CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-411

PHARMACOLOGY REVIEW

PHRMACOLOGY/TOXICOLOGY COVER SHEET

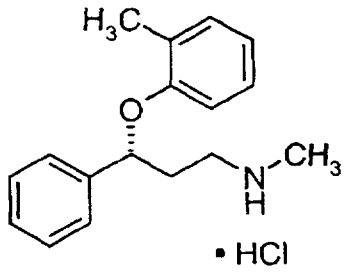
NDA number: 21411
Review number: 001
Sequence number/date/type of submission: 000/Oct 12, 2001
Information to sponsor: Yes (x) No ()
Sponsor and/or agent: Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

Manufacturer for drug substance: all drug substance lots submitted to the NDA have been manufactured at the Tippecanoe, IN facility of Eli Lilly.

Reviewer name: Ikram M. Elayan
Kathy Haberny (Genotoxic and Safety Pharmacology studies)
Division name: Neuropharmacological Drug Products
HFD#: 120
Review completion date: August 5, 2002

Drug:

Trade name: Straterra
Generic name: atomoxetine hydrochloride
Code name: LY139603
Chemical name: benzenepropanamine, N-methyl-gamma-(2-methylphenoxy), hydrochloride, (-)
CAS registry number: 82248-59-7
Mole file number:
Molecular formula/molecular weight: $C_{17}H_{21}NO.HCl/291.82$
Structure:



Relevant INDs/NDAs/DMFs: IND —

Drug class: norepinephrine reuptake inhibitor

Indication: treatment of attention deficit/hyperactivity disorder (ADHD) in children, adolescents, and adults.

Clinical formulation: capsules 5, 10, 18, 25, 40, and 60-mg

Route of administration: oral

Proposed use: treatment of ADHD in children, adolescents, and adults.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

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Executive Summary

I. Recommendations

A. Recommendation on Approvability

From a pharmacology/toxicology point of view, the NDA is approvable with commitment to perform phase IV studies (genotoxicity, reproductive segment II in rats, and juvenile studies) to qualify impurities (compound

B. Recommendation for Nonclinical Studies:

Two impurities were found in the drug substance, compound and compound Compound was found to be qualified based on its presence up to in some of the lots used in animal studies (genotoxic studies, 3-month toxicity study in mice, segment II rabbit, and juvenile studies). Compound was found not to be qualified since its levels in batches used in non-clinical studies did not reach the proposed level. Therefore, a decision was made by the division to ask for phase IV commitment studies as detailed here:

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Commitments from sponsor to perform these studies are still pending. In a response from Eli Lilly dated July 16, 2002, the sponsor has agreed with the FDA to conduct juvenile studies with doses of atomoxetine spiked with up to of the compound However, the sponsor does not agree with the FDA requirement for genotoxic studies to qualify the impurity because the levels of this impurity in the genotoxic studies are higher than those in humans ("concentrations of tested in the in vitro genetic toxicity studies were higher than in humans"). We do not agree with the sponsor since the concentration of the impurity in the genotoxicity studies is not decided on the basis of the fold difference from the human dose. As for the segment II (teratology) studies, we asked that these studies be done if results of the studies reviewed here indicate something of a concern. The sponsor responded by saying "We acknowledge

FDA's decision, and are confident that there are no findings of concern in the completed study". Based on the findings in rabbits (abnormalities in major blood vessels) a study with rats treated with atomoxetine spiked with the impurity [redacted] will be needed.

Upon their final review, CMC team have indicated that solvent [redacted] present in the manufacturing process of the drug substance was not included in the final drug substance specifications (see page 33 and 91 of the CMC review in DFS). CMC has requested a specification limit for this solvent along with other residual solvents with justification and reflective batch data (See CMC Review, Deficiency Letter comment #5). Upon receipt of the information from the sponsor, CMC will decide if the specification and acceptance criteria proposed for this solvent require toxicological qualification, then non-clinical toxicity studies will be requested from the sponsor.

C. Recommendations on labeling: proposed labelings are included here

[redacted]

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Pharmacodynamics: in *in vitro* studies in membranes expressing the human NE, DA and serotonin transporters, atomoxetine inhibited the NE transporter with a Ki value that was 16 times lower than that for the serotonin and 270 times lower than that for the DA transporter. Of the two major metabolites, the 4-hydroxymetabolite had similar effects on the NE transporter as the parent. In addition to an increase in NE levels, *in vivo* microdialysis studies in rats indicated an increase in DA levels in the prefrontal cortex as a result of treatment with atomoxetine by IP and local injection into the brain structure. The increase in DA is probably caused by the blockade of the NE transporter since NE neurons in the prefrontal cortex were shown to accumulate DA. In addition, an increase in serotonin in the prefrontal cortex and striatum was seen with IP injection of atomoxetine, which could be due to "lost specificity" at these drug levels, as stated by the sponsor. This might be a possibility since lower doses did not have an effect on serotonin.

Pharmacokinetic: the drug plasma levels were increasing with dose and there were no differences between sexes in treated animals (except mice). Some decline was seen in rats with repeated treatment but this was not confirmed in later studies. Young rats had higher plasma levels compared to mature animals while the opposite was observed in dogs. Oral bioavailability was low in mouse (5%) and rats (4%) and moderate in rhesus monkeys (45%) and dogs (74%). In rats the drug was rapidly distributed to tissues and it was excreted in milk and radioactivity transferred through the placenta after treatment with radioactive atomoxetine in pregnant females. Atomoxetine is extensively metabolized and the two major metabolites were 4-hydroxyatomoxetine and N-desmethylatomoxetine. In humans aromatic hydroxylation appears to be mainly mediated by CYP2D6. Two minor metabolites that were detected in humans but not in animals were hydroxy-carboxyatomoxetine-O-glucuronide and 2,4-dihydroxyatomoxetine. The major phase I metabolic pathways were aromatic ring hydroxylation, benzylic/aliphatic hydroxylation, and N-demethylation. Subsequent glucurodination of the hydroxylated metabolites was the only phase II metabolic pathway to result in conjugation in mouse, rat, and human. In addition to glucurodination, dogs also expressed O-sulfation as phase II metabolism. The major route of elimination was through urine even though some radioactivity was seen in feces of animals treated with radioactive atomoxetine, which appeared to be due to biliary elimination of radioactive metabolites. The drug is highly bound to proteins (98.7%) in humans and animals (82% in mice, 96.7% in beagle dogs). The drug strongly induces CYP2B and weakly CYP1A and CYP3A in mice. Slight increases in CYP450 levels were seen in rats treated with 50 mg/kg with induction in CYP1A and 2B observed and an increase in total CYP450 levels was seen in male beagle dogs.

General toxicology: in multiple dose studies in rats (1 month to 1 year) orally administered atomoxetine in diet [up to 50 mg/kg, ~4x the maximum recommended human dose (MRHD) based on a mg/m^2] or by gavage (up to 160 mg/kg) produced decreases in body weight, body weight gain, and food consumption. In addition some clinical signs such as salivation, soiling, and respiratory effects were seen in some of these studies. No serious side effects were noticed however the liver seemed to be affected by treatment with some changes observed such as mottling, pallor, and increased incidence of focal vacuolization. In dogs treated with atomoxetine up to 16 mg/kg for one year tremors and mydriasis were observed. No apparent effect on body weight and food consumption was observed even though anorexia was described as part of the clinical signs. In a study with IV administration of the drug (up to 10 mg/kg) decreased food consumption but not body wt were observed. Tremors and hyperactivity were seen at high dose.

Safety Pharmacology:

A Core Battery of GLP Safety Pharmacology studies on the effects of tomoxetine HCL (LY139603) on vital functions were performed, including evaluation of central nervous system (CNS), cardiovascular system (CV) and respiratory system (RS) parameters. The primary pharmacodynamic studies demonstrated that tomoxetine HCl is a selective

norepinephrine reuptake inhibitor. The adverse effects associated with norepinephrine reuptake inhibition, commonly observed in other drugs in this therapeutic class, can include agitation, seizures, sedation, hypotention, anticholinergic effects, weight gain, sexual effects and cardiac effects.

The undesirable pharmacodynamic effects of tomoxetine, with relevance to human safety, were observed in the central nervous and cardiovascular systems in the preclinical safety pharmacology studies. The neurological effects of very high doses in mice included deaths associated with clonic convulsions (300 and 400 mg/kg dose, 13.5X and 18X the MRHD of 1.8 mg/kg on a BSA basis), myoclonic jerking, decreased body temperature, decreased motor activity, lethargy, irritability, leg weakness, jerky gait, exophthalmos, and piloerection, tremors (particularly when walking), grasping loss, pinna reflex, mydriasis, lacrimation, vibrissal response, analgesia, placing loss, decreased abdominal tone, corneal loss, and righting loss. Tomoxetine had no effects on locomotion at doses of up to 30 mg/kg PO, suggesting a low potential for producing psychomotor stimulation in clinical use. A dose-related increase in hexobarbital sleeping time was observed at doses of 6.26-50 mg/kg PO. Decreased weight and rectal temperature, and antagonism of hypothermia by apomorphine are expected effects of norepinephrine uptake inhibition. In young beagle dogs administered 4-16 mg/kg PO tomoxetine HCl for 4 weeks (0.8X-2.6X the MRHD in poor metabolizers and 2.4X-7.6X the MRHD in extensive metabolizers on an AUC basis), pupillary light reflex was decreased, and the incidence of mydriasis increased with dose, but no other treatment-related effects were observed in the neurological examination. Tomoxetine increased cocaine-like responding rates at IP doses of 5-50 mg/kg, and produced seizures at 50 mg/kg IP in rats, but was without cocaine discriminative stimulus or behavioral effects, and produced no seizures at up to 10 mg/kg IM in monkeys.

Based on the known pharmacology of norepinephrine, norepinephrine reuptake inhibition may induce cardiovascular effects of increased cardiac stroke volume, arrhythmias and coronary blood flow, increased systolic, mean arterial, diastolic and mean pulmonary blood pressure, increased total peripheral resistance, and increased respiration. Appropriate *in vitro* and *in vivo* assessments were made to address the potential by tomoxetine HCl to induce repolarization and conductance abnormalities, and to evaluate effects on blood pressure, heart rate, and the electrocardiographic parameters. The safety pharmacology studies on cardiovascular toxicity were conducted in isolated canine purkinje fibers, human embryonic kidney cells transfected with the HERG clone to express I_{Kr} channels, anesthetized beagle dogs, and conscious mongrel dogs. Additionally, potential cardiovascular toxicity was evaluated in a 4-week oral capsule toxicity and toxicokinetic study in young beagle dogs.

