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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-427

Pharmacology Review(s) #3

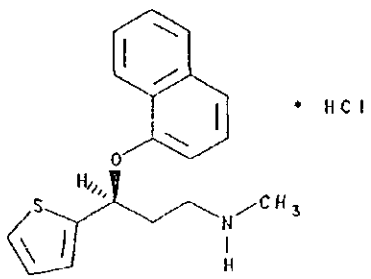
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-427.
Review number: 1.
Sequence number/date/type of submission: N-000 / 13 November, 2001 / Original submission.
Information to sponsor: Yes (X) No ( ).
Sponsor (or agent): Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285; (317) 276-2000.
Manufacturer for drug substance: Eli Lilly and Company, Tippecanoe Laboratories, Lafayette, IN 47909.

Reviewer Name: Linda H. Fossom
Division Name: Neuropharmacological Drug Products
HFD# 120
Review Completion Date: September 5, 2002.

Drug:

Code Name: Compound LY248686 HCl (LY246916); this is the S(+) enantiomer.
Generic Name: **duloxetine hydrochloride**.
Trade Name: Cymbalta™.
Chemical Name: see below.
CAS Registry Number: 116539-59-4.
Molecular Formula/ Molecular Weight: see below.
Structure:



(+)-N-Methyl-gamma-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride M.Wt 333.883

Relevant INDs/NDAs/DMFs: IND [redacted] IND 38,838 (HFD-120/depression/enteric coated tablets), IND [redacted] IND [redacted]

Drug Class: Inhibitor of monoaminergic (5-HT and NE) reuptake pumps.

Indication: Depression.

Clinical formulation: gelatin capsule containing enteric-coated pellets of duloxetine (to prevent acid hydrolysis in stomach); proposed dosage forms of 20, 30, [redacted] 60 mg.

Route of administration: oral.

Proposed use: Treatment of Major Depression, dosing at 60 mg once daily, although labeling states that [redacted]

In proposed labeling, [redacted]

Disclaimer: Tabular and graphical information is excerpted directly from the Sponsor's submission where feasible and cited as such.

It should also be noted that most of the pre-clinical studies submitted in support of the current NDA were recently reviewed (review dated September 8, 1998) by Karen Davis-Bruno, for the original submission of IND [redacted]. I have independently reviewed the pivotal studies in detail for the current review.

Executive Summary

I. RECOMMENDATIONS

A. Recommendation on Approvability:

Approvable, if the issues outlined below and detailed at the end of this review are adequately addressed.

B. Recommendations for Nonclinical Studies:

[These recommendations are explained in somewhat more detail at the end of this review.]

1. Both 4-OH-duloxetine glucuronide conjugate (which represented ~30% of total human systemic exposure and at most 0.1-fold coverage in rats) and 5-OH, 6-MeO-duloxetine sulfate (12% of human exposure, but undetected in plasma of any toxicology species) should be qualified according to ICH Q3A Guideline on Impurities (1996). Qualification should include: 1) *in vitro* genotoxicity testing, specifically the Ames test and *in vitro* chromosomal aberration test, and 2) a Segment II reproductive toxicity study, because the patient population will include women of child-bearing potential. It should be noted that intravenous, rather than oral, dosing would probably be necessary to achieve adequate plasma concentrations of these molecules.

2. With regard to — impurities in the drug substance that have specifications — requiring qualification: 1) data be submitted showing that the amounts of these impurities in the lots of drug substance used in pivotal toxicology studies (e.g., *in vitro* genotoxicity tests (Ames and chromosomal aberrations) and a segment II reproductive toxicity study) qualify — impurities; or 2) specifications for — impurities be lowered to — % so that qualification is not an issue; or 3) — impurities be qualified.

3. The *in vitro* chromosomal aberration test, part of the standard test battery according to the current ICH Guidance for Industry, S2B Genotoxicity, 1997, was inadequate and should be repeated. In the submitted study, duloxetine was negative for 4-hr treatment, with and without metabolic activation. However, the study was not valid by current standards, because this negative finding should have verified with a study using continuous treatment with duloxetine (without activation) for ~24 hr (1.5 cell doubling times), in accordance with the current ICH Guidance. Additionally, 200 metaphases, rather than only 100 as in the original study, should be analyzed at each concentration.

The first 2 recommendations should be adequately addressed before approval.

C. Recommendations on Labeling:

[These labeling recommendations are compared with the Sponsor's proposed labeling at the end of this review.]

CLINICAL PHARMACOLOGY/Pharmacodynamics

[

]

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogensis

Duloxetine was administered in the diet to mice and rats for 2 years. In female mice receiving duloxetine at dietary doses of approximately 140 mg/kg/day [times the maximum recommenede human dose [MRHD] on a mg/m^2 basis), there was an increased incidence of hepatocellular adenomas and carcinomas; the no-effect level was approximately 50 mg/kg [times the MRHD on a mg/m^2 basis). Tumor incidence was not increased in male mice receiving duloxetine at dietary doses up to approximately 100 mg/kg/day [times the MRHD on a mg/m^2 basis).

In rats, dietary doses of duloxetine up to approximately 27 mg/kg/day in females [times the MRHD on a mg/m^2 basis) or approximately 36 mg/kg/day in males [times the MRHD on a mg/m^2 basis) did not increase the incidence of tumors.

Mutagenesis

Duloxetine was not mutagenic in the *in vitro* bacterial reverse mutation assay (Ames test) and was not clastogenic in an *in vivo* chromosomal aberration test in mouse bone marrow cells [

] Additionally, duloxetine was not genotoxic in an *in vitro* mammalian forward gene mutation assay in mouse lymphoma cells or in an *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes, and did not induce sister chromatid exchange in Chinese hamster bone marrow *in vivo*.

Impairment of Fertility

Duloxetine administered orally to either male or female rats prior to and throughout mating at daily doses up to 45 mg/kg — times the maximum recommended human dose [MRHD] on a mg/m^2 basis) did not alter mating or fertility.

Pregnancy

Pregnancy Category C

In animal reproduction studies, duloxetine has been shown to have adverse effects on embryo/fetal and postnatal development.

When duloxetine was administered orally to pregnant rats and rabbits during the period of organogenesis, there was no evidence of teratogenicity at doses up to 45 mg/kg/day — times the maximum recommended human dose [MRHD] on a mg/m^2 basis, in rats and rabbits, respectively). However, fetal weights were decreased at this dose, with a no-effect level of 10 mg/kg — times the MRHD on a mg/m^2 basis, in rats and rabbits, respectively).

When duloxetine was administered orally to pregnant rats throughout gestation and lactation, the survival of pups to 1 day postpartum and pup body weights at birth and during the lactation period were decreased following maternal exposure to 30 mg/kg/day —times the MRHD on a mg/m^2 basis), with a no-effect level of 10 mg/kg. Furthermore, behaviors consistent with increased reactivity, such as increased startle response to noise and decreased habituation of locomotor activity, were observed in pups following maternal exposure to 30 mg/kg/day. Post-weaning growth and reproductive performance of the progeny were not affected adversely by maternal duloxetine treatment.

There are no adequate and well-controlled studies in pregnant women, therefore duloxetine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Labor and Delivery

Nursing Mothers

Pediatric Use

DRUG ABUSE AND DEPENDENCE

Physical and Psychological dependence

II. SUMMARY OF NONCLINICAL FINDINGS

A. Brief Overview of Nonclinical Findings: See "Summary and Conclusions" in section IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS, at the end of this review, below.

D. Pharmacologic Activity:

Duloxetine inhibits the reuptake of serotonin and norepinephrine.

E. Nonclinical Safety Issues Relevant to Clinical Use:

Duloxetine has potential for deleterious effects on embryo/fetal development. Duloxetine and/or its metabolites: 1) adversely affected embryo/fetal development in rats and rabbits, but was not teratogenic; 2) crossed the placental barrier in pregnant rats; and 3) was excreted in the milk of lactating rats.

III. ADMINISTRATIVE

A. Reviewer signature:	Linda H. Fossom *{see appended electronic signature page}*
B. Supervisor signature:	Barry Rosloff *{see appended electronic signature page}*

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: *In vitro*, duloxetine potently inhibits the reuptake of serotonin and norepinephrine, and has lower affinity for dopamine reuptake (seeTable 1, below). Although some metabolites of duloxetine have significant affinity for the reuptake transporters, the major metabolites circulating in human plasma, 4-OH-duloxetine-glucuronide and 5-OH, 6-MeO-duloxetine sulfate, do not (Ki's >3000 nM; see Table 2, below). *In vivo*, duloxetine blocks serotonin and norepinephrine depletion by reuptake transporter substrate releasers, such as p-chloroamphetamine and 6-OH-dopamine, consistent with reuptake transporter inhibition by duloxetine.

Table 1. Sponsor's table showing *in vitro* binding affinities for duloxetine at monoamine reuptake transporters. [Excerpted directly from Pdsumm.pdf, page 3, this submission.]

Measurement	Duloxetine K_i, nM
[^{3}H]-Paroxetine binding - human SERT	0.8 ± 0.04
[^{3}H]-Nisoxetine binding - human NERT	7.5 ± 0.3
[^{3}H]-Win35428 binding - human DART	240 ± 23
[^{3}H]-Paroxetine binding - rat SERT	0.5 ± 0.1
[^{3}H]-Nisoxetine binding - rat NERT	3.6 ± 0.3
[^{3}H]-5-HT uptake synaptosomes	4.6 ± 1.1
[^{3}H]-NE uptake synaptosomes	16 ± 2.9
[^{3}H]-DA uptake synaptosomes	369 ± 38
[^{3}H]-5-HT uptake platelets - human	0.20 ± 0.04

Abbreviations: SERT = serotonin transporter, NERT = norepinephrine transporter, and DART = dopamine transporter. Synaptosomal uptake of [^{3}H]-5-HT, [^{3}H]-NE, and [^{3}H]-DA was in synaptosomes from rat cerebral cortex, hypothalamus, and striatum, respectively. All values were determined from three or more independent experiments with a least six concentrations of drug in triplicate (Nonclinical Pharmacology Report 10 and Nonclinical Pharmacology Report 54).

Table 2. Sponsor's table showing *in vitro* binding affinities for metabolites of duloxetine at monoamine reuptake transporters. [Excerpted directly from Pdsumm.pdf, page 11, this submission.]

Compound	[3H]-Paroxetine (5-HT)	[3H]-Nisoxetine (NE)	[3H]-Mazindol (DA)
	K_i, nM		
4-hydroxy-duloxetine (550399)	63.9[b]	97.1[b]	130[b]
5-hydroxy-duloxetine (550381)	9.6[b]	18.4[b]	240.5[b]
6-hydroxy-duloxetine (550391)	1.06[b]	4.72[b]	163.5[b]
6-hydroxy-5-methoxy-duloxetine (550406)	3.66[b]	235.5[b]	353[b]
5-hydroxy-6-methoxy-duloxetine (550407)	266[a]	920[a]	2814[a]
Duloxetine-5,6-diol I	120[a]	695[a]	7275[a]
Duloxetine-5,6-diol II	31.7[a]	554[a]	>10μM[a]
4-hydroxy-duloxetine glucuronide (550408)	> 10μM[a]	>10μM[b]	3509[b]
6-hydroxy-duloxetine glucuronide (594422)	1459[a]	5455[a]	>10μM[a]
5-hydroxy, 6-methoxy-duloxetine (5) sulfate (581920)	3118[a]	>10μM[a]	>10μM[a]
5-hydroxy-6-methoxy-duloxetine (5) glucuronide (609122)	>10μM[a]	>10μM[a]	>10μM[a]
Dihydroxy-duloxetine glucuronide	>10μM[a]	>10μM[a]	>10μM[a]

Data from Nonclinical Pharmacology Report 30.
[a] N = 1.
[b] N = 2.

Mechanism of action: The mechanism of action of duloxetine as an anitdepressant is unknown. As for other currently approved antidepressants, the actual mechanism of action is probably through (as yet unidentified/unexplained) compensatory mechanisms initiated by the direct effect(s) of the drugs. For duloxetine, the initiating effect is assumed to be blockade of reuptake of serotonin and norepinephrine, which results in enhanced serotonergic and noradrenergic activities in the CNS.

Secondary pharmacodynamics: Duloxetine has been determined to have low affinity (Ki > 1000 nM) for other neuronal receptors and bindings sites *in vitro* (see Table 3, below).

Table 3. Sponsor's table showing neuronal receptor and binding sites for which duloxetine has low affinity (Ki > 1000 nM). [Excerpted directly from Pdsumm.pdf, pages 6-7, this submission.]

Receptor (Species)	Receptor (Species)
Neurotransmitter Receptors	
Adenosine, A1 (R)	Glutamate, NMDA, Glycine(Strychnine-insensitive) (R)
Adenosine, A2 (B)	Glycine, Strychnine-sensitive (R)
Adrenergic, α1A (R)	Histamine, H2 (GP)
Adrenergic, α1B (R)	Melatonin (C)
Adrenergic, α2A (H)	Muscarinic, M1 (H)
Adrenergic, α2B (M)	Muscarinic, M2 (H)
Adrenergic, β1 (R)	Nicotinic (α-bungarotoxin insensitive) (R)
Adrenergic, β2 (R)	Opiate, Delta 1 (R)
Dopamine, D1 (R)	Opiate, Kappa 1 (GP)
GABA A, Agonist Site (B)	Opiate, Mu (R)
GABA A, Benzodiazepine, Central. (B)	Opiate, Non-selective (R)
Glutamate, AMPA Site (R)	Sigma 1 (GP)
Glutamate, Kainate Site (R)	Sigma 2 (GP)
Glutamate, NMDA Agonist Site (R)	
Ion channels	
Calcium Channel, Type L (R)	Glutamate, NMDA, PCP (R)
Calcium Channel, Type N (R)	Potassium Channel, ATP-Sensitive (R)
GABA, Chloride, TBOB Site (R)	Potassium Channel, Ca^{++} Active, Volt Sensitive (R)
Glutamate, Chloride Site (R)	Sodium, Site 1 (R)
Glutamate, MK-801 Site (R)	Sodium, Site 2 (R)
Second messengers	
Adenylate Cyclase, Forskolin (R)	NOS (Neuronal-Binding) (R)
Inositol Triphosphate (R)	Protein Kinase C, PDBu (M)
Transporters	
Choline transporter (R)	Adenosine transporter (R)
GABA transporter (R)	
Brain/gut peptides	
Cholecystokinin, CCK1 (CCKA) (M)	Neurokinin, NK3 (NKB) (R)
Cholecystokinin, CCK2 (CCKB) (M)	Neuropeptide, NPY1 (H)
Neurokinin, NK1 (R)	Neurotensin (R)
Neurokinin, NK2 (NKA) (H)	Somatostatin, Non-selective (R)

Abbreviations: R = rat, M = mouse, GP = guinea pig, B = bovine, and H = human.

[a] <50% inhibition of receptors by 1000 nM concentration of duloxetine (from Nonclinical Pharmacology Report 54).

Labeling recommendations:

The mechanism of the antidepressant action of duloxetine in humans is believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS. Preclinical studies have shown that

duloxetine is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a weak inhibitor of dopamine reuptake. Duloxetine has no significant affinity for dopaminergic, adrenergic, cholinergic or histaminergic receptors *in vitro*. Pharmacologic activity at these receptors is hypothesized to be associated with the various anticholinergic and sedative effects seen with other psychotropic drugs. Duloxetine does not inhibit monoamine oxidase (MAO).

[NB Paul Andreason, the Medical Officer for this submission, noted hypertension as a potential risk, with 24% of patients taking 120 mg/d experiencing elevated blood pressure, compared with 9% of patients on placebo. Although the binding affinities noted above wouldn't necessarily predict cardiovascular side effects, the potentiation of norepinephrine's effects by duloxetine (by blockade of reuptake) is consistent with this side effect.]

II. SAFETY PHARMACOLOGY:

Only selected safety studies are briefly summarized here; most were recently reviewed (review dated September 8, 1998) by K. Davis-Bruno, for the original submission of IND

Neurological effects: Acute testing of doses up to 30 mg/kg by oral gavage in male CD-1 mice: irritability, increased vocalization, and mydriasis at doses ≥10 mg/kd. No evidence of proconvulsant activity in mice: no change in convulsive threshold with pentylenetetrazol and increased threshold with electro-shock (at 30 mg/kg; enhanced at 10 mg/kg with 5 days of dosing); prolongation of hexibarbital sleep times (attenuated with 5 days of dosing). No effects on muscle tone, coordination, or passive-avoidance learning. However, there was more rapid habituation of ambulatory and non-ambulatory spontaneous activity after 10 and 30 mg/kg acutely but slower habituation of acoustic startle response in mice that were treated with 10 mg/kg/day for 5 days.

Cardiovascular and pulmonary effects: Because of the recent concern regarding cardiovascular toxicity of antidepressants, I examined these safety studies for evidence that might cause concern, such as prolonged QTc intervals or *in vitro* activity (e.g., inhibition of HERG channels).

