

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-733

PHARMACOLOGY REVIEW(S)



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

MEMO TO PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-733
SERIAL NUMBER: N000
DATE RECEIVED BY CENTER: 03/03/2004
DRUG NAME: duloxetine hydrochloride (Cymbalta®)
INDICATION: —

SPONSOR: Eli Lilly
DOCUMENTS REVIEWED: 2 of 57 volumes
REVIEW DIVISION: Division of Anesthetic, Critical Care, and
Addiction Drug Products (HFD-170)

PHARM/TOX REVIEWER: Suzanne R. Thornton-Jones, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob Rappaport, M.D.
PROJECT MANAGER: Lisa Malandro

Date of review submission to Division File System (DFS): 01 September 2004

Sponsor's proposed label (yellow highlighted areas are proposed Sponsor changes):

Pharmacodynamics

Although the mechanism of the antidepressant and central pain inhibitory action of duloxetine in humans is unknown, it. The antidepressant action is believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS,¹

the central nervous system. Preclinical studies have shown that duloxetine is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a less potent inhibitor of dopamine reuptake. Duloxetine has no significant affinity for dopaminergic, adrenergic, cholinergic or histaminergic receptor

in vitro. Duloxetine does not inhibit monoamine oxidase (MAO). Duloxetine undergoes extensive metabolism, but the major circulating metabolites have not been shown to contribute significantly to the pharmacologic activity of duloxetine.

Assessment of label changes:

¹ The inclusion of the mechanism of action for the pain pathway is a correct statement and is acceptable for inclusion in the label. Rewording of the statement is, however, required.

accurate, it is not relevant to the actual mechanism of action of the duloxetine. In this light this statement should be removed or rewritten.

Suggested labeling: (double underlined text are corrections to label)

Although the exact mechanism of the antidepressant and central pain inhibitory action of duloxetine in humans is unknown, the antidepressant and pain inhibitory actions are 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 less potent inhibitor of dopamine reuptake. Duloxetine has no significant affinity for dopaminergic, adrenergic, cholinergic, histaminergic

Duloxetine does not inhibit monoamine oxidase (MAO). Duloxetine undergoes extensive metabolism, but the major circulating metabolites have not been shown to contribute significantly to the pharmacologic activity of duloxetine.

Reviewer Signature Suzanne R. Thornton-Jones, Ph.D.

Date: 09/01/2004

Supervisor Signature R. Daniel Mellon, Ph.D.

Concurrence Yes X No

Date: 09/01/2004

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Suzanne Thornton-Jones
9/1/04 11:54:15 AM
PHARMACOLOGIST

R. Daniel Mellon
9/3/04 11:41:12 AM
PHARMACOLOGIST

I concur with the labeling recommendations. However, final labeling negotiations have not been completed to date.



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PHARM/TOX REVIEWER: Suzanne R. Thornton-Jones, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob Rappaport, M.D.
PROJECT MANAGER: Lisa Malandro

Date of review submission to Division File System (DFS): 13 August 2004

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on acceptability.

The NDA can be approved from a pharmacology/toxicology perspective.

B. Recommendation for nonclinical studies.

None.

C. Recommendations on labeling.

The Sponsor is proposing a 60 mg/day
indication.

for this

1 Page(s) Withheld

 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 ✓ § 552(b)(5) Draft Labeling

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings.

Confirmatory studies showed that duloxetine hydrochloride is a serotonin and norepinephrine uptake inhibitor both *in vitro* and *in vivo*. Duloxetine treatment reversed mechanical allodynia in the Seltzer and Chung neuropathic pain animal models, and had effects on locomotor activity, TF latency, and formalin test.

CD-1 mice and Fisher F344 rats given duloxetine hydrochloride for 1-month in the diet and in pregnant New Zealand white rabbits given duloxetine hydrochloride orally during gestation days 7-19 duloxetine showed an increase, though not proportional, in duloxetine and glucuronide conjugated 4-hydroxy duloxetine metabolite. Exposure to duloxetine was decreased with repeat dosing indicating enzyme induction. The glucuronide conjugated 4-hydroxy duloxetine exposure was higher than duloxetine.

B. Pharmacologic activity.

Duloxetine hydrochloride is a serotonin (5-HT) and norepinephrine (NE) uptake inhibitor.

C. Nonclinical safety issues relevant to clinical use.

Liver toxicity, as evidenced by increases in liver enzymes, in both the clinical and non-clinical study result and a potential of duloxetine/ethanol interaction leading to further liver enzyme elevation were observed.

The mitochondrial beta-oxidation study was submitted to the IND (62,536) that correlates with this NDA and after review it does appear that duloxetine hydrochloride and its major human metabolites when given at comparable plasma levels to cultured rat hepatocytes may lead to mitochondrial beta-oxidation.

The issues of liver toxicity either as a direct affect of duloxetine or via its potential interaction with ethanol can be addressed in the label and through post-marketing surveillance. HFD-120 (NDA 21-427) in August 2004 approved Cymbalta with appropriate labeling precautions for these liver toxicities and I find this an acceptable approach.

Reviewer Signature Suzanne R. Thornton-Jones, Ph.D.

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrence Yes X No

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW**2.6.1 INTRODUCTION AND DRUG HISTORY**

NDA NUMBER: 21-733
REVIEW NUMBER: 1
SEQUENCE NUMBER/DATE/TYPE OF SUBMISSION: N000/03 March 2004
INFORMATION TO SPONSOR: Yes () No (X)
SPONSOR: Eli Lilly
 Lilly Corporate Center
 Indianapolis, IN 46285
MANUFACTURER FOR DRUG SUBSTANCE : Eli Lilly and Company, Tippecanoe
 Laboratories, Lafayette, IN
REVIEWER NAME: Suzanne R. Thornton-Jones, Ph.D.
DIVISION NAME: DACCADP
HFD #: 170
REVIEW COMPLETION DATE: 09 August 2004
DRUG:
TRADE NAME: Cymbalta®
GENERIC NAME (LIST ALPHABETICALLY): S(+)-duloxetine hydrochloride
CODE NAME: LY248686
CHEMICAL NAME: (+)-N-methyl-gamma-(1-naphthalenyloxy)-
 2-thiophenepropane-amine hydrochloride
CAS REGISTRY NUMBER: 116539-59-4
MOLE FILE NUMBER: not specified
MOLECULAR FORMULA/MOLECULAR WEIGHT: C₁₈H₁₉NOS •HCl/333.883
STRUCTURE:



RELEVANT INDs/NDAs/DMFs: IND 37,071; 38,838: — . 62,536;
 NDA 21-427: —
DRUG CLASS: serotonin and norepinephrine re-uptake
 inhibitor
INTENDED CLINICAL POPULATION: —

CLINICAL FORMULATION: Clinical formulation: capsules containing enteric-coated pellets
 of duloxetine hydrochloride equivalent to 20, 30, or 60 mg duloxetine and inactive
 ingredients: FD&C Blue No. 2, gelatin, — hydroxypropyl

methylcellulose acetate succinate, sodium lauryl sulfate, sucrose, sugar spheres, talc, titanium dioxide, triethyl citrate, iron oxide yellow.