Tomoxetine HCl decreased the maximum rate of rise of the action potential (Vmax) in isolated canine cardiac purkinje fibers at a concentration of 10^{-5} M, suggesting a potential for interference with cardiac conduction, although tomoxetine was half as potent as the approved antidepressant drug amitriptyline in this effect. Blockade of the I_{Kr} (HERG) by tomoxetine and the metabolites N-desmethyltomoxetine and 4-hydroxytomoxetine in transfected human embryonic kidney cells at clinically relevant concentrations suggested a potential for Q-T prolongation and predisposition to the occurrence of ventricular arrhythmias, and signaled the need for further assessment in an *in vivo* model. In anesthetized dogs, intravenous tomoxetine increased heart rate to a lesser extent, and

increased respiratory rate to a similar extent compared to intravenous amitriptyline. There was no effect by tomoxetine on the QRS duration, but a negative dromotropic effect, with prolongation of the P-R interval, was observed after both intravenous tomoxetine and intravenous amitriptyline. Tomoxetine increased the Q-TC interval as much as 22%-32% at 10 mg/kg IV in the anesthetized dogs, with and without pretreatment with atropine and propranolol, although the changes were not statistically significant. Oral tomoxetine had no effects on heart rate, respiratory rate, and ECG parameters at single doses up to 16 mg/kg (5X the MRHD of 1.8 mg/kg on a BSA basis) in conscious dogs, and after daily oral administration at up to 16 mg/kg (2.6X the MRHD in poor metabolizers and 7.6X the MRHD in extensive metabolizers on an AUC basis), for 4 weeks in young beagle dogs. However, the results of *in vitro* and anesthetized dog studies indicate that careful ECG monitoring should be conducted in the clinical setting.

Tomoxetine HCl had no effects on respiratory function at 10-100 mg/kg PO (.9X-9X the MRHD of 1.8 mg/kg on a BSA basis) in rats. The renal effects in rats were similar to those observed by the antidepressant drugs imipramine and desipramine, and included mild, dose-related diuresis and decreased osmolality, without changes in the concentrations of sodium, potassium and chloride at 10 and 50 mg/kg PO in the rats. Creatinine excretion was increased and concentration decreased at 50 mg/kg PO (4.5X the MRHD on a BSA basis). No agonist effects were observed in isolated guinea pig ileum and rabbit jejunum at concentrations up to 10^{-5}M, and there were no effects on gastrointestinal motility in mice. Also, tomoxetine had no effects on immune response, measured by alteration of hemagglutinin concentration in response to sheep red blood cell antigen injection, at oral daily doses of 1.6-25 mg/kg (.01X-1X the MRHD of 1.8 mg/kg on a BSA basis) for 10 days in male mice. Evaluation of receptor-binding showed poor affinity by tomoxetine and the p-hydroxy and N-desmethyl metabolites for muscarinic, alpha1-adrenergic, alpha2-adrenergic, beta-adrenergic, serotonin (5-HT_2), histamine (H_1), $GABA_A$ and benzodiazepine receptors, and dopamine (D_1 and D_2) receptors, suggesting little potential for interaction with the sympathetic and parasympathetic control of peripheral organ function. There were no effects at concentrations up to 1 mcM in isolated guinea pig smooth (ileum) and cardiac muscle tissue bath preparations, and no inhibition of acetylcholine-induced (ileum) and isoproterenol-induced (atria) contractions.

Genetic toxicology:

Tomoxetine HCl was negative in two Ames tests, evaluating the mutagenic potential in the bacterial strains *Salmonella typhimurium* and *Escherichia coli.* The first bacterial assay was a modification of the Ames test using a gradient plate technique that 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 or 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 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 negative in three *in vitro* assays and in two *in vivo* assays for chromosomal damage in mammalian cells. There was no evidence of unscheduled DNA synthesis at concentrations of 0.5-1000 nmoles/ml 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. 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. 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 chromatid exchanges in the bone marrow of Chinese hamsters at doses of 3.25-13 mg/kg PO. The dose selection was not supported by data indicating toxicity in the sister chromatid study, and therefore, the *in vivo* study is deemed inadequate.

Carcinogenicity: two replicate studies in both rats and mice were conducted with a total of 60 animals/group/sex in each species. Drug was administered in food containing, 0.0, 0.03, 0.1 or 0.3% tomoxetine in mice and 0.0, 0.01, 0.03, or 0.1% tomoxetine in rats. These levels provided an estimated (since no food intake was measured) time-weighted average daily dose of 0, 34, 120 or 436 mg/kg in M mice and 0, 34, 124, 479 mg/kg in F mice (~20x MRHD based on mg/m^2 value). However, if a decrease in food consumption in these mice is assumed, these doses will be about 10% less as discussed in the review of that study. In rats these levels provided time-weighted average daily doses of 0, 4, 13, or 43 mg/kg for M and 0, 5, 15, or 51 mg/kg for F (~4x MRHD based on mg/m^2 value, 1.4x in EM and 0.15x in PM based on AUC value). Body wt was decreased in M and F mice at the MD and HD. In rats there was a slight decrease in body wt in M at the HD and at all doses in F. A decrease in food consumption was seen at MD early in the study and at HD throughout the study in M rats and at all doses

throughout the study in F. No measurement of food consumption was done in mice, but this was assumed to be decreased based on a subsequent study. As discussed later in this review (carcinogenicity section) it was felt that decrease palatability was not a reason for the decrease in food consumption. No major changes in hematology or clinical chemistry were seen in mice. In rats, an increase in glucose levels was seen in M and an increase in BUN was seen in M at MD and HD and at all doses in F. Pathological changes were seen in the liver in mice and rats and these included nodular changes (M and F mice) and whole tissue alteration (F mice) and liver "lesions" in M rats at the HD and histologically, vacuolation in both M and F rats. Increased incidence of cataracts in M and F rats at HD. The incidence of fibroadenoma of the mammary gland in F rats appeared to decline with treatment and the incidence of mononuclear cell leukemia in M and F rats was also decreased with treatment. No clear increases in tumor incidences were seen in either mice or rats.

Reproductive and developmental toxicology: one study in rats consisted of 4 substudies: 1) a fertility study where males treated for 10 weeks prior to mating and throughout two mating trials were mated with females treated with atomoxetine for two weeks prior to mating and throughout one breeding trial. 2) Females pretreated with the drug for two weeks prior to mating with males from the fertility study throughout one breeding trial, designated as F0 females of the delivery component, were allowed to deliver and rear their progeny. 3) A teratology study, where females were treated with atomoxetine for two weeks prior to mating with males of the fertility study to gestation day 20 when they were terminated and their fetuses were examined for teratological and prenatal drug effects. 4) A postnatal study for behavioral and fertility assessment of the F1 generation. CD rats were used (20/sex/group for all studies except for the postnatal study, 16-19/sex/group). Treated animals received 0, 0.01, 0.03, and 0.06% atomoxetine in their diets which provided a time-weighted average doses of 0, 7, 20, and 40 mg/kg/day for males and 0, 7, 20 and 41 mg/kg/day for females (~3.6x MRHD based on mg/m^2 value). Body wt and food consumption was measured for all parental animals in F0 and F1 generation. Reproduction and progeny measurements were performed for F0 females of the delivery component. Reproduction and fetal measurements (external, visceral, and skeletal) were performed for the F0 females of the teratology component. Behavioral and fertility assessments were performed for the F1 generation. No mortality was observed in any of the parental animals in F0 or F1 generation. No serious clinical signs were observed but higher incidences of "injury" (no details about their nature were provided) in F0 males were noticed. Decreases in body wt were seen in F0 males and females of both the delivery and teratology component mostly at MD and HD. No decreases in body wt were seen in F1 generation. Decreases in food consumption were seen in F0 generation of both males and females but not in F1 generation. There was no drug effect on mating index, fertility index, or precoital period in both the teratology and delivery components. There was no drug effect on gestation length, litter size, or live birth index in the delivery component. A slightly higher incidence of early resorptions in the teratology study at HD compared to the control. Some effects on physical development, or "morphological development" as called by the sponsor, in the progeny of females of the delivery component were seen as delayed incisor eruption and eye opening in the MD and HD animals compared to control. A 7% decrease in female fetal wt at HD was seen

in the teratology component. There was no drug effect on visceral or external parameters in fetuses of the teratology component. A slight increase in the incidence of incomplete ossification of several bones was noted. No drug effect was seen on behavior, fertility, or mating indexes, nor on production parameters in F1 generation. No drug effect on body wt of progeny (F2 generation) or sex ratio.
In another basic fertility study, 10 Wistar rats/sex/group were treated with 0, 0.02, 0.04, or 0.08% tomoxetine in the diet which provided a time weighted average daily doses of approximately 15, 29, or 57 mg/kg for males and 12, 23, or 46 mg/kg for females. Females were treated two weeks premating through lactation (daily doses were higher during lactation due to increased food consumption, values were approximately 24, 48, 83 mg/kg/day) while males were treated for 10 weeks prior to mating. A decrease in body weight and food consumption was observed in both males and females. No drug effect on fertility, mean gestation length, or gestational survival. There was a decrease in the survival of the progeny of females treated with MD and HD (% survival was 96, 92, 63, and 42% in the control, LD, MD, and HD respectively). Most of the progeny mortality was seen prior to postpartum day seven. The mean body weights of the HD progeny were lower than the control group on postnatal days 1 and 7. A slight decrease was still seen on postpartum days 14 and 21 but it was not statistically significant. According to the sponsor "no noteworthy necropsy findings in the 31 progeny that died prior to postpartum day 21".
In a teratology study in Wistar rats, 25 females/group were treated with 0, 25, 60, and 150 mg/kg/day tomoxetine (up to 13.5 MRHD on a mg/m^2 basis) orally by gavage from gestation day 6 to 15 (organogenesis period). One female at HD died and decreases in body wt and food consumption were seen at HD. There was no effect for the drug on reproduction parameters (# of corpora lutea, implantations, and live fetuses), however, a slightly higher incidence of resorptions was seen at MD and HD compared to control. No fetal anomalies (external, visceral, and skeletal) were increased with drug treatment.

Teratology studies were performed in rabbits according to ICH guidelines requiring fertility studies in a second mammalian species.
New Zealand White time-mated female rabbits (20/group) were treated with 0, 10, 30, and 100 mg/kg/day atomoxetine (2.6x MRHD in EM and 0.3x in PM based on AUC value, and 18 times the MRHD based on mg/m^2 value) orally by gavage on gestation day 7 through 19. Animals were observed for survival and clinical signs and body weight and food consumption were measured. The uterus and ovaries were weighed and the number of corpora lutea, implantations, and preimplantation loss were evaluated. Fetal wt, gender, and morphology were evaluated. Live fetuses were evaluated for external, visceral, and skeletal anomalies. There was no mortality or abortions reported. Slight decreases in body wt at HD and statistically significant decreases in food consumption at MD and HD. A slight increase in early resorptions was seen in females treated with HD. A slight decrease in percent of live fetuses was seen at HD. Female fetal weight at HD was slightly decreased. Even though not statistically significant, the number of fetuses with malformations/litter at MD and HD was increased. These were mainly male fetuses. The malformations (as called by the sponsor) observed were cardiovascular and mostly an increase in the incidence of "atypical origin of the common carotid artery" and absence of subclavian artery at HD. The incidences were greatly above the historical

control values. Other cardiovascular anomalies which were observed in fetuses of treated animals (one fetus from HD group for each anomaly) included enlarged heart, small heart ventricle, absent heart papillary muscle, focal thinning of the heart ventricle, and fused aorta/pulmonary artery. Some of these anomalies (absent heart papillary muscle and focal thinning of ventricle) were seen in the same fetus of the control group while the other anomalies (enlarged heart, small ventricle, and fused aorta/pulmonary artery) were not seen in control. However, the small number of affected fetuses in the treated groups might argue against the significance of these findings. Some skeletal deviations that were observed included incomplete ossification of the forepaw (HD).