Cardiovascular safety studies included: 1) *in vivo* studies in rats and dogs; and 2) *in vitro* studies in isolated cardiac muscles (guinea pig atria) and human cardiac ion channels (from isolated human atrial myocytes or HERG cells).

Key findings:

- Increased heart-rate, decreased stroke volume and P-R interval, but no apparent effects on QTc in anesthetized Beagles given iv infusions up to a dose (10 mg/kg) that probably achieved blood levels of duloxetine similar to those in humans given MRHD of 60 mg BID. [Additionally, there were no clear effects on heart-rate determined before and 2 hr after oral dosing in 6-mo and 1-yr studies in Beagles; but complete EKGs were not performed. More importantly, there were no cardiac or pulmonary histopathologic findings in the 6-mo and 1-yr dog studies that would indicate cardiac or pulmonary toxicity after chronic administration.]

- Increased respiratory rate, MPAP, wedge pressure, and pulmonary resistance in anesthetized Beagles given iv infusions up to a dose (10 mg/kg) that probably achieved blood levels of duloxetine similar to those in humans given MRHD of 60 mg BID. [No effect of this dose on MPAP, HR or MAP in conscious mongrel dogs.] [I talked with Shari Targum, Medical Officer, and Feemie Williams, Senoir Staff Fellow, in Cardio-Renal regarding whether these results would signal concern for primary pulmonary hypertension (PPH), as was seen with fenfluramine. These acute findings in anesthetized dogs suggest a direct vasoconstriction, which would likely be independent of any PPH, which is a chronic condition. I checked for cardiac or pulmonary histopathologic findings in the 6-mo and 1-yr dog studies and none were noted.]

- *In vitro* potency at blocking HERG channels was low (IC_{50} = 5.5 μM). While this is not an obvious cause for concern regarding QT prolongation, there are examples of drugs that cause QT prolongation at similar low *in vitro* potencies, such as sotalol (class III antiarrhythmic) and fluoroquinolone antibiotics (John Koerner, Pharmacologist, Division of Cardio-Renal Drug Products, personal communication).

In vivo studies:

- — : (Nov 1989) maleate in male S-D rats, with chronic indwelling cannula in right femoral artery (for pulse rate and arterial blood pressure): oral gavage at 7, 20, 60, and 170 mg/kg (in 10% acacia); monitored for HR and MAP. Maybe slight (~14%) decreases in HR at 60 and 170 mg/kg; no effects on MAP.

- — : (January, 1990) maleate iv in (morphine sulfate, Brevital, and α-chloralose) **anesthetized Beagle** dogs at duloxetine doses of 0.4, 2, and 10 mg/kg as 50-min infusion + 30 min more of monitoring.

Duloxetine infusion resulted in decreased minute volume (↓~20% at MD and ↓5-10% at HD, vs ↑10-20% in vehicle controls, throughout infusion and subsequent 30 min) and **increased respiratory rate** during infusion (peaking at ↑45% at LD, 49% at MD, and 93% at HD, vs <1% decrease in controls at the end of infusion). This was accompanied by **increased mean pulmonary arterial pressure** (↑12% at LD (ns: not significant), 116% at MD and 136% at HD, vs ↓15% in vehicle controls at 20 min), **increased pulmonary capillary wedge pressure** (↑56% at LD (ns), 75% at MD and 203% at HD, vs ↑15% in vehicle controls at 20 min), **and increased pulmonary resistance** (↑44% at LD, 183% at MD and 102% at HD, vs ↓24% in vehicle controls at 10 min), that peaked at 10-20 min and dissipated by end of infusion; **increased HR** (↑12% at MD (ns), 62% at HD, vs <1% in vehicle controls at 20 min), **decreased stroke volume** (↓18% at HD, vs ↑3% in vehicle controls at 30 min), and **decreased P-R interval** (↓17% at HD, vs ↑5% in vehicle controls at 20 min). There was **no apparent effect on QTc** (msec/$sec^{1/2}$, Bazett's correction, which over-corrects when HR is increased, so negative results in this case are convincing).

- Tox19: (April, 1992) HCl (2 mg/kg as 50 min infusion) iv and (10 mg/kg) po on arterial pressure in **conscious mongrel dogs** (serotonin creatinine as positive control): no effect on mean pulmonary arterial pressure (positive control worked); no effect on heart rate; no effect on mean arterial pressure (except for (25% PEG 300) vehicle infusion-induced increase).

- Tox48 and ADME66: (November, 2000; [ ] HCl (Lot no. 019JD0), 30-min infusion (4 (HD) or 5% mannitol in sterile water for injection; 5 ml/kg/d, alternating right and left cephalic veins, saphenous vein if necessary) of 0, 1, 2.5, or 5 mg/kg/d for 14 d; in **Beagle dogs** ([ ] ~7 mos old, 7.2-12.7 kg; 3/sex/dose): EKG predosing and within 30 min of finishing dosing on day 14 or 15; TK on days 1, and 10 (HD) or 14; predosing, ~15 and 29 min into dosing, and 1, 2, 4, 6, 8, 12, and 24 hr after dosing from jugular vein; necropsy of HD at day 10 (sacrificed early due to irritation at infusion site), other groups after 15 days. There were

no effects on HR or QT_C (no other EKG parameters were presented, e.g., PR-interval that was decreased at 10 mg/kg in an earlier acute study in anesthetized Beagles) at doses up to 2.5 mg/kg/d (5 mg/kg/d group was sacrificed early without EKG). The systemic exposure values for duloxetine in this study are displayed in Table 4, below. Human (AUC) exposures (see Table 12, below) following 60 mg QD or BID were approximately equal to the 2.5 or 5 mg/kg iv doses in dogs, respectively.

Table 4. Systemic exposure to duloxetine after single or repeated daily intravenous dosing of Beagle dogs. [Sponsor's table, excerpted directly from this submission.]

	Administered Dose (mg/kg/day)					
Parameter	1		2.5		5	
Sex	M	F	M	F	M	F
Day 1						
C_{max} (ng/mL)	454 ± 83	410 ± 87	1097 ± 87	947 ± 234	1703 ± 308	1657 ± 380
$AUC_{0-\infty}$ (ng•hour/mL)	459 ± 22	404 ± 27	1163 ± 94	1291 ± 58	2166 ± 39	2550 ± 375
T_{max} (hour)	0.48	0.48	0.48	0.48	0.41	0.41
Half-life (hour)	2.31	1.29	2.16	2.22	2.41	2.96
Half-life Range (hour)	1.68-2.92	1.05-1.98	1.91-2.76	1.98-2.49	2.075-3.45	2.02-4.50
Day 10/14[a]						
C_{max} (ng/mL)	585 ± 324	480 ± 62	1074 ± 67	945 ± 226	1556.1[b]	1923[c]
$AUC_{0-\tau}$ (ng•hour/mL)	523 ± 65	435 ± 19	1140 ± 96	1312 ± 79	2077[b]	2791[c]
$AUC_{0-\infty}$ (ng•hour/mL)	523 ± 66	436 ± 19	1140 ± 96	1312 ± 79	2077[b]	2793[c]
T_{max} (hour)	0.48	0.41	0.48	0.48	0.48	0.48[c]
Half-life (hour)	3.03	2.14	1.89	1.93	2.62	3.05[c]
Half-life Range (hour)	2.41-5.28	2.02-2.35	1.60-2.17	1.84-1.98	1.98-3.89	2.83-3.32

Abbreviations: M = male; F = female; SD = standard deviation; C_{max} = maximum observed plasma concentration; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from 0 to infinity; $AUC_{0-\tau}$ = area under the plasma concentration-time curve from 0 to τ, where τ is the dosing interval.

a Samples were collected on Day 14 for the 1-and 2.5-mg/kg dose groups, and on Day 10 for the 5-mg/kg dose group.

b Samples were collected from only 2 dogs on this day; so the standard deviations were not calculated.

c One of the 3 dogs, (Dog H05838), did not receive the full dose, so the data from this dog were not used in calculation of the means.

In vitro studies:

- — maleate in isolated cardiac muscles: Suppressed force and rate of spontaneous contraction in gp atria and inhibited ISO effects at 100 uM, but not 10 uM.

- Tox45: (June 1999) **Human cardiac ion channel** blocking profile of duloxetine (LY248686 hydrochloride). Performed by [ ]. Currents were: cardiac sodium I_{Na}, transient outward potassium I_{to}, and sustained I_{sus}, inwardly rectifying K I_{K1}, from isolated human atrial myocytes and HERG (I_{Kr} stably transfected in HEK293 cells). Duloxetine concentrations of 0.18, 1.8, 9 (HERG only), 18 and 36 (HERG only) uM. Pacing rate was 0.1 Hz.

At 18 uM, inhibited INa (↓32%) and Ito (↓24%). Inhibited HERG 27%at 1.8 uM and 100% at 36 uM; IC50 was 5.5 uM. There was no apparent rate-dependency of the inhibition at 0.18 uM duloxetine and 1, 2, or 3 Hz.

Renal effects: In female mice, duloxetine (at oral doses 3-20 mg/kg, as the HCl) increased sodium excretion, with a slight increase in serum potassium at ≥10 mg/kg; with no effect on urine volume. In female S-D rats, duloxetine (at oral doses 7-170 mg/kg, as the maleate) increased urine output and sodium and chloride excretion at 20 and 60 mg/kg, but these parameters were decreased at 170 mg/kg. [In her review of IND —, K. Davis-Bruno noted that animal studies suggest that duloxetine may increase urinary bladder capacity and increase periurethral striated muscle EMG activity.]

Gastrointestinal effects: Duloxetine (at oral doses up to 30 mg/kg) did not alter gastrointestinal transit time for a charcoal meal in mice. More interestingly, duloxetine decreased body weights and food consumption in oral gavage (reproductive toxicity) studies in CD Sprague-Dawley rats using daily doses similar, on a mg/kg basis, to the dietary ones used in the carcinogenicity study (specifically, 45 mg/kg in males and 30 mg/kg in females). A similar effect was also seen in a pharmacology study of meal-fed obese Zucker rats treated with duloxetine by intraperitoneal injection (7.2 mg/kg). Although there is apparently no data to allow a comparison of the systemic exposures achieved at toxic doses by different routes of administration, it seems likely that decreased food consumption and decreased body weight would limit doses (at least in rats) regardless of route.

Abuse liability: The potential for dependence upon duloxetine was studied in Rhesus monkeys and rats (Tox41.pdf; Sept, 1996). In Rhesus monkeys, duloxetine (unlike the positive control diazepam) did not suppress barbital withdrawal signs (doses up to at least 1.6 mg/kg iv). Furthermore, duloxetine was not self-administered by monkeys trained to self-administer pentobarbital. In rats, there were no withdrawal signs, such as weight loss or altered food intake or hyper-reactivity, when duloxetine was withdrawn after 4 weeks of dietary administration (at doses up to 50 mg/kg/day).

Other: Immune response was tested in male CD-1 mice; duloxetine (as the maleate) at oral doses of 5-130 mg mg/kg/day for 10 days no effect on primary antibody production (microtiter, hemaggutination procedure; ıs antigen) (see Special Toxicology Section, below).

Safety pharmacology summary and conclusions: Duloxetine is a centrally, as well as peripherally, acting drug and was active in animal models that are predictive of antidepressant and antinociceptive (chronic pain) activities in humans. Although convulsions were seen at high doses in acute toxicity studies, duloxetine did not appear to be pro-convulsant; it did not potentiate pentylenetetrazol-induced seizures and appeared to decrease sensitivity to electrogenic seizures in mice. Although potential for cardiac toxicity can not be ruled out completely, there is no pre-clinical evidence that would indicate a problem. Limited testing in rats and monkeys did not indicate abuse liability; duloxetine did not substitute for barbiturate in self-administration paradigm, did not block barbiturate withdrawal signs, and did not produce withdrawal signs after discontinuation of repeated (4-week) dosing. While not strictly a safety issue, duloxetine decreased food consumption in animal species (e.g., rats, mice, dogs).

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters: Duloxetine (as the maleate salt) was rapidly (Tmax of 1-1.5 hr) and essentially completely absorbed (82% and 100%) following oral administration to rats and dogs (see Table 5, below). Especially at this low dose (5 mg/kg) duloxetine was extensively metabolized, with unchanged duloxetine accounting for only 7% and 1% of total systemic exposure for 24 hr after administration to rats and dogs, respectively. Duloxetine was highly bound to plasma proteins: *in vitro* binding was 94-97% in mice, rats, dogs, and humans at duloxetine concentration of 150 ng/ml (ADME62.pdf). In humans, duloxetine bound to both albumin and α1-acid glycoprotein.

Table 5. Sponsor's table showing "Pharmacokinetics of duloxetine and radioactivity in mice, rats, and dogs after a single 5 mg/kg dose of ^{14}C-duloxetine maleate." [Excerpted directly from Pksumm.pdf, Table 3, page 13.]

	Rats (n = 3)		Dogs (n = 3)		Mice[d]
Parameter	IV	Oral	IV	Oral	Oral
C_{max} (µg equiv/mL or µg/mL)					
Radioactivity	6.481	0.597	7.285	1.698	1.591
Duloxetine	4.678	0.053	5.819	0.022	0.069
T_{max} (hours)					
Radioactivity	0.017	1.5	0.017	2 to 3	1.5
Duloxetine	0.017	1.5	0.017	1 to 1.5	1.5
AUC (µg equiv•hr/mL or µg•hr /mL)					
Radioactivity	13.05	10.72	53.08	76.73	10.38
Duloxetine	2.06	0.43	4.20	0.19	0.25
Half-life (hours)					
Radioactivity					
α	7	7	6.4	4	4.6
ß	23	122	32	52	27
γ	NC	NC	92	93	NC
Duloxetine	2.5	1.5	3.3	4	2.1
Biotransformation[a] (%)	75	93	81	99	97
Absorption (%)[b]		82		100	
Bioavailability[c] (%)		21		5	
ADME Report No.	7	5	16	14	3

Abbreviations: IV = intravenous, C_{max} = maximum observed plasma concentration, T_{max} = observed sampling time of C_{max}, NC = not calculated; AUC = the area under the plasma concentration-time curve from the first sample collection to the time of the last sample with concentrations above the quantitation limit of the assay; n = Number of rats per timepoint or number of dogs per study.

[a] Plasma radioactivity area under the plasma concentration-time curve (AUC_{0-24hr}) minus plasma duloxetine AUC_{0-24hr} as a percentage of the plasma radioactivity AUC_{0-24hr}.

[b] Oral plasma radioactivity AUC as a percentage of the intravenous plasma radioactivity AUC.

[c] Oral plasma duloxetine AUC as a percentage of the intravenous plasma duloxetine AUC.

[d] There were 10 animals per time point, but the plasma was pooled to yield a single sample per time point.

Special PK study: I am including a detailed review of this study, because it is such a beautiful demonstration of how dietary studies can be a good way to achieve continuous exposure in animals

when the half-life of the drug is short. A similar study in mice was also submitted (Tox49.pdf), but I will not review it here.

Study Tox50 (September 2001; performed at [redacted] # 6180-319; details of TK results were reported in ADME78.pdf) was a **13-wk dietary TK study in Fischer 344 rats** (F-344/ [redacted] 24/sex/dose) using duloxetine HCl (lot no 019JD0; [redacted] potency→ [redacted] pure duloxetine HCl; dietary doses of 0.01, 0.02, and 0.05 (females only) or 0.08 (males only), no controls) and determining systemic exposure to duloxetine (AUC, Cmax) at days 2, 33, and 96 (blood drawn every 4 hr for 24 hr, 3/sex/dose/time point). There were no deaths and no clinical signs noted. Body weights were significantly decreased throughout dosing in HD males (BW ↓17%, BW gain ↓25% at week 14) and HD females (BW ↓14%, BW gain ↓31% at week 13) compared with LD groups; food consumption was similarly reduced. [NB In other studies, low doses of duloxetine tended to increase body weights, so the relative decreases at the HD noted in this study may be exaggerated.] The TK parameters are presented in the Sponsor's table (Table 6), below.

Table 6. Systemic exposure parameters for Fischer 344 rats treated for up to 14 weeks with dietary duloxetine HCl. [Excerpted directly from this submission; Tox50.pdf.]