ROUTE OF ADMINISTRATION: oral

PROPOSED DOSE: 60 mg/day

BACKGROUND: Duloxetine hydrochloride is an inhibitor of monoaminergic (5-HT and NE) uptake for the indication of pain associated with peripheral diabetic neuropathy. Duloxetine was originally submitted to HFD 120 (NDA 21-427) for the indication of depression and received an Approvable (AE) decision in August 2002. During the pharmacology/toxicology review HFD-120 identified the following approvability issue: 'with regard to the drug substance impurities, a specification of not more than — has been proposed.' The following additional information was requested: 1) indicate the amounts of — present in lots of drug substance used for pivotal toxicology studies (i.e., genotoxicity, carcinogenicity, and reproduction studies); 2) indicate the amounts of — present in lots of drug substance used in the animal reproduction studies; and 3) if the analytical data requested do not adequately qualify these impurities per the ICH Q3A Guidance, we suggest that you lower the specification limit for each impurity to not more than 0.1% or qualify the impurities. The issue was resolved at a teleconference held on December 18, 2002, FDA Division of Neuropharmacologic Drug Products agreed that the information provided in the briefing document was sufficient to qualify — at the proposed specification of not more than — in duloxetine drug substance. Concerning the qualification of — the FDA indicated that the reproduction studies were adequate to qualify this impurity. At the request of Dr. Fossom, Lilly provided certificates of analysis to demonstrate that the lot of duloxetine drug substance (F58-KYO-152) used in the genetic and repeat dose studies contained —. These studies include a mouse lymphoma unscheduled DNA synthesis, sister chromatid exchange, Ames test, and 3-month rat and 3-month dog repeat dose studies. All non-clinical issues were resolved at the end of the second review cycle for NDA 21-427 in 2003.

To date, the Sponsor is conducting an *in vitro* toxicology study to assess the inhibitory effects of duloxetine hydrochloride and the two major human metabolites on mitochondrial β -oxidation in rat hepatocytes.

If the *in vitro* assessment is negative that no additional *in vivo* assessments will be necessary. However, if the results are positive, then additional *in vivo* assessments will be necessary.

The mitochondrial beta-oxidation study was submitted to the IND (62,536) that correlates with this NDA and after review it does appear that duloxetine hydrochloride and its major human metabolites when given at comparable plasma levels to cultured rat hepatocytes may lead to mitochondrial beta-oxidation.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: No new studies were submitted.

Study Title	Document/ Study no.	Volume
Effect of duloxetine (LY248686 hydrochloride) on the norepinephrine, serotonin, and dopamine transporter binding site	NCPR55	4
Effect of S-duloxetine (LY248686 hydrochloride) on N-methyl scopolamine (NMS) binding to human muscarinic M1 through M5 receptors	NCPR56	4
In Vivo Receptor Occupancy of the Norepinephrine and Serotonin Transporters by LY248686 Hydrochloride in Sprague Dawley Rats Using Non-radiolabeled Versions of the Ligands McNER and DASB	NCPR59	4
Occupancy of the serotonin and norepinephrine transporters by duloxetine hydrochloride in rat cortex	CNS455	4
Comparison of effects of duloxetine and other dual transporter inhibitors on extracellular monoamine levels in rats	NCPR63	4

LY248686 hydrochloride occupancy of cortical serotonin transporter sites in Sprague Dawley rats measured using citalopram as a tracer	NCPR60	4
In vivo occupancy of striatal dopamine reuptake sites by oral LY248686 hydrochloride administration in Sprague Dawley rats	NCPR58	4
Blockade of the a-methyl-m-tyrosine-induced depletion of rat cortical norepinephrine concentrations by duloxetine	NCPR57	4
Effect of duloxetine hydrochloride (LY248686) on extracellular levels of 5-hydroxytryptamine and noradrenaline in the rat medial prefrontal cortex	NCPR61	4
Update on effects of duloxetine.HCl (LY246916) in the partial sciatic nerve ligation and L5/L6 spinal nerve ligation models of neuropathic pain in Sprague Dawley rats	CNS465	4
Additional studies on effects of acute systemic and central administration of duloxetine.HCl (LY246916) in the formalin test of persistent pain and the rotorod test in rats	CNS466	4
Effects of duloxetine.HCl (LY246916) in a model of acute nociceptive pain in Sprague Dawley rats	CNS467	4
Quantification of _____ in rabbit, rat, and mouse plasma using _____	ADME report 93	4
Quantification of LY248686 (duloxetine) in rabbit plasma using _____	ADME report 79	4
Quantification of _____ in rabbit, rat, and mouse plasma using _____	DM-089-02-S	4
Plasma exposure of CD-1 mice to duloxetine and the glucuronide conjugate of 4-hydroxy duloxetine following daily dietary doses of 0.01%, 0.03%, or 0.08% of the diet as duloxetine in the hydrochloride form for 1 month	ADME report 94	5
Plasma exposure of Fischer 344 rats to duloxetine and the glucuronide conjugate of 4-hydroxy duloxetine following daily dietary doses of 0.01%, 0.02%, 0.05%, or 0.08% of the diet as duloxetine in the hydrochloride form for 1 month	ADME report 95	5
Plasma exposure of duloxetine (LY248686) and metabolites in pregnant New Zealand white rabbits following multiple oral doses of 2, 10, or 45 mg duloxetine/kg/day as the hydrochloride salt on gestation day 7 through gestation day 19	ADME report 92	5

Studies not reviewed within this submission (previously reviewed under NDA 21-427 unless otherwise indicated):

Study Title	Document/ Study no.	Reference
Pharmacology		
LY248686, A New Inhibitor of Serotonin and Norepinephrine Uptake	NCPR 10	
Comparative Affinity of Duloxetine and Venlafaxine for Serotonin and Norepinephrine Transporters <i>In vitro</i> and In	NCPR 54	

Vivo, Human Serotonin Receptor Subtypes, and Other Neuronal Receptors -		
Inhibition by Duloxetine on Synaptosomal Uptake of [3H]-5-HT, [3H]-Norepinephrine and [3H]-Dopamine	NCPR 52	
Inhibition of 3H-Paroxetine Binding by Fluoxetine and Duloxetine in Human, Rat, Mouse, Guinea Pig, and Pig Cortical Membranes	NCPR 29	
The Effect of Duloxetine and its Putative Metabolites on Binding to Serotonin, Norepinephrine, and Dopamine Human Transporters	NCPR 30	
LY248686, Its as Inhibitors of Serotonin Uptake in Human Platelets	NCPR 14	
Inhibition of Serotonin and Norepinephrine Uptake by	NCPR 16	
Effects of on Uptake of Serotonin and Norepinephrine <i>In vitro</i> and <i>Ex Vivo</i>	NCPR 15:	
LY248686, An Inhibitor of Serotonin and Norepinephrine Uptake in Synaptosomal Preparations of Rat Brain	NCPR 27	
Affinities of LY248686, Its for the Serotonin Uptake Site in Cortical Membranes of Rat Brain	NCPR 28	
Receptor Pharmacology Study of Duloxetine Metabolites Using Radiolabeled Receptor Binding	NCPR 49	
The Lack of Affinity of LY248686 for Neurotransmitter Receptors	NCPR 40	
Inhibition of Radioligand Binding by Duloxetine (LY248686) to Human Serotonin Receptor Subtypes and Other Neuronal Receptors	NCPR 51	
Effects of duloxetine on p-Chloramphetamine-Induced Serotonin Depletion, Monoamine Metabolite Levels, and Corticosterone Levels in Urine in Rat	NCPR 33	
Inhibition of Serotonin and Norepinephrine Uptake by LY248686, In Vivo	NCPR 31	
Inhibition of Serotonin and Norepinephrine Uptake <i>Ex Vivo</i> by LY248686 Hydrochloride and Its	NCPR 17	
LY248686 Hydrochloride Inhibits <i>Ex Vivo</i> 3H-Serotonin Uptake Into Rat Platelets, But Not <i>Ex Vivo</i> 3H-Dopamine Uptake Into Rat Striatal Homogenates	NCPR 18	
Effects of Duloxetine, an Antidepressant Drug Candidate, on Concentrations of Monoamines and Their Metabolites in Rats and Mice	NCPR 19	
Blockade by Duloxetine of Serotonin and Norepinephrine Transporter Specific Neurotoxins in Mice	NCPR 32	