A second study in rabbits was performed probably to follow up on the findings in the major blood vessels, even though this was not explicitly expressed by the sponsor. The study was performed in an outside site where 22/sex/group artificially inseminated New Zealand White rabbits were treated orally by gavage with 0, 10, 30, 100 and 150 mg/kg atomoxetine from gestational day (GD) 7-19. Survival, clinical observation, food consumption, and body wt were evaluated. Maternal parameters including: the number of corpora lutea, the number of implantations, the number and location of all fetuses, and early and late resorptions were evaluated in the dams. Fetal examination included external, visceral, and skeletal parameters. There was one death at 30, one death at 100, and 3 deaths at 150 mg/kg groups. The death at the 30 mg/kg dose was considered drug unrelated. The animals at the 150 mg/kg dose were terminated early due to toxicity. A slight decrease in body wt was at 100 mg/kg and a larger decrease at 150 mg/kg. Decreases in food consumption were seen at the 100 and 150 mg/kg. Some variations (as called by the sponsor) in the origin of the carotid artery were seen in the treated group compared to the control. Left carotid artery was originating from the brachicephalic trunk in 6(5), 5(2), 3(3), and 11(9) fetuses (litter) in the control, 10, 30, and 100 mg/kg group. The sponsor indicated that it was within the historical control data. However the values presented (0-31.5%) were for the category "major blood vessel variation" and not specifically for this type of variation (origin of carotid artery).

An earlier teratology study (1982) of compound LY139603 administered orally to Dutch Belted rabbits is included here, however since the number of animals in this study were not sufficient the previously reviewed studies were conducted. In this study, artificially inseminated female Dutch Belted rabbits (15/group) were treated with 0, 25, 50, and 100 mg/kg/day tomoxetine orally by gavage from gestation day 6 to 18. One rabbit from the control group died on gestation day 24. Body wt did not appear to be affected while food consumption was decreased. No effects on the following reproduction parameters: corpora lutea, implantations, and live fetuses. However, there appeared to be an increase in resorptions at HD compared to the control group. The sponsor did not consider this as drug related. There was no effect for the drug on fetal wt. External, visceral, and skeletal examinations did not indicate a drug effect.

Special toxicology studies (juvenile animal studies): studies were done in young rats treated from postnatal day (PND) 10. Those studies were performed to investigate the effect of the drug on fertility, reproduction, and neurobehavioral development in response to drug treatment at a young age.

In one study, 10-day old rats were treated with 0, 1, 10, and 50 mg/kg/day tomoxetine ($\leq$13x MRHD in EM and $\leq$1.5x MRHD in PM based on AUC values) from PND 10 to

PND 84. A companion blood level study was conducted. Slight decreases in body wt were seen in M and F at HD. Slight decreases in food consumption were seen in M and F at HD. Decreases in vaginal patency were observed in F with treatment (1.3, 1.4, and 2.2 days delay in the vaginal patency in the LD, MD, and HD groups in comparison to the control). In M there was a delay in the onset of preputial separation with treatment, the average postnatal days for preputial separation were 42.3, 42.8, 43.7, and 44.9 in control, LD, MD, and HD group respectively (not statistically significant at LD). The cauda epididymal wt was decreased with treatment at the MD and HD. The number of sperms/cauda were decreased at MD and HD, however, when expressed per gram of tissue, there was no apparent drug effect. Slight decreases in the absolute wt of some organs in M (heart, spleen, prostate, and adrenals) and F (spleen, ovaries, and pituitary) at HD. No drug effect on histopathology or femoral length. In the toxicokinetic study it was apparent that there was decreased plasma levels in matured animals in comparison to the young.

The effect of the drug on fertility and reproduction was tested in 10-day old rats (20/sex/group) that were treated with 0, 1, 10, and 50 mg/kg/day from PND 10 through maturation (approximately 77 days), through mating and until gestation day 6 (females) or prior to termination (males). Animals were observed daily; food consumption and body wts were evaluated. Mating performance and fertility were evaluated (time to mating, mating index, and fertility index). Two M at HD died (one was due to gavage error). Increased activity and muscle tremors were seen at HD in both M and F. Body wt was decreased at HD in M and a slight decrease was seen in F at HD but was not statistically significant at all times. There are no drug effects on mating performance and fertility. There was a decrease in the number of corpora lutea at HD. There was less implantations at HD but there was no drug effect on % of conceptuses, pre- or post-implantation loss.

In another study the effect of the drug on physical and neurobehavioral development was examined in 10-day old rats that were treated with 0, 1, 10, and 50 mg/kg atomoxetine by gavage through PND 84. On PNDs 11 through 12 rats were examined for positive signs of incisor eruption and on PNDs 15 through 18 for positive signs of eye opening (these were called "developmental landmarks" by the sponsor). On PND 19 and again on PND 55 auditory startle habituation was monitored, on PNDs 15, 30, and 60 activity levels were monitored, and on PND 20 and 83 rats were tested in a passive avoidance task (neurobehavioral development). In addition animals were observed daily for survival and general physical condition. Body wt and food consumption were measured at certain days. One M at HD died on Test Day 45, the sponsor considered the death drug unrelated. Intermittent tremors were observed at HD in 1 M and 1 F. Leg weakness was observed in 2 F at HD. Increased activity was seen in 4 M and 3 F at HD. Body wt was decreased in M and F at HD throughout the study. Decreases in food consumption were observed in M at HD and to a lesser extent in F. A slight delay in the onset of incisor eruption in M at MD and HD and only at HD in F. However, all animals reached this developmental landmark at PND 12. Eye opening was not affected by drug treatment. On day 19, a slightly higher peak auditory startle response in M at all doses compared to those seen in the control group. These changes were not statistically significant. There was an increase in activity in M at MD and HD and in F at HD on day 15. No meaningful drug effects on the passive avoidance test were observed.

B. Pharmacologic Activity: see pharmacodynamic section earlier

C. Nonclinical Safety Issues Relevant to Clinical Use:

From both *in vitro* [decreased Vmax of action potential in isolated canine cardiac purkinje fibers and blockade of the I_{kr} (HERG) channel] and *in vivo* (increased Q-T by 22-32% in anesthetized dogs) it appears that atomoxetine had a potential for Q-TC prolongation. However, other *in vivo* studies (single oral administration in conscious dogs and oral administration for 4 weeks in conscious young dogs) did not show an effect on heart rate, respiratory rate, or ECG parameters. In light of all these findings it is recommended that careful ECG monitoring be conducted in the clinical setting.

Another effect of atomoxetine that was observed generally is the decrease in body weight and food consumption in treated animals (mainly rodents) compared to control. These changes might have been the potential cause of several variable observations such as delayed onset of puberty in young animals together with delayed physical development in young animals. In addition to its effect on young animals, effects were also noticed on the progeny of pregnant animals treated with the drug where a decrease in fetal survival and weight of fetuses was observed compared to control animals. Even though not directly proven, all these effects might reflect an effect of the drug on "growth". Since this drug is indicated to young population, close monitoring of growth in this population is recommended.

Due to the abnormalities observed in pregnant rabbits treated with atomoxetine during organogenesis (atypical origin of the carotid artery and missing subclavian artery) it is recommended that atomoxetine not be given to women of child bearing potential.

III. Administrative

A. Reviewer signature:

B. Supervisor signature: Concurrence-
Non-concurrence-
(see memo attached)

C. cc.list:

TABLE OF CONTENTS-PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMCOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: atomoxetine inhibited the human NE transporter with a Ki value of 5.36 nM which was 16 times lower than for the serotonin transporter (Ki = 87 nM) and 270 times lower than that for the dopamine (DA) transporter (Ki = 1451 nM), as measured by an *in vitro* assay. Of the two major metabolites 4-hydroxyatomoxetine (424478) exhibited similar, yet slightly lower (5x lower), affinity for the human NE transporter (Ki = 25.2 nM) in comparison to the N-desmethylatomoxetine (137877) which exhibited a 146 fold lower affinity (Ki = 780 nM). However, the 4-hydroxymetbolite exhibited a slightly higher affinity (2.4x) for the serotonin transporter compared to the parent. See the following table.

Table 1: [^{3}H]-Radioligand Binding to Membranes Expressing Human Monoamine Transporters

	K_i, nM		
	[^{3}H]-paroxetine 5HT	[^{3}H]-nisoxetine NE	[^{3}H]-mazindol DA
Atomoxetine	87.0 ± 9.0	5.36 ± 0.22	1451 ± 30.1
N-desmethyl-atomoxetine (137877)	361 ± 67	780 n = 1	-
4-hydroxy-atomoxetine (424478)	35.8 ± 1.05	25.2 n = 1	-
N-desmethyl-4-hydroxy-atomoxetine (440032)	-	976 n = 1	-

N = 3 or more separate determinations, unless otherwise noted.

Similar findings were observed in rat hypothalamic synaptosomes with atomoxetine and its 4-hydroxy metabolite having the same inhibitory effect on the NE transporter (Ki value = 4.47 nM for atomoxetine and 3 nM for 4-hydroxyatomoxetine). Generally, similar effects on the DA and serotonin transporter to those seen in humans. See the following table for Ki values in rats.

Table 1: **K_i values for Atomoxetine (LY139603), 4-Hydroxyatomoxetine (LY424478) and N-Desmethylatomoxetine (LY137877) for Uptake of ^{3}H-Serotonin, ^{3}H-Norepinephrine, and ^{3}H-Dopamine in Rat Brain Synaptosomes**

Inhibition of Monoamine Uptake (Ki, nM)			
Compound	^{3}H-Serotonin in Cortex	^{3}H-Norepinephrine in hypothalamus	^{3}H-Dopamine in striatum
Atomoxetine	152.23 ± 18.65 n=3	4.47 ± 0.77 n=4	657.60 ± 22.30 n=4
4-Hydroxyatomoxetine	42.65 ± 2.93 n=3	3.00 ± 0.25 n=5	575.95 ± 62.34 n=3
N-Desmethylatomoxetine	649.14 ± 88.73 n=3	92.12 ± 10.29 n=4	1430.68 ± 242.83 n=4

Table adapted from Table 1 in Nonclinical Pharmacology Report 10.

In vivo microdialysis studies in Sprague Dawley rats indicated that atomoxetine increases extracellular levels of NE and DA in the prefrontal cortex when the drug was administered intraperitoneally (IP, starting at 0.1 mg/kg up to 10 mb/kg) and locally through the dialysis probe. The sponsor has indicated that NE neurons in this structure are responsible for substantial accumulation of DA and therefore an increase in DA levels was seen as a result of blockade of the NE transporter. The drug did not increase extracellular DA levels in the striatum or the nucleus acumbens, a finding that could support the previous observation in the frontal cortex, since little NE neurons are found in these two structures. In addition this finding that atomoxetine does not increase DA levels in the striatum or the nucleus acumbens might be important for studying the rewarding effects of atomoxetine since stimulant drugs usually increase DA levels in these structures.