Summary Table						
	Administered Dose					
Parameter[a]	Group 1		Group 2		Group 3	
Sex	M	F	M	F	M	F
% Diet	0.01	0.01	0.02	0.02	0.08	0.05
Day 2						
C_{max} (ng/mL)	30.4	28.7	121.9	139.4	784.4	418.1
$AUC_{0\text{-}24\ hr}$ (ng•hour/mL)	405	290	1717	1772	10759	5482
AUC %CV	13.2	18.5	15.4	15.5	9.8	13.6
T_{max} (hour)	20	20	20	20	16	20
Day 33						
C_{max} (ng/mL)	29.6	51.7	178.0	318.9	931.5	654.9
$AUC_{0\text{-}24\ hr}$ (ng•hour/mL)	313	628	2554	4503	18623	13272
AUC %CV	15.8	15.2	11.9	10.4	5.6	6.8
T_{max} (hour)	16	20	20	16	20	20
Day 96						
C_{max} (ng/mL)	16.4	35.8	140.3	229.7	836.8	714.4
$AUC_{0\text{-}24\ hr}$ (ng•hour/mL)	200	437	1827	3362	17488	13535
AUC %CV	11.0	12.1	13.3	9.6	4.0	6.8
T_{max} (hour)	16	20	20	16	20	0[b]

Abbreviations: M = male; F = female; C_{max} = the maximum observed plasma concentration; T_{max} = the time at which C_{max} was reached; $AUC_{0\text{-}24hr}$ = the area under the plasma concentration-time curve from 0 hours (8:00 a.m.) to 24 hours (8:00 a.m. the following day); AUC %CV = the percent coefficient of variation associated with the AUC determination.

a Parameter values were calculated from the grouped data in which there were three rats per time point.

b C_{max} was reached at the first 8:00 a.m. sample on the day of the toxicokinetic sampling (designated as time 0).

Average daily doses across the study were 6.9, 13, and 52 mg/kg for males and 7.7, 15, and 37 mg/kg for females, at LD, MD, and HD, respectively. Doses in all groups decreased during the study, however, AUCs increased, particularly between days 2 and 33, except for LD males. There was not a clear, simple sex-related difference. I have included the plasma curves in the figure below (Figure 1, below) because it is the first time I've seen plasma curves for dietary dosing. Plasma levels appear to peak at ~16-20 hr, which is midnight to 4 am; the nadir is at 8 hr, which is 4 pm; and levels are increasing at the next time point, 12 hr/8 pm. [Lights were on a 12-hr cycle, but on-time was not specified; I'd assume it was ~7 am; and that the rats began to eat when the light went out about ~7 pm.] This data suggests that plasma levels measured at 8-10 am, as in the 3-mo and 3-yr dietary studies, probably well reflect average plasma levels, at least after ~4 weeks (i.e., 33 days) of dosing. Pooled plasma samples were also analyzed for the presence of metabolites and the summary table is presented below (Table 7, below).

Figure 1. Plasma curves for duloxetine levels in Fischer 344 rats treated for up to 14 weeks with dietary duloxetine HCl. Values are means ± standard deviations (n=3/sex/time point); days 2, 33, and 96 of dosing are depicted in the left, middle, and right panels, respectively; circles are LD, triangles are MD, and squares are HD, open symbols are males, solid symbols are females; time 0 is 8:00 am. [Graphs excerpted directly from this submission.]

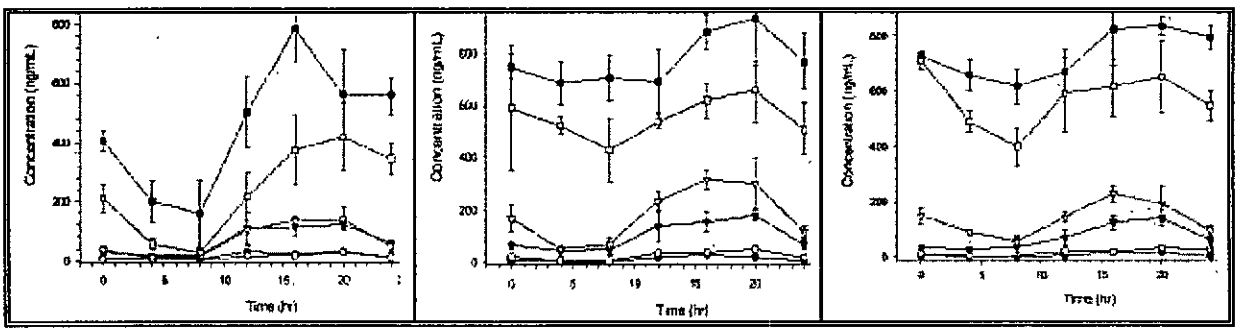


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Table 7. Sponsor's summary table showing qualitative profiling of metabolites in pooled plasma samples from 3-mo dietary study Tox50. It is not clear which day or days the pooled samples represent. [Table excerpted directly from this submission.]

Table 1: Rat Plasma Samples (6160-319)

Analyzed Samples (pooled*)	Observed Compound				
	Duloxetine	Glucuronide conjugate of 5-hydroxy	Glucuronide conjugate of 4-hydroxy	Glucuronide conjugate of 6-hydroxy	Desaminomethyl acid
Female High Dose, 4 & 8 hr	+	+	+	+	+
Female High Dose, 12 & 16 hr	+	+	+	+	+
Female High Dose, 20 & 24 hr	+	+	+	+	+
Male High Dose, 4 & 8 hr	+	+	+	+	+
Male High Dose, 12 & 16 hr	+	+	+	+	+
Male High Dose, 20 & 24 hr	+	+	+	+	+
Female Low Dose, 4 & 8 hr	+	+	+	+	+
Female Low Dose, 12 & 16 hr	+	+	+	+	+
Female Low Dose, 20 & 24hr	+	+	+	+	+

+ = observed in the
* = plasma from 3 animals at 2 time points were pooled

Distribution: Results of distribution studies in male rats were not remarkable, with highest amounts of radioactivity found in the gastrointestinal tract and liver, but amounts greater than plasma also found in lung, kidney, spleen, pituitary, Hardarian gland and prostate, and measurable amounts in brain. Of more interest, when duloxetine (45 mg/kg, ^{14}C-labeled, as the HCl salt) was administered to pregnant CD rats on day 12 or day 18 of gestation, radioactivity crossed the placenta, and could be found in embryonic tissue (GD12) or fetuses (GD18). Finally, when duloxetine (5 mg/kg, ^{14}C-labeled, as the HCl salt) was administered to lactating rats, peak plasma and milk concentrations of radioactivity (1 hr after dosing) were 1 and 0.5 µg-equiv/g, respectively; demonstrating that duloxetine and/or its metabolites is excreted in milk of lactating rats.

Metabolism: Because duloxetine is so extensively metabolized and the major molecular species circulating in humans appear to be poorly covered in the toxicology studies, I have examined in some detail the evidence for the amounts of circulating metabolites in both humans and animals.

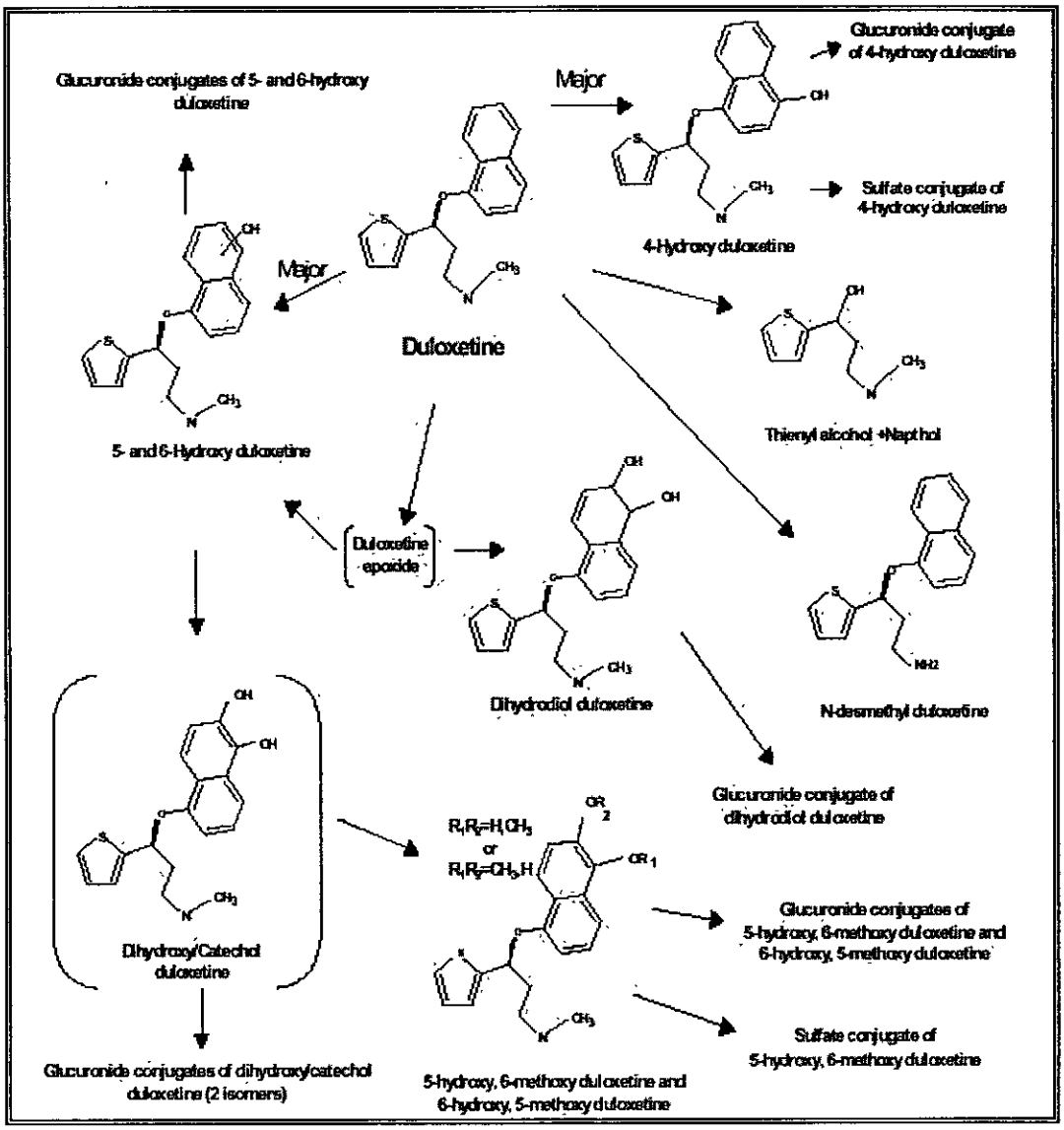
The Sponsor has identified the glucuronide conjugate of 4-OH-duloxetine and the sulfate conjugate of 5-OH, 6-MeO-duloxetine as the major circulating metabolites in humans. This designation appears to be based upon the fraction of total radioactivity peak area (not the fraction of total radioactivity) from a pooled 10-hr plasma sample in study SAAZ: duloxetine accounted for 4%, 4-OH-duloxetine-glucuronide for 47%, and 5-OH, 6-MeO-duloxetine-sulfate for 22%; 2 other metabolites, dihydroxy/catechol-duloxetine-glucuronide and 6-OH, 5-MeO-duloxetine-glucuronide,

accounted for 14 and 13%, respectively. I have tried to get a better idea of how much of the systemic exposure in humans is accounted for by (or due to) these metabolites (see below).

Summary/Discussion of human metabolism:

As in other animal species, duloxetine was heavily metabolized in humans, with 16 metabolites identified by [ ] from nonhydrolyzed and hydrolyzed urine samples. The proposed biotransformation pathways for duloxetine in humans are summarized in the Sponsor's figure, below.

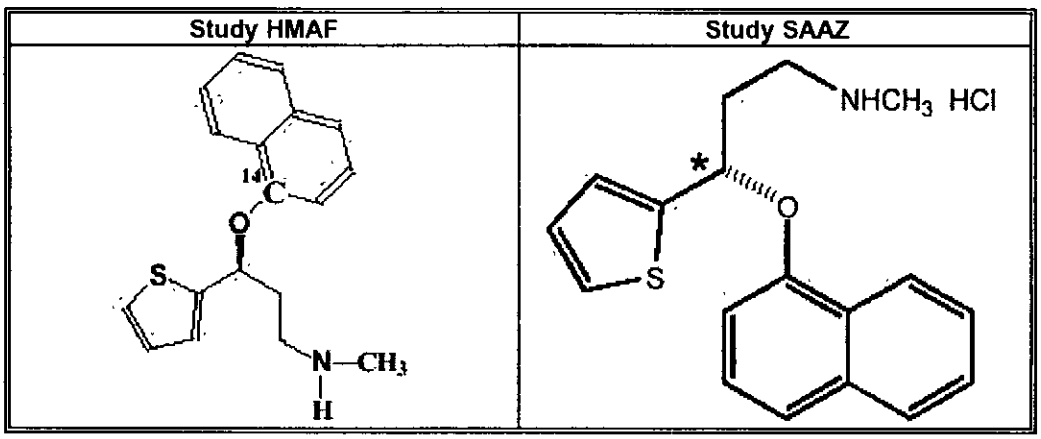
Figure 2. Proposed biotransformation pathways for duloxetine in humans. The compounds in brackets are postulated intermediates that have not been isolated. {Excerpted directly from this submission.}



With regard to the systemic exposure of humans to duloxetine and its metabolites, I found **3 studies** that looked at *in vivo* metabolism of duloxetine in humans:

1) **F1J-LC-HMAF** (1992; 1999 report): single oral dose (20 mg, enteric coated, 50 uCi 14C; 4 normal males), measured/calculated Cmax, AUC for duloxetine and total radioactivity;
2) **2) F1J-LC-SAAZ** (1998; 2000 report): single oral dose (20.2 mg, enteric coated, 100.6 uCi 14C; 4 normal volunteers, 3 males, 1 female), measured/calculated Cmax, AUC for duloxetine and total radioactivity; 90.5% of radioactivity recovered in 312 hr, with 72.0% excreted in urine; >11 metabolites that were excreted primarily in urine, as conjugates; metabolites found in plasma [redacted] as: a) gluc conj of 4-OH-dulox, b) gluc conj of methyl catechol dulox, c) gluc conj of gluc conj of catechol dulox, and d) sulfate conj of methyl catechol dulox;
3) **3) F1J-LC-HMBN** (2001): single 60 mg dose and QD and BID dose at steady state, measured Cmax, AUC for duloxetine and "its 2 major circulating metabolites," glucuronide conjugate of 4-OH-duloxetine (aka 55408) and sulfate conjugate of 5-OH, 6-MeOH-duloxetine (aka 581920), which were analyzed/quantified by [redacted]

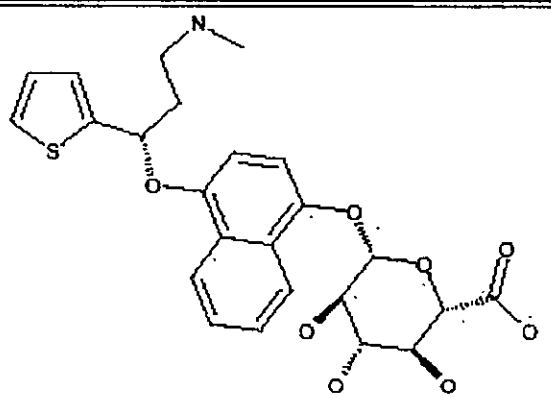
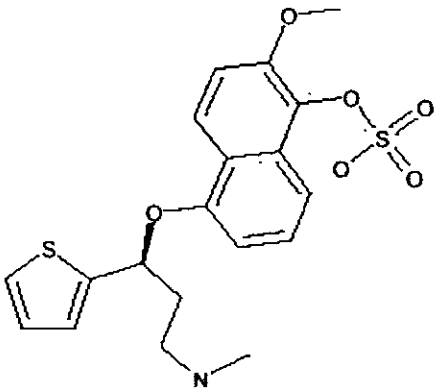
Figure 3. Structural formulas for radiolabeled duloxetine, with "^{14}C" or "*"showing position of ^{14}C. [Structures excerpted directly from Sponsor's submission.]



In 2 studies (HMAF and SAAZ), unchanged duloxetine accounted for only ~3% of the total radioactivity circulating in humans ($AUC_{0-\infty}$) after a single 20-mg dose (see table, below). In one of these studies (SAAZ), ^{14}C-labeled metabolites of duloxetine were identified and quantified in urine, but only at 10 hr in blood/plasma. Approximately 68% of the radioactivity was excreted in urine within 72 hr and 92% of this could be accounted for among 11 identified metabolites; with the 2 most prevalent being 4-OH-duloxetine-glucuronide (30% of urinary excretion) and 5-OH, 6-MeO-duloxetine-sulfate (22% of urinary excretion). Duloxetine and 4 metabolites were identified in 10-hr samples of plasma: 4-OH-duloxetine-glucuronide (M6), methyl catechol [subsequently identified as 5-OH, 6-MeO-]duloxetine-sulfate (M7), catechol-duloxetine-glucuronide (M9), and methyl catechol-duloxetine-glucuronide (M10) were approximately 12-fold, 5.5-fold, 3.5-fold, and 3.2-fold duloxetine,

respectively. It should be noted that the relative amounts of these metabolites were measured at a single time point and do not necessarily represent total systemic exposures. A more complete analysis of the 2 most prevalent metabolites was performed in study HMBN (see below).

Figure 4. Structures of metabolites identified by the Sponsor as major species circulating in humans. [Excerpted directly from Sponsor's submission; file 906DM19601.pdf.]