Study Title	Document/ Study no.	Reference
Effects of Duloxetine Hydrochloride on Concentrations of Monoamines and Their Metabolites in Rats and Mice	NCPR 36	
Effect of Duloxetine Hydrochloride as an Inhibitor of Norepinephrine Reuptake in Rats	NCPR 50	
Effects of Duloxetine, a New Serotonin and Norepinephrine Uptake Inhibitor, on Extracellular Monoamine Levels in Rat Frontal Cortex	NCPR 22	
Simultaneous Increases of Extracellular Monoamines in Microdialysates from Hypothalamus of Conscious Rats by Duloxetine, a Dual Serotonin and Norepinephrine Uptake Inhibitor	NCPR 46	
Duloxetine Dose Dependently Elevates Serotonin and Norepinephrine and Decreases 5-Hydroxyindoleacetic Acid and 4-Hydroxy-3-Methoxyphenylglycol in the Hypothalamus as Measured Via Microdialysis in Rat	NCPR 21	
Effect of Duloxetine Hydrochloride on Monoamine Concentrations in Rat Hypothalamic Microdialysates	NCPR 34	
Effect of Duloxetine Hydrochloride and 5-HT1A Antagonist WAY 100635 on 5-HT, 5-HIAA, and NE Levels in Rat Hypothalamic Microdialysates	NCPR 35	
Enhancement of the Duloxetine-Dependent Elevation of Extracellular 5-HT Levels in Hypothalamus by Continuous Infusion of the Antagonist at Beta-Adrenergic/5-HT1A Receptors, +/-Pindolol, in Rats	NCPR 41	
Beta-adrenergic/5-HT1A Antagonists Potentiate the Duloxetine-Dependent Elevation of Extracellular 5-HT Levels in Rat Hypothalamus	NCPR 47	
Effects of Duloxetine (LY248686) Hydrochloride in Acute Pain Tests, the Carrageenan Test, and the Capsaicin Test in Mice and Rats -	NCPR 01/ T-88-01	N21-427/ I62,536
Effects of the Concomitant Administration of Duloxetine (LY248686) Hydrochloride and Ibuprofen in the Writhing Test in Mice and the Carrageenan Test in Rats	NCPR 02/ T-88-02	N21-427/ I62,536
Effects of Duloxetine Hydrochloride in the Formalin Test of Persistent Pain in the Rat	NCPR 03/ SI-1	N21-427/ I62,536
Effects of Duloxetine Hydrochloride in the Partial Sciatic Nerve Ligation Model of Neuropathic Pain (Seltzer Model) and the L5/L6 Spinal Nerve Ligation Model of Neuropathic Pain (Chung Model) in Rats	NCPR 04/ SI-2	N21-427/ I62,536
Effects of Duloxetine Hydrochloride on Neurological Function in the Rotorod Test in Rats	NCPR 05/ SI-3	N21-427/ I62,536
LY248686 Hydrochloride Administration Failed to Produce Analgesia in a Rat Model of Chronic Pain	NCPR 48	

Study Title	Document/ Study no.	Reference
Lack of Effects of Duloxetine on Punished Responding Behavior in Pigeons	NCPR 37	
Effects of Duloxetine HCl on Anti-Depressant Like Responses in the Tail Suspension Test Using CF-1 Mice	NCPR 38	
Behavior Observations and Effects of Duloxetine as Serotonin and Norepinephrine Uptake Inhibitors	NCPR 09	
Behavioral and Electroencephalographic Properties of Duloxetine (LY248686), a Reuptake Inhibitor of Norepinephrine and Serotonin, in Mice and Rats	NCPR 24	
Antidepressant Behavioral Effects by Dual Inhibition of Monoamine Reuptake in the Rat Forced Swimming Test	NCPR 25	
Blockade of the Serotonin and Norepinephrine Uptake Processes by Duloxetine: <i>In vitro</i> and <i>In Vivo</i> Studies in the Rat Brain	NCPR 23	
Suppression of Food Intake by an Inhibitor of Serotonin/Norepinephrine Uptake (LY248686), Fluoxetine and Nisoxetine in Food Deprived Rats	NCPR 07	
Effects on Food Intake, Body Weight, Brain Levels of Monoamines and Metabolites and Beta-Adrenergic Receptor After Chronic Administration of Duloxetine (LY248686) Hydrochloride in Rats	NCPR 20	
Suppression of Food Intake in Meal-Fed Rats by LY248686, the Inhibitor of Serotonin and Norepinephrine Uptake (— and 255485, Maleate Salt)	NCPR 39	
The Absence of Tolerance to Anorectic Effects in Meal-Fed Rats During Chronic Treatment With LY248686, a Mixed Serotonin/Norepinephrine Uptake Inhibitor	NCPR 43	
The Suppression of Body Weight Gain and Food Intake During the Chronic Treatment of Meal-Fed Obese Zucker Rats with LY248686 Maleate, a Mixed Serotonin and Norepinephrine Uptake Inhibitor	NCPR 11	
The Effect of LY248686, a Mixed Serotonin/Norepinephrine Reuptake Inhibitor, on Appetite, Body Weight Gain and Glucose Intolerance of Obese and Streptozotocin Diabetic Obese Zucker Rats	NCPR 13	
Effects of Treatment with LY248686 Maleate, a Mixed Serotonin/Norepinephrine Uptake Inhibitor, on Food Intake and Body Weight in Meal-Fed Lean and Obese Rats	NCPR 12	
The Effects of LY248686 on Food Consumption, Body Weight and Heat Production in Viable Yellow Obese Mice (VY/WFL-Avy/a)	NCPR 06	
Suppression of Saccharin Solution Consumption by — in Rats	NCPR 08	

Study Title	Document/ Study no.	Reference
Suppression of Alcohol Intake and Food Intake by LY248686 (A New Inhibitor of Serotonin and Norepinephrine Uptake), Fluoxetine and Nisoxetine in High-Alcohol-Drinking Rats	NCPR 42	
<i>In vitro</i> Effects of Duloxetine on Potassium-Evoked Release of 3H-Norepinephrine and on the Contractile Responses of Urethral and Bladder Tissues from Female Rabbits	NCPR 44	
Effects of Duloxetine (LY248686), a Combined Serotonin and Norepinephrine Re-uptake Inhibitor, on Central and Peripheral Neural Control of Lower Urinary Tract Function in the Female Cat	NCPR 26	
Effects of Duloxetine, a Combined Serotonin and Norepinephrine Re-uptake Inhibitor, on Central Neural Control of Lower Urinary Tract Function in the Chloralose-Anesthetized Female Cat	NCPR 45	
Safety pharmacology		
<i>In vitro</i> Studies of LY248686 Maleate in the Smooth and Cardiac Muscles of Sprague Dawley Rats and Hartley Albino Guinea Pigs	GenPharmRpt 16	
Human Cardiac Ion Channel Blocking Profile of Duloxetine (LY248686) Hydrochloride	Tox Rpt 45	
Cardiovascular Effects of LY248686 Maleate in Male Sprague-Dawley Rats	GenPharmRpt 14	
Cardiovascular Effects of LY248686 Maleate Administered Intravenously in Anesthetized Beagle Dogs	GenPharmRpt 15:	
An Acute Study of Aortic and Pulmonary Arterial Pressure Effects of LY248686 Hydrochloride Administered Orally and Intravenously in Conscious Mongrel Dogs	ToxRpt 19:	
CNS Behavioral Effects of LY248686 Maleate Administered Orally in the Male CD-1 Mouse	GenPharmRpt 13:	
The Acute Behavioral Profile of LY248686 Hydrochloride when Administered Orally to Male CD-1 Mice	GenPharmRpt 2	
The Behavioral Profile of Duloxetine Hydrochloride (LY248686 Hydrochloride) Following Multiple (5-Day) Oral Administration in Male CD-1 Mice	GenPharmRpt 3	
Acute Effects of Orally Administered LY248686 Hydrochloride upon Charcoal Meal Transit in the Gastrointestinal Tract of the Male CD-1 Mouse	GenPharmRpt 1	
A Study of the Acute Effects of LY248686 Maleate by Oral Administration on Urine and Electrolyte Excretion in Female Sprague-Dawley Rats	GenPharmRpt 17	
A Renal Pharmacology Study in Female Fischer 344 Rats Given a Single Gavage Dose of Duloxetine Hydrochloride (LY248686 Hydrochloride)	GenPharmRpt 4	
Dependence study on LY248686 in rhesus monkeys and rats	Tox report 41	

