: no apparent drug-related tumor findings, however, concerns about adequacy of doses used are expressed.

Study number: two studies were conducted study M03583 and M03683 (replicate studies)

Volume #, and page #: vol. 46-49, toxRpt21 page 1

Conducting laboratory and location: Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Date of study initiation: study M03583 was initiated on November 2, 1983 and study M03683 was initiated on November 15, 1983.

GLP compliance: yes

QA report: Yes (x) No ()

Drug, lot #, and % purity: the followings were obtained from the analytical characterization sheets provided in Appendix D.

866-1G8-070 Nov. 2, 1983-Dec. 26 1984, 99.8% by — (assayed 4-5-83), sponsor indicated that potency was 99.2%

866-1G8-071 Dec. 26, 1984-Aug 6, 1985, 98.9% by — (assayed 4-5-83)

866-83F-249 Aug. 6, 1985 to study termination, 98.9% by GC (assayed 9-15-81)

See following table for other values of impurities assayed at different dates.

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TABLE 1 TEST ARTICLE PURITY VALUES FOR TOMOXETINE FOR STUDIES M03583 AND M03683.

<u>Assay Date</u>	<u>Lot Number</u>	<u>Purity (%)</u>
05/03/84	866-1G8-070	99.12
01/15/85	866-1G8-071	100.3
05/11/85	866-1G8-071	98.7
08/09/85	866-83F-249	101.4
11/27/85	866-83F-249	99.6

CAC concurrence: see CAC report in the addendum

Study Type: 2-year bioassay

Species/strain: mice B6C3F1

Number/sex/group, age at start of study: 30/sex/dose (for each study), 5-6 weeks old

Animal housing: 3/cage. Animals from the two studies (two different shipments of animals) were housed in different rooms; however, according to the sponsor all other aspects (supplier, strain, and experimental protocol) were identical for the two studies.

Formulation/vehicle: test article was administered in a mash feed containing 0.0, 0.03, 0.1, or 0.3% tomoxetine. The sponsor stated that diets were prepared every two weeks and were stored at room temperature in closed containers until used.

Drugs stability/homogeneity: the compound seems to be fairly stable (for 3-4 months) since no major changes in purity were noticed with time (see compound purity summarized previously in table 1 for the same batch at different times). Homogeneity of the compound in the diet was tested and the values indicated acceptable levels of homogeneity. As for the concentration of the test article in the diet, it was reported that the concentrations of the compound found in the 0.03% and 0.1% diet levels prepared on July 9, 1985, were 10 and 12% below the theoretical levels, respectively. The sponsor indicated that there was no explanation for this deviation and that the proper preparation techniques were used. This was not observed in the other preparations (the 0.03% preparation performed on 03-06-84 was 8% less than the theoretical level). The levels of the compound in the diet appeared to be stable for at least 4 weeks.

Methods:

Doses: dietary concentration of 0.0, 0.03, 0.1 or 0.3%. These values provided estimated time-weighted average daily doses of 0, 33.6, 120.1 or 436 mg/kg for M and 0, 33.7, 124.1, or 479 mg/kg for F, respectively. These values were calculated using actual body weight data from the replicate studies combined and historical control food consumption data (See following table in Appendix G provided by the sponsor).

The actual amount of consumed food by mice was not measured, therefore the actual levels of exposure to the compound are not known. The sponsor did not address this issue in the report of this study, however, a later study was conducted to predict the amount of food consumed by mice treated with similar drug levels (see the section "the adequacy of the carcinogenicity study" later in the review for more details).

Basis of dose selection: according to the sponsor, the doses for this study were chosen based on the findings of a subchronic study in which 0.025, 0.1, or 0.4% tomoxetine were administered for three months. According to the sponsor no drug-related mortality or toxicity was observed in that study except at the HD where a decrease in body wt and body wt gain, an increase in relative liver weight, increased hepatic microsomal enzyme activity, and increased incidence of diffuse hepatocyte vacuolization were observed. Accordingly, the sponsor chose the doses for this study on the basis that the highest dose will produce measurable toxic response but it will not increase treatment-related mortality. (According to the reviewer's examination of the data, the decrease in body wt at the HD was ~13% in M and ~9% in F, no deaths were observed, and no other major findings).