4-OH-DULOXETINE-GLUCURONIDE	5-OH, 6-MeO-DULOXETINE-SULFATE
	

In a recent study using unlabeled duloxetine (HMBN), 2 major circulating metabolites, as well as unchanged duloxetine, were quantified in human plasma after a 60-mg dose. The AUC for unchanged duloxetine agreed well with previous studies using 20-mg dose: the 60-mg dose gave an AUC for duloxetine that was 3- to 4-fold that following the 20-mg dose. The systemic exposures (AUC) to the major metabolites were much higher than for parent drug: 4-OH-duloxetine-glucuronide was 9-fold and 5-OH, 6-MeO-duloxetine-sulfate was 4-fold duloxetine. [These relative exposures to the metabolites agree well with the 10-hr values determined in study SAAZ, above.]

Table 8. Systemic exposures to duloxetine and 2 major metabolites (and total ^{14}C from duloxetine); values are AUCs, either 0-∞ for single dose studies (HMAF, SAAZ) or steady-state for repeated dose study (HMBN).

STUDY (YEAR)	DOSE	AUC, ng.hr/ml			
		duloxetine	Total ^{14}C	4-OH-D-GLUC	5-OH, 6-MeO-D-Sulf
HMAF (1992)	20 mg	226	8230	ND	ND
SAAZ (1998)	20 mg	257	8770	ND	ND
HMBN (2001)	60 mg	845	ND	7520	3180

Table 9. Relative amounts of duloxetine and its 2 major metabolites in human plasma as determined in 3 studies (see Error! Reference source not found., **above).**

STUDY (YEAR)	DOSE	AUC, ng.hr/ml		
		Duloxetine % of total	4-OH-D-GLUC vs dulox	5-OH, 6-MeO-D-Sulf vs dulox
HMAF (1992)	20 mg	2.75%	ND	ND
SAAZ (1998)	20 mg	2.7%	(12X)*	(5.5X)*
HMBN (2001)	60 mg	ND	9X	4X

*These values do not represent AUCs, but only relative concentration at a 10-hr time point.

Conclusions: Combining the results of these 3 studies, I estimate that unchanged duloxetine accounts for only 3% of the total systemic (plasma) exposure to drug (based upon 2 studies), with 4-OH-duloxetine-glucuronide and 5-OH, 6-MeO-duloxetine-sulfate accounting for another 27% (9 X 3%) and 12 % (4 X 3%), respectively. These 3 molecular species probably account for ~40% of the systemic exposure to orally administered duloxetine HCl (at least at doses up to 60 mg).

Two other metabolites of duloxetine were identified at a single (10-hr) time point after duloxetine administration: catechol-duloxetine-glucuronide and methyl-catechol-duloxetine-glucuronide (i.e., probably 5-OH, 6-MeO-duloxetine). Although the systemic exposure to these metabolites was not quantified, it seems unlikely that they could each account for more than 10% of total systemic exposure, since at the 10-hr time point their levels were ~3-fold duloxetine.

In vitro reports [1996; ADME45.pdf]: inhibition of CYP3A and 2D6; [2000; ADME64.pdf]: CYP2C9, 1A2.

[2001; ADME70.pdf]: ultimate NMR identification of 5-OH, 6-MeO-duloxetine-O-sulfate (not 5-MeO-, 6-OH-duloxetine-O-sulfate) as a major (circulating and urinary) human metabolite of duloxetine (samples from study F1J-LC-SAAZ, 2000).

Metabolism in animal species:

Duloxetine was extensively metabolized in the animal species used for toxicological evaluation, however, the major (most prevalent) metabolites circulating in humans were not well covered by in the animals. [It should be noted that *in vitro* metabolism (ADME67.pdf) showed similar metabolite patterns for rat, dog, monkey, and human liver microsomes.]

Dogs: In dogs, the predominant circulating form of duloxetine was des(aminomethyl) acid metabolite, with desmethyl duloxetine, dihydrodiol duloxetine, a glucuronide conjugate of 5-hydroxy-duloxetine, and cysteinylhydroxy-duloxetine also found. Twelve metabolites were found in urine, with dihydrodiol-duloxetine and cysteinylhydroxy-duloxetine predominating. Duloxetine was the predominant form found in feces.

Rats: In rats, the predominant circulating forms of duloxetine were unchanged duloxetine, des(aminomethyl)acid duloxetine, 4-OH-duloxetine-glucuronide, and 6-OH-duloxetine-glucuronide (and 5-OH-duloxetine-glucuronide, in the 3-mo study, but not after a single dose). The predominant metabolites in urine were glucuronide conjugates of 4-OH-duloxetine and 6-OH-duloxetine. In feces, the major identified metabolites were the thienyl alcohol, 4-OH-duloxetine, 5-OH-duloxetine, and 6-OH-duloxetine. In bile from cannulated rats, the major metabolites were glucuronide conjugates of 6-OH-duloxetine (most abundent), 4-OH-duloxetine, and 5-OH-duloxetine, and dihydrodiol duloxetine. Fifteen metabolites were identified in plasma, urine and feces. In the 3-mo study, 4-OH-duloxetine-sulfate plasma concentrations were estimated from levels of duloxetine (measured in that study) and assuming that duloxetine is 45% and the metabolite is 2% of all circulating molecular species (see Table 11, below).

Mice: In mice, the predominant circulating forms of duloxetine were 4-OH-duloxetine as the glucuronide conjugate, 6-OH-duloxetine as the glucuronide conjugate, and the des(aminiomethyl) acid metabolite. In urine, 12 metabolites, in addition to duloxetine, were identified, with the predominant being 4-OH-duloxetine as the glucuronide conjugate, 6-OH-duloxetine as the glucuronide conjugate, and 6-OH-duloxetine. In feces the predominant metabolites were duloxetine, 6-OH-duloxetine, and 4-OH-duloxetine. In the 3-mo study, 4-OH-duloxetine-sulfate plasma concentrations were estimated from levels of duloxetine (measured in that study) and assuming that duloxetine is 1% and the metabolite is 43% of all circulating molecular species (see Table 11, below).

Comparative exposures to "major" human circulating metabolites: The 4 major human metabolites were conjugates (sulfate or glucuronide) of oxidation products of duloxetine (with or without O-methylation). There was very little quantitative data provided for these metabolites in animals in this submission. I have reorganized the Sponsor's summary table to show the presence of these 5 major metabolites, regardless of whether they are free or conjugated (see Table 10, below).

Table 10. Reorganization of Sponsor's table (Table 11, page 38, from this submission, Pharmtox/Pharmsum/Pksumm.pdf) of the identification in animals (+ sign indicates that the metabolite was detectable) of the 4 most prevalent metabolites of duloxetine found in human plasma. I have included the % of total human exposure (i.e., the AUC for each metabolite as a % of the AUC for total radioactivity) that I calculated for each (see section, above). The shaded cells show apparent complete lack of circulating metabolites in animals.

SPECIES	COMPART-MENT	METABOLITE (CONJUGATE)				
		Duloxetine	4-OH-Dulox	5-OH, 6-MeOH-	6-OH, 5-MeOH-	DiOH-Dulox
Human	Plasma	3% (free)	27% (GLUC)	12% (SO_4)	<10% (GLUC)	<10% (GLUC)
Dog	Plasma	+				
Rat	Plasma	+	+(G)			
Mouse	Plasma	+	+(G)			
Dog	Urine		+(G)			[+(G)]*
Rat	Urine		+(G)	+(G)		+(G)
Mouse	Urine	+	+(G & free)	+(G & free)	+(free)	
Dog	Feces	+	+(free)	+(free)	+(free)	
Rat	Bile		+(G)	+(G)		+(G)
Mouse	Feces	+	+(free)			

*: catechol-duloxetine exists in different isomeric forms that show different retention times on HPLC; the 2 found in dog urine are apparently not the same isomer that was seen in human plasma, since the Sponsor listed them under "metabolites identified in animals, but not in humans."

Summary of comparative metabolism findings:

1. 4-OH-duloxetine-sulfate conjugate (the major circulating form in humans) was also detectable in the plasma of rats and mice, but not dogs. The amount of this metabolite was estimated in the plasma of rats and mice from the 3-mo dietary studies (see below). There appeared to be a considerable safety margin in mice for a 60-mg human dose at steady-state, but no safety margin in rats (<0.1).

2. 5-OH, 6-MeO-duloxetine, which circulated as the sulfate conjugate in humans, was not detected in any form in the plasma of dogs, rats or mice. However, there was evidence that this metabolite was synthesized in all 3 animal species: a) the glucuronide conjugate was present in urine of rats and mice and the free metabolite was present in mouse urine; b) the metabolite was detected in feces of dogs, as free metabolite, and bile of rats, as the glucuronide.

3. 6-OH, 5-MeO-duloxetine, which circulated as the glucuronide conjugate in humans, was also not detected in any form in the plasma of dogs, rats or mice. However, there was also some evidence that this metabolite was synthesized in mice and dogs: the free metabolite was present in mouse urine and dog feces.

4. Dihydroxy-duloxetine (aka catechol-duloxetine) exists in different isomeric forms that show different retention times on HPLC. The isoform found in human plasma, as the glucuronide conjugate, was not detected in any form in the plasma of dogs, rats, or mice. This isoform, as the glucuronide

conjugate was detected in rat urine and bile; 2 other isoforms, also as glucuronide conjugates, were detected in dog urine.

Table 11. Sponsor's table comparing systemic plasma metabolite (only 4-OH-duloxetine-glucuronide) exposures of animals (rats, mice, and dogs) to that for humans. [Excerpted directly from this submission, page 41, Pharmtox/pharmsum/Pksumm.pdf.]

Table 13: Comparison of the Systemic Plasma Metabolite Exposure of Animals to that for Humans

Dose	Duration of Dosing (Days)	Mean $AUC_{0\text{-}24hr}$ (ng•hr/mL) Glucuronide Conjugate of 4-hydroxy duloxetine	Metabolite Ratio $AUC_{0\text{-}24hr}$ (Animal/Human at 60 mg QD)	Metabolite Ratio $AUC_{0\text{-}24hr}$ (Animal/Human at 60 mg BID)
Mouse				
0.01% Diet (14 to 21 mg/kg)	93	34637[b]	5.0	2.7
0.03% Diet (44 to 51 mg/kg)	93	398137[b]	57.1	30.8
0.08% Diet (126 to 205 mg/kg)	93	619179[b]	88.9	47.9
Rat				
0.01% Diet (5 to 6 mg/kg)	96	14[c]	<0.1	<0.1
0.02% Diet (11 to 12 mg/kg)	96	115[c]	<0.1	<0.1
0.05% Diet (29 mg/kg)	96	601[c]	<0.1	<0.1
0.08% Diet (42 mg/kg)	96	777[c]	0.1	<0.1
Dog (Metabolite not detected in dog plasma)				
Humans (Mean Data from Study HMBN)				
60 mg QD	Steady State	6968		
60 mg BID[a]	Steady State	12932		

[a] The $AUC_{0\text{-}\tau}$ at steady state which was $AUC_{0\text{-}12hr}$ was multiplied by a factor of 2 to get $AUC_{0\text{-}24hr}$.

[b] These values are estimates calculated from the duloxetine AUC values assuming that duloxetine is 1% of all the circulating related compounds and that the glucuronide conjugate of 4-hydroxy duloxetine is 43% of all circulating related compounds in mice.

[c] These values are estimates calculated from the duloxetine AUC values assuming that duloxetine is 45% of all the circulating related compounds and that the glucuronide conjugate of 4-hydroxy duloxetine is 2% of all circulating related compounds in rats.

Table 12. Sponsor's table showing "Comparison of the systemic plasma duloxetine exposure of animals to that for humans." [Excerpted directly from Pksumm.pdf, Table 8, page 21.]

Dose	Duration of Dosing (Days)	Mean $AUC_{0\text{-}24hr}$ (ng•hr/mL)		Ratio $AUC_{0\text{-}24hr}$ (Animal/Human at 60 mg QD)		Ratio $AUC_{0\text{-}24hr}$ (Animal/Human at 60 mg BID)	
		Males	Females	Males	Females	Males	Females
Mouse (ADME Report 73)							
0.01% Diet (14 to 21 mg/kg)	93	758	853	1.1	0.5	0.5	0.2
0.03% Diet (44 to 51 mg/kg)	93	7988	10530	11.3	6.7	5.6	2.7
0.08% Diet (126 to 205 mg/kg)	93	15611	13188	22.1	8.4	11.0	3.4
Rat (ADME Report 78)							
0.01% Diet (5 to 6 mg/kg)	96	200	437	0.3	0.3	0.1	0.1
0.02% Diet (11 to 12 mg/kg)	96	1827	3362	2.6	2.1	1.3	0.9
0.05% Diet (29 mg/kg)	96	NA	13535	NA	8.6	NA	3.5
0.08% Diet (42 mg/kg)	96	17488	NA	24.8	NA	12.3	NA
Dog (Toxicology Report 32, Toxicology Report 33)							
3 mg/kg	93	320	592	0.5	0.4	0.2	0.2
10 mg/kg	93	2237	2030	3.2	1.3	1.6	0.5
30 mg/kg	93	11279	15735	16.0	10.0	8.0	4.1
3 mg/kg	367	505	147	0.7	<0.1	0.4	<0.1
10 mg/kg	367	2035	572	2.9	0.4	1.4	0.1
30 mg/kg	367	13016	13560	18.4	8.7	9.2	3.5
Humans (Mean Data from Study HMBN)							
60 mg QD	Steady State	706	1567				
60 mg BID[a]	Steady State	1418	3862				

[a] The $AUC_{0-\tau}$ at steady state which was $AUC_{0\text{-}12hr}$ was multiplied by a factor of 2 to get $AUC_{0\text{-}24hr}$.
NA = Not applicable since this dose was not administered.

Excretion: At low doses (5-10 mg/kg) in rats and dogs, duloxetine (total radioactivity) was excreted ~25-30% in urine and ~60-70% in feces, after both oral and intravenous dosing. In monkeys, a 5 mg/kg oral dose was excreted ~60% in urine and ~30% in feces. In humans, a 20 mg/kg oral dose was excreted 70-80% in urine and 15-20% in feces.

PK/TK summary and conclusions: See overall summary in Detailed Conclusions and Recommendations section XI, below.

IV. GENERAL TOXICOLOGY:

[All studies were performed at Eli Lilly and Co, unless otherwise noted.]

A. Rats

1. *Summary of studies shorter than 6 months in duration*

The Sponsor submitted several acute, subacute and subchronic studies in rats.

- The acute oral toxicity of duloxetine was tested in 2 studies in Fischer 344 rats, one using the maleate salt and the other using the hydrochloride salt.

  - **Acute toxicity** of LY248686 **[duloxetine] maleate** [lot F58KY0152] administered orally [gavaged as suspension in 10% aqueous acacia] to [8-9-week old] Fischer 344 rats [ ] (Study no. R24788; report Tox06.pdf, December, 1988):

Median lethal doses were 595 mg/kg duloxetine for males and 493 mg/kg for females. Clinical signs included tremors and hyper-responsiveness at all doses, ataxia at ≥700 mg/kg in males and ≥500 in females, and clonic convulsions at 1000 mg/kg in males and at ≥700 mg/kg in females.

  - **Acute toxicity** of LY248686 **[duloxetine] hydrochloride** [Lot 619NK0] administered orally [gavaged as suspension in 10% aqueous acacia] to [8-9-week old] Fischer 344 rats [ ] (Study nos. R33791 (males) and R04792 (females); report Tox21.pdf, April, 1992):

Median lethal doses were 491 mg/kg duloxetine for males and 279 mg/kg for females. Clinical signs included tremors, salivation, hyper-responsiveness, with clonic convulsions were observed in several rats prior to death. Hunched posture, perineal soiling, and chromodacryorrhea were also noted.

- A **1-mo [dieatary] subchronic toxicity study** in [6-7-week old, 10/sex/group] Fischer 344 rats [ ] given diets containing LY248686 **[duloxetine] hydrochloride** (Compound 246916) [Lot 508NK0; duloxetine concentrations in feed of 0, 0.005, 0.02, and 0.08%]. (Study no. R10790; report Tox14.pdf, January 1991):

There were no deaths and no treatment-related clinical signs. Body weights and body weight gains were decreased at 0.08% and sporadically at 0.02% in females. Changes in hematology and clinical chemistry were minimal; histopathology was limited to minimal to slight centrilobular hepatic lipidosis in HDM (and liver phopholipid phosphorous was slightly (20%) elevated in HD males and females).

- A **[3-mo] subchronic toxicity study** and blood level study in [5-6-week old, 20/sex/group and 12/sex/group, respectively] Fischer 344 rats [ ] given diets containing LY248686 **[duloxetine] maleate** [Lot F58-KY0-152; duloxetine concentrations in feed of 0, 0.005, 0.01,

0.03, and 0.08%]. (Study nos. R20187 (toxicity) and R23287 (blood levels); report Tox05.pdf; January 1989):

There were no deaths or treatment-related clinical signs. Body weights were decreased at 0.03 and 0.08% doses, however, HDM returned to near control values by the end of the study; food consumption was also decreased at 0.03 and 0.08% in both males and females throughout the study. Average daily doses of duloxetine were 3.2, 6.6, 19, and 50 mg/kg for males and 3.8, 7.7, 22, and 60 mg/kg for females. TK did not contribute new information useful for this review; duloxetine and the N-desmethyl metabolite (~4-10-fold lower than duloxetine) were quantified from samples drawn between 8-10 am on days 2, 16, 44, and 96. [NB No drug-related histopathology of male sex organs was noted; specifically no hypospermatogenesis.]