Another interesting finding in the microdialysis studies was an increase in serotonin levels that was seen in the frontal cortex when atomoxetine was given IP at high doses (3 and 10 mg/kg). The sponsor stated that at this "relatively high dose" the selectivity for NE inhibition is "probably lost". However when the drug was administered directly through the dialysis probe (0.34 and 1.03 μM) into the rat prefrontal cortex this effect on serotonin was not observed. The proposal that at those dose levels (3 and 10 mg/kg, IP) selectivity to the NE transporter is lost might be possible since lower doses (0.1-1 mg/kg, IP) did not have an effect on serotonin.

A NovaScreen test was performed where the effect of atomoxetine and its two major metabolites on different receptors and transporters was evaluated (adrenergic, cholinergic, adenosine, dopaminergic, gabaergic, glutamatergic, histamine, serotonin receptors, calcium channels, potassium channels, and a variety of other channels and receptors). At a concentration of 1 μM atomoxetine, the sponsor considered only those inhibitions that were >50%. At that concentration, atomoxetine inhibited sigma 1

receptor (51.4%). At 1 μM the 4-hydroxy metabolite inhibited opiate delta 1 (52.4%), kappa 1 (58.7%) and Mu (65.5%) receptors, the serotonin 5HT6 receptor (57.1%). At 1 μM the desmethoxy metabolite inhibited the MK-801 glutamate receptor by 55% (three experiments with different results 84%, 36%, and 46%), the voltage sensitive calcium activated K channel (50.8%) and the sodium site 2 receptor (77.9%).

According to an earlier study in rat cerebral cortex synaptosomes, the Ki value for the inhibition of the NE transporter by atomoxetine was 2 nM. Therefore, the inhibitory effects of atomoxetine at the previously mentioned receptors is at least 500x (2 nM vs. 1 μM conc.). As for the effect of the 4-hydroxy metabolite, the Ki values for the inhibition of the previously mentioned receptors were 300, 95, and 422 nM at the Delta 1, Kappa 1, and Mu opiate receptors, respectively. Thus the difference was at least 10x (for the Kappa 1 receptor). The Ki values for the effect of the desmethoxy metabolite on the other receptors were not provided.

Mechanism of action: as gathered from the data presented, atomoxetine appears to inhibit mainly the NE transporter with a much lower affinity for the DA and serotonin transporters.

Drug activity related to proposed indication: the sponsor has indicated that even though the etiology of ADHD is unclear, studies have shown that medications with noradrenergic and/or dopaminergic activity are effective in the treatment of ADHD. Therefore, the sponsor proposed that compounds that increase NE levels would be clinically useful in the treatment of ADHD. Atomoxetine increases NE levels by inhibiting the reuptake carrier that pumps NE back into the neurons, therefore the net result is an increase in the extracellular levels of NE.

Secondary pharmacodynamics: no secondary pharmacodynamics were proposed, however, the *in vivo* effects on DA and serotonin in the rat as seen by microdialysis might indicate some secondary pharmacodynamic effects.

Pharmacology summary: atomoxetine inhibits mainly the NE transporter resulting in an increase in NE levels. The effect of the drug on other transporters (DA and serotonin) was much less (16x less for the human serotonin and 270x less for the human DA transporters, as measured by the Ki values). Of the two major metabolites 4-hydroxyatomoxetine exhibited similar yet slightly lower affinity for the NE transporter. In vivo microdialysis studies indicated an increase in DA levels in the prefrontal cortex in the rat which is probably caused by the blocked of the NE transporter since NE neurons in that brain structure were shown to accumulate DA. The increase in serotonin in the prefrontal cortex and striatum that was seen with IP injection of atomoxetine could be due to "lost specificity" at those drug levels as indicated by the sponsor which can argue against the selectivity of the compound for the NE transporter. The compound and its two major metabolites (4-hydroxyatomoxetine and desmethoxyatomoxetine) were found to minimally affects a variety of receptors.

Pharmacology conclusions: from the reports included it appears that there is minimal effect of atomoxetine on other transporters and a variety of other receptors compared to its effect on the NE transporter.

II. SAFETY PHARMACOLGY:

Reviewed by Kathleen Haberny

Neurological effects:

Study title: EFFECTS OF LY139603 IN MICE

Key study findings:

- Deaths associated with clonic convulsions observed at 400 mg/kg PO LY139603 (2/3 mice)
- Treatment-related effects on motor activity, behavior and sensory motor reflex were decreased motor activity, irritability, leg weakness, jerky gait, exophthalmos, piloerection, observed at doses of ≥50 mg/kg PO; tremors (particularly when walking), grasping loss, pinna reflex, mydriasis, lacrimation at doses of ≥100 mg/kg PO; increased respiration, vibrissal response, analgesia, placing loss, decreased abdominal tone, corneal loss, righting loss at 200 or 400 mg/kg PO
- Reduced weight gain at all doses from 6.25-59 mg/kg PO
- Decreased apomorphine-induced hypothermia at 3.125-12.5 mg/kg PO
- No effect on pentylenetetrazole-induced tonic extensor convulsions; increased ECS50 and decrease in tonic extensor convulsions induced by electroconvulsive shock at 50 mg/kg PO
- Reduced writhing in response to acetic acid injection at 25 mg/kg but not 50 mg/kg PO, suggesting analgesia but probably not pharmacologically relevant
- Dose-related increase in hexobarbital sleeping time from 6.26-50 mg/kg PO

Study no: [redacted]
Volume # 28, and page #: (not paginated)
Conducting laboratory and location: Lilly Research Laboratories, Division of Eli Lilly and Company, Indianapolis, IN 46206
Date of study initiation: March, 1981
GLP compliance: yes (x) no ( )
QA report: yes (x) no ()
Drug Tomoxetine HCl (LY139603), **lot #** 525-U332-0720, **radiolabel** Not applicable, **and % purity:** Certificate of analysis not provided in this submission
Formulation/Vehicle: Water
Negative Control: 0.9% physiological saline
Species/strain: Male albino mice (n=10/dose in all but the observation study at 3/dose)
Age: Adult
Weight: 18-26 g
Doses in administered units: 0, 1.56, 3.125, 6.25, 12.5, 18.0, 25.0, 50.0 mg/kg for all parameters except the observation study at 25, 50, 100, 200, and 400 mg/kg

Route, form, volume, and infusion rate: Oral, dissolved in deionized water, at 0.01 cc/gm body weight (except at 400 mg/kg volume was 0.02 cc/gm)

Methods: Male albino mice [redacted] were individually housed and fed standard Rodent Laboratory Chow [redacted]. The mice were fasted for 3 hours before dosing. The following parameters were measured:

General observations: After dosing, the mice were placed in an 18"x18' observation pan. The following effects were monitored for 7 days post-dosing, and scored: death, time of death, motor activity, irritability, respiration, stay center, position, leg weakness, writhing, ataxia, arch/roll, gait, ptosis, exophthalmos, piloerection, tremors, ears back, face washing, rigid stance, tail erection, convulsions, corneal loss, righting loss, miosis, mydriasis, lacrimation, salivation, pink skin color, cyanosis, vasodilatation, squeak when handled, and defensive behavior.

Additionally, the following tests were performed: analgesia (tail pressure), irritability (sound and touch), pinna reflex, placing, corneal reflex, vibrassal response (total closure scored as 3), grasping test (dowel rod grasp with ability to stay on top of rod, total inability to hold on scored as 3), abdominal tone (response to dropped steel ball on abdomen, maximum loss of tone scored as 3).

The effects were scored as ½ (questionable), 1 (slight), 2 (moderate), and 3 (marked).

Weight Gain of Fasted Mice: Mice were fasted 17 hours and then administered saline or LY139603 at 6.25, 12.5, 25, and 50 mg/kg PO (n=10/dose). The mice were fed for 1 hour, beginning 30 minutes after dosing. Water was available *ad libitum* throughout the assay.

Weights were measured at baseline (beginning of fast), at the time of dosing, and 1 hour after the availability of food. The experiment was replicated once.

Apomorphine Hypothermia: The mice were administered LY139603 at 1.56, 3.125, 6.25 and 12.5 mg/kg PO (n=5/dose) or saline vehicle, followed by apomorphine HCl (10 mg/kg IP), thirty minutes later. Rectal temperature was measured at LY139603 administration, at apomorphine administration, and at 30 minutes after apomorphine administration. The experiment was replicated once.

Pentylenetetrazole-Induced Convulsions: The mice were administered LY139603 at doses of 6.25, 18, or 50 mg/kg PO or saline vehicle (n=5/dose) followed by pentylenetetrazole (70.8, 79.4, 89.1, 100, and 112 mg/kg IP, n=3 LY139603 groups/pentylenetetrazole dose or 15/pentylenetetrazole dose), 30 minutes later. The mice were observed for tonic extensor convulsions for 1 hour after pentylenetetrazole administration.

Electroconvulsive Shock-Induced Convulsions: The mice were administered LY139603 at doses of 6.25, 18, or 50 mg/kg PO, or saline vehicle (n=5/dose), followed by electroshock (11.2, 12.6, 14.2, 15.0, 18.0, 20.0 milliamps, 0.1 second, via corneal

electrodes, n=3 LY139603 groups/shock intensity or 15/shock intensity), 30 minutes later. Tonic extensor convulsions were recorded and the current intensity (ma) required to produce the convulsions in 50% mice (ECS_{50}) was determined.

Acetic Acid-Induced Writhing (analgesia): The mice were administered LY139603 at doses of 6.25, 25.0, and 50 mg/kg PO, or saline vehicle (n=3/group), followed by acetic acid (0.55%, 0.01 cc/gm IP) 25 minutes later. The mice were observed in clear observation chambers, and the numbers of writhes were counted for 5 minutes by 2 independent observers per mouse.

Hexobarbital Sleeping Time: The mice were administered LY139603 at doses of 6.25, 12.5, 25, and 50 mg/kg PO, or saline vehicle (n=10/dose). Hexobarbital sodium (100 mg/kg IP) was administered at 48 hours, 24 hours, or 30 minutes after dosing with LY139603. Sleeping time (duration of loss of righting reflex from time of hexobarbital administration to recovery of righting reflex) was measured in one-minute increments.

Results:

General observations: The results of the general observations are presented in the following table. Parameters for which there were treatment-related effects are presented, only.