Study Title	Document/ Study no.	Referenc e
PK/TK		
The Synthesis of (S)-(+)-N-Methyl-G-(Naphthalenyloxy)-2-Thiophenepropanamine-[1-14C] Hydrochloride(LY248686-[14C] HCl)	ADME Rpt 1	
Amendment 1: Method of Determination of LY248686 in Plasma by High Performance Liquid Chromatography with Fluorescence Detection	ADME Rpt 2	
High Performance Liquid Chromatographic Determination of LY248686 Hydrochloride in Plasma (AM-AA-TA-T014-AA-992)	ADME Rpt 80	
Determination of LY248686 in Plasma by HPLC with UV Detection (GL718DP-010-AA.0)	ADME Rpt 81	
Determination of LY248686 in Plasma by HPLC with UV Detection (GL718DP-010-AB.0)	ADME Rpt 82	
Quantitation of LY248686 (Duloxetine) in Mouse Plasma Using	ADME Rpt 83	
Quantitation of LY248686 (Duloxetine) in Rat Plasma Using	ADME Rpt 84	
Quantitation of LY248686 (Duloxetine) in Dog Plasma Using	ADME Rpt 85	
Long Term Stability for the Determination of LY248686 (Duloxetine) in Mouse Plasma (142917 S1)	ADME Rpt 86	
Long Term Stability for the Determination of LY248686 (Duloxetine) in Rat Plasma (142918 S1)	ADME Rpt 87	
Long Term Stability for the Determination of LY248686 (Duloxetine) in Dog Plasma (145432 S1)	ADME Rpt 88	
An Asymmetric Synthesis of Duloxetine Hydrochloride, a Mixed Uptake Inhibitor of Serotonin and Norepinephrine, and Its C-14 Labeled Isotopomers	ADME Rpt 89	
Plasma Pharmacokinetics of Radioactivity and LY248686 in Mice Following Oral Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 3	
Plasma Pharmacokinetics of Radioactivity and LY248686 in Rats Following Oral Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 5	
Plasma Pharmacokinetics of Radioactivity and LY248686 in Rats Following Intravenous Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 7	

Study Title	Document/ Study no.	Referenc e
Comparison of Radioactivity and LY248686 Plasma Concentrations in Rats Administered a Single 5 mg/kg (Base Weight) Oral Dose of 14C- LY248686 Maleate or 14 C- LY248686 Hydrochloride	ADME Rpt 9	
Plasma Concentrations of LY248686, 1-Naphthol and — in Rats Given a Single 5 mg/kg Dose of LY248686 or —	ADME Rpt 29	
Whole Blood Concentrations of Radioactivity in F344 Rats after a Single 5, 10 or 20 mg/kg (Base Weight) Oral Dose of 14C-LY248686 Hydrochloride	ADME Rpt 32	
Plasma Pharmacokinetics of Radioactivity and LY248686 in Nonfasted Female F-344 Rats Following a Single Oral 5 mg/kg (Base Weight) Dose of 14C-LY248686 Hydrochloride	ADME Rpt 38	
Plasma Pharmacokinetics of Radioactivity and LY248686 in Fasted Male F-344 Rats Following (Base Weight) Dose of 14C- LY248686 Hydrochloride a Single Oral 5 mg/kg	ADME Rpt 39	
Plasma Pharmacokinetics of Radioactivity and LY248686 in Mongrel Dogs Following Oral Administration of 5 mg/kg (Base Weight) of 14C- LY248686 Maleate	ADME Rpt 14	
Pharmacokinetics of Radioactivity and LY248686 in Dogs Given a Single 5 mg/kg (Base Weight) Intravenous Dose of 14C- LY248686 Maleate	ADME Rpt 16	
Comparison of Radioactivity and LY248686 Plasma Concentrations in Dogs Administered a Single 5 mg/kg (Base Weight) Oral Dose of 14C-LY248686 Maleate or 14C- LY248686 Hydrochloride	ADME Rpt 18	
A Blood Level Study in CD-1 Mice Given Duloxetine Hydrochloride (LY248686 Hydrochloride) in the diet for up to 18 Months (Study M01393)	ADME Rpt 43	
Plasma Exposure of CD-1 Mice to Duloxetine Following Daily Dietary Doses of 0.01%, 0.03% and 0.08% of the Diet as Duloxetine in the Hydrochloride Form for 3 Months	ADME Rpt 73	
LY248686: Plasma Concentration Data for Two Week Dietary Rat Study R09387	ADME Rpt 21	
LY248686: Plasma Concentration Data for Three Month Rat Dietary Study R23287	ADME Rpt 23	
A Chronic/Oncogenic Study in Fischer 344 Rats Given Duloxetine Hydrochloride (LY248686 Hydrochloride) in the Diet for 2 Years (Study R03893)	ADME Rpt 44	
Blood Concentrations of Radioactivity In Rats Following Repeated Oral Administration of 14C-LY248686 Hydrochloride	ADME Rpt 46	

Study Title	Document/ Study no.	Referenc e
Plasma Pharmacokinetics of Duloxetine (LY248686) in Fischer 344 Rats Following Single and Multiple Intravenous Infusion Doses of 1, 5, and 10 mg/kg Duloxetine as the Hydrochloride Salt for up to 2 Weeks	ADME Rpt 65	
LY248686: Plasma Concentration Data for Two Week Dog Study D04687	ADME Rpt 22	
Plasma Concentrations of LY248686 And --- in Dogs after Seven 30 mg/kg/day Oral Doses of LY248686 Maleate	ADME Rpt 28	
Plasma Pharmacokinetics of Duloxetine (LY248686) in Dogs Following Single and Multiple Intravenous Infusion Doses of 1, 2.5, and 5 mg/kg Duloxetine as the Hydrochloride Salt for up to 2 Weeks	ADME Rpt 66	
Tissue Concentrations of Radioactivity in Rats after a Single 5 mg/kg (Base Weight) Oral Dose of 14C-LY248686 Maleate	ADME Rpt 12	
Correlation of the Inhibition of Serotonin Uptake in Cerebral Cortex Homogenates With Plasma and Cortex Concentrations Of LY248686 and Radioactivity after A Single 20 mg/kg (Base Weight) Oral Dose 14C-LY248686 Maleate to Rats	ADME Rpt 20	
Protein Binding of LY248686 in Rat, Dog and Human Plasma	ADME Rpt 31	
Amendment 1: Intestinal Absorption Site of 14C-Duloxetine Hydrochloride in Rats	ADME Rpt 34	
Amendment 1: Tissue Concentrations of Radioactivity in Rats after a Single 5 mg/kg (Base Weight) Oral Dose of [14C-Naphthyl]-Duloxetine Hydrochloride	ADME Rpt 35	
Determination of <i>In vitro</i> and Ex Vivo Protein Binding of Radioactivity Derived from 14C-Duloxetine in Rat Plasma	ADME Rpt 41	
Tissue Distribution of Radioactivity in Rats Following Repeated Oral Administration of 14C-LY248686 Hydrochloride	ADME Rpt 48	
Whole-body Autoradiographic Distribution of 14C-LY248686 Hydrochloride in Rats	ADME Rpt 49	
Tissue Distribution of Radioactivity in Rats Following Single Oral Administration of 14C-LY248686 Hydrochloride	ADME Rpt 50	
Whole-body Autoradiographic Distribution of 14C-LY248686 Hydrochloride in Rats Following Single Oral Administration of 14C-LY248686 Hydrochloride	ADME Rpt 52	
Milk Excretion of Radiocarbon in Lactating Rats Following a Single Oral 5-mg/kg Dose of [14C] Duloxetine ([14C]LY248686) Administered as the Hydrochloride Salt	ADME Rpt 53	
Whole-body Autoradiographic Tissue Distribution and Placental Transfer of Radiocarbon Following the Administration of a Single Oral 45 mg/kg Dose of 14C-Duloxetine (LY248686) as the Hydrochloride Salt to Pregnant CD Rats on Gestation Day18	ADME Rpt 54	