2. ***Study title: A chronic toxicity study and blood level study in Fischer 344 rats given duloxetine hydrochloride (LY248686 hydrochloride) for 6 months.***

Key study findings:

- No mortality;
- Decreased body weights at HD (0.08% in diet; ~50 mg/kg/day);
- Decreased food consumption in MD females and at HD;
- Increased liver enzymes (ALT and AST) at MD and HD;
- Organ toxicities: liver (increased weight and increased incidence and severity of midzonal vacuolation in HD males), prostate (decreased weight at HD);
- Induction of liver P450 at HD.

Study no: Studies R10890 (main study) and R32291 (blood level arm).
Volume #, and page #: Tox31.pdf; 421 pages; April 1993.
Conducting laboratory and location: Lilly Research Laboratories, Greenfield, IN.
Date of study initiation: 8/10/90-2/6/91 (termination) for main study; 10/31/91-4/30/91 (termination) for blood level arm.
GLP compliance: yes, see page 3.
QA report: yes, see pages 2-3.
Drug, lot #, and % purity: duloxetine hydrochloride, lot nos. 508KNO, [redacted] as duloxetine (used in main study), and 619NK0, [redacted] as duloxetine (used in blood level arm).
Formulation/vehicle: dietary; prepared every 2 weeks; assayed 3-4 times during each study, results average 96.8% of nominal levels (range 90-100%).

Methods (unique aspects):
Dosing:

Species/strain: male and female Fischer 344 rats, [redacted]

#/sex/group (main study): 10/sex/group.
Satellite groups used for toxicokinetics: 3/sex/group, no controls.

Age: initially 5-6 weeks old.
Weight: initially ~123 g for males and ~102 g for females in main study; ~135 for males and ~102 for females in TK arm.
Housing: individually, in suspended stainless steel cages; with food (standard mash diet — and tap water *ad libitum*; 12-hr light/dark cycle, changing at 6 am and 6 pm.
Doses in administered units: oral, dietary, *ad libitum*; 0, 0.005, 0.02, or 0.08% concentrations of duloxetine → average daily doses of duloxetine of approximately 0, 3, 12, or 47 mg/kg.
Route, form, volume, and infusion rate: dietary, *ad libitum*; 0, 0.005, 0.02, or 0.08% concentrations of duloxetine.

Observations and times:

Clinical signs: daily; with weekly examination of muscle tone, condition of pelage, color and appearance of eyes, respiration, posture, excreta, locomotion, and presence of external lesions or growths.
Body weights: weekly.
Food consumption: weekly.
Ophthalmoscopy: prior to start of study and twice during the study, midway and at termination; under tropicamide/reduced illumination; fundus, adnexa, cornea, anterior chamber, iris, and lens were examined; fundus was evaluated by indirect ophthalmoscopy.
EKG: not performed.
Hematology: at ~ month 3 (not fasted; from orbital sinus) and at termination (fasted; from aorta).
Clinical chemistry: at ~ month 3 (not fasted; from orbital sinus) and at termination (fasted; from aorta).
Urinalysis: at ~ month 3 and near termination; 5-hr collection.
Gross pathology: see Histopathology Inventory Table 14, below.
Organs weighed: see Histopathology Inventory Table 14, below.
Histopathology: see Histopathology Inventory Table 14, below.
Toxicokinetics: blood from 3/sex/dose on days 2, 92, and 183, between 8-10 am.
Other: *In vitro* liver P450 activities: p-nitroanisole O-demethylase, 7-ethoxyresorufin O-deethylase, benzphetamine N-demethylase, and total P-450; liver samples on 5/sex/dose at necropsy. Phospholipid (viz., total phospholipid phosphorous) concentrations in lung and liver; samples from 5/sex/dose at necropsy.

Results:

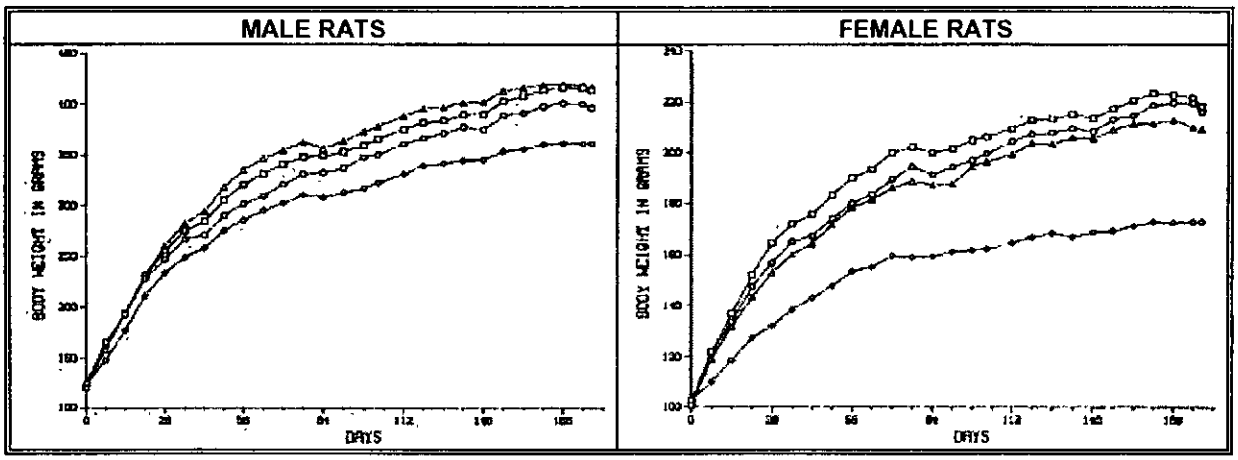
Mortality: no deaths.

Clinical signs: limited to dose-related increased incidence of chromodacryorrhea in MD and HD females from week 18 through week 26 (i.e., the end of the study).

Body weights: Body weights for HD groups were decreased compared with control throughout the study (see Figure 5, below); at the end of the study HD males weighed 9% less than controls and HD females weighed 20% less than controls. After 3-mo of dosing, HD males had gained 11% less weight

than controls (187 g vs 211 g) and HD females had gained 36% less than controls (58 g vs 92 g). There was a tendency for LD and MD males and LD females to weigh slightly more than controls.

Figure 5. Mean body weights in male and female Fischer 344 rats given duloxetine HCl in the diet at amounts equivalent to duloxetine concentrations of 0% (circles), 0.005% (squares), 0.02% (triangles), or 0.08% (diamonds). [Sponsor's graphs excerpted directly from this submission.]



Food consumption: Daily food consumption (not graphed by the Sponsor) was decreased in MD females and HD males and females throughout the study. Mean daily food consumption throughout the study was decreased in MD females (↓6%) and HD males (↓9%) and females (↓19%). Food consumption was slightly increased in LD females, beginning at approximately week 4 and continuing throughout the study; their mean daily food consumption throughout the study was increased ~3%. Efficiency of food utilization (EFU; see Sponsor's graphs, below) was decreased in HD males during the first week of dosing, but had returned to control values by weeks 2-3; EFU in HD females was decreased throughout the study.

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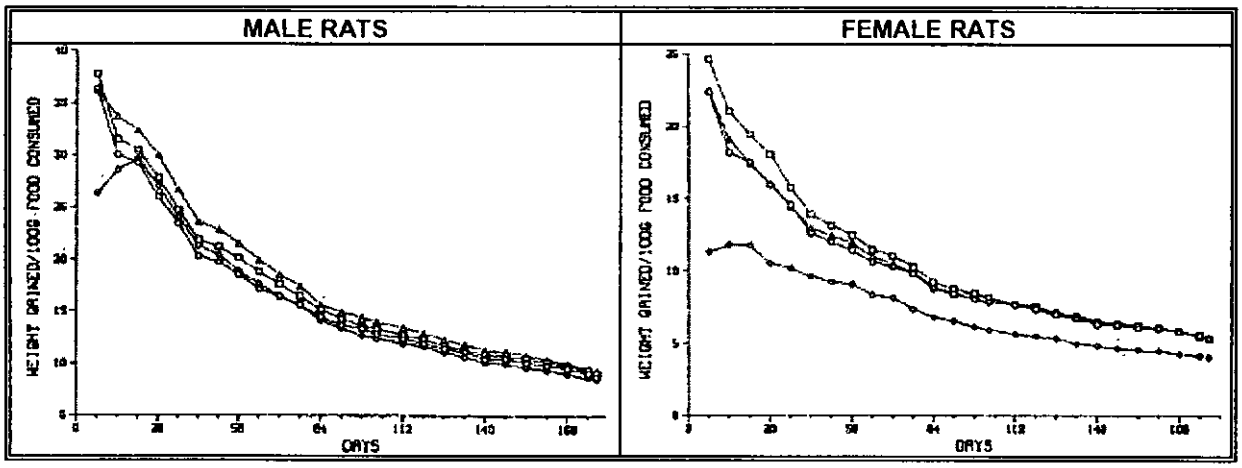
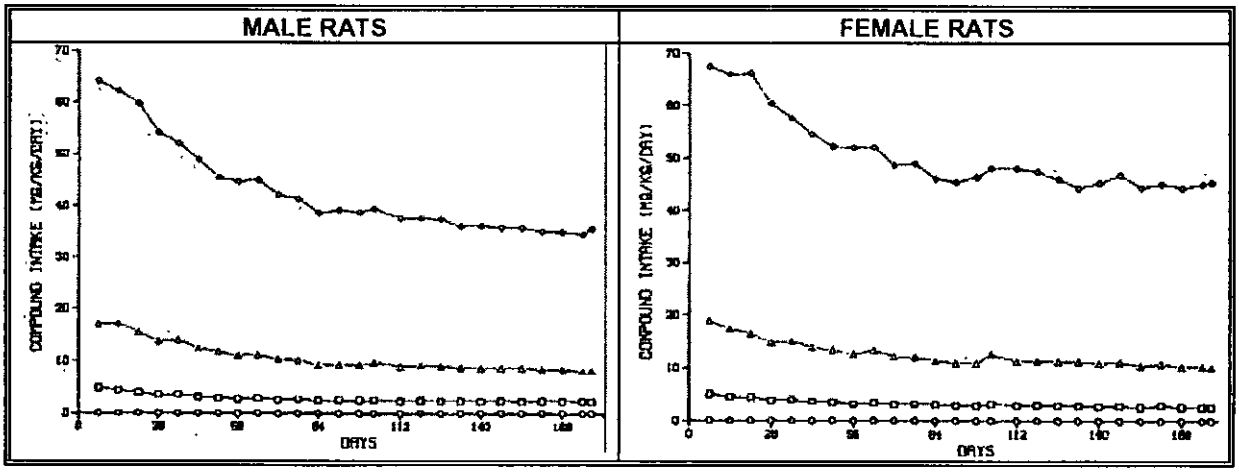
Figure 6. Mean cumulative efficiency of food utilization per week in male and female Fischer 344 rats given duloxetine HCl in the diet at amounts equivalent to duloxetine concentrations of 0% (circles), 0.005% (squares), 0.02% (triangles), or 0.08% (diamonds). [Sponsor's graphs excerpted directly from this submission.]



Drug intake: Dietary drug intake was fairly stable for LD and MD groups throughout the study and for HD groups after ~7 weeks of dosing (see figure, below). Average daily doses were 0, 2.9, 11, and 44 mg/kg for males and 0, 3.6, 14, and 59 mg/kg for females.

Figure 7. Mean duloxetine intake per week in male and female Fischer 344 rats given duloxetine HCl in the diet at amounts equivalent to duloxetine concentrations of 0% (circles), 0.005% (squares), 0.02% (triangles), or 0.08% (diamonds). [Sponsor's graphs excerpted directly from this submission.]



Ophthalmoscopy: The examining veterinarian found a low incidence of minor abnormalities, but no treatment-related effects.

Hematology: Treatment-related changes in hematology parameters were minimal. At the end of the study (but not midway), HD males and females had minimally lowered (2-4%) red blood cell values [specifically, RBC count (females only), hemoglobin (both), and MCV and MCH (males only)], compared with controls. There were no effects on white blood cell parameters. Clotting parameters were slightly affected at HD: HD males had slightly shortened (15%) APTT at the end of the study, with no changes in PT or thrombocyte count; APTT was also slightly (7%) shortened in HD females and PT was minimally (2%) increased.

Clinical chemistry (see table, below): There were increases in liver enzymes indicative of liver toxicity: ALP was increased in MD females, and HD males and females at 3 and 6 mos, ALT was increased in MD (at 3 and 6 mo) and HD (at 3 mo only) males, AST was increased in HD males and females at 3 mo only. There were several treatment-related changes in clinical chemistry parameter, mostly at HD, and probably attributable to decreased food intake and decreased body weights: decreased glucose, cholesterol and triglyceride levels at 6 mo, not 3 mo; decreased total protein in HDF at 3 and 6 mo, with decreased albumin at 3 and 6 mo, and decreased globulin 6 mo; decreased ALT and AST in HD females at 6 mo; slightly decreased creatinine in HD females at 6 mo (muscle wasting). Total bilirubin was also decreased at HD at 6 mo, possibly because of the decrease in albumin, its binding protein. Potassium was increased in MD females and HD males and females at 6 mo, which might indicate inadequate excretion through the kidneys. Upon histopathological examination, there was increased incidence of minimal progressive glomerulonephrosis in HD males, however, there was no histopathology in kidneys from HD females (see below).

Table 13. Clinical chemistry parameters that were altered in male and female Fischer 344 rats given duloxetine HCl in the diet at amounts equivalent to duloxetine concentrations 0.005%, 0.02%, or 0.08% for up to 6 mo. Values show increases or decreases relative to those for control groups.

PARAMETER	SEX	DIETARY DOSE					
		0.005%		0.02%		0.08%	
		3 mo	6 mo	3 mo	6 mo	3 mo	6 mo
ALP	M					↑30%	↑10%
	F	↑ 10%		↑10%	↑30%	↑50%	↑70%
ALT	M			↑25%	↑30%	↑50%	
	F						↓22%
AST	M					↑30%	
	F					↑40%	↓22%
Total protein	M						
	F					↓8%	↓18%
Albumin	M						↑7%
	F					↓12%	↓16%
Globulin	M						
	F						↓22%
Glucose	M						↓16%

PARAMETER	SEX	DIETARY DOSE					
		0.005%		0.02%		0.08%	
		3 mo	6 mo	3 mo	6 mo	3 mo	6 mo
	F						↓10%
Cholesterol	M						↓22%
	F				↓9%		↓19%
Triglycerides	M						↓30%
	F						↓54%
Creatinine	M						
	F						↓9%
Total bilirubin	M						↓25%
	F						↓20%
Potassium	M						↑13%
	F				↑15%		↑22%

Urinalysis: Urine pH was increased (~1 pH unit, not dose-related) in all drug-treated groups (except MD females) at 3 mos, but not at 6 mos. In HD females, specific gravity (↑2%), K concentration (↑80%), and creatinine concentration (↑80%) were increased and volume (↓40%), was decreased at 3 mo, but not at 6 mo. Urine volume was also decreased (↓50%) in HD males at 3 mo. Since the decrease in urine volume was compensated for by increased concentrations of potassium and creatinine, these changes were probably due to decreased water consumption (assumed to accompany the decreased food consumption) rather than kidney toxicity.

Organ weights: **Liver** weights were increased in HD males (absolute weight ↑18%, relative to body weight ↑29%, relative to brain ↑18%, vs controls). The absolute weights of several organs were decreased in the HD groups (spleen and prostate in males, liver, heart, spleen (MDF also), adrenals (MDF also), but brain weights were essentially identical across treatment groups, with brains from males slightly heavier than females (1.92 g vs 1.75 g). When the weights of these organs were normalized to brain weight, only **prostate** was significantly decreased in males [↓20% at HD, ↓16% at MD (not statistically significant), and ↓8% at LD (not statistically significant), vs controls]. **In females**, the relative (to brain) weights of several organs were **decreased: liver** (↓14% at HD), **heart** (↓17% at HD, ↓8% at MD), **spleen** (↓24% at HD, ↓12% at MD), and **adrenals** (↓19% at HD, ↓10% at MD, ↓9% at LD), but not kidneys, ovaries or uterus.