General Observations in Mice Administered LY139603*

	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Deaths					2
Time of death					8-40 min
Decreased motor activity		3	3	3	1
Irritability		3	3	3	3
Increased respiration					2
Position (F-H/C)					1
Leg weakness		2/1	3/3	3/4	3/6
Jerky gait		1	3	3	3
Exophthalmos		1/0.5	3/2.5	3/3	3/6
Piloerection		1/0.5	3/5	3/6	3/6
Tremors			3/1.5 (walking)	3.3 (walking)	3/8
Convulsions					2 clonic
Vibrissal response					1/1
Analgesia				3/2	3.3.5
Grasping Loss			3.1.5	3/4	3/7
Placing Loss					2/4
Decreased Abdominal Tone					1/1
Pinna Reflex			1 ↑		2 ↑ 1/2↓

Corneal Loss				2/2	1/1
Righting Loss					2/5
Mydriasis			2/1	3/3.5	2/5
Lacrimation			1/1	2/3	1/2
Cyanosis				3 (slight)	
Squeaked			3		
Defensive			1	1	

*The results are presented as number of animals exhibiting effect/sum of maximum scores for each mouse; n=3/dose

The deaths at 400 mg/kg followed clonic convulsions. The behavioral effects peaked at 20-30 minutes and resolved by 2 hours at 200 mg/kg and by 3 days at 400 mg/kg.

Weight Gain of Fasted Mice: There were no differences in mean initial weight and mean weight loss after a 17-hour fast among the groups. Mean weight gain was significantly reduced at 50 mg/kg (0.70 g) compared to saline control (1.56 g) in the first experiment ($p = 0.012$), and at all doses (1.09, 0.70, 0.95, and 0.86 g at 6.25, 12.5, 25, and 50 mg/kg, respectively) in the second experiment ($p = 0.020$, <0.001, 0.003, and 0.001, respectively). The mean gain/loss ratio showed a comparable reduction in the LY139603-treated mice.

Apomorphine Hypothermia: The results of the rectal temperature measurements are presented in the following table:

	Experiment 1			Experiment 2		
Dose LY139603	Mean Temp Change 1[a]	Mean Temp Change 2[b]	Overall Temp Change	Mean Temp Change 1[a]	Mean Temp Change 2[b]	Overall Temp Change
0.000	+0.09	-3.87	-3.78	+0.17	-4.40	-4.23
1.560	+0.01	-3.11	-3.10	-0.54*	-1.44*	-1.98*
3.125	-0.02	-2.15*	-2.17	-0.40	-0.78*	-1.18*
6.250	+0.09	-1.44*	-1.35	-0.42	-0.38*	-0.80*
12.500	-0.33	-0.73*	-1.06	-0.70*	+0.29*	-0.41*

[a]Temp Change 1 = Temperature change after LY139603 treatment
[b]Temp Change 2 = Temperature change after apomorphine treatment
*$p \leq 0.01$, compared to saline control

Pentylenetetrazole-Induced Convulsions: There were no treatment-related effects on pentylenetetrazole-induced tonic convulsions.

Electroconvulsive Shock-Induced Convulsions: LY139603 decreased the number of electroconvulsive shock-induced convulsions in a dose-related manner, and increased the ECS_{50} at 50 mg/kg PO. The results are presented in the following table:

Percent Mice with Electroconvulsive Shock-Induced Convulsions

	Dose LY139603 (mg/kg PO)			
Shock level (ma)	**0.00**	**6.25**	**18.00**	**50.00**
11.2	7	13	Not evaluated	Not evaluated
12.6	27	20	20	7
14.2	27	20	20	20
16.0	27	47	33	7
18.0	60	73	47	47
20.0	100	80	80	40
D. Mean %	48	48	40	24
P Value (compared to 0 dose mean)	reference	0.635	0.182	<0.001
ECS_{50}	16.1 ma	16.1 ma	17.3 ma	21.0 ma

Acetic Acid-Induced Writhing: There were no dose-related effect on number of writhes, although writhing was significantly reduced at 25 mg/kg LY139603 (30.8 writhes compared to 51.0 in the controls, p value 0.049).

Hexobarbital Sleeping Time: The results of the measurements of latency to recovery of righting reflex are presented in the following table:

LY139603 Treatment (mg/kg)			N	Mean sleeping time	P-value
-48 hours	**-24 hours**	**-30 minutes**			
0.000	0.000	0.000	10	44.1	reference
0.000	0.000	6.250	10	54.8	.151
6.250	6.250	6.250	10	57.8	.067
0.000	0.000	12.500	10	57.3	.078
12.500	12.500	12.500	10	57.9	.065
0.000	0.000	25.000	10	58.6	.053
25.000	25.000	25.000	10	68.1	.002
0.000	0.000	50.000	10	82.8	<.001
50.000	50.000	50.000	10	76.4	<.001

There was a significant dose-related increase in hexobarbital sleeping time, with no effect as a function of number of treatments.

Summary of individual study findings: There were deaths associated with clonic convulsions in 2/3 animals at the 400 mg/kg dose. The treatment-related effects of LY139603 on motor activity, behavior, and sensory motor reflex were decreased motor activity, irritability, leg weakness, jerky gait, exophthalmos, and piloerection, observed at doses of 50 mg/kg and above, tremors (particularly when walking), grasping loss, pinna reflex, mydriasis, and lacrimation at doses of 100 mg/kg and above, and increased respiration, vibrissal response, analgesia, placing loss, decreased abdominal tone, corneal

loss, and righting loss at 200 or 400 mg/kg. Reduced weight gain was observed at all doses from 6.25-59 mg/kg, and decreased apomorphine-induced hypothermia was observed at 3.125-12.5 mg/kg. Although there were no effects on pentylenetetrazole-induced tonic extensor convulsions, LY139603 increased the ECS_{50} and decreased the tonic extensor convulsions induced by electroconvulsive shock at 50 mg/kg. LY139603 attenuated writhing in response to acetic acid injection at 25 mg/kg but not at 50 mg/kg PO. A dose-related increase in hexobarbital sleeping time was observed from 6.26-50 mg/kg LY139603. Decreased weight and rectal temperature, and antagonism of hypothermia by apomorphine are expected effects of norepinephrine uptake inhibition.

Cardiovascular effects:

Study title: ELECTROPHYSIOLOGICAL EFFECTS OF LY139603 ON CANINE CARDIAC PURKINJE FIBERS

Key study findings:

- Upstroke of the action potential, action potential duration and amplitude decreased by tomoxetine HCl (LY139603), its (+) isomer (93627) and the racemate (139602)
- Tomoxetine (LY139603) was half as potent as amitriptyline in reducing upstroke velocity and action potential duration, but more potent than the (+) isomer in inhibiting upstroke velocity
- The results suggest decreased potential for interference with cardiac conduction by LY139603 compared to amitriptyline

Study no: ———

Volume # 28, and page #: (not paginated)

Conducting laboratory and location: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Date of study initiation: Not provided in this submission

GLP compliance: yes ( ) no (x)

QA report: yes () no (x)

Drug Tomoxetine HCl [LY139603, (-) isomer], 139602 [(+)optical isomer of LY139603], 93627 (racemate of LY139603), **lot #** Not provided in this submission, **radiolabel** Not applicable, **and % purity:** Not provided in this submission

Formulation/Vehicle: Test article dissolved in distilled water or dilute (0.01N) HCl with dilution in Tyrode's solution

Species/strain: Adult Mongrel dogs (n=7:LY139603, 139602, 93627; n=5:amitriptyline, sex not provided in this submission)

Weight: 8-15 kg

Doses in administered units: 10^{-5} M LY139603 (concentration selected based on results for racemate), 10^{-5} M 139602, 10^{-6} to 10^{-5} M Amitriptyline, 10^{-6} to $5x10^{-5}$ M 93627, in distilled water or dilute HCl (0.01N) before dilution in Tyrode's solution

Methods: The dog's hearts were removed under sodium pentobarbital anesthesia. The distal right or left bundle branches were isolated and superfused with modified Tyrode's

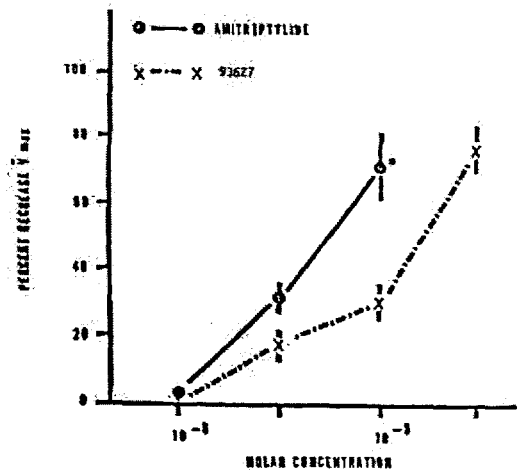
solution (156.7 mEq/L Na^+, 4.0 mEq/L K^+, 1.0 mEq/L Mg^{2+}, 4.0 mEq/L Ca^{2+}, 145.9 mEq/L Cl, 1.8 mEq/L $H2PO_4^-$, 18.0 mEq/L HCO_3^-, 5.5 mM glucose) at 35°C. The tissues were stimulated with square-wave pulses (4-6 V, 0.5 msec duration, cycle length 1,000 msec) using stainless-steel electrodes. At steady state (1.5 hours after tissue perfusion, 40 min after addition of drug to bath), purkinje fiber intracellular action potentials were recorded using glass microelectrodes connected to high-impedance unity gain electrometer. The following parameters were measured:

Vmax: Maximum rate of rise of the action potential

APD_{50}, APD_{70}, APD_{95}: Action potential duration at 50%, 70%, and 95% full repolarization, respectively, (sampling interval 50 mcsec for 10 msec after stimulus artifact and 1 msec for next 800 msec)

The means were determined, and differences calculated, using a paired-sample t-test comparing treated and control means, and an unpaired t-test comparing absolute differences between drug responses.

Results: The results of the measurements of maximum rate of rise of the action potential are presented in the following figure. There was a dose-related increase in the percent decrease in Vmax (rate of rise of the antracellularly recorded action potential) by amitriptyline and 93627 (racemic mixture of LY139603 and 139602).



A 50% decrease in the Vmax was induced by 93627 at $1.7x10^{-5}$ M and by amitriptyline at $5.3x10^{-6}$ M. At the concentration of 10^{-6} M, amitriptyline decreased the APD_{70} and APD_{95} (action potential duration at 70% and 95% full repolarization), and the action potential amplitude to a greater extent than did 93627, with the difference reaching statistical significance at the 95% level.

Effects of Amitriptyline and 93627 on the Canine Purkinje Fiber Action Potential (mean ±S.E.)				
	Amplitude (mv)	Vmax (V/S)	Em (mv)	APD_{95} (msec)
Control	132 ± 2	585 ± 80	-91 ± 1	376 ± 26

Amitriptyline ($3x10^{-6}$M)	124 ± 4**	408 ± 61**	-87 ± 2	231 ± 9**
Control	130 ± 2	515 ± 48	-91 ± 1	387 ± 21
93627 ($3x10^{-6}$M)	126 ± 2	418 ± 29*	-86 ± 1*	351 ± 17**

*p<0.05; **p<0.01

Both the (-) isomer LY139603 and the (+) isomer 139602 decreased Vmax, APD_{95} and the action potential amplitude, with a slightly greater effect by the (-) isomer LY139603, except that the decrease in Vmax was significantly greater by LY139603 (52.7±6.3%) than by 139602 (22.4±4.0%), at the concentration of 10^{-5} M.