Study Title	Document/ Study no.	Referenc e
Amendment 1: Quantitative Whole Body Autoradiographic Disposition of [14C] Duloxetine in Male and Female Fischer 344 Rats After a Single Oral 5 mg/kg Dose as the Hydrochloride Salt	ADME Rpt 55	
<i>In vitro</i> Protein Binding of 14C-Duloxetine in Mouse, Rat, Dog, and Human Plasma at a Concentration of 150.2 ng/mL	ADME Rpt 62	
Whole-body Autoradiographic Disposition of 14C-Hydrochloride Following Oral Administration to a Fischer 344R	Tox Rpt 23	
Placental Transfer of Radiocarbon Following the Administration of a Single Oral Dose of 14C-Duloxetine (LY248686 Hydrochloride) to Pregnant CD Rats	Tox Rpt 29	
Identification of Metabolites in Plasma, Urine, and Feces from Mice Following a Single Oral Dose of 10 mg/kg 14C-Duloxetine as the Hydrochloride Salt	ADME Rpt 71	
Metabolism of LY248686 in the Rat	ADME Rpt 13	
Enzyme Induction in Rats by Compound LY255485. Study R20187	ADME Rpt 24	
Enzyme Induction in Rats by LY248686 Hydrochloride. Study R10790	ADME Rpt 25	
Identification of Metabolites in Plasma, Urine, and Feces from Female Fischer Rats Administered a Single Oral Dose of 10 mg/kg 14C-Duloxetine as the Hydrochloride Salt	ADME Rpt 60	
Metabolites in the Bile, Urine and Plasma After Oral Administration of [14C-alkyl]- LY248686 to Rat	ADME Rpt 68	
Metabolites in the Bile, Urine and Plasma After Oral Administration of [14C-naphthyl]-LY248686 to Rat	ADME Rpt 69	
Determination of the Biliary Excretion and Metabolism of Duloxetine in Female Fischer 344 Rats Following a Single Oral Dose of 10 mg/kg 14C-Duloxetine as the Hydrochloride Salt	ADME Rpt 76	
Enzyme Induction in Dogs by Compound LY248686. Study D01488	ADME Rpt 26	
Enzyme Induction in Dogs by Compound LY248686 Hydrochloride. Study D04590	ADME Rpt 27	
Amendment 1: Identification of Metabolites in Plasma, Urine, and Feces from Female Dogs Administered a Single Oral Dose of 5 mg/kg 14C-Duloxetine as the HCl Salt	ADME Rpt 61	
Amendment 1: Determination of the Biliary Excretion and Metabolism of Duloxetine in Male Beagle Dogs Following a Single Oral Dose of 5 mg/kg 14C-Duloxetine as the Hydrochloride Salt	ADME Rpt 74	
Disposition of Radioactivity and Identification of Metabolites in Plasma, Urine, and Feces from Female Cynomolgus Monkeys Following a Single Nasogastric Dose of 5 mg/kg 14C-Duloxetine as the Hydrochloride Salt	ADME Rpt 75	

Study Title	Document/ Study no.	Referenc e
Metabolism of LY248686 to 1-Naphthol <i>In vitro</i>	ADME Rpt 30	
The <i>In vitro</i> Metabolism of LY248686 and Thienyl Alcohol	ADME Rpt 67	
Elimination of Radioactivity by Mice Following Oral Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 4	
Elimination of Radioactivity from Mice Following a Single Oral Dose of 10 mg/kg Duloxetine as the Hydrochloride Salt	ADME Rpt 59	
Elimination of Radioactivity by Rats Following Oral Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 6	
Elimination of Radioactivity in Rats after Intravenous Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 8	
Amendment 1: Comparison of Elimination of Radioactivity in Rats Administered a Single 5 mg/kg (Base Weight) Oral Dose of 14C-LY248686 Maleate or 14C-LY248686 Hydrochloride	ADME Rpt 10	
Biliary Excretion of Radioactivity after a Single 5 mg/kg (Base Weight) Oral Dose of 14C LY248686 Maleate to Rats	ADME Rpt 11	
Enterohepatic Elimination and Reabsorption of Radioactivity in Rats Administered 14C-LY248686 Hydrochloride	ADME Rpt 33	
Elimination of Radioactivity in Rats after a Single 5 mg/kg (Base Weight) Oral Dose of [14C-Naphthyl]-Duloxetine Hydrochloride	ADME Rpt 36	
Elimination of Radioactivity by Nonfasted Female F-344 Rats Following a Single Oral Dose of 5 mg/kg (Base Weight) of 14C-LY248686 Hydrochloride	ADME Rpt 37	
Urinary and Fecal Excretion of Radioactivity in Rats Following Repeated Oral Administration of 14C-LY248686 Hydrochloride	ADME Rpt 47	
Biliary Excretion of Radioactivity in Rats Following Single Oral Administration of 14C-LY248686 Hydrochloride	ADME Rpt 51	
Elimination of Radioactivity from Female F344 Rats Following a Single Oral Dose of 10 mg/kg 14C-LY248686 as the HCl Salt	ADME Rpt 56	
Elimination of Radioactivity by Mongrel Dogs Following Oral Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 15	
Elimination of Radioactivity by Dogs Following Intravenous Administration of 5 mg/kg (Base Weight) of 14C-LY248686 Maleate	ADME Rpt 17	
Amendment 1: Comparison of Elimination of Radioactivity in Dogs Administered a Single 5 mg/kg (Base Weight) Oral Dose of 14C-LY248686 Maleate or 14C-LY248686 Hydrochloride	ADME Rpt 19	

Study Title	Document/ Study no.	Reference
2-Week Intravenous Toxicity Study with Duloxetine (LY248686) Hydrochloride in Dogs	Tox Rpt 48	
<u>Genetic toxicology</u>		
The Effect of LY248686 Maleate on the Induction of Reverse Mutations in Salmonella typhimurium and Escherichia coli Using the Ames Test	Tox Rpt 4	
The Effect of LY248686 Maleate on Induction of Forward Mutation at the Thymidine Kinase Locus of L5278Y Mouse Lymphoma Cells	Tox Rpt 1	
The Effect of LY248686 Maleate on the Induction of DNA Synthesis in Primary Cultures of Adult Rat Hepatocytes	Tox Rpt 2	
The Effect of LY248686 Maleate on <i>In vitro</i> Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters	Tox Rpt 3	
The Effect of LY248686 Hydrochloride on the Induction of Reverse Mutations in Salmonella typhimurium and Escherichia coli Using the Ames Test	Tox Rpt 24	
The Effect of LY248686 Hydrochloride on the <i>In vitro</i> Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells	Tox Rpt 30	
The Effect of LY248686 Hydrochloride on the <i>In vivo</i> Induction of Micronuclei in Bone Marrow of ICR	Tox Rpt 20	
<u>Carcinogenicity</u>		
Oncogenic and Blood Level Studies in CD-1 Mice Given Duloxetine in the Diet for Their Life	Tox Rpt 43	
A Chronic/Oncogenic Study in Fischer 344 Rats Given Duloxetine in the Diet for 2 Years	Tox Rpt 44	
<u>Reproductive toxicology</u>		
A 14-Week Male Fertility Study of LY248686 Hydrochloride Administered by Oral Gavage to CD Rats	Tox Rpt 25	
A 10-Week Female Basic Fertility Study of LY248686 Hydrochloride Administered by Oral Gavage to Female CD Rats	Tox Rpt 28	
A Developmental Toxicity Study of LY248686 Hydrochloride Administered Orally to CD Rats	Tox Rpt 26	
A Developmental Toxicity Study of LY248686 Hydrochloride Administered Orally to New Zealand White Rabbits	Tox Rpt 27	
A Fertility and Developmental Toxicity Study of Duloxetine Hydrochloride (LY248686 Hydrochloride) Administered Orally to Female CD Rats	Tox Rpt 35	
<u>Special toxicology</u>		
A Study of the Immune Response in CD-1 Mice Treated Orally with LY248686 Maleate	GenPharmRpt 18	
Antigenicity Study of LY248686 in Guinea Pigs	Tox Rpt 40	
Antigenicity Study of LY248686 in Mice	Tox Rpt 42	

2.6.2 PHARMACOLOGY:

Supplemental pharmacology studies were submitted for review that were conducted after filing of NDA 21-247. The findings do not provide any significantly new information but used new ligands or techniques. All results and were confirmatory of previous findings.