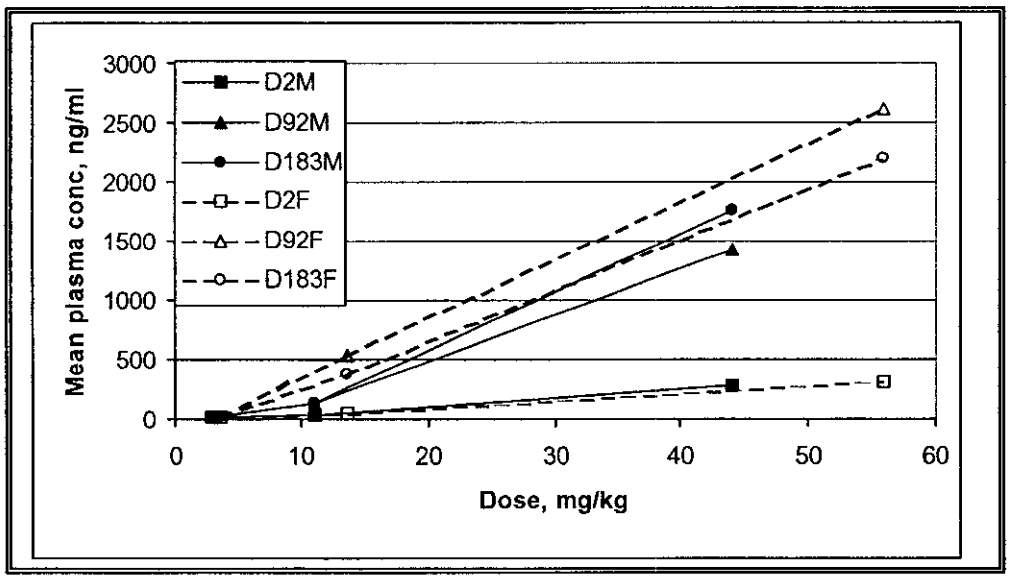
Gross pathology: Gross pathological findings were limited to liver in males (hepato-diaphragmatic nodule in 2/10 and whole tissue alteration in 5/10 at HD) and testes (2/10 noted as small in both MD and HD groups).

Histopathology: ***kidney:*** increased incidence of minimal progressive glomerulonephrosis in HD males only (7/10 vs 3/10 controls, 2/10 LD, and 3/10 MD); *liver:* increased incidence and severity of midzonal vacuolation in males only (0/10 controls and LD; 9/10 slight at MD, 10/10 moderate at HD); *heart:* decreased incidence of minimal multifocal progressive cardiomyopathy in males (4/10 controls, LD, and MD vs 1/10 HD; only 1/10 controls in females); *testes:* bilateral hypospermatogenesis in 2/10 MD and HD males, vs 1/10 controls and 0/10 LD); *adrenals:* unilateral slight focal fibrosis of the

capsule (1/10 HD females, vs 0/10 other groups). [NB Mammary glands were only examined in 5 females and no males.]

Toxicokinetics: Plasma levels of duloxetine increased fairly linearly with dose (see figure, below), with apparent accumulation between days 2 and 92, but not much more between days 92 and 183; there was no dramatic difference between males and females.

Figure 8. Plasma levels of duloxetine in male (solid symbols) and female (open symbols) Fisher 344 rats treated for 2, 92, or 183 days with duloxetine (HCl) at dietary levels of 0.005%, 0.02%, and 0.08%, providing average daily doses of 2.9, 11, and 44 mg/kg for males and 3.6, 14, and 56 mg/kg for females. [Graph of Sponsor's summary data; mean, n=3/sex/dose/time point; blood was collected between 0800 and 1000 hrs.]



Liver P450 activity: Induction of P450 activities was confined to HD groups. Total P450 activity was increased 50% in HD males and 80% in HD females after 6 mo of dosing. The more specific activities also showed induction: 7-ethoxyresorufin O-deethylase activity was ~4-fold control values in HD males and females, p-nitroanisole O-demethylase activity was increased 40% in HD males, and benzphetamine N-demethylase was increased 30% in HD males and 50% in HD females. These results are consistent with induction of hepatic cytochrome P440 isoforms, including, but not necessarily limited to, CYP1A1 and CYP2B.

Lung and Liver Phospholipid concentrations: no effect: concentrations of phospholipid phosphorus in livers and lungs of HD rats were not different from those in controls.

B. Dogs

1. *Summary of studies shorter that 1 year in duration*

- The **acute oral toxicity** of duloxetine was tested in 2 studies in Beagle dogs, one using the maleate salt and the other using the hydrochloride salt.

  - Acute toxicity of [100 mg/kg] LY248686 [duloxetine] maleate [lot 503NK8] administered orally [in gelatin capsules] to [12-13-mo old] Beagle dogs [; 2/sex]. (Study no. D01588; report Tox09.pdf, March, 1989):

All dogs survived. Clinical signs included emesis (4/4), mydriasis (2/4), and slow pupillary light response and slight intermittent tremors in a single dog; all recovered within 28 hr.

  - Acute toxicity of [50 or 100 mg/kg] LY248686 [duloxetine] hydrochloride [lot 619NK0] administered orally [in gelatin capsules] to [18-26-mo old] Beagle dogs [1/sex/dose]. (Study no. D06591; report Tox22.pdf, April 1992):

All dogs survived; clinical signs were similar to the previous study and occurred at both doses.

- The **subchronic oral toxicity** of duloxetine was tested in 2 studies in Beagle dogs, one for 3 mo using the maleate salt and the other for 1 mo using the hydrochloride salt.

  - A **1-month subchronic toxicity** study of LY248686 [duloxetine] hydrochloride (Compound 246916) [Lot 508NK0] administered orally [in gelatin capsules] to [9-12-mo-old] Beagle dogs [ ][at daily doses of duloxetine of 0, 3, 10, or 30 mg/kg; 3/sex/dose]. (Study no. D04590; report Tox15.pdf, January 1991):

There were no deaths; dose-related clinical signs were emesis and mydriasis. There were no clear effects on body weights or food consumption. No treatment-related changes in hematology, clinical chemistry, or urinalysis parameters. Liver p450 (benzphetamine N-demethylase) was induced (~2-fold controls) and relative liver weights were increased in HD females only. No gross or histopathology changes.

  - A **subchronic toxicity** studyin Beagle dogs given daily oral doses of LY248686 [duloxetine] maleate for **three months**. (Compound 246916) [Lot 508NK0] administered orally [in gelatin capsules] to [8-mo-old] Beagle dogs [ ][at daily doses of duloxetine of 0, 3, 10, or 30 mg/kg; 4/sex/dose]. (Study no. D01488; report Tox12.pdf, July, 1989):

There were no deaths and the only significant toxicological effects were decreased food consumption, abnormal stools, emesis, and mydriasis.

- A **chronic toxicity study** of duloxetine hydrochloride administered orally to Beagle dogs for **6 months**. (Compound 246916) [Lot 508NK0 and 521NK0] administered orally [in gelatin capsules] to [5mo-old] Beagle dogs [ ] [at daily doses of duloxetine of 0, 3, 10, or 30 mg/kg; 4/sex/dose]. (Study no. D07690; report Tox32.pdf, March, 1993):

There were no deaths and the only significant toxicological effects were emesis, and mydriasis, and decreased pupillary light response; slight induction of P450 CYP2B (benzphetamine N-demethylation) and CYP 1A1 (EROD) at MD and HD; slight (24%) increase in liver phospholipid phosphorous concentrations in HD males.

EKGs (Lead II) were performed pre-study and before and 2 hr after dosing on day 2, and at months 1 and 3, and near termination (6-mo); EKG waveforms (10-sec samples) were captured directly using a portable, automated system.; heart rates were calculated by computer-determined average R-R interval for a 10-sec sample or directly from the EKG using a 10-sec interval..

The Sponsor concluded that "electrocardiogram evaluation indicated that duloxetine did not cause any disturbances in cardiac rhythm, conduction, or alterations in heart rate." They noted that 1 LD dog had a single occurrence of a premature ventricular complex pre-dosing on day 2, but that this finding is occasionally seen in normal lab Beagles and was not considered treatment related. However, numerical values were only provided for heart rate (in a summary table and as individual animal data), not for QT intervals, etc.

2. ***Study title: A chronic toxicity study of LY248686 [duloxetine] hydrochloride administered orally to Beagle dogs for 1 year.***

Key study findings:
- No deaths;
- Mydriasis, and slow or incomplete pupillary light response;
- Emesis;
- Decreased food consumption at HD;
- Liver toxicity limited to slightly increased amount of secondary lysozomes at HD;
- Slight induction of CYP 2B at HD.

Study no: Study D07790.
Volume #, and page #: EDR file Tox33.pdf, 523 pages.
Conducting laboratory and location: Lilly Research Laboratories, Greenfield, IN.
Date of study initiation/termination: October 22, 1990 / October 25, 1991.
GLP compliance: yes, see page 3.
QA report: yes, see pages 2-3.

Drug, lot #, and % purity: duloxetine hydrochloride; Lot 521NK0 (October 22, 1990-April 30, 1991 and Lot 619NK0 (May 1, 1991-end of dosing); [ ]duloxetine, respectively.
Formulation/vehicle: oral gelatin capsule.

Methods:

Dosing:

Species/strain: male and female Beagle dogs [ ]
#/sex/group (main study): 4/sex/dose.
Satellite groups used for toxicokinetics or recovery: none; TK done on main study dogs.
Age: 5 mo.
Weight: males, 7.8±0.6 kg; females, 6.7±0.6 kg.
Housing: individually; fed ~300 g — Certified Canine Diet — each morning after dosing, with [ ] Diet, a supplemental meat diet, offered to any dogs that would not eat in an attempt to stimulate appetite; tap water *ad libitum*; 12-hr light/dark cycle, changing at 0600 and 1800 hr;
Doses in administered units: 0, 3, 10, and 30 mg/kg/d.
Route, form, volume, and infusion rate: packed in gelatin capsules; prepared weekly, adjusted for most recent body weight for each dog.

Observations and times:

Clinical signs: several times each day; physical exams before study initiation, at 6 mo and near termination (~1 yr); and neurological exams before study initiation and just prior to dosing at 6 mo and just prior to dosing near termination (~1 yr).
Body weights: recorded at weekly intervals.
Food consumption: estimated visually each day, with any changes in appetite noted.
Ophthalmoscopy: before study initiation, at 6 mo, and near termination (~1 yr).
EKG: (Lead II) pre-study and before and 2 hr after dosing on day 2, and at months 1 and 3, 6, 9 and near termination (12 mo); EKG waveforms (10-sec samples) were captured directly using a portable, automated system.; heart rates were calculated by computer-determined average R-R interval for a 10-sec sample or directly from the EKG using a 10-sec interval.
Hematology: before study initiation, and at week 1 and months 1, 3, 6, 9, and near termination (~1 yr).
Clinical chemistry: before study initiation, and at week 1 and months 1, 3, 6, 9, and near termination (~1 yr); after ~12-16 hr fasting.
Urinalysis: before study initiation, and at week 1 and months 1, 3, 6, 9, and near termination (~1 yr).
Gross pathology: see Histopathology Inventory Table 14, below.
Organs weighed: see Histopathology Inventory Table 14, below.
Histopathology: see Histopathology Inventory Table 14, below.
Toxicokinetics: on days 8, 30, 93, 183, 274, and 367; at 0, .05, 1, 2, 4, 8, 12, and 24 hr after dosing; plasma from whole venous blood; assayed for duloxetine concentration by HPLC.
Other: At necropsy, liver and lung phospholipid concentrations (control and HD, only), *in vitro* hepatic enzyme activity (all dogs), and hepatic total porphyrin concentrations (2 controls and 3 HD) to characterize pigmented granules observed during histopathological exam.

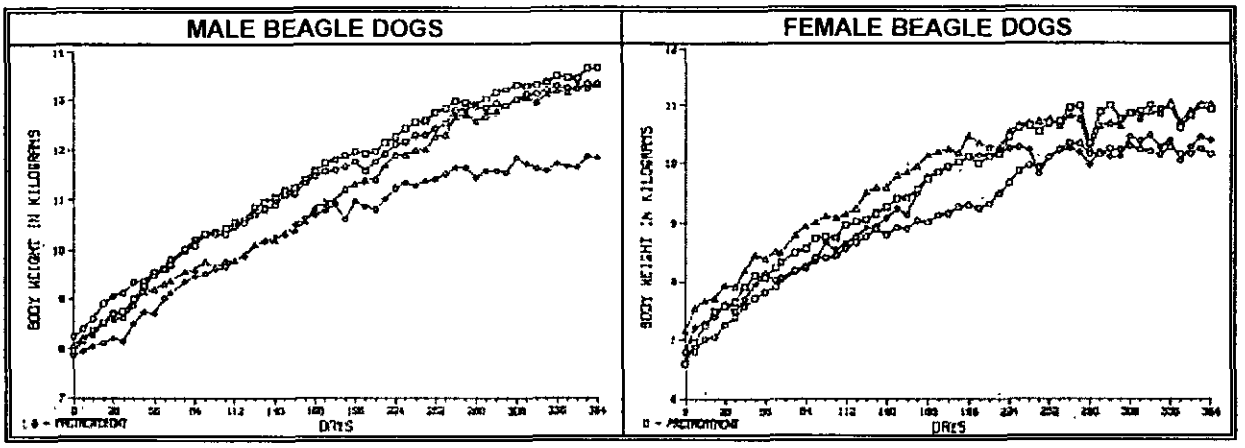
Results:

Mortality: All dogs survived to termination.

Clinical signs: Clinical signs attributable to duloxetine treatment were: 1) mydriasis (dilation of pupil); 2) slow and/or incomplete pupillary light response; and 3) emesis; and 4) rigidity and salivation at HD.

Body weights and Food Consumption: Mean body weights for HD males increased more slowly after about 6 mo of dosing (see figure, below). However, this represented low body weight gain in 2/4 HD males, compared with only 1/4 control males. The Sponsor says that food consumption was decreased in most dosed females (especially at HD) and HD males (but data was not provided).

Figure 9. Duloxetine appeared to decrease body weight gain in male, but not female, Beagles. Symbols denote: controls (circles), LD (squares), MD (triangles) and, HD (diamonds). [Sponsor's graphs excerpted directly from this submission.]



Ophthalmoscopy: As in shorter studies, there were dose-related, apparently reversible, increases in incidence and severity of mydriasis and incomplete light response.

Electrocardiography: The Sponsor concluded that "evaluation of the electrocardiograms indicated that duloxetine did not cause any disturbances in cardiac rhythm, conduction, or alterations in heart rate." They noted that 2 dogs (1 LD and 1 HD) had a single occurrence of a missing ventricular complex following a P wave, but that this finding is occasionally seen in normal lab Beagles and was not considered treatment related. However, numerical values were only provided for heart rate (in a summary table and as individual animal data), not for QT intervals, etc.

Hematology: Findings were limited to a slight (10-20%) increase in activated partial thromboplastin time at HD, which was evident in males and females from the first week of dosing through the end of the study.

Clinical chemistry: Findings were limited to slightly increased serum triglycerides at HD.

Urinalysis: No treatment-related findings.

Organ weights: Ovary weights (absolute and relative) tended to be increased at HD; this was due to 1/4 HD females (#243653) whose ovary weight, even when normalized to body weight, was nearly 3-fold the average control. [No findings were noted for the ovaries of this dog on histopathological examination; it did have a focal cyst in its pituitary, but no other details were given.]

Gross pathology: No remarkable findings.

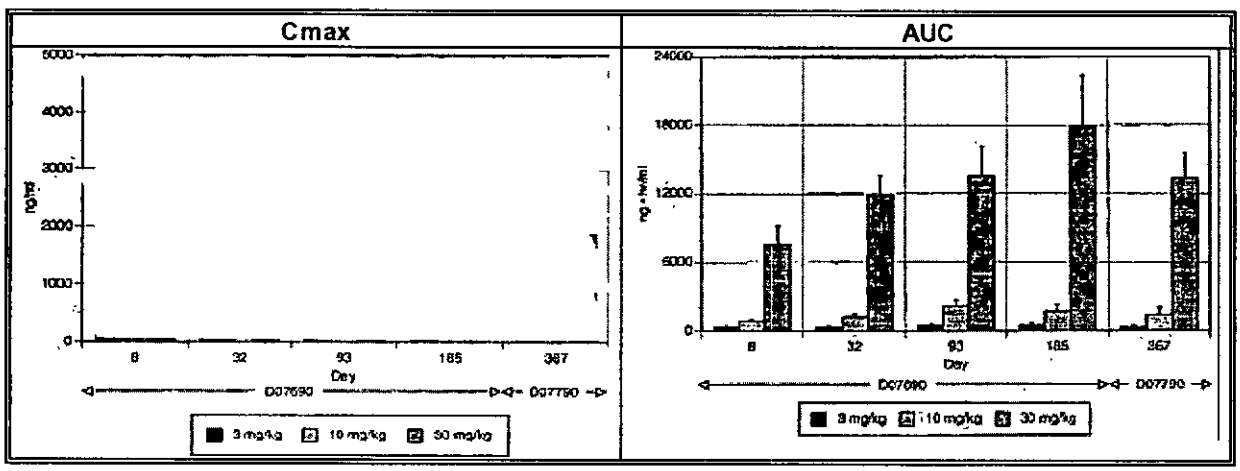
Histopathology: Treatment-related findings were limited to increased incidence of slight hepatocellular pigmentation especially in HD females (3/4, compared with 0 or 1/4 in other groups). Further investigation determined that these were secondary lysozomes that contained lipofuscin. I agree with the Sponsor that these are probably a consequence of a small increase in hepatic metabolism following duloxetine administration.