Effects of LY139603 and 139602 on the Canine Purkinje Fiber Action Potential (mean ±S.E.)				
	Amplitude (mv)	Vmax (V/S)	Em (mv)	APD_{95} (msec)
Control	122.2 ± 2	587 ± 75	-90 ± 1	382 ± 13
139602 (10^{-5}M)	114 ± 3*	443 ± 57**	84 ± 2*	324 ± 13**
Control	122 ± 2	568 ± 56	-92 ± 1	379 ± 13
LY139603 (10^{-5}M)	107 ± 3**	264 ± 35**	-87 ± 3	298 ± 9**

*p<0.05; **p<0.01

Summary of individual study findings: The racemic mixture (Compound 93627) was less potent than amitriptyline in decreasing the Vmax, resting membrane potential and action potential duration at 95% full repolarization. To determine if there is a difference in potency by the isomers of 93627, the (-) and (+) isomers LY139603 and 139602, respectively, were tested. The (-) isomer LY139603 was 2X more potent than the (+) isomer 139602 in decreasing the Vmax, and decreased amplitude, membrane resting potential, and APD_{95} to a similar extent compared to the (+) isomer.

Study title: HUMAN CARDIAC I_{Kr} (HERG) BLOCKING PROFILE OF LY139603, COMPOUND 137876, AND COMPOUND 424478

Key study findings:

- HERG blockade was significantly increased in a dose-related manner 10%-94% at all LY139603 concentrations tested, from 10 nM-10mcM at the pacing rate of 0.1 Hz (IC_{50}=0.869 mcM); no effect of pacing rate at 1, 2, an 3 Hz
- The metabolites increased the mean %HERG block in a dose related manner at 0.1 Hz from 19%-98% at 0.88-88 mcM N-desmethyltomoxetine (IC_{50}=5.71 mcM), and from 10%-100% at 0.88-264 mcM 4-hydroxytomoxetine (IC_{50}=20.0 mcM); no effect of pacing rate from 1-3 Hz at constant concentration of 88 nM for either compound
- The results of this study indicate a risk of HERG blockade, and increased potential for QT prolongation, at clinically relevant doses; predicted HERG blockade is 21% in extensive and 31% in poor metabolizers.

Study no: LLY99_10, LLY99_11, LLY99_12 ([redacted]
Volume # 28, and page #: (not paginated)
Conducting laboratory and location: [redacted]

Date of study initiation: November 15, 1999 (LY139603), January 17, 2000 (137877 and 484305)
GLP compliance: yes ( ) no (x)
QA report: yes ( ) no (x)
Drug, lot #, % purity:

Tomoxetine HCl (LY139603) / Lot # 037JD9
Compound 137877 (hydrochloride salt of the metabolite N-desmethyltomoxetine (Compound 137876) / Lot # 687-XY9-41C
Compound 484305 (hydrochloride salt of the metabolite 4-hydroxytomoxetine (Compound 424478) / Lot # Z53-LLI-118A
% purity: Not provided in this submission

Formulation/Vehicle: Test article in powdered form dissolved in de-ionized H_2O or DMSO
Species/strain: Human embryonic kidney (HEK293) cells transfected with human I_{Kr} (HERG) channels, and isolated human atrial myocytes obtained during cardiac surgery
#/sex/group: 4
Satellite groups used for toxicokinetics or recovery: Not applicable
Concentrations tested: 0.01-10 mcM (LY139603) and 0.1-100 mcM (424478 and 137876)

Methods:
Human embryonic kidney cells (HEL293) were transfected with the HERG clone to express I_{Kr} channels, and maintained in standard culture medium (MEM, Earle's salts, nonessential amino acids, sodium pyruvate, penicillin, streptomycin, fetal bovine serum). The standard external bathing solution during recording of the HERG current was composed of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11 mM dextrose, and 10 mM HEPES (pH adjusted to 7.4 with NaOH). The assay used the whole-cell patch clamp method to measure ionic current, with an Axopatch amplifier. The internal pipette solution was composed of 130 mM KCl, 1 mM $MgCl_2$, 10 mM NaATP, 5 mM EGTA, 5 mM HEPES (pH to 7.2 with KOH). After stabilization (3-7 minutes) at 37 ± 1 °C, superimposition of three current traces to voltage pulses at 0.1 Hz was accepted establishment of stable current. LY139603, Compound 137876, or Compound 424478 was added to the external solution and I_{Kr} currents were recorded at voltage pulses up to +10 mV (500 ms) applied from holding potentials of -75 mV. Rate-dependence was evaluated by determining the percent blockade by 100 nM LY139603 at the pacing rates of 0.1, 1.2, and 3 Hz. HERG tail currents were measured at steady state after repolarization (-40 mV, 500 ms). The percent reduction of current amplitude was determined by comparing the current at steady-state with baseline current amplitude before the drug was added to the medium. The IC_{50} values were determined.

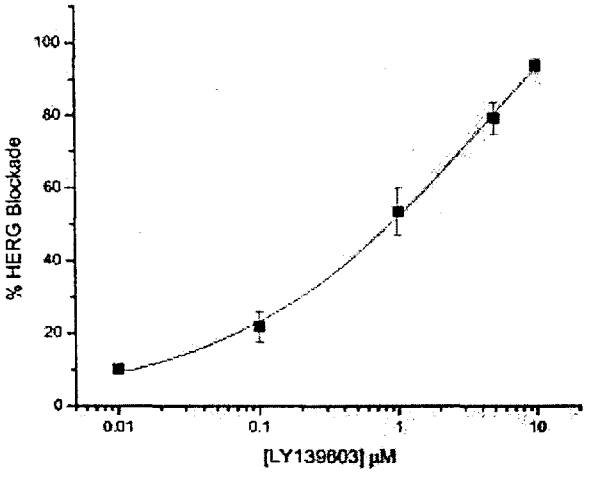
Results: The results of the HERG block measurements for LY139603 are presented in the following table:

Mean (±SEM) % Block of HERG by LY139603*				
10 nM	100 nM	1 mcM	5 mcM	10 mcM
10.2 ± 1.5	21.9 ± 4.2	53.5 ± 6.4	79.2 ± 4.3	93.5 ± 1.9

*Pacing rate 0.1 Hz.

The reduction in amplitude of HERG was statistically significant compared to control values at all LY139603 concentrations tested, from 0.01-10 mcM. The IC_{50} for blockade of HERG by LY139603 at 0.1 HZ was 0.869mcM. The evaluation of rate-dependence showed additional HERG blockade of 3.9 ± 1.1%, 4.1 ± 0.6%, and 1.9 ± 0.8% percent at 1 Hz, 2 Hz, and 3 Hz, respectively.

Dose-Response Curve for HERG Block by LY139603 at 0.1 Hz
(Data are Mean ± SEM)



The results of the HERG block by Compounds 137876 and 424478 are presented in the following table:

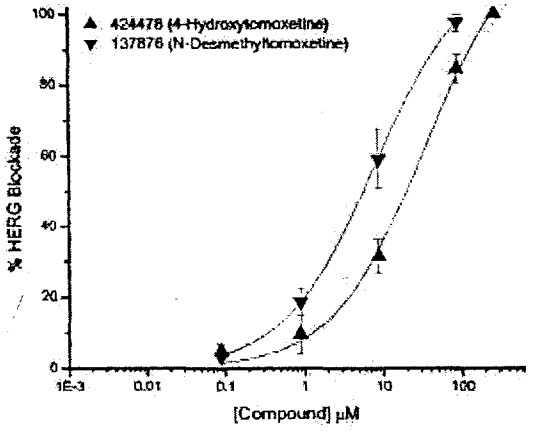
Mean (± SEM) % HERG Block at 0.1 Hz					
Compound	88 nM	0.88 mcM	8.8 mcM	88 mcM	264 mcM
137876	3.3 ± 2.0	18.6 ± 3.9*	59.1 ± 8.4*	97.6 ± 2.4*	Not performed
424478	4.7 ± 1.9	9.5 ± 5.2*	31.4 ± 4.7*	84.4 ± 3.9*	100 ± 0*

*Statistically significant compared to controls

The IC_{50} values for blockade of HERG by Compounds 137876 and 424478 were 5.71 and 20.0 mcM, respectively, at 0.1 HZ. Holding the concentration of Compound 137876 to 88 nM, the percent block of HERG was 0.9 ± 0.6, 1.1 ± 0.7, and 1.1 ± 0.7 at 1 Hz,

2Hz, and 3 Hz, respectively. At the concentration of 88 nM, the percent block of HERG by Compound 424478 was 0.6 ± 0.4%, 1.0 ± 0.5%, and 2.5 ± 0.9% at 1 Hz, 2 Hz, and 3 Hz, respectively. There were no statistically significant differences from control values at 0.1 Hz, in percent block as a function of pacing rate up to 3 Hz for either compound.

Dose-Response Curves for HERG block by 137876 and 424478 at 0.1 Hz (Data are means ± SEM)



Summary of individual study findings: HERG blockade was significantly increased at all LY139603 concentrations tested (10.2%, 21.9%, 53.5%, 79.2%, and 93.5% at 0.01, 0.1, 1, 5, and 10 mcM, respectively) at the pacing rate of 0.1 Hz (IC_{50}=0.869mcM). There was no effect of pacing rate at the concentration of 88 nM (HERG block 3.9 ± 1.1%, 4.1 ± 0.6%, and 1.9 ± 0.8% percent at 1 Hz, 2 Hz, and 3 Hz, respectively). The mean % HERG block also increased with concentration of the metabolites N-desmethyltomoxetine (Compound 137876, 18.6%, 59.1%, 97.6% at 0.88, 8.8, and 88 mcM, respectively) and 4-hydroxytomoxetine (Compound 424478, 9.5%, 31.4%, 84.4%, and 100% at 0.88, 8.8, 88, and 264 mcM, respectively). The IC_{50} values for blockade of HERG by Compounds 137876 and 424478 were 5.71 and 20.0 mcM, respectively, at 0.1 HZ. Holding the concentration of Compound 137876 to 88 nM, the percent block of HERG was 0.9 ± 0.6%, 1.1 ± 0.7%, and 1.1 ± 0.7% at 1 Hz, 2Hz, and 3 Hz, respectively. At the concentration of 88 nM, the percent block of HERG by Compound 424478 was 0.6 ± 0.4%, 1.0 ± 0.5%, and 2.5 ± 0.9% at 1 Hz, 2 Hz, and 3 Hz, respectively. There were no statistical differences from control values in percent block as a function of pacing rate up to 3 Hz for either Compound.

The expected maximum unbound plasma LY139603 concentration at the highest clinical dose (1.8 mg/kg/d) is expected to be 0.016-0.082 mcM in extensive metabolizers and

0.108-0.219 mcM in poor metabolizers. The results of this study indicate a risk of HERG blockade, and increased potential for QT prolongation, at clinically relevant doses. At these concentrations, the predicted HERG blockade is 21% in extensive and 31% in poor metabolizers. Although the concentrations tested spanned a broad range producing a concentration-response curve, and exceeded the anticipated maximal therapeutic plasma concentration, appropriate positive control articles were not used. However, the positive, dose-related effects observed in this study validate the responsiveness of the test system, confirming the presence of the I_{Kr} channels.