In vitro binding studies: Duloxetine binding was assessed using membrane preparations of HEK-293 cells expressing human norepinephrine transporter (hNET), h-serotonin transporter (hSERT), and h-dopamine transporter (hDAT) sites. Duloxetine (LY248686 hydrochloride) was a potent inhibitor ($K_i = 1.45 \pm 0.2$ nM) of the hSERT transporter receptor sites, hNET ($K_i = 18.2 \pm 0.8$ nM) transporter receptor sites, but was a weak inhibitor of hDAT transporter sites ($K_i = 247 \pm 12$ nM). Duloxetine is a reported dual reuptake inhibitor of both serotonin and norepinephrine with very little activity on the dopamine transporter. This study confirms previous results. Duloxetine, as expected, had very little muscarinic activity with K_i values $>20,000$ nM when assessed using human M1-M5 expressed receptors.

In vivo binding studies: LY248686 hydrochloride was found, *in vivo*, to occupy brain serotonin and norepinephrine transporters following i.v. (0.01-10 mg/kg) and oral (30 mg/kg) administration in Sprague Dawley rats. New "tracers", MeNER (methyl-norethyl reboxetine, 3 μ g/kg) which illuminates norepinephrine transporter (NET) sites and DASB (N,N-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine, 10 μ g/kg) which illuminates serotonin transporter sites, were used. LY248686 hydrochloride pretreatment blocked the binding of the MeNER tracer to hypothalamic NET sites with an estimated ED_{50} of 0.2 mg/kg (i.v.) and at a dose of 30 mg/kg (oral). Cortical SERT sites binding of DASB were blocked by LY248686 hydrochloride pretreatment with an estimated ED_{50} of 0.08 mg/kg (i.v.) and at a dose of 30 mg/kg (oral). LY248686 hydrochloride occupied both brain SERT and NET sites in the Sprague Dawley rat prefrontal cortex after its peripheral administration (i.v. and oral).

Citalopram, thought to be a potentially useful tracer for SERT sites in rats but not humans, was used to determine the cortical SERT binding in fasted Sprague Dawley rats. LY248686 hydrochloride (oral) occupied SERT sites with an estimated ED_{50} of 0.1 mg/kg. For reasons that are not well understood, this potency is much greater than that seen for LY248686 hydrochloride in subsequent studies using the better established SERT site tracer, DASB.

LY248686 hydrochloride pretreatment (oral) did not block the binding of the nomifensin, a receptor occupancy tracer for dopamine transporter (DAT) sites. The study results confirm that LY248686 hydrochloride does not have significant occupancy of DAT sites.

In microdialysis studies in male Sprague Dawley rats where extracellular concentrations of serotonin (5-HT) and norepinephrine (NE) in prefrontal cortex were quantified duloxetine (15 mg/kg, i.p.) increased, approximately equally, serotonin and norepinephrine extracellular concentrations.

The depletion of rat cortical norepinephrine concentrations after α -methyl-m-tyrosine (α -MMT) administration into rats was antagonized by pretreatment with duloxetine (LY248686) with an ED₅₀ of 15.7 mg/kg, 14.9 mg/kg, and 2.3 mg/kg for oral, i.p., and s.c., respectively. The data show duloxetine, regardless of the route of administration, antagonizes the depletion of rat cortical norepinephrine produced by α -MMT

Oral administration of duloxetine (LY248686) produced a dose-dependent increase in extracellular levels of 5-HT and NE in the rat medial prefrontal cortex at doses ≥ 10 mg/kg. However, while the effect of duloxetine on 5-HT levels was maximal at 10 mg/kg, this dose was submaximal for its effects on NE. The difference in the dose required for a maximal 5-HT and NA response suggests that duloxetine is more efficacious as an inhibitor of 5-HT reuptake *in vivo*.

Neuropathic pain *in vivo* animal models: Duloxetine HCl (LY246916) had previously been shown to effectively reverse mechanical allodynia behavior in the partial sciatic nerve ligation model (Seltzer model) and the L5/L6 spinal nerve ligation model (Chung model) of neuropathic pain after intraperitoneal and oral administration. Overall the studies reviewed in this NDA extend the doses previously studied.

Duloxetine HCl (LY246916) (5, 10, 20, and 30 mg/kg orally) showed dose-dependent reversal of mechanical allodynia behavior as graded by von Frey filaments in the partial sciatic nerve ligation (Seltzer model) by 4 hours after administration. Reversal of mechanical allodynia was also observed following i.p. (10 and 20 mg/kg) administration in this model.

Duloxetine HCl (LY246916) (10, 20, and 30 mg/kg orally) in the L5/L6 spinal nerve-ligated (Chung model) rats as graded by von Frey filaments showed dose-dependent reversal of mechanical allodynia behavior by 30 mins at a dose of 10 mg/kg dose but at 2 hrs with a dose of 30 mg/kg. Effects persisted for 6 hours after administration. Duloxetine HCl (LY246916) (50, 100, and 200 nmoles intra-cisternally) showed reversal of mechanical allodynia at 30 mins after concentrations ≥ 50 nmoles with maximal effects at 4 hrs at a concentration of 100 nmoles. Subcutaneous (30 mg/kg) administration in this model also showed reversal of mechanical allodynia.

Duloxetine HCl (LY246916) has previously been shown to be effective in the formalin model of persistent pain in rats. In the current study, duloxetine was tested in an automated version of the rat formalin test in Sprague Dawley male rats as follows: 1) systemic administration [subcutaneous (s.c.), intraperitoneal (i.p.), or oral gavage (p.o.) doses ranging from 3 to 50 mg/kg] in order to determine the dose response and time course of effects by different routes; and 2) local central and peripheral administration [intracerebroventricular (i.c.v., 60 to 200 nmoles), intra-cisternal (1 to 100 nmoles), intrathecal (i.t., 100 to 300 nmoles) and intraplantar (200 nmoles)]. The effects of duloxetine following i.p. and oral administration in the automated formalin test are consistent with data reported earlier using the manual formalin method. The significant effects of duloxetine after central administration,

especially intra-cisternal administration, and the lack of effects after direct intraplantar administration suggests that the systemic effects of duloxetine likely occurs within the central nervous system. Overall, the results continue to provide consistent evidence for the efficacy of duloxetine in the formalin model of persistent pain.

In order to determine the specificity of the effects of duloxetine in the formalin test, duloxetine was also evaluated in the rotorod test after oral (3, 10, 30 mg/kg p.o.), and intra-cisternal administration (60 and 100 nmoles). Duloxetine did not cause motor impairment in the rotorod test after oral administration, but had a small, short-lived and reversible affect on performance when administered intra-cisternally at a concentration of 100 nmoles.

Duloxetine HCl (LY246916) was studied in the tail-flick test in Harlan Sprague Dawley rats after oral (3, 10, 20, 30 mg/kg) and intraperitoneal (3, 10, 15, 20, 30 mg/kg) administration. A small but statistically significant increase in tail-flick latency was observed at doses ≥ 20 mg/kg, orally, 1 hr after administration. Tail-flick latency was increased following i.p. administration at 30 mins for all doses.

2.6.3 PHARMACOLOGY TABULATED SUMMARY: No new studies were submitted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS:

Three new PK/TK studies and three analytical method reports were submitted to NDA 21-733 since the original filing of NDA 21-247. The new PK/TK studies provide systemic exposure information for the duloxetine and its glucuronide conjugate, 4-hydroxy duloxetine (also called 550408) in mice and rats, and in pregnant rabbits.