Restriction paradigm of dietary restriction studies: no restriction.

Route of administration: dietary

Frequency of drug administration: ad libitum daily for two years

Dual controls employed: one control group was used with each replicate study.

Interim sacrifices: no interim sacrifice was performed

Satellite PK or special study group(s): none.

Deviations from original study protocol: studies were conducted and reported in 1986, however, they were reopened (1997-1998?) in order to conduct further statistical analysis (see the following section for details).

Statistical methods: according to the sponsor, in the previously reported results (1986), the tumor incidence was analyzed statistically but data were not adjusted for survival and the methods did not include Peto's analysis, which according to the sponsor was not available at that time. Therefore, the studies were re-evaluated statistically to address these deficiencies.

Observations and times:

Clinical signs: animals were examined daily for general physical condition and behavior. A detailed examination which included muscle tone, condition of pelage, color and appearance of eyes, respiration, posture, excreta, locomotion and the presence of external lesions or growth were performed weekly.

Body weights: animals were weighed weekly for the first three months and every other week thereafter.

Food consumption: not measured.

Hematology: performed at termination of the study. Mice were fasted overnight and blood samples were obtained by cardiac puncture. The following parameters were evaluated: RBC, Hb, packed cell volume, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, erythrocyte morphology, and total and differential leukocyte counts.

Clinical Chemistry: performed at study termination. The following parameters were evaluated: glucose concentration, urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, and alanine transaminase.

Organ weights: at necropsy the weights of the following organs were recorded: brain, liver, kidneys with adrenals attached, heart, spleen, testes, and uterus with ovaries attached. Relative organ weights to body weight and to brain weight were also calculated.

Gross pathology: all animals were necropsied. The gross examination included general physical condition, body orifices, and external and internal organs and tissues.

Histopathology: performed for all animals at necropsy with the following tissues collected for histopathologic examination: kidney, liver, heart, lung, spleen, thymus, lymph node, salivary gland, pancreas, stomach, duodenum, jejunum, ileum, colon, ovary, uterus, adrenal, thyroid, testis, prostate, skin, mammary gland, skeletal muscle, urinary bladder, bone, bone marrow, eye, cerebrum, cerebellum, brain stem, pituitary, and tissues containing gross lesions.

Toxicokinetics: none.

Results

Mortality: deaths occurred at an earlier time (starting in week 3 of treatment) in the HD males in comparison to controls (first observed in week 64) in both replicate studies. By week 56 about 18% of M at HD (combined studies) died or were killed moribund in comparison to none in the control. By week 83 about 23% of M at the HD were killed or died moribund in comparison to 8% in the control and by week 104 about 32% of M at HD and 27% control M were killed or died moribund (combined studies). At study termination the percent survival of M at the HD in one of the replicate studies (study # M03683) was less than that of the control group (66.7% in treatment vs. 83.3% in control). That was not the case for the other replicate study (study # M03583), where the percent survival of M at the HD were comparable to those of the control at study termination (70% for treatment vs. 63.3% for control). As a result, the sponsor considered this decrease in survival observed in M at HD in the combined study as drug unrelated since it was seen in one of the replicate studies but not the other. According to the sponsor, some of the deaths (4 incidences) that occurred in M at the HD were attributed to fighting among cage mates. This decrease in survival at HD was not observed in F, neither at the level of the individual study or at the level of the combined studies. The following table is a summary table of survival percentages at study termination for both M and F provided by the sponsor:

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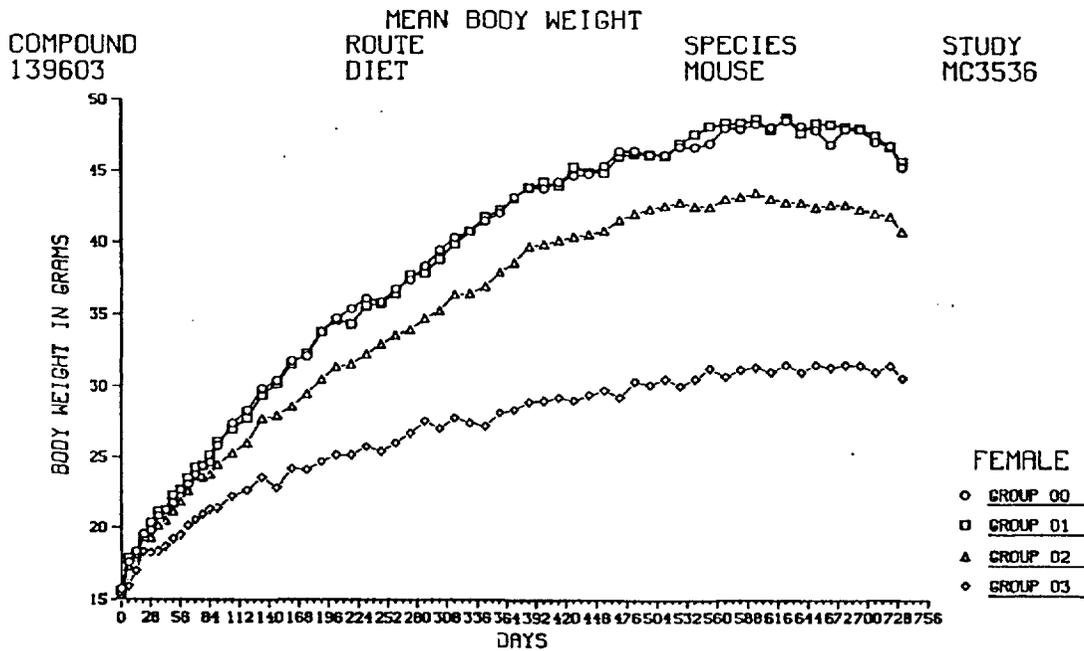
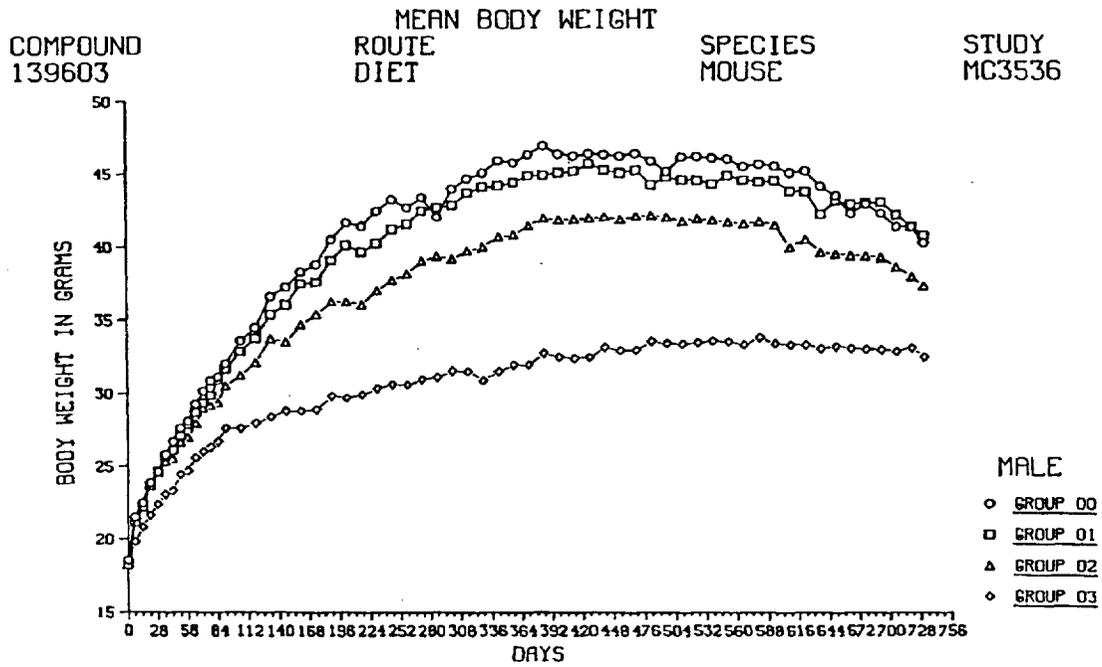
	<u>Treatment Group</u>	<u>M03583</u>	<u>M03683</u>	<u>Replicates Combined</u>
<u>MALES</u>	00	63.3	83.3	73.3
	01	66.7	86.7	76.7
	02	90.0	80.0	85.0
	03	70.0	66.7	68.3
<u>FEMALES</u>	00	80.0	70.0	75.0
	01	76.7	66.7	71.7
	02	83.3	86.7	85.0
	03	83.3	80.0	81.7

The overall survival rate for both sexes for both studies were 74.2, 74.2, 85, and 75% for mice fed 0, 0.03, 0.1, and 0.3% tomoxetine, respectively.