Study title: CARDIOVASCULAR AND RESPIRATORY EFFECTS OF INTRAVENOUS ADMINISTRATION OF LY139603 IN ANESTHETIZED DOGS

Key study findings:

- LY139603 **increased heart rate** (28%-38% at 4-10 mg/kg), **respiration rate** (43%-76% at 4-10 mg/kg), **P-R interval** (14%-18% at 8-10 mg/kg and 30 post-infusion), **the corrected Q-T interval** (19%-22% at 6-10 mg/kg), **slightly increased PCO_2** (at 2 mg/kg and 30 minutes after infusion), and **decreased arterial blood pH** (<1%-1%) at 2-6 mg/kg in non-pretreated (atropine and propranolol) dogs;
- In the pretreated dogs (atropine and propranolol), LY139603 **increased the PCO_2** (4%-7% at 4-10 mg/kg and 11%-13% post-infusion), and **increased the corrected Q-T interval** (7-32% at all doses)
- Amitriptyline **increased heart rate** (62%-116% at 2-10 mg/kg) without pretreatment with atropine and propranolol; **increased respiration rate** (37%-76% at 2-10 mg/kg and post-infusion without pretreatment and 14%-23% at 8-10 mg/kg and 30 in post-infusion with pretreatment); **decreased the Stroke Work Index** (45%-54% at 4-10 mg/kg and post-infusion without pretreatment and 16%-24% at 4 mg/kg and 60 minutes post-infusion with pretreatment); **decreased Pulmonary Capillary Wedge Pressure** (11%-56% at 4-10 mg/kg, and post-infusion without pretreatment, and 29%-71% at 2-10 mg/kg and post-infusion with pretreatment); **increased Minute Volume** (7%-27% at all doses and post-infusion) without pretreatment; **decreased arterial blood pH** (1% at 2-8 mg/kg) without pretreatment; **increased the P-R interval** (up to 13% at 8-10 mg/kg without pretreatment and 36% at 10 mg/kg with pretreatment); **increased the QRS duration** (8%-16% at 4-10 mg/kg and post-infusion with dose relationship without pretreatment and 14%-72% at all doses and post-infusion with dose relationship with pretreatment); **decreased the Q-T interval** (8%-11% at 6-10 mg/kg) without pretreatment and **increased the Q-T interval** (8%-24% at all doses and post-infusion) with pretreatment
- In comparison, amitriptyline decreased blood pressure 6%-8% and peripheral vascular resistance 11%-23%, while LY139603 increased blood pressure 4%-15% (significant difference at the 10 mg/kg doses only) and peripheral vascular resistance 0.4%-18% (significant difference at 30 minutes post-dose only) in the absence of pretreatment; greater heart rate increase, stroke work index decrease, pulmonary capillary wedge pressure decrease, and minute volume increase (non-pretreated dogs) were observed following amitriptyline than after LY139603 administration, and greater increase in respiration rate and increase in minute volume (pretreated dogs)

observed following LY139603 than following amitriptyline (pretreated dogs) administration

- In the ECG evaluation, significant increase in P-R interval by LY139603 but not amitriptyline without pretreatment, and increased P-R interval by amitriptyline only in pretreated animals; QRS duration increased by amitriptyline only in non-pretreated and pretreated dogs, Q-T interval increased by LY139603 and decreased by amitriptyline without pretreatment, moderately increased by both drugs to similar extent with pretreatment

Study no: ——

Volume # 29, and page #: (not paginated)

Conducting laboratory and location: Lilly Research Laboratories, Eli Lilly and Company, 307 East McCarty Street, Indianapolis, IN 46285

Date of study initiation: May 13, 1980

GLP compliance: yes (x) no ( )

QA report: yes ( ) no (x)

Drug, lot #: LY139603 / Lot # 525-U332-072D

Amitriptyline / Lot # L-720, 101-01X22

Radiolabel not applicable, **and % purity:** Certificates of analysis not provided in this submission

Formulation/Vehicle: Drug dissolved in 0.9 % saline

Dosing:

Species/strain: Beagle dog ——

#/sex/group: 2, except 4 in the group that received amitriptyline after atropine and propranolol pretreatment

Satellite groups used for toxicokinetics or recovery: Not applicable

Age: 10-13 months

Weight: 6.5-14.5 kg

Doses in administered units: 2, 4, 6, 8, 10 mg/kg

Route, form, volume, and infusion rate: Intravenous infusion in the right saphenous vein over 50 minute period, at 5 mg/ml, 0.2 mg/kg/min, for total cumulative dose of 10 mg/kg,

Methods: The dogs were anesthetized with thiopental (10 mg/kg IV) and alpha-chloralose (anesthesia maintenance) and were placed on heating pads to maintain body temperature. The dogs were administered intravenous infusions of LY139603, the positive control article amitriptyline (2, 4, 6, 8, 10 mg/kg), or the negative control article saline, with and without pretreatment with atropine (1 mg/kg) and propranolol (2 mg/kg) for cholinergic and beta-adrenergic blockade. The following measurements were made at baseline, 10, 20, 30, 40, and 50 minutes during infusion, and at 30 and 60 minutes after infusion:

Heart rate; Respiratory rate, inspiratory flow using a —— pneumotachograph and —— strip-chart recorder; **minute volume** (respiratory rate X inspiratory volume);

Femoral artery blood pressure via intra-arterial cannula filled with heparin in the left femoral artery, using a [redacted] pressure transducer and a [redacted] recorder; **Cardiac output** by the thermal dilution method using Swan-Ganz catheter and Instrumentation Laboratory cardiac output computer; **Pulmonary arterial pressure and wedge pressure** through the same cannula; **Arterial blood pH, P_{02}, and HCO_3** using Instrumentation Laboratory pH/Blood Gas Analyzer; **Lead II ECG** using subdermal needle electrodes and [redacted] recorder [**P-R, QRS, Q-T** intervals manually from strip-charts, Q-T interval corrected for heart rate changes (Q-T interval / square root of R-R interval)].

The following calculations were made:

Body surface area ($BSA=W^{2/3}/10^3$), **stroke work index** (SWI=CO/HRxBSA X (MP-PCWP) X 0.0136 where CO=cardiac output, HR=heart rate, MP=mean arterial pressure, and PCWP=pulmonary capillary wedge pressure), **peripheral systemic resistance** (PVR=MPx1332/Q where MP=mean pressure, and Q=blood flow), **pulmonary vascular resistance** (PVR=(P-PCWP) X 1332/Q where P=pulmonary pressure and Q=blood flow). Statistical analyses were performed using Duncan's multiple range test for mean changes from pre-drug values and Student's t-test for mean changes from pre-drug values in the pretreated dogs.

Results:

LY139603, at intravenous doses of 2-10 mg/kg, had no effects on peripheral vascular resistance, cardiac output, stroke work index, mean pulmonary pressure, pulmonary vascular resistance, pulmonary capillary wedge pressure, minute volume, and arterial blood HCO_3 in the anesthetized dogs without and with pretreatment with atropine and propranolol. In the ECG evaluation, LY139603 had no effects on the QRS Duration in the non-pretreated and pretreated dogs. Amitriptyline had no effects on cardiac output, mean pulmonary pressure, and pulmonary vascular resistance.

The following effects were observed, with significant differences between the mean change from pre-drug (baseline) for the treatment vs. mean change from pre-drug for the saline control (percents given are mean change from pre-drug value):

LY139603

LY139603 increased **heart rate** (Table 1) 31%, 38%, 28%, and 31% at doses of 4, 6, 8, and 10 mg/kg, respectively, and 26% at 30 minutes after termination of the infusion in the non-pretreated dogs, but there were no effects on heart rate in the atropine and propranolol pretreated dogs. **Mean blood pressure** was increased 15% at 10 mg/kg. **Respiration rates** were increased 43%, 51%, 63%, and 76% at doses of 4, 6, 8, and 10 mg/kg IV, respectively, in the non-pretreated dogs, but resolved after termination of the infusion (Table 2). Increased respiration in the pre-treated dogs did not reach statistical significance. LY139603 decreased **arterial blood pH** <1% at 2 mg/kg and 1% at 4, and 6 mg/kg compared to baseline (Table 3) in the non-pretreated but not in the pretreated dogs. **Arterial blood PCO_2** was slightly, but significantly (compared to saline) increased

at 2 mg/kg and at 30 minutes after termination of infusion, only, in the non-pretreated dogs, and increased 4%-7% at 4-10 mg/kg and 11%-13% post-infusion in the pretreated dogs.

In the ECG evaluation, LY139603 increased the **P-R interval** 14% and 18% at 8 and 10 mg/kg IV, respectively, and 10% at 30 minutes after the termination of infusion compared to baseline values in the non-pretreated dogs (Table 4), but not in the dogs pretreated with atropine and propranolol. The **corrected Q-T interval** was increased by LY139603 19%, 21%, and 22%, at 6, 8, and 10 mg/kg IV, respectively, and 21% at 30 minutes after termination of infusion compared to baseline, in the absence of pretreatment (Table 5). The **corrected Q-T interval** was increased by LY139603 from 7%-32% at all doses and timepoints after termination of infusion compared to baseline with pretreatment, but the changes did not reach statistically significance with respect to differences from pre-test values or when compared to the changes in the saline group.

Amitriptyline

Amitriptyline increased **heart rate** by 62%, 116%, 113%, 107%, and 103%, at 2, 4, 6, 8, and 10 mg/kg, respectively, and 70% and 52% at 30 and 60 minutes, respectively, after the termination of infusion in the non-pretreated dogs (Table 1). There was no effect by amitriptyline on heart rate in the dogs pretreated with atropine and propranolol for cholinergic and beta-adrenergic blockade. **The Stroke Work Index** was decreased by amitriptyline (45%-54%) at 4-10 mg/kg and at both timepoints after termination of infusion in the non-pretreated dogs (Table 6), and (16%) at 4 mg/kg and (24%) at 60 minutes after the end of infusion in the pretreated dogs. Amitriptyline decreased **Pulmonary Capillary Wedge Pressure** by 11%-56% at doses from 4-10 mg/kg and at both post-infusion timepoints without pretreatment, and 29%-71% at 2-10 mg/kg and post infusion with pretreatment, but the changes were not reported to be statistically significant. The **respiration rate** was increased by amitriptyline 37%-76% at all doses and for 30 and 60 minutes post-infusion without pretreatment (Table 2), and 14%-23% at 8 and 10 mg/kg and 30 minutes post-infusion with pretreatment with atropine and propranolol.

Amitriptyline also increased **Minute Volume** by 7%-35% at all doses and timepoints without pretreatment. **Arterial blood pH** was decreased by amitriptyline 1% at 2-8 mg/kg in the absence of pretreatment (Table 3). **Arterial blood PCO_2** was slightly, but significantly (compared to saline) increased at 2 mg/kg, only. Amitriptyline decreased arterial blood PO_2 6% and 27% at 30 and 60 minutes, respectively, after the termination of infusion in the pretreated dogs.