1-month dietary mouse study:

CD-1 mice received duloxetine hydrochloride at 0.01%, 0.03%, or 0.08% in the diet for 1-month. TK was evaluated on study days 1 and 30. The $AUC_{(0-24hr)}$ values for duloxetine appeared to increase with dose, but the increase was not dose proportional. C_{max} and $AUC_{(0-24hr)}$ values were comparable at the 0.01% and 0.03% groups for male and female mice on both study days. At 0.08% male mice C_{max} and $AUC_{(0-24hr)}$ were higher than the female mice values on both study days. $AUC_{(0-24hr)}$ values for duloxetine were lower on SD30 than on SD1 indicating probable enzyme induction.

The exposure to the 4-hydroxyduloxetine glucuronide conjugate (4-OH) metabolite appeared to increase with increasing doses of duloxetine. Plasma concentrations of the 4-OH metabolite were consistently higher than duloxetine in all dose groups in both male and female mice. In the 0.01% group, the AUC values of the 4-OH metabolite was generally 10 to 15 fold higher than the AUC exposure to duloxetine. AUC exposure to the 4-OH metabolite in the 0.03% group were generally 6- to 12-fold higher, and were 3-to 7-fold higher in the 0.08% group. The concentrations of the 4-OH metabolite were similar on SD30 and SD1 in all dose groups. The exposure

of male mice to the 4-OH metabolite was consistently higher than the exposure in female mice, especially in the 0.08% group. Other metabolites of duloxetine found in mouse plasma include the glucuronide conjugate of 6-hydroxy duloxetine and the des (aminomethyl) acid metabolite.

Summary Table for Duloxetine

Parameter ^a	Sex	Administered Dose					
		0.01% Diet		0.03% Diet		0.08% Diet	
		M	F	M	F	M	F
Day 1							
C_{max} (ng/mL)		78.8	66.2	261.7	240.4	904.3	810.5
$AUC_{(0-24hr)}$ (ng·hour/mL)		1077.7	1039.3	4466.8	3677.4	15167.7	10871.2
AUC CV (%)		15.8	14.3	10.8	7.4	15.9	19
Day 30							
C_{max} (ng/mL)		56.1	51.1	192.6	185.4	575.0	433.4
$AUC_{(0-24hr)}$ (ng·hour/mL)		730.5	857.2	2907.1	2686.5	8698.2	6129.1
AUC CV (%)		13.6	17.3	14.3	14.7	10.2	15.9

Abbreviations: M = male; F = female; C_{max} = the maximum observed plasma concentration; $AUC_{(0-24hr)}$ = the area under the plasma concentration-time curve from 0 hours (8:00 AM) to 24 hours (8:00 AM) the following day; AUC CV = Coefficient of variation associated with the area under the plasma concentration-time curve.

^a = Parameter values were calculated from the grouped data in which there were usually 3 mice per time point.

Summary Table for the Glucuronide Conjugate of 4-Hydroxy Duloxetine

Parameter ^a	Sex	Administered Dose					
		0.01% Diet		0.03% Diet		0.08% Diet	
		M	F	M	F	M	F
Day 1							
C_{max} (ng/mL)		884.9	689.7	1737.0	1118.0	3030.3	2175.7
$AUC_{(0-24hr)}$ (ng·hour/mL)		16475.4	13384.1	32821.5	23483.5	60571.3	42171.8
AUC CV (%)		8.5	8.6	9.3	9.1	8.2	7.9
Day 30							
C_{max} (ng/mL)		844.1	722.6	1559.3	1361.0	3201.7	2088.7
$AUC_{(0-24hr)}$ (ng·hour/mL)		15638.8	13930.1	33918.9	26848.3	61549.3	40369.3
AUC CV (%)		7.1	5.7	7.5	5.3	6.7	4.7

Abbreviations: M = male; F = female; C_{max} = the maximum observed plasma concentration; $AUC_{(0-24hr)}$ = the area under the plasma concentration-time curve from 0 hours (8:00 AM) to 24 hours (8:00 AM) the following day; AUC CV = Coefficient of variation associated with the area under the plasma concentration-time curve.

^a = Parameter values were calculated from the grouped data in which there were usually 3 mice per time point.

Rat study:

Fisher F344 rats received duloxetine hydrochloride at 0.01%, 0.02%, or 0.08% for male rats and 0.01%, 0.02%, or 0.05% for female rats in the diet for 1-month. TK was evaluated on study days 1 and 30. The $AUC_{(0-24hr)}$ values for duloxetine appeared to increase with dose, but the increase was not dose proportional. $AUC_{(0-24hr)}$ values were higher on SD30 than SD1 in male rats at a dose of 0.08% and in all dose groups for females indicating probable enzyme induction. Exposure in male rats at 0.01% and 0.02% were comparable on SD1 and

SD30. There were no consistent differences in the exposure to duloxetine between male and female rats in the 0.01% and 0.02% groups.

The exposure to the 4-OH metabolite appeared to increase with increasing doses of duloxetine. Plasma concentrations of the 4-OH metabolite were consistently higher (1.6- to 9.1-fold) than duloxetine in the 0.01% and 0.02% groups in both male and female rats. The 4-OH metabolite concentrations were similar or less than the duloxetine concentrations in the 0.08% group in both male and female rats. The AUC_(0-24hr) values for the 4-OH metabolite were higher on SD30 than on SD1 in all dose groups for the females and in the 0.08% group males. Female rats in all dose groups tended to have higher exposure to the 4-OH metabolite than male rats.

Summary Table for Duloxetine

Parameter ^a	Administered Dose					
	Group 1		Group 2		Group 3	Group 4
	0.01		0.02		0.05	0.08
% Diet						
Sex	M	F	M	F	F	M
Day 1						
C _{max} (ng/ml)	17.0	12.3	135.0	115.1	334.2	617.4
AUC _(0-24hr) (ng·hour/ml)	299.1	220.9	1904.3	1518.7	5849.1	10345.1
AUC CV (%)	19.1	10.8	8.9	22.7	10.2	12.9
Day 30						
C _{max} (ng/ml)	20.7	33.1	149.4	227.1	721.6	878.6
AUC _(0-24hr) (ng·hour/ml)	254.3	463.1	2240.6	3030.8	12866.7	18404.1
AUC CV (%)	31.8	18.9	16.5	14.4	6.9	5.2

Abbreviations: M = male; F = female; C_{max} = the maximum observed plasma concentration; AUC_(0-24hr) = the area under the plasma concentration-time curve from 0 hours (8:00AM) to 24 hours (8:00AM the following day); AUC CV = Coefficient of variation associated with the area under the plasma concentration-time curve.

^a Parameter values were calculated from the grouped data in which there were usually 3 rats per time point.

Summary Table for the Glucuronide Conjugate of 4-Hydroxy Duloxetine

Parameter ^a	Administered Dose					
	Group 1		Group 2		Group 3	Group 4
	0.01		0.02		0.05	0.08
% Diet						
Sex	M	F	M	F	F	M
Day 1						
C _{max} (ng/ml)	93.0	89.4	154.2	215.3	322.6	347.7
AUC _(0-24hr) (ng·hour/ml)	1610.9	2006.0	3126.9	4184.0	6986.9	6944.7
AUC CV (%)	10.9	8.5	5.6	10.4	10.6	6.1
Day 30						
C _{max} (ng/ml)	86.6	161.9	202.1	266.9	500.6	494.0
AUC _(0-24hr) (ng·hour/ml)	1619.5	2730.1	3802.6	5913.7	11294.7	10113.7
AUC CV (%)	9.2	6.4	7.1	5.5	6.6	4.3

Abbreviations: M = male; F = female; C_{max} = the maximum observed plasma concentration; AUC_(0-24hr) = the area under the plasma concentration-time curve from 0 hours (8:00AM) to 24 hours (8:00AM the following day); AUC CV = Coefficient of variation associated with the area under the plasma concentration-time curve.

^a Parameter values were calculated from the grouped data in which there were usually 3 rats per time point.