Liver P450 activity: Slight (~50% increase) induction of CYP 2B (p-nitroanixole O-demethylation and/or benzphetamine N-demethylation) at MD and HD.

Lung and Liver Phospholipid Concentrations: A slight (20%) increase in phospolipid concentration in livers of HD males only.

Toxicokinetics: Systemic exposure to duloxetine increased with dose, however, there was a non-linear increase from 10 to 30 mg/kg doses (see figure, below). There was no clear evidence of accumulation or decreased exposures following repeated treatment, which is not unexpected with the short half-life (2-3 hr). There were no consistent sex-related differences.

Figure 10. Systemic exposures (Cmax and AUC) to duloxetine in Beagles (male and female) treated for up to 1 yr with duloxetine (HCl) at oral doses of 3, 10, and 30 mg/kg/day. [Sponsor's graphs, excerpted directly from this submission. The 1-yr values are from this study; the values from 1 week to 6 mo are from the previous 6-mo study.]



C. Mice

The Sponsor submitted acute and subchronic studies in mice.

- **Acute [oral gavage] toxicity of LY248686 [duloxetine] maleate** [lot F58KY0152] administered orally [gavaged as suspension in 10% aqueous acacia] to [4-5-week old] CD-1 mice (Study nos. M34188 and M24188; report Tox07.pdf, December, 1988):

The median lethal doses were 397 mg/kg for males and 303 mg/kg for females; the lowest lethal dose was 365 mg/kg for males (3/5 died within 2 hr of dosing) and 250 mg/kg for females (1/5 died within 4 hr of dosing). All males given 700 or 1000 mg/kg died within 2 hr of dosing (9/10 within 1hr). Acute clinical signs (during the 1st day after treatment) were CNS-related, including hyperactivity, tremors, ataxia, clonic convulsions, and salivation, and showed dose-related severity in survivors.

- A subchronic **[3-mo, dietary] toxicity study** and blood level study in CD-1 mice [ ]; 5-6 weeks old; 10/sex/dose for main study] given diets containing **duloxetine hydrochloride** [lot 619NKO; dietary duloxetine concentrations of 0, 0.02, 0.04, and 0.08%] for 3 months. (Study nos. M01692 (main study) and M01792 (blood levels); report Tox34.pdf, October, 1993):

This study served as range-finding for the 2-yr carcinogenicity study. Relevant details of this study are presented in the Methods section of that study, along with findings from a 2-week study whose study report was not included in this submission.

D. Monkeys

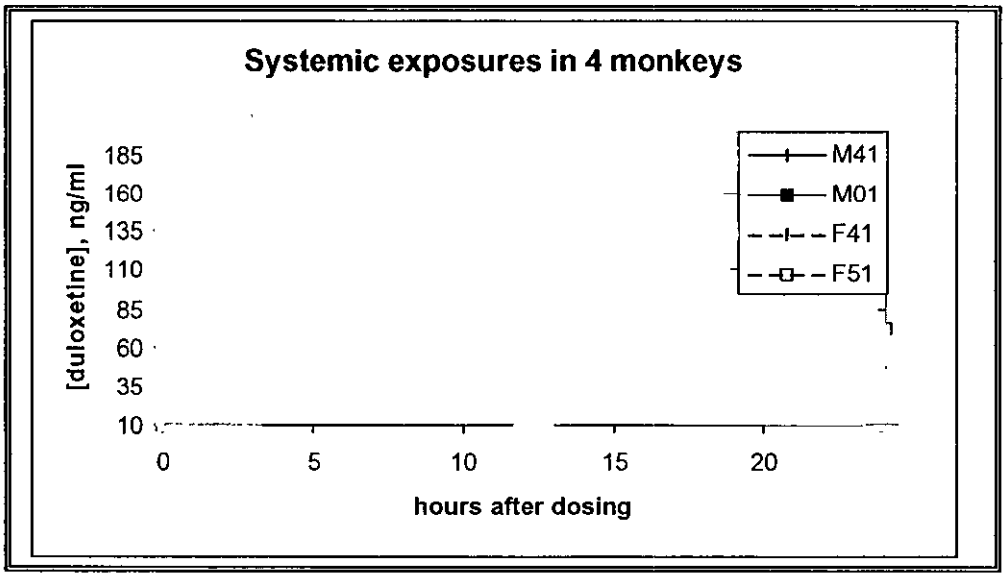
The Sponsor submitted only a single, acute study of toxicity of a single oral dose of duloxetine, as the maleate salt, in monkeys.

- **Acute toxicity of [100 mg/kg] LY248686 [duloxetine as the] maleate [salt]** [lot F58-KYO-152] administered nasogastrically [suspended in 10% aqueous acacia] to **Rhesus monkeys** [2/sex]. (Study no. P01088; report Tox11.pdf, April, 1989):

There were no deaths, but 3/4 monkeys vomited (with apparent loss of drug in 2) and 2 monkeys had decreased appetite; all appeared normal after 24 hr. Plasma levels of duloxetine were determined for up to 24 hr after dosing (see figure, below). [Three monkeys vomited (M41 at ~3 hr, F41 at ~2 hr and F51 at 6hr; although white crystals of drug were reportedly seen in vomitus of 2/3, their identities were not disclosed in the Study Report. It should also be noted that the monkey that didn't vomit had the lowest levels of duloxetine.] It appears that females achieved higher levels of duloxetine than males. The Sponsor concluded that only low amounts of duloxetine were detectable throughout the time course of sampling and that this indicated that duloxetine maleate is poorly absorbed in the monkey after

nasogastric administration. However, it seems equally likely that duloxetine may be extensively metabolized in monkeys, as in other species. In study HMBN (2001), the AUC in humans given a 60-mg dose was 845 ng.hr/ml, or an average plasma level of ~35 ng/ml. This is certainly higher than the exposure of M01 (see graph, below), the only monkey that didn't vomit, so if vomiting is a limiting toxicity, then I agree that exposure coverage for duloxetine might not be feasible, at least using this formulation.

Figure 11. Plasma curves for duloxetine in individual Rhesus monkeys after a single, nasogastric dose of 100 mg/kg (as the maleate salt). [Graphed from data presented by the Sponsor in Table 3 of study Tox11, page 20. I have plotted values below the limit of quantification (LOQ) as the LOQ= — ug/ml. (I assume that the high value at 0 hr for M41 is an error.) I have truncated the monkey identification numbers, but M denotes males and F denotes females.]



Toxicology summary and conclusions: See overall summary in Detailed Conclusions and Recommendations section IX.

Table 14. Histopathology Inventory for NDA 21-427.

Study	6-mo	1-yr	2-yr	2-yr
Species	rat	dog	mouse	rat
Adrenals	X*	X*	X	X*
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)	X	X	X	X
Brain	X*	X*	X*	X*
Cecum			X	X
Cervix			X	X

Study	6-mo	1-yr	2-yr	2-yr
Species	rat	dog	mouse	rat
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis			X	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder		X	X	
Gross lesions	X	X	X	X
Harderian gland	X		X	X
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X	X	X
Lymph nodes, cervical	X+	X+	X+	X+
Lymph nodes mandibular	X+	X+	X+	X+
Lymph nodes, mesenteric	X+	X+	X+	X+
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves				
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X	X
Parathyroid	X	X	X	X
Peripheral nerve	X	X	X	X
Pharynx				
Pituitary	X	X*	X	X
Prostate	X*	X*	X	X*
Rectum			X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X		X	X
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X	X*
Sternum				X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X	X	X	X
Thyroid	X*	X*	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X*

Study	6-mo	1-yr	2-yr	2-yr
Species	rat	dog	mouse	rat
Zymbal gland				
Standard List				

X: histopathology performed.
*: organ weight obtained.
+: particular lymph node not specified.

V. GENETIC TOXICOLOGY:

A. *In vitro* bacterial mutation (Ames) tests.

1. *Study title: The effect of LY248686 [duloxetine] hydrochloride on the induction of reverse mutations in Salmonella typhimurium and Escherichia coli using the Ames test.*

Key findings: Valid and negative. However, the Sponsor should be reminded (for future submissions) that according to FDA/CFSAN 2000 Redbook,"2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S9 mix. If 2-aminoanthracene is used, each batch of S9 should also be characterized with a mutagen that requires metabolic activation by microsomal enzymes, *e.g.*, benzo(a)pyrene, dimethylbenzanthracene)."

Study no: 920225AMS3512; Report no. 24.
Study type: Ames test.
Volume #, and page #: EDR file Tox24.pdf.
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN 46140.
Date of study initiation: 2/25/92; completed 2/27/92.
GLP compliance: yes, see p 3.
QA reports: yes, see p 2.
Drug, lot #, and % purity: 246916 (duloxetine hydrochloride), lot no. 619NKO, [redacted] as HCl salt (HPLC, 11/21/90).

Formulation/vehicle: dissolved in distilled water.

Methods:

Strains/species/cell line: S. typhimurium strains TA1535, TA100, TA1537, TA98; E. coli strain WP2uvrA$^-$; adequate selection.

Dose selection criteria:

Basis of dose selection: preliminary toxicity test in TA100 strain; and precipitation test. Range finding studies: 0, 50, 100, 500, 1000, 2000, 3000, 4000, and 5000 µg/plate, TA100 strain (duplicate plates), resulted in slight decrease in colonies at 100 µg/plate (without activation) and essentially complete cytotoxicity at ≥500 µg/plate (with or without activation).

Test agent stability: prepared fresh on day of use, not stored.

Metabolic activation system: S9 fraction from livers of male rats treated with Aroclor1254 (500 mg/kg) 5 days before sacrifice; 0.5 ml per 2.65 ml total top agar solution.

Controls:

Vehicle: distilled water.

Negative controls: distilled water.

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), 2-nitrofluorene (2NF), 9-aminoacridine (9AmAc), and 2-aminoanthracene (2AA); 2 concentrations each.

Comments: 2AA was the only positive control used with metabolic activation; historical controls were provided for vehicle and positive controls.

Exposure conditions:

Incubation and sampling times: 48 hr in top agar at ~37C.

Doses used in definitive study: 0, 25, 50, 100, 200, 400 µg/plate of LY248686, duloxetine base.

Study design: plate incorporation assay.

Analysis:

No. of replicates: triplicates.

Counting method: revertant colonies were counted using an —— Automated Colony Counter, on 86% of plate; values were corrected to 100% of plate.

Criteria for positive results: dose-related increase in revertants, with at least 2 consecutive doses producing at least a doubling (TA98, TA100, WP2urva-) or tripling (TA1535, TA1537) of control value.

Summary of individual study findings:

Study validity: generally valid: 5 amounts of drug (2-fold spacing) with highest amount decreasing number of revertants (i.e., cytotoxic) for each strain; triplicate plates; strong positive controls; but only 2AA used as positive control for S9-activated samples. According to FDA/CFSAN 2000 Redbook,"2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S9 mix. If 2-aminoanthracene is used, each batch of S9 should also be characterized with a mutagen that requires metabolic activation by microsomal enzymes, *e.g.*, benzo(a)pyrene, dimethylbenzanthracene)."

Study outcome: Negative (see Sponsor's table, below).

Table 15. Duloxetine hydrochloride was negative in the Ames test to amount that was cytotoxic (i.e., decreased the number of revertants). [Sponsor's table, exerpted directly from this submission.]

		Colony Counts (Mean ± SD)[a]				
Treatment	µg/plate	TA1535	TA1537	TA98	TA100	WP2uvrA⁻
		Test without Metabolic Activation				
Water[b]	0.05 ml	16 ± 4	8 ± 1	29 ± 2	158 ± 34	49 ± 9
Water[c]	0.05 ml	14 ± 4	7 ± 1	26 ± 5	214 ± 17	53 ± 3
246916	25	9 ± 1	7 ± 1	20 ± 3	163 ± 12	49 ± 6
	50	12 ± 2	10 ± 1	26 ± 2	147 ± 19	56 ± 8
	100	9 ± 5	8 ± 1	25 ± 5	106 ± 7	33 ± 2
	200	7 ± 4	7 ± 5	24 ± 13	39 ± 6	22 ± 3
	400	0 ± 0	0 ± 1	11 ± 2	16 ± 6	8 ± 1
ENNG[d]	5	164 ± 14			802 ± 27	1218 ± 54
ENNG[d]	10	863 ± 127			1645 ± 112	1650 ± 32
9AmAc[d]	50		259 ± 7			
9AmAc[d]	100		1195 ± 61			
2NF[d]	0.5			325 ± 20		
2NF[d]	5			1359 ± 224		
		Test with Metabolic Activation				
Water[b]	0.05 ml	17 ± 3	7 ± 2	34 ± 5	203 ± 12	62 ± 7
Water[c]	0.05 ml	15 ± 3	8 ± 2	33 ± 3	233 ± 18	47 ± 4
246916	25	14 ± 1	9 ± 4	35 ± 7[e]	207 ± 15	55 ± 7
	50	12 ± 4	7 ± 3	34 ± 2[e]	213 ± 9	58 ± 6
	100	10 ± 2	4 ± 1	33 ± 3	194 ± 16	48 ± 6
	200	12 ± 2	4 ± 3	31 ± 2	166 ± 16	26 ± 7
	400	4 ± 2	0 ± 1	10 ± 1	14 ± 4	12 ± 2
2AA[d]	1.25	131 ± 9	44 ± 2	729 ± 88	1081 ± 15	
2AA[d]	2.5	242 ± 12	97 ± 12	1600 ± 167	1922 ± 15	
2AA[d]	5					195 ± 23
2AA[d]	10					543 ± 25

[a]Mean ± standard deviation of counts from triplicate plates.
[b]Sterile water served as the vehicle control for the tester strain plated at the initiation of plating.
[c]Sterile water served as the vehicle control for the tester strain plated at the termination of plating.
[d]Positive control compounds: ENNG (N-ethyl-N'-nitro-N-nitrosoguanidine), 9AmAc (9-aminoacridine), 2NF (2-nitrofluorene), 2AA (2-aminoanthracene).
[e]Mean of two plates, one plate not counted due to contamination.

2. ***Study title: The effect of LY248686 [duloxetine] maleate on the induction of reverse mutations in Salmonella typhimurium and Escherichia coli using the Ames test.***

Key Findings: This preliminary study confirms the negative findings from the pivotal study reviewed above.

Methods: *Study nos.* 870824AMT3007 (prelim), 870831AMS3007 (without activation), 870914AMS3007 (± activation, some problematic pinpoint colonies), 870921AMS3007 (± activation, some problematic pinpoint colonies), 880404AMS3007 (replicate plating study of pinpoint colonies from study 870921); studies initiated 8/24/87, 8/31/87, 9/14/87, 9/21/87, 4/4/88; Electronic file Tox04.pdf; GLP and QA.

Compound 255485 (duloxetine maleate), lot no. F58-KYO-152, — , pure; vehicle DMSO; S.typhimurium strains TA1535, TA1537, TA98, TA100, E.coli strain WP2uvrA-; same preliminary toxicity test in TA100 and precipitation test as in study reviewed above; toxicity at ≥500 ug/plate without activation (60% survival at 500, 10% at 1000) and at ≥2000 ug/plate with activation (80% survival at 2000, 10% at 3000) in TA100, no precipitation up to 5000 ug/plate.

In the "definitive" studies, doses of 0, 22, 45, 89, 178, and 357 µg/plate of duloxetine base were used without activation and doses of 0, 89, 178, 357, 714, and 1428 µg/plate were used with activation. Cytotoxicity was clear at the 357-µg dose for all S. typhimurium strains and marginal for the E. coli strain without activation; cytotoxicity was apparent at 714-µg dose with activation, assuming that the pinpoint colonies observed at high concentrations were artifact. [When these pinpoint colonies were replated onto complete medium plates vs minimal medium plates, essentially none of them could grow without supplements (but all could grow with supplements), i.e., they were not true revertants; whereas (normal sized) colonies from positive or negative controls could all grow on minimal medium plates, i.e., they were true revertants.]

Results: There was no evidence of mutagenicity for any doses of duloxetine, however, only 3-4 doses were useable for each strain; this serves as the equivalent of a preliminary study and confirms the negative findings from the pivotal study reviewed above.

B. Study title: The effect of LY248686 [duloxetine] hydrochloride on the *in vitro* induction of chromosomal aberrations in Chinese hamster ovary cells.

Key findings: Negative for chromosomal aberrations after 4-hr treatment with or without activation, **but** only 100 (not 200) metaphases were counted per treatment **and** these negative findings should have been followed up with ~24-hr treatment without activation.