In the ECG evaluation, amitriptyline increased the **P-R interval** up to 13% at 8 and 10 mg/kg without pretreatment (Table 4), and 36 % at 10 mg/kg with pretreatment with atropine and propranolol. The differences from saline-induced increases were statistically significant at doses of 2 and 4 mg/kg in the pretreated dogs, only. The **QRS duration** was increased by amitriptyline by 8%, 11%, 15%, 16% at 4, 6, 8, and 10 mg/kg IV, respectively, without pretreatment, and 14%-72% at all doses and 17% at 30 minutes

after the termination of infusion with pretreatment. The **Q-T interval** was decreased, although not significantly, by 8%-11% at 6-10 mg/kg and increased 2%-3% at both timepoints after the end of infusion without pretreatment (Table 5). After pretreatment with atropine and propranolol, amitriptyline increased the Q-T interval 8%-24% at all doses and post-infusion timepoints.

Comparison between the effects of LY139603 and Amitriptyline

In the absence of pretreatment with atropine and propranolol, amitriptyline decreased arterial **blood pressure** by 6%-8% at doses of 6-10 mg/kg and at 30 minutes after infusion termination, whereas LY139603 increased blood pressure 15% at 10 mg/kg. The changes by amitriptyline were significantly different from those by LY139603 at 10 mg/kg and at 30 minutes post-infusion. In the pretreated dogs, amitriptyline decreased arterial blood pressure 14%-33% at all doses and 8% at 30 minutes post-infusion, while blood pressure was slightly increased at all doses and post-infusion timepoints in the LY139603 treated animals. The observed difference in blood pressure between the amitriptyline-treated and LY139603-treated groups was significant at all timepoints except pre-drug and 60 minutes post-infusion.

Although significant changes from pre-drug were not reported, **peripheral vascular resistance** was decreased in the non-pretreated dogs 11%-23% by amitriptyline at all doses and 30 minutes after termination of infusion. In comparison, peripheral vascular resistance was increased in the non-pretreated dogs 0.4%-18% by LY139603 at 6, 8, and 10 mg/kg and at both post-infusion timepoints and by saline 2%-40% at all timepoints. The difference between the LY139603 and amitriptyline groups was significant at 30 minutes after the end of infusion. In the dogs pretreated with atropine and propranolol, peripheral vascular resistance was increased by both the LY139603 and amitriptyline to a similar extent.

Heart rate was increased to a significantly greater extent by amitriptyline than by LY139603 in the non-pretreated dogs, at all doses and post-infusion timepoints (Table 1). The decrease in **Stroke Work Index** by amitriptyline was significantly greater when compared to that in the LY139603-treated dogs at all dose levels and timepoints except 2 mg/kg. Although not reported to be significant, either by comparison to baseline, to saline or between LY139603 and amitriptyline, there was a considerable decrease in **pulmonary capillary wedge pressure** by amitriptyline in both the atropine and propranolol pretreated and in the non-pretreated dogs, at all doses and at both post-infusion timepoints, that was not observed in the LY139603 treated dogs. There were no differences in the increase in **respiration rate** by LY139603 and amitriptyline in the non-pretreated dogs (Table 2). However, after atropine and propranolol pretreatment, the increase in respiration by amitriptyline was 47%-58% lower than that observed following LY139603 at 8 and 10 mg/kg and at 30 minutes after the end of the infusion. Amitriptyline increased **minute volume** to a greater extent than did LY139603 in the non-pretreated dogs, whereas LY139603 increased minute volume to a greater extent in the pretreated dogs. Amitriptyline and LY139603 decreased **arterial blood pH** to a similar extent (1%) at the lower doses in the non-pretreated dogs (Table 3). Slightly

decreased **arterial blood PCO_2** and increased **arterial blood PO_2** were observed in both treated and saline control groups, both with and without pretreatment. There was a slight increase in **arterial blood HCO_3** by LY139603 and decrease by amitriptyline at all doses and timepoints with atropine and propranolol pretreatment.

In the ECG evaluation, the **P-R interval** was increased to a similar extent by LY139603 and amitriptyline in the absence of pretreatment with atropine and propranolol, but the increase was statistically significant compared to saline controls in the LY139603-treated dogs, only. In the pretreated animals, the P-R interval was significantly increased by amitriptyline and not by LY139603, at doses of 2 and 4 mg/kg compared to saline controls. The **QRS duration** was increased by amitriptyline only, compared to saline controls, in both non-pretreated and pretreated dogs. Without pretreatment, the **Q-TC interval** was increased 19%-22% by LY139603 at all doses and post-infusion, and decreased 8%-11% at all doses by amitriptyline. However, after atropine and propranolol pretreatment, the Q-T interval corrected was increased 7%-32% by LY139603 and 8%-24% at all doses and post-infusion timepoints, without significant differences between the treatments.

Summary of individual study findings: LY139603 increased heart rate (28%-38% at 4-10 mg/kg), respiration rate (43%-76% at 4-10 mg/kg), P-R interval (14%-18% at 8-10 mg/kg and 30 post-infusion), the corrected Q-T interval (19%-22% at 6-10 mg/kg), slightly increased PCO_2 (at 2 mg/kg and 30 minutes after infusion), and decreased arterial blood pH (<1%-1%) at 2-6 mg/kg in the non-pretreated (atropine and propranolol) dogs. In the pretreated dogs, LY139603 increased the PCO_2 (4%-7% at 4-10 mg/kg and 11%-13% post-infusion), and increased the corrected Q-T interval (7-32% at all doses).

Amitriptyline increased heart rate (62%-116% at 2-10 mg/kg without pretreatment with atropine and propranolol), increased respiration rate (37%-76% at 2-10 mg/kg and post-infusion without pretreatment and 14%-23% at 8-10 mg/kg and 30 in post-infusion with pretreatment), decreased the Stroke Work Index (45%-54% at 4-10 mg/kg and post-infusion without pretreatment and 16%-24% at 4 mg/kg and 60 minutes post-infusion with pretreatment), decreased Pulmonary Capillary Wedge Pressure (11%-56% at 4-10 mg/kg, and post-infusion without pretreatment and 29%-71% at 2-10 mg/kg and post-infusion with pretreatment), increased Minute Volume (7%-27% at all doses and post-infusion without pretreatment), decreased arterial blood pH (1% at 2-8 mg/kg without pretreatment), increased the P-R interval (up to 13% at 8-10 mg/kg without pretreatment and 36% at 10 mg/kg with pretreatment), increased the QRS duration (8%-16% at 4-10 mg/kg and post-infusion with dose relationship without pretreatment and 14%-72% at all doses and post-infusion with dose relationship with pretreatment), decreased the Q-T interval (8%-11% at 6-10 mg/kg without pretreatment), and increased the Q-T interval (8%-24% at all doses and post-infusion with pretreatment).

Table 1

The Effects of LY139603, Amitriptyline and Saline on Heart Rate in Anesthetized Dogs

Time (min)	Dose (mg/kg, i.v.)	Saline Heart Rate (Mean ± S.E.)	LY139603 Heart Rate (Mean ± S.E.)	Amitriptyline Heart Rate (Mean ± S.E.)
0	Pre-drug	70 ± 6	68 ± 3	86 ± 14
10	2	66 ± 6	84 ± 6	139 ± 30[ab]
20	4	66 ± 5	89 ± 8[a]	186 ± 15[ab]
30	6	64 ± 5	94 ± 5[a]	184 ± 14[ab]
40	8	61 ± 7	87 ± 4[a]	178 ± 12[ab]
50	10	60 ± 4	84 ± 4[a]	175 ± 10[ab]
80		51 ± 9	86 ± 10[a]	146 ± 12[ab]
110		56 ± 8	66 ± 4	131 ± 19[ab]

LY139603 or amitriptyline was infused at 0.2 mg/kg min for 50 min. Doses given are cumulative. Saline was given at a volume rate equal to that administered to the drug groups (0.02 ml/kg min). Values for LY139603 and amitriptyline are the means (± S.E.) from 4 dogs. Significant differences in mean changes from pre-drug values compared to saline group (a) or between LY139603 group and amitriptyline group (b) were determined by Duncan's multiple range test ($P = 0.05$).

Table 2

The Effects of LY139603, Amitriptyline and Saline on Respiration Rate in Anesthetized Dogs

Time (min)	Dose (mg/kg, i.v.)	Saline Respiration Rate (Mean ± S.E.)	LY139603 Respiration Rate (Mean ± S.E.)	Amitriptyline Respiration Rate (Mean ± S.E.)
0	Pre-drug	6.0 ± 0.6	8.6 ± 1.2	7.0 ± 1.0
10	2	6.6 ± 0.5	10.2 ± 1.3	9.6 ± 1.3[a]
20	4	6.8 ± 0.5	12.3 ± 1.5[a]	11.4 ± 0.7[a]
30	6	6.9 ± 0.6	13.0 ± 1.4[a]	12.0 ± 0.2[a]
40	8	7.0 ± 0.3	14.0 ± 1.5[a]	12.3 ± 0.3[a]
50	10	6.0 ± 0.7	15.1 ± 2.0[a]	11.8 ± 0.4[a]
80		6.3 ± 0.9	11.3 ± 0.8	10.8 ± 0.5[a]
110		5.5 ± 1.0	10.5 ± 0.6	11.2 ± 0.7[a]

LY139603 or amitriptyline was infused at 0.2 mg/kg min for 50 min. Doses given are cumulative. Saline was given at a volume rate equal to that administered to the drug groups (0.02 ml/kg min). Values for LY139603 and amitriptyline are the means (± S.E.) from 4 dogs. Significant differences in mean changes from pre-drug values compared to saline group (a) or between LY139603 group and amitriptyline group (b) were determined by Duncan's multiple range test (P = 0.05).

Table 3
The Effects of LY139603, Amitriptyline and Saline on Arterial Blood pH in Anesthetized Dogs

Time (min)	Dose (mg/kg, i.v.)	Saline pH (Mean ± S.E.)	LY139603 pH (Mean ± S.E.)	Amitriptyline pH (Mean ± S.E.)
0	Pre-drug	7.25 ± 0.03	7.25 ± 0.01	7.21 ± 0.02
10	2	7.25 ± 0.02	7.22 ± 0.01[a]	7.17 ± 0.02[a]
20	4	7.25 ± 0.04	7.21 ± 0.01[a]	7.18 ± 0.02
30	6	7.26 ± 0.03	7.21 ± 0.01[a]	7.18 ± 0.02[a]
40	8	7.25 ± 0.03	7.23 ± 0.01	7.18 ± 0.02
50	10	7.26 ± 0.03	7.24 ± 0.02	7.23 ± 0.05
80		7.26 ± 0.03	7.21 ± 0.02	7.21 ± 0.03
110		7.31 ± 0.04	7.23 ± 0.01	7.21 ± 0.02

LY139603 or amitriptyline was infused at 0.2 mg/kg min for 50 min. Doses given are cumulative. Saline was given at a volume rate equal to that administered to the drug groups (0.02 ml/kg min). Values for LY139603 and amitriptyline are the means (± S.E.) from 4 dogs. Significant differences in mean changes from pre-drug values compared to saline group (a) or between LY139603 group and amitriptyline group (b) were determined by Duncan's multiple range test (P = 0.05).