Rabbit study:

Pregnant New Zealand rabbits received duloxetine hydrochloride daily via oral gavage at doses of 2, 10, or 45 mg/kg during gestation days (GD) 7-19. TK was evaluated on GD19. T_{max} for duloxetine and the 4-OH metabolite was 1 hour except for 1 animal (Animal 38242) which had a T_{max} of 2 hours. The plasma concentrations of the 4-OH metabolite were much higher than the duloxetine concentrations. These data indicate that duloxetine is rapidly and extensively metabolized in rabbits. Both the mean C_{max} and $AUC_{(0-24\text{ hr})}$ values for duloxetine increased with increasing doses, but the increases were not proportional to the dose increases. The exposure of the rabbits to the 4-OH metabolite also increases with increasing doses of duloxetine.

The plasma concentrations of the sulfate conjugate of 5-hydroxy, 6-methoxy duloxetine were usually below the lower limit of quantitation of g/mL. Quantifiable concentrations of this metabolite were only found in 8 samples with concentrations below 3 ng/mL indicating little exposure to this metabolite.

Other metabolites detected in the rabbit plasma but not quantitated were dihydrodiol of duloxetine, the glucuronide conjugate of dihydrodiol duloxetine, the glucuronide conjugate of dihydroxy duloxetine (the same isomer as that found in human plasma), the glucuronide conjugate of 5-hydroxy, 6-methoxy duloxetine and the glucuronide conjugate of 6-hydroxy, 5-methoxy duloxetine.

Gestation Day 19 Parameters ^a	Administered dose (mg/kg/day)		
	2	10	45
<u>Duloxetine</u>			
C_{max} (ng/mL)	26 ± 9.6	27.8 ± 6.6	60.5 ± 14.9
$AUC_{(0-24\text{ hr})}$ (ng·hr/mL)	11.9 ± 1.9	94.3 ± 31.3	367.0 ± 57.1
<u>Glucuronide conjugate of 4-hydroxy duloxetine</u>			
C_{max} (ng/mL)	16.4 ± 5.5	300.8 ± 124.0	3285.3 ± 665.6
$AUC_{(0-24\text{ hr})}$ (ng·hr/mL)	93.0 ± 22.5	822.8 ± 195.7	9100.4 ± 1071.0

Abbreviations: C_{max} = maximum observed plasma concentration; $AUC_{(0-24\text{ hr})}$ = area under the plasma concentration-time curve from 0 hour to 24 hours.

^aValues are mean values ± standard deviation (N = 4 animals/group).

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: No new information.

Genetic toxicology: No new information. 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*.

Carcinogenicity: 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 there was an increased incidence of hepatocellular adenomas and carcinomas; the no-effect level was approximately 50 mg/kg. Tumor incidence was not increased in male mice receiving duloxetine at dietary doses up to approximately 100 mg/kg/day. In rats, dietary doses of duloxetine up to approximately 27 mg/kg/day in females or approximately 36 mg/kg/day in males did not increase the incidence of tumors.

Reproductive toxicology: Duloxetine administered orally to either male or female rats prior to and throughout mating at daily doses up to 45 mg/kg did not alter mating or fertility. 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. However, fetal weights were decreased at this dose, with a no-effect level of 10 mg/kg. 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, 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.

Special toxicology: No new information.

2.6.6.2 Single-dose toxicity: No new studies were submitted.

2.6.6.3 Repeat-dose toxicity: No new studies were submitted.

2.6.6.4 Genetic toxicology: No new studies were submitted.

2.6.6.5 Carcinogenicity: No new studies were submitted.

2.6.6.6 Reproductive and developmental toxicology: No new studies were submitted.

2.6.6.7 Local tolerance: No new studies were submitted.

2.6.6.8 Special toxicology studies: No new studies were submitted.

2.6.6.9 Discussion and Conclusions:

Confirmatory studies showed that duloxetine hydrochloride is a serotonin and norepinephrine uptake inhibitor both *in vitro* and *in vivo*. Also confirmed prior findings of reversal of mechanical allodynia in the Seltzer and Chung neuropathic pain animal models, affected locomotor activity, TF latency, and formalin test.

CD-1 mice and Fisher F344 rats given duloxetine hydrochloride for 1-month in the diet and in pregnant New Zealand white rabbits given duloxetine

hydrochloride orally during gestation days 7-19 duloxetine showed an increase, though not proportional, in duloxetine and glucuronide conjugated 4-hydroxy duloxetine metabolite. Exposure of duloxetine was decreased with repeat dosing indicating enzyme induction. The glucuronide conjugated 4-hydroxy duloxetine exposure was higher than duloxetine.

2.6.6.10 Tables and Figures: Not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY: Not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

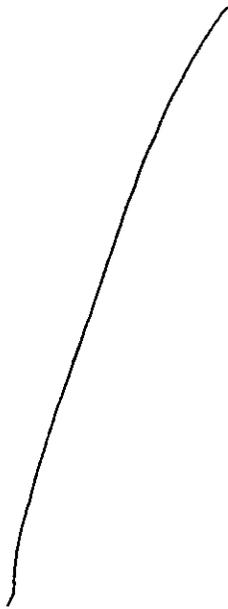
No significant new information is contained in this NDA. Included in this NDA are confirmatory pharmacology studies and PK data for duloxetine and its major glucuronide metabolites from 1-month dietary studies in mice and rats, and in pregnant rabbits. All pertinent information and reviews can be found in Dr. Fossom's Pharmacology and Toxicology Review of NDA 21-427 from HFD-120. HFD-120 approved NDA 21-427 on 03 August 2004 for major depressive disorder.

Unresolved toxicology issues: There are no unresolved toxicology issues that are pertinent to the approval of this NDA. See Executive Summary for unresolved issues for NDA 21-556 (HFD-580).

Recommendations: None at this time.

Suggested labeling:

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY



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 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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/s/

Suzanne Thornton-Jones
8/13/04 10:55:01 AM
PHARMACOLOGIST

R. Daniel Mellon
8/13/04 10:57:25 AM
PHARMACOLOGIST
I concur

45 DAY MEETING CHECKLIST
NDA 21-733

FILEABILITY:

On initial overview of the NDA application:

PHARMACOLOGY AND TOXICOLOGY:

- (1) On its face, is the pharmacology section of the NDA organized in a manner to allow substantive review to begin? **Yes.**
- (2) Is the pharmacology section of the NDA indexed and paginated in a manner to allow substantive review to begin? **Yes.**
- (3) On its face, is the pharmacology section of the NDA legible so that substantive review can begin? **Yes.**
- (4) Are all required (*) and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity*, effects on fertility*, juvenile studies, acute adult studies*, chronic adult studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetics studies, etc)? **No non-clinical studies were submitted for this NDA, but the Sponsor provides a cross-reference to NDA 21-427 which is held by the Sponsor.**
- (5) If the formulation to be marketed is different from the formulation used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies using the marketed product or to explain why such repetition should be required? **Not applicable.**
- (6) Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in either mg/m² or comparative serum/plasma levels) and in accordance with 201.57? **Yes.**
- (7) Has the sponsor submitted all special studies/data requested by the Division during Pre-submission discussions with the sponsor? **—**
- (8) On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted rationale to justify the alternative route? **Yes.**
- (9) Has the sponsor submitted a statement(s) that all the pivotal Pharm/Tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? **No.**
- (10) Has the sponsor submitted a statement(s) that the Pharm/Tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns? **No.**
- (11) From a pharmacology perspective, is this NDA fileable? If "no", please state below why it is not. **Yes**

Reviewer Signature Suzanne R. Thornton-Jones, Ph.D. Date: 19 April 2004

Supervisor Signature R. Daniel Mellon, Ph.D. Date: 19 April 2004

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Suzanne Thornton-Jones
4/19/04 04:15:33 PM
PHARMACOLOGIST

R. Daniel Mellon
4/19/04 05:39:48 PM
PHARMACOLOGIST
I concur