Study no: 920422CAB3512 (with and without activation) and 920617CAB3512 (with activation, only).
Study type: *In vitro* chromosomal aberration assay.
Volume #, and page #: Electronic file Tox30.pdf; paper volume 1.25, 140 pages.
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN 46140.
Date of study initiation: 4/16/92 and 6/17/92.
GLP compliance: yes, see p 3.
QA reports: yes, see p 2.
Drug, lot #, and % purity: Compound 246916 [duloxetine hydrochloride], lot no. 619NKO, — duloxetine HCl (HPLC, 11/21/90).

Formulation/vehicle: dissolved in sterile — water.

Methods:

Cell line: CHO cells [ ] originally obtained from [ ]

Dose selection criteria:

Basis of dose selection: preliminary toxicity test, with and without activation; survival presented for concentrations from 8-24 ug/ml without activation (~25% decreased survival at 10-12 ug/ml, ~50% decrease at 18-20 ug/ml, and ~75% decrease at 24 ug/ml); survival presented for concentrations from 80-240 ug/ml with activation (~30% decreased survival at 120 ug/ml and essentially no survival at the 6 concentrations ≥140 ug/ml).

Range finding studies: see above.

Test agent stability: dissolved on day of use, not stored.

Metabolic activation system: from male Fischer 344 rats treated with Aroclor 1254; ~10% final concentration on cells.

Controls:

Vehicle: sterile — water.

Negative controls: sterile — water.

Positive controls: cyclophosphamide (with activation) and mitomycin C (without activation); sterile solutions in — water.

Comments: historical control data for mean % aberrations for vehicle and positive controls, with and without activation, from ~16 recent studies (1990-1992) were provided.

Exposure conditions:

Incubation and sampling times: 4-hr exposure to drug, with or without activation, then ~19-hr further incubation without drug.

Doses used in definitive studies: 0, 8, 12, 16, 18, and 20 µg/ml (study 920422CAB3512) without activation (aberration data provided for 8, 12, and 18, only); 0, 110, 115, 120, 125, and 130 µg/ml (study 920422CAB3512, but toxicity too great) and 45, 60, 75, 90, 105, and 120 µg/ml (study 920617CAB3512, used for aberration determination) with activation (aberration data provided for 75, 90, and 105, only).

Analysis:

No. of replicates: triplicates, of which 2 were treated with colcemid and used to determine chromosomal aberrations and the 3rd was used to determine cytotoxicity.

Counting method: 50 metaphases from each treatment culture (100/concentration) and 25 metaphases from each positive control culture were read; only cells with 19-23 chromosomes were scored; aberrations were scored up to a maximum of 10/cell.

Criteria for positive results: Quoted from submission, below:

"

> Criterion for a positive response: A compound will be determined to be a clastogen when a concentration-related increase in chromosome aberrations is observed in which the number of aberrations is statistically greater than that of the concurrent control value as determined by a trend test for binomial distribution (Margolin, et al., 1986).

"

Summary of individual study findings:

Study validity: valid, except that 200 metaphases/treatment should have been counted (not 100); and negative findings after 4-hr drug exposure (± activation) should have been followed up by ~24-hr exposure without activation.

Study outcome: Negative for chromosomal aberrations after 4-hr treatment with or without activation, **but** only 100 (not 200) metaphases were counted per treatment **and** these negative findings should have been followed up with ~24-hr treatment without activation. [Additionally, there were increases in % of cells with diplochromosomes at the high concentration of duloxetine ± activation; however, this is not considered by the Sponsor or the Agency to indicate chromosomal aberration.]

Table 16. Sponsor's tables showing results of 4-hr treatment with duloxetine, without (upper panel) or with (lower panel) metabolic activation, on occurrence of chromosomal aberrations in CHO cells. [Cell survival compared with negative control, determined in sister cultures, showed ~5% decreased survival at 8 ug/ml, ~50% decreased survival at 12 ug/ml and ~60% decreased survival at 18 ug/ml without activation; 10% decreased survival at 75 ug/ml, ~15% at 90 ug/ml, and ~55% at 105 ug/ml (and 95% decrease at 120 ug/ml) with activation.]

Table 1.2. Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated with LY248686 Hydrochloride in the Absence of Metabolic Activation. Study 920422CAB3512 (Results from Individual Cultures).

Treatment	Cells Scored	Number and Type of Aberration[a]															No. of Aberrations Per Cell	% Cells with Aberrations (TA)[b]	% Cells with Aberrations (TAG)[b]	% Cells with >1 Aberrations	% Cells with Diplochromosomes
		g		ctb	cte				csb	cse			other								
		TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT						
Controls:																					
Vehicle:																					
Water	50																0	0	0	0	0
	50									1			1				0.04	4	4	0	0
Positive:																					
MMC[a] 0.5 µg/ml	25			2	2	2	1		3	1					1		0.84	32***	32	16	5
Test Compound: LY248686																					
8 µg/ml	50									1							0.02	2	2	0	2
	50								1								0.02	2	2	0	0
12 µg/ml	50								1								0.02	2	2	0	3
	50								2								0.04	4	4	0	5
18 µg/ml	50																0	0	0	0	14
	50																0	0	0	0	12

[a]Abbreviations: g=gap; ctb=chromatid break; cte=chromatid exchange; csb=chromosome break; cse=chromosome exchange; TG=chromatid gap; SG=chromosome gap; TB=chromatid break; SB=chromosome break (includes acentric fragment); DM="double minute" fragment; ID=interstitial deletion; TR=triradial; QR=quadriradial; CR=complex rearrangement; D=dicentric; R=ring chromosome; CI=chromosome intrachange; PU=pulverized chromosome; GT=greater than 10 aberrations; MMC=Mitomycin C.
[b]TA=Percent cells with aberrations excluding gaps. TAG=Percent cells with aberrations including gaps.
***Significantly greater than the vehicle controls.

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Table 2.2. Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Treated with LY248686 Hydrochloride in the Presence of Metabolic Activation, Study 920617CAB3512 (Results from Individual Cultures).

Treatment	Cells Scored	Number and Type of Aberration[a]														No. of Aberrations Per Cell	% Cells with Aberrations (TA)[b]	% Cells with Aberrations (TAG)[b]	% Cells with >1 Aberrations	% Cells with Diplochromosomes
		g		ctb	cte				csb					other						
		TG	SG	TB	TR	QR	CR	ID	SB	D	R	CI	DM	PU	GT					
Controls:																				
Vehicle:																				
Water	50								1							0.02	2	2	0	0
	50								1							0.02	2	2	0	1
Positive:																				
CP[c] 7.5 µg/ml	25				2		2		10							0.56	40***	40	8	5
Test Compound: LY248686																				
75 µg/ml	50								1							0.02	2	2	0	1
	50								1							0.02	2	2	0	1
90 µg/ml	50								1							0.02	2	2	0	4
	50								2							0.04	4	4	0	3
105 µg/ml	50								1							0.02	2	2	0	13
	50								2							0.04	4	4	0	7

[a]Abbreviations: g=gap; ctb=chromatid break; cte=chromatid exchange; csb=chromosome break; cse=chromosome exchange; TG=chromatid gap; SG=chromosome gap; TB=chromatid break; SB=chromosome break (includes acentric fragment); DM="double minute" fragment; ID=interstitial deletion; TR=triradial; QR=quadriradial; CR=complex rearrangement; D=dicentric; R=ring chromosome; CI=chromosome intrachange; PU=pulverized chromosome; GT=greater than 10 aberrations; CP=cyclophosphamide.
[b]TA=Percent cells with aberrations excluding gaps. TAG=Percent cells with aberrations including gaps.
***Significantly greater than the vehicle control.

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C. Study title: The effect of LY248686 [duloxetine] hydrochloride on the *in vivo* induction of micronuclei in bone marrow of ICR mice.

Key findings: Valid and negative, with high dose approximately half the LD50.

Study no: 911118MNT3512; toxicology report no. 20.
Study type: mouse *in vivo* micronucleus assay.
Volume #, and page #: electronic file Tox20.pdf; paper volume 1.25, 32 pages.
Conducting laboratory and location: Lilly Research Labs, Greenfield, IN 46140.
Date of study initiation:
GLP compliance: yes, see p 3.
QA reports: yes, see p 2.
Drug, lot #, and % purity: Compound 246916 (duloxetine hydrochloride), lot no. 619NKO, — pure (HPLC, 11/21/90).

Formulation/vehicle: suspended in 10% aqueous acacia.

Methods:

Strains/species: male and female ICR [— (ICR))] mice, from [redacted] ~ 8 weeks old.

Dose selection criteria:

Basis of dose selection: high dose was ~50% the median lethal dose for males and ~73% that for females, determined in a preliminary study (see below).

Range finding studies: Study 911112MTT3512: doses of 0, 125, 250, 500, and 1000 mg/kg, daily for 2 days, 5/sex/dose: at 125 mg/kg, all had tremors and hyperactivity after each dose (normal within 24 hr); at higher doses, deaths were observed after the first dose (see table, below); LD50 (median lethal dose) was ~300 mg/kg (females maybe slightly more sensitive than males).

Table 17. Range-finding study for duloxetine HCl (oral gavage) in mice; 2 days of dosing, but deaths occurred after 1st dose. [Excerpted directly from Sponsor's submission, but without footnotes.]

Chemical Treatment	Dose (mg/kg)	No. Dead/No. Dosed[a]	
		Males	Females
LY248686	125	0/5[b]	0/5[b]
LY248686	250	0/5[c]	2/5[c]
LY248686	500	4/5[d]	5/5[d]
LY248686	1000	5/5[e]	5/5[e]
Vehicle (10% Acacia)	20 ml/kg	0/5[f]	0/5[f]
Estimated MLD[g] (males)		385.55 mg/kg	
Estimated MLD (females)		259.72 mg/kg	
Estimated MLD (males/females combined):		329.52 mg/kg	

Test agent stability: suspensions prepared fresh daily.
Metabolic activation system: *in vivo.*
Controls:
Vehicle: 10% aqueous acacia.
Negative controls: 10% aqueous acacia.
Positive controls: cyclophosphamide (25 mg/kg), suspended in 10% aqueous acacia.
Comments: included table of historical control values.
Exposure conditions:
Incubation and sampling times: mice were killed 24 hr after 2nd dosing.
Doses used in definitive study: oral daily doses of 0, 47.5, 95, and 190 mg/kg for 2 consecutive days (20 ml/kg).
Study design: bone marrow streaks were prepared from femur marrow, 4 streaks/femur/mouse; streaks from 1 femur/mouse were fixed, stained with Wright's and Wright's-Giemsa, analyzed, those from the other were held in reserve.
Analysis:
No. of replicates: ~1000 PCEs plus NCEs per mouse to determine bone marrow toxicity; 1000 PCEs/mouse were evaluated for micronuclei (MNs); 5 mice/sex/treatment.
Counting method: microscopy, with ' cell counter.
Criteria for positive results: Quoting directly from the report:

"

A chemical is judged to have induced a positive response when a dose-related increase in micronucleated PCE is observed in which the number of micronucleated PCE is statistically greater than the concurrent control value as determined by a trend test for Poisson distribution (Tamura et al., 1990). The Mantel-Haenszel chi-square test (Mantel, 1963) is used to pool the inference across sexes.

"

Summary of individual study findings:

Study validity: Valid: negative control within historical control value, positive control gave 9-fold and 4-fold increase in micronuclei, versus negative control, in males and females, respectively.
Study outcome: Negative: duloxetine HCl did not increase micronuclei at doses up to ~half the LD50; these doses did not produce bone marrow toxicity, as evidenced by NCE/PME ratio (see table of study results, below).

Table 18. Duloxetine was not clastogenic in an *in vivo* mouse bone marrow micronucleus test. [Excerpted directly from this submission.]

Sex	Treatment[a,b]	Dose (mg/kg)	PCE/NCE[c] Ratio	MPCE[d] per 1000 PCE	Trend Test two-tailed p-value
Male					
	Vehicle[e]	20 ml/kg	1.0 ± 0.5	1.4 ± 1.7	
	LY248686	47.5	0.9 ± 0.3	1.0 ± 1.7	
	LY248686	95	0.9 ± 0.2	1.4 ± 1.5	
	LY248686	190	1.2 ± 0.4	2.0 ± 1.2	
					0.36
	CP[f]	25	0.7 ± 0.2	13.2 ± 6.4**	
Female					
	Vehicle[e]	20 ml/kg	1.4 ± 0.3	1.6 ± 1.5	
	LY248686	47.5	1.3 ± 0.5	1.0 ± 1.4	
	LY248686	95	1.2 ± 0.3	1.6 ± 1.1	
	LY248686	190	1.2 ± 0.3	0.8 ± 1.3	
					0.42
	CP[f]	25	1.2 ± 0.3	6.6 ± 4.4**	
				pooled[g] p = 0.90	

[a]Two equal treatments ~24 hours apart with harvest ~24 hours after the second treatment.
[b]Values are mean ± SD for 5 animals/treatment group.
[c]PCE: polychromatic erythrocyte; NCE: normochromatic erythrocyte.
[d]MPCE: micronucleated polychromatic erythrocytes.
[e]10% aqueous acacia given in a dose volume of 20 ml/kg.
[f]Cyclophosphamide (CP) served as the positive control.
[g]Pooled: Mantel-Haenszel pooled across sex.
**Significantly greater than the vehicle control ($p<.01$) as determined by a chi-square analysis.

D. Additional genotoxicity studies

1. ***Tox01.pdf: [June 1988] The effect of LY248686 maleate [F58-KYO-152] on the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells.***

Methods: Only a 4-hr treatment was performed, with or without metabolic activation, and both large and small colonies were included in the total count (automated ——— Counter, colonies from plates treated with solvent control were used to set the size discrimination and sensitivity for colonies). Cytotoxic concentrations were identified in an initial assay, with and without activation.

Results: (See Sponsor's table, below.) Without activation, 8 concentrations, from 0.25 to 10 ug/ml, did not increase the mutation frequency compared with DMSO vehicle control; in this study 10 ug/ml decreased survival 88% and in the pilot study concentrations of 10 and 25 ug/ml (and higher) completely suppressed survival. With activation, 6 concentrations, from 2.5 to 15 ug/ml, did not increase the mutation frequency compared with DMSO vehicle control; in this study 15 ug/ml decreased survival 78% and in the pilot study 10 ug/ml decreased survival 33% and 25 ug/ml (and higher) completely suppressed survival. Positive controls (EMS, without activation, and 3-MC, with activation) gave robust increases in mutation frequency.

[NB Both large and small colonies were apparently counted, however, only the combined counts were presented in this report. Nonetheless, the combined totals were so low, that it would be impossible for either large (mutations) or small (chromosomal aberrations) to have been increased in these study results. This could serve as evidence for lack of induction of chromosomal aberrations *in vitro*; however, the 24-hr treatment without activation would still be necessary for an adequate study.]

Conclusion: **Negative but inadequate**: Negative with 4-hr treatment with or without metabolic activation, but should have followed these results with 24-hr treatment without activation.

Table 19. Sponsor's summary of results for the mouse lymphoma assay with duloxetine maleate. [Excerpted directly from this submission.]

TABLE 5. A SUMMARY OF RESULTS FOR THE MOUSE LYMPHOMA FORWARD MUTATION ASSAY WITH COMPOUND LY255485, STUDY 871007MLA3007[a].

Treatment	Concentration (µg/ml)	Percent Total Survival[b]	Mutation Frequency[c]		Mutation Index[d]
NON-ACTIVATED TEST					
LY255485[i]	10	12	3.5		1.5
	8	32	2.8		1.2
	6	65	3.0		1.3
	4	68	3.6		1.6
	2	80	1.8		0.8
	1	76	2.7		1.2
	0.5	76	2.9		1.3
	0.25	87	3.1		1.3
DMSO[e]	(1%)	100	2.1		
DMSO[e]	(1%)	100	2.4	2.3[g]	(1.0)
DMSO[e]	(1%)	100	2.5		
EMS[f]	620	22	82.8		36.0
ACTIVATED TEST					
LY255485[i]	20	[h]	--		--
	17.5	[h]	--		--
	15	22	3.4		1.5
	12.5	30	2.7		1.2
	10	34	2.7		1.2
	7.5	39	2.9		1.3
	5	52	2.5		1.1
	2.5	54	2.7		1.2
DMSO[e]	(1%)	100	2.6		
DMSO[e]	(1%)	100	2.1	2.6[g]	(1.0)
DMSO[e]	(1%)	100	1.9		
3MC[f]	2	32	19.1		8.7

[a] Consult Appendix D for calculations.
[b] (Suspension growth) x (relative cloning efficiency).
[c] TK+/- mutants per 1 x 10^7 colony forming cells.
[d] (Mutation frequency of treated culture)/(control mutation frequency).
[e] Solvent control. [f] Positive control. [g] Mean of solvent controls.
[h] Insufficient suspension growth, culture not cloned.
[i] LY255485 is the maleate salt of LY248686. When corrected for the potency of the free base, the doses are equivalent to 7.1, 5.7, 4.3, 2.9, 1.4, 0.7, 0.4, and 0.2 µg/ml in the non-activated test and 14.3, 12.5, 10.7, 8.9, 7.1, 5.4, 3.6, and 1.8 µg/ml in the activated test.