Clinical signs: few clinical observations were reported in drug-treated group and not in the controls. For example aggressiveness was reported in M at the HD (5 total for both studies) and none in the control. The sponsor considered this observation toxicologically insignificant because of the low incidence and stated that this strain of mice is frequently aggressive.

Body weights: for combined studies, mean body wt at termination for M were decreased by 7 and 19% and mean body wt gain was decreased by 12 and 36% in MD and HD in comparison to control, respectively. In F at study termination, mean body wt was decreased by 10 and 32% and mean body wt gain was decreased by 14 and 48% in the MD and HD groups in comparison to control, respectively. According to the sponsor's statistical analysis, changes in body wt were observed around day 40 of the study at the MD in M and around day 90 at the MD in F while changes at the HD were observed from the first week of the study in both M and F. See the following figures for the combined studies (this effect on body wt and body wt gain was fairly consistent in both replicate studies).

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Food consumption: not determined.

Hematology: minor decreases (3%) in HB and PCV at HD in F. Decreases in leukocytes (23, 14, and 34% at LD, MD, and HD, respectively) in M. The sponsor stated that these changes are within the historical control values except for those that were associated with inflammation, degeneration, or neoplasia. No historical values were provided. At the individual data level, some values were high even though no clinical signs (i.e. signs that might indicate an inflammation) were observed in these animals.

Clinical Chemistry: no drug related changes were observed.

Organ weights: some differences between the replicate studies were observed (absolute wt of the spleen in M and F). Variability was seen at the individual data level. As a result, a decrease in absolute wt of spleen was seen in M at HD in the combined studies but this was seen as a decrease in one of the replicate studies (M03583) and an increase in the other study (M03683). In contrast, an increase in the absolute wt of the spleen in F was observed at HD but was seen as a decrease in one of the replicate studies (M03583) and an increase in the other study (M03683). Those changes were also reflected on the relative wt of the spleen to bdwt in both M and F. Decreases (ranged between 15-31%) in the absolute wt of the liver at all doses were observed in the combined studies in M and at HD (14%) in F (no contradicting findings in the replicate studies). This was seen as a decrease (15%) in the relative wt of the liver to bdwt in M at the HD and as an increase (25%) in F at HD in the combined studies (contradicting findings in the replicate studies only in M).

Gross pathology; Whole tissue alteration was described in the liver of F in the drug treated groups but not in the control in study #M03683 (0/30 in control, 3/30 at LD, and 6/30 at HD). In study M03583, whole tissue alteration in F liver was seen in the control 1/30 at MD 2/30 and HD 1/30. When the two studies were combined, the incidence was 1/60 in control, 3/60 in LD, 2/60 in MD and 7/60 in HD. Whole tissue alteration in the spleen was described for F at the MD (1/30) and HD (3/30) and none was described for control or at LD while 1/30 M at the HD and 0/30 of the control group were described (study #M03583, this gross finding was not seen in study M03683).

Histopathology:

Non-neoplastic: liver vacuolation was observed in both control and treated animals, with the incidence being higher in the treatment groups most of the time, even though it did not follow a dose related response (5/60 controls, 12/60 LD, 8/60 MD, and 3/60 HD in M and 5/60 control, 6/60 LD, 2/60 MD and 11/60 HD in F).

Neoplastic: hepatocellular carcinomas were observed in F only in the treatment group and none in the control (1/60 at MD and 1/60 at HD). These tumors were observed both in the control and drug treated groups in M, however, overall their incidences in treatment groups were similar to or lower than those in controls. Accessory ocular adenocarcinoms were seen in M at the MD and HD (1/60 at MD and 1/60 at HD) and none in the control. A summary of benign and malignant tumors is provided by the sponsor in the following tables 3